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Nutritional, Anti-nutritional and Phytochemical Profile of the Leaves and Fruits of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell

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Abstract

Synsepalum dulcificum (Sapotaceae) also known as a miracle berry is an evergreen shrub that is native to tropical West Africa. This berry has an attribute of modifying sour taste into a sweet taste. This study investigated the nutritional, anti-nutritional and phytochemical compositions of the leaves and whole fruit of *Synsepalum dulcificum*. Proximate composition (moisture content, crude protein, fats, crude fiber, carbohydrate and ash content); Mineral composition (calcium, magnesium, potassium, sodium, zinc, iron, copper and manganese); Anti-nutritional factors (oxalate, phytate, nitrate) and Qualitative and Quantitative phytochemicals assays (flavonoids, phenol, alkaloids, saponin, glycoside and tannin) were determined using standard methods. Results shows the following trend for Proximate composition: carbohydrate (40.38 and 17.44)%, crude fiber (17.58 and 3.63)%, crude protein (8.42 and 10.26)%, ash content (2.87 and 3.95)%, crude fat (1.97 and 2.32)% and moisture content (28.78 and 62.40)%; Mineral content: macronutrient - calcium (4100 and 1395) mg/kg, magnesium (300.8 and 2678.8) mg/kg, potassium (2500 and 2900) mg/kg, sodium (1986 and 1531) mg/kg, micronutrients - zinc (52 and 17) mg/kg, iron (243 and 102) mg/kg, copper (48 and 89) mg/kg, manganese (44 and 34) mg/kg; Anti-nutrients: oxalate (31.5 and 28.5) mg/100g, phytate (59.9 and 34.7) mg/100g, nitrate (180 and 95) mg/100g; Phytochemicals: alkaloid (0.90 and 1.37)%. flavonoids (0.12 and 0.58)%, cardiac glycosides (0.06 and 0.14)%, tannins (0.65 and 0.34)%, phenol (0.147 and 0.231)% and anthraquinones (0.006 and Not Detected)% for the leaves and whole fruits respectively. *S. dulcificum,* leaves, and whole fruit could potentially be a good source of nutrients with minimal levels of anti-nutritional factors thus, safe for human consumption.

Keywords

Synsepalum dulcificum, Proximate, Anti-nutrition, Mineral Elements, Phytochemicals

1. Introduction

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves or other parts of plants that interplay with nutrients and dietary fiber and capable of preventing and treating most oxidative related diseases [1-2]. Some of the well-known phytochemicals include; lycopene found in tomatoes, isoflavones in soy and flavonoids in fruits. Most phytochemicals are known to possess many protective properties which makes them vital to both plants and animals. Some of these properties include anti-inflammatory, anti-oxidant, anti-microbial, antiviral, and anti-carcinogenic properties [3].

Anti-nutrients or anti-nutritional factors are compounds or substances which act to reduce nutrient intake, digestion, absorption, and utilization or produce other adverse effects. These anti-nutrients are potentially harmful in that they reduce growth and healing by affecting the availability of nutrients to the body especially if present in high levels [4-5]. The major anti-nutrients including phytate, oxalates, nitrate, lectins, trypsin inhibitor, protease inhibitors, chlorogenic acid, and amylase inhibitors have an adverse effect on health via inhibition of protein digestion, growth, iron, and zinc absorption.

Synsepalum dulcificum (Schumach. & Thonn.) Daniell is a

plant commonly known as miracle fruit. It is an evergreen shrub of the Sapotaceae family. It originates from West and West-Central tropical Africa. It is about 2-5m (6 to 15 feet) high with the leaves measuring up to 5-10cm in length, 2-3.7cm in width and are glabrous below. It has many slender branches which are clustered at the end of the branchlets. The flowers are white, fruits are about 2-2.5cm long and are red when ripe and each fruit contains one seed [6]. The active component of the berry is the glycoprotein called miraculin, which has no taste in itself [7]. When the fruit pulp is licked it miraculously turns sour foods such as lemons, limes, vinegar, etc. subsequently consumed to taste sweet because miraculin is said to bind to some receptors on the taste buds to activate the sweet receptors hence resulting in a perception of sweet taste [6]. This action of miraculin is usually favored at a low pH (acidic) like that resulting from the ingestion of sour food [8], and the effect lasts until the protein is washed away by saliva up to about an hour. It is called Agbayun (Yoruba, Nigeria), asowa (Twi, Ghana) and ele (Ewe, Ghana). It has also been found useful among diabetics and dieters because studies have shown that the fruit has the ability to improve insulin sensitivity [9-10], and also due to its characteristic of being a low-calorie sweetness enhancer, limiting energy intake [11]. In addition to the value of the fruit as a taste modifier, the skin, pulp, and seeds of miracle berries have been shown to have antioxidant properties, hence, possible benefits to human health [12].

Previous studies have demonstrated the nutritional and phytochemical potential of this plant. Jeremiah *et al.* [13] showed the proximate and mineral composition of the seeds of *S. dulcificum*; Osabor *et al.* [14] reported the proximate and phytochemical contents of the leaves and roots of *S. dulcificum*; Agblekpe *et al*. [15] reported the nutritional values of the three parts of the miracle fruits from Togo; the nutritional composition of the pulp of the berry from Anambra state and Imo was also reported by Nkwocha, [16] and Njoku *et al.* [17] respectively. A detailed review on the health benefit of the berry was presented by Mangla & Kohli, [18], while the toxicological evaluation of the extract of the berry via oxidative stress and hepatotoxicity markers in rats suggests that the berry could boost antioxidant defense and hepatoprotective activity [19].

In the present study, we present the findings on the comparative nutritional, antinutritional and phytochemical composition of the leaves and whole berry of *S. dulcificum* from Ibadan, Nigeria.

2. Materials and Methods

2.1. Sample Collection and Preparation

This study was carried out at the Federal College of Forestry, Ibadan, Nigeria. *S. dulcificum* leaves and fruits were harvested from the plant stands at plant physiology nursery of the Forestry Research Institute of Nigeria and identified by a taxonomist at the taxonomy section of the Institute. The leave samples were air-dried on a cabinet dryer, milled to powdery form and stored in an airtight container for later analysis. The berries were analyzed after crushing together with the pulp and seeds.

2.2. Proximate Analysis

Proximate parameters (protein, ash, moisture, fats, fiber, and carbohydrate,) of the powdered sample and miracle berry were determined using standard procedures as described by the Association of Official Analytical Chemists [20]. The protein content of the samples was determined by the micro-Kjeldhal method, moisture content was determined by oven drying to constant weight, ash content was determined using muffle furnace, fat content was extracted with ether using soxhlet apparatus while carbohydrate was estimated by difference.

2.3. Mineral Analysis

Mineral contents of the samples were determined according to the method used by Oshodi *et al.* [21] with slight modifications. From 2g of powdered and fruit samples, ash residues obtained from the proximate analyses were digested with 10ml of 3M HCl after which the digest was made up to 50ml with deionized water. Salts of the metals to be analyzed were used to make standards and relevant lamps were fixed for the instrumental analysis. This was done for Mg, Ca, K, Na Zn, Cu, Fe, and Mn respectively. The Flame Photometer was used for Na and K.

2.4. Anti-nutritional Analysis

The anti-nutritional analysis for oxalate of the leave and fruit samples was determined by redox titration with potassium permanganate in acidic medium (H_2SO_4) as previously used by Agbaire, [22]; phytate was determined by spectrophotometer in a procedure described by Pearson, [23]; nitrate content in the test samples was determined by the colorimetric method of Sjoberg and Alanka [24].

2.5. Phytochemical Analysis

Qualitative phytochemical screening of the leaves and fruits was determined using the standard methods previously used by Boye *et al.* [25]; and Omoruyi *et al.* [26], while quantitative analyses estimating the quantities of saponin, alkaloids, tannin, phenol, cardiac glycoside, anthraquinone, and flavonoids were determined based on methods of analyses described by AOAC [27].

2.6. Statistical Analysis

Quantitative data were expressed as Mean and Standard deviation of triplicate measurement; Student's t-test was used to determine a significant difference between the mean of measured parameters of leaves and fruits at $p < 0.05$, Microsoft Excel was used for statistical data analysis.

3. Results and Discussion

The result of the proximate analysis for both leaves and

fruit of *S. dulcificum* is presented in Table 1 with the parameters like crude protein, crude fat, carbohydrate, crude fiber and ash having varying quantities both in fruit and leaves. The result for the mineral analysis (macro and micronutrients) are expressed in Table 2. The anti-nutrient content of *S. dulcificum* is presented in Table 3, while the phytochemical screening and analysis are presented in Table 4 and 5 respectively. All the results are expressed as the mean and standard deviation of triplicate measurement.

3.1. Proximate Composition

The proximate composition of any plant material measures the nutritional benefits that are potentially derived from such plant. Protein, a nitrogen-containing class of food has been shown to be essential for the survival of humans and animals [28]. In this study, the protein content was found to be significantly higher ($p < 0.05$) in whole fruit (10.26%) than in leaves (8.42%). However, the levels though slightly higher are comparable with 7.75% reported by Nkwocha *et al*. [16] in the pulp of *S. dulcificum*. This value is higher than that reported for some edible fruits such as *Spondias mombin* (2.6%) and *Dialium guineense* (8.30%) thus, suggesting that the berry is a good source of protein. The protein level in the leaves is also comparable with 6.62% reported by Osabor *et al*. [14], however, this is much lower than the 12.00 - 28.08% range widely reported for *M. oleifera* [29-30] and other leafy vegetables [31-33].

The fat content of 2.32% in the fruit obtained in this study is significantly higher than that in the leaves, however, this value is lower than the 3.26% reported in pulp of *S. dulcificum* [16] and the average of 7.56% reported by Agblekpe *et al.* [15] in the skin, pulp and seeds of the miracle berry. The fat content in leaf (1.97%) is comparably lower than the levels in the leaves as published by Osabor *et al.* [14] and also to that generally reported for *moringa* and some other vegetables [29-33]. Carbohydrates or Saccharides are known to be the most abundant biological molecules. They play vital roles in the body as sources of energy as well as structural materials [28]. The carbohydrate content in both leaf and fruit are 40.38% and 17.44% respectively with significant differences ($p < 0.05$) however, the value obtained in the whole fruit is comparable with 18.84% reported in the pulp of *S. dulcificum* by Nkwocha *et al*. [16]. The carbohydrate content in leaves in this study compares well with that obtained in a similar study [14], the level is also in consonance with that generally reported for *M. oleifera* [29] and *Costus afer* [34]. This suggests that this plant can be used as a source of carbohydrates. Crude fiber is a measure of the quantity of indigestible carbohydrate components present in the plant. It has little food value but provides the bulk necessary for proper peristaltic action in the intestinal tract [35]. A significant difference exists in the crude fiber content of the fruits and leaves. The crude fiber of the fruit (3.63%) in this study is lower than 6.24% reported by Nkwocha *et al.* [16] for the pulp. However, the leaves also contain higher crude fiber (17.58%) which is comparable with the range reported for some leafy medicinal plants of nutraceutical importance such as *Ocimum gratissimum*, *Parquetina nigrescens*, *Vernonia amygdalina* [36]. The ash content is a measure of the total amount of minerals present within a food substance. The ash content of both leaf and fruit are 2.87% and 3.95% respectively, the levels in the leave is quite lower than 6.70% reported in the leaves by Osabor *et al.* [14], this is also far below the range 8.00% - 10.00% found in *M. oleifera* [29] and 8% reported for scent leaf and *Hibiscus esculentus* [37], however, the ash content in the whole fruit is a little lower than the 4.36% obtained in pulp by Nkwocha, [16]. Moisture content is used to predict the stability and susceptibility of a material to microbial decomposition, thus, low moisture content of food would enhance its longevity, storage stability by avoiding mold growth and biochemical reactions. The moisture content of the whole fruit (62.40%) is significantly higher than that on the leaves; the high moisture content of the whole fresh fruit indicates that it is a perishable commodity justifying its rapid degradation after harvesting. However, this value, although higher, is comparable with the range of 45.12-59.55% reported for the fruit pulp [16-17] and 38.08% in the seeds [13]. The moisture content of the leaf sample indicates the presence of moisture in a considerable amount which is lower than that obtained by Osabor *et al.* [14] in the leaves.

Table 1. *Proximate analysis for both leaves and fruit of S. dulcificum.*

Parameter (%)	Leaf	Fruit
Crude Protein	8.42±0.72a	10.26±1.46b
Crude Fat	1.97±0.426a	2.32±0.63b
Carbohydrate	40.38±0.57a	17.44±0.99b
Crude Fiber	17.58±0.42a	3.63±0.35b
Ash Content	2.87±1.24a	3.95±0.25b
Moisture	28.78±1.13a	62.40±1.38b

Means with same different alphabet in the same rows are significantly different at $p < 0.05$.

3.2. Mineral Composition of *Synsepalum dulcificum*

Minerals constitute important nutrients essential for the maintenance of certain physiochemical processes that are very vital to life. Calcium is vital in the formation of bones and teeth as well as regulation of nerve and muscle functions and blood functions, magnesium helps in the maintenance of acid-base equilibrium, plays important role in the metabolism of carbohydrate, protein and lipids, sodium and potassium are important in acid-base balance, cell membrane function and osmotic pressure regulation [38].

The mineral composition of *S. dulcificum* leaves and fruits as shown in Table 4 shows a significant difference between the mineral contents in both leaves and whole fruits. All the macronutrients are needed in large quantities and are very important to the health of humans. The leaves were found to contain higher levels of macro and micro mineral than the whole fruits. The levels of calcium, potassium, magnesium, and sodium reported in the leaves and whole fruits in this study are widely at variance with that reported by Osabor *et al.* [14] and Jeremiah *et al.* [13] where the macro elements

were found in trace amount. Similarly, in the whole fruit, higher concentration of calcium was obtained in this study compared to that reported by Nkwocha, [16] for *S. dulcificum* pulp which contains 100ppm; other microminerals like iron, zinc copper and manganese in this study is comparatively higher compared with those reported for other parts of the plant [13-14, 39]. The mineral composition of the leaves and whole fruits of *S. dulcificum* is lower than those in the leaves of *Azadirachta indica, Leea guineensis* and *Parkia biglobosa* in our previous study where we reported some macro and micro mineral composition [40]. The other Micro minerals like Manganese, Copper, Iron (Fe) and Zinc are present in small quantities and they also play important roles in metabolic activities.

Table 2. *Mineral composition of the leaves and fruits of Synsepalum dulcificum.*

Minerals (mg/kg)	Leaf	Fruit
Macro Elements		
Calcium (Ca)	4100±282a	1395±354b
Magnesium (Mg)	3000.8±131a	2678.8±88b
Potassium	2500±156a	2900±127b
Sodium (Na)	1986±250a	1531±231b
Micro Elements		
Zinc (Zn)	52±4a	17±1b
Iron (Fe)	243±25a	102±2b
Copper (Cu)	48±2a	89±18b
Manganese (Mn)	44±10a	34±6b

Means with different alphabets in the same rows are significantly different at $p < 0.05$.

3.3. Anti-nutrients

Anti-nutrients are undesirable chemical compounds naturally found in plants, which deter the absorption of nutrients in humans; phytate and oxalate are particularly known to form chelates with mineral elements thereby forming poorly soluble compounds which are not readily absorbed from the gastrointestinal tract [41]. In this study, the phytate value of both leaves and fruit are 59.9mg/100g and 34.7mg/100g respectively, this is much lower compared to that reported in the pulp of *S. dulcificum* [17, 39] as well as that reported in the leaves of common medicinal plants like *O. gratissimum, M. scandens* and *L. guineensis* as well as *Dacryodes edulis* where high phytate contents were reported [42-43]. Also, the values for oxalate content in leaves and fruits (31.5mg/100g and 28.5mg/100g) are much lower than those in *Musa sapentum* with a value of 0.72mg/g [44], *Butryospernum parkii* with 1.5mg/g [45], *Spondias mombin* with 0.9mg/g [46]. The range of phytate and oxalate obtained in the leaves and whole fruits of *S. dulcificum* is higher than those reported for common edible leafy vegetables such as *Vernonia amygdalina*, *Teiferia occidentalis, Talinum triangulare* and *Amaranthus spinosus* [22]. Some plants are known to accumulate nitrate into their body tissues such as leaves, roots, stems, and fruits; generally, vegetables and fruits containing nitrate concentrations in the range of 1000 – 5000 mg/kg are classified between high to very high nitrate content plant [47]. The result obtained for nitrate in this study is very much within the very low nitrate content range. Thus, the phytate, oxalate, nitrate content in *Synsepalum dulcificum* fruit and leaves might not pose any health hazard and may not need significant post-harvest processing to reduce the concentration of the antinutritional factors.

Table 3. *Quantitative anti-nutrients for leaves and fruit of S. dulcificum.*

Anti-Nutrients (mg/100g)	Leaves	Fruit
Oxalate	31.5±0.022a	28.5±0.011a
Phytate	59.9±0.019a	34.7±0.013b
Nitrate	180±0.036a	95±0.020b

Means with same alphabets in the same rows are not significantly different at $p < 0.05$.

3.4. Qualitative and Quantitative Phytochemical Composition of *Synsepalum dulcificum*

Phytochemicals in human beings are reported to promote good health and prevent disease development [48]. The qualitative and quantitative phytochemical constituents of the leaves and the whole fruit of *S. dulcificum* was assessed in this study. The phytochemical constituents of the leaves and whole fruits of *S. dulcificum* shows the presence of alkaloids, tannins, flavonoids, cardiac glycosides, saponins and anthraquinone (Table 4). Quantitatively, the result (Table 5) shows the presence of alkaloids, saponin, flavonoids, tannin, polyphenols, and anthraquinone in different quantities. The presence of these compounds may give credence to its local usage for the management of oxidative stress induced ailments. Alkaloids have been shown to have several pharmacological actions such as anti-microbial, antifungal and anti-inflammatory effects; and it also acts as an anti-hypertensive agent [49]. In this study, alkaloid is the phytochemical with the highest concentration (0.90% and 1.37%) for both the leaves and fruit respectively, in which the fruit had the significantly ($p < 0.05$) higher concentration. This is comparatively lower in the work of Sango *et al.* [50] which had a high level of alkaloid for both *S. nigrum* and *C. gynandra.* The leaves and fruits of the berry's saponin content (0.14 and 0.69%) was higher when compared with 0.65mg/100g observed in *S. dulcificum* pulp [16], however, it is lower than 2.80% reported in the leaves by Osabor *et al.* [14] The value of tannin in the test samples for leaves and fruits (0.647 and 0.34%) was higher than the tannin content of 0.03% in Africa star apple [51] and the range of 0.101-0.133% observed in some lesser known Nigerian fruits as reported by Bello *et al.* [52]. Tannin has been used traditionally for the treatment of diarrhea, hemorrhage, and detoxification [53]. The value of flavonoids obtained in the samples is considerably low both in the leaves and fruits of *S. dulcificum.* Phenolic compounds and flavonoids which are widely distributed in plants are known to exert multiple biological effects including antioxidant, free radical scavenging, anti-inflammatory and anti-carcinogenic effects

[54]. Cardiac glycosides had been reported to be effective in the treatment of congestive heart failure and regulation of heartbeat [55]. The result obtained for cardiac glycosides for leaves and fruit were found to be 0.06% and 0.14% respectively, these levels are lower when compared to that reported in the leaves (4.40%) and roots (4.20%) *S. dulcificum* [14].

Table 4. *Phytochemical screening of the leaves and fruits of Synsepalum dulcificum.*

Phytochemical	Leaf	Fruit
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	+
Tannin	+	+
Glycoside	+	+
Phenolics	+	+
Anthraquinone	+	-

Key.
+ = Present.
= Absent.

Table 5. *Quantitative Phytochemical compositions of the leaves and fruits of S. dulcificum.*

Phytochemical (%)	Leaf	Fruit
Alkaloid	0.90±0.03a	1.37±0.12b
Flavonoid	0.12±0.01a	0.58±0.08b
Saponin	0.14±0.03a	0.69±0.04b
Tannin	0.65±0.01b	0.34±0.01a
Glycoside	0.06±0.001a	0.14±0.001b
Phenolics	0.147±0.02a	0.231±0.03b
Anthraquinone	0.006±0.001	ND

Means with same alphabets in the same rows are not significantly different at $p < 0.05$.

4. Conclusion

This study has shown that the miracle berry, which is known for its ability to turn sour things to taste sweet also contains in the leaves and whole berry fruit some information on the proximate, minerals, anti-nutritional and phytochemical contents of the fruit and leaves of *S. dulcificum.* The plant is a rich source of nutrients and phytochemicals which are important antioxidants source for medicinal purposes. The plant owing to its high ash content especially in the leaves may be a good source of mineral elements such as calcium and magnesium. The leaves and fruit can contribute to the nutrient requirements and health management of man and livestock. The anti-nutrients observed for both fruits and leaves were all below their respective lethal doses, thus, the leaves and fruits can be safely utilized as food and ingredient in food processing.

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