



Chemical Profile of Leaves and Roots of Miracle Fruit (*Synsepalum dulcificum*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author VNO designed the study, managed the literature searches and performed the statistical analysis. Author RAE wrote the protocol and wrote the first draft of the manuscript. Author MUN managed the laboratory analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Proximate analysis, elemental analysis and phytochemical analysis were conducted on leaves and roots of *S. dulcificum* (miracle fruit). The results of proximate analysis of the leaves sample showed the following compositions: protein (6.62±0.02)%, crude lipid (12±2.00)%, crude fibre (17.5±0.50)%, moisture (40.30±1.53)%, ash (6.70±2.00)% and carbohydrate (57.60±0.01)%, while that of roots samples showed protein (5.6±0.07)%, crude lipid (7.5±1.4)%, crude fibre (20.30±1.53)%, moisture (29.2±1.06)%, ash (8.00±1.56)%, and carbohydrate (58.60±0.01)%. Meanwhile the phytochemical screening analysis of the leaves of *S. dulcificum* showed the following concentrations in the leaf samples; alkaloids (0.6±0.20)%, saponins (2.80±0.20)%, flavonoids (2.8±0.2)%, polyphenols (3.52±0.10)% cardiac glycosides (3.44±0.20)%. Similarly, the root samples exhibited the following concentrations: saponins (0.6±0.02)%, polyphenols (4.30±0.10)%, anthraquinones (0.02±0.12)% and cardiac glycosides (4.40±0.20)%. On the other hand, the mineral elements concentrations of the leaf samples were as follows; Ca(0.001±0.00) mg/100 g, Cr(0.0006±0.00) mg/100 g, Fe(0.0029±0.01) mg/100 g, Zn (0.0095±0.00) mg/100 g Cu (0.00082±0.01) mg/100 g while that of root samples were recorded thus; Ca(0.00134±0.01) mg/100 g, Cr(0.00073±0.01) mg/100 g,

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Zn(0.0097±0.01) mg/100 g Fe(0.00025±0.01) mg/100 g and Cu (0.007±0.01) mg/100 g. Generally, the leaf samples of *S. dulcificum* were rich in carbohydrate and moisture while the roots were rich in carbohydrate, moisture and fibre. Ash, fat and protein also showed remarkable concentration. Essential mineral elements were present at required concentration thus making *S. dulcificum* an important source of phytomedicine in the study area.

Keywords: *Miracle fruit S. dulcificum*; mineral content; chemical profiling.

1. INTRODUCTION

Right from the creation, plants and its products derived from parts of plants such as stem bark, leaves, fruits and seeds have been parts of phytomedicine, thus indicating that any part of a plant may contain useful active compounds [1]. Medicinal plants constitute the main source of raw pharmaceuticals and health care products [2]. Mandal [3] pointed out that extraction and characterization of several active phyto-compounds from green plants have given birth to some high activity profile drugs. Edem and Dosunmu [4] also reported that plants are primary sources of medicines, food, shelters and other items used by human every day. While Beroumand and Deokule [5] identified fruits as sources of minerals, fibre and vitamins which also provide essential nutrients for the human health.

Thus, *S. dulcificum* has also been found as an important source of phytomedicine. *S. dulcificum* is commonly known as miracle fruit. It is known as Mkpapun by the Ibibio and Efik people of Akwa Ibom and Cross River States, Azimomo by the Benin people of Edo State and Igbayum by the Yoruba people of Western Nigeria. *S. dulcificum*, is a plant with a berry that when eaten, causes sour foods (such as lemons and limes) consumed to taste sweet [6]. The sweetening property is due to miraculin (a glycoprotein) which is used commercially as sugar substitute. Apart from miracle fruit, it is also commonly known as miracle berry. But *S. dulcificum* should not be confused with two other plants species *Gymnema sylvestre* and *Thaumatococcus daniellii* referred to as miracle fruit which also affect perception of taste.

S. dulcificum (sapotaceae) is an ever green shrub native to tropical West Africa, and the fruits, and red berries have the property of modifying sour taste into sweet taste remarkably. The active material of the berry is the glycoprotein, miraculin, which has no taste in itself [7]. The most outstanding property of the fruit is its effect on the taste buds

of the tongue that causes every sour or acid foods eaten to taste very sweet. The test-modifying effect last up to two hours or more, causing acid food substances such as sour lime, lemon, grape juice and even vinegar to taste sweet [8].

Chen [7] pointed out that the sweetness intensity is reduced by 0.02% citric acid after 0.4% purified miraculin solution is held in the mouth is equivalent to that of about 0.3% sucrose. The fruits are small approximately 2-3 cm long ellipsoid berries that are bright red when riped and composed of a thin layer of edible pulp surrounding a single seed [9]. The leaves are thin, papery, leathery and evergreen. It is reported that African here used *S. dulcificum* to sweeten a sour gruel made from stale bread, soured palm win and pito, a sour alcoholic beverage made from fermented grain. It has also been used before eating a certain type of sour corn bread [10-12]. The present studies deals with the analysis of *S. dulcificum* obtained from Akwa Ibom State Nigeria with a view of obtaining its nutritional potentials and Therapeutic utility.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The fresh leaf and root samples of *S. dulcificum* were collected from Ibot village in Mkpapun Local Government Area of Akwa Ibom State. The samples were identified in the Department of Botany University of Calabar, stored in a polyethylene bag and dried at 160°C in an oven. After drying, both leaves and roots were blended to powder with a manual blender. The blended samples were put in a desiccator and labeled accordingly and stored in a desiccator.

2.2 Sample Extraction

Petroleum ether and distilled water were used to extract each of leaf and root samples. In each process, 20 g of the powdered samples were

washed and packed into extraction thimble and fitted into soxhlet extractor. The samples were Soxhlet extracted for three (3) hours. The petroleum ether and water extracts obtained were put in the reagent bottles. Each bottle was distinctively labeled and kept in the laboratory for phytochemical screening.

2.3 Digestion of Sample

Five grams (5 g) of the powdered samples were accurately weighed into a conical flask. 25 ml of concentrated nitric acid was added to the sample. 5 ml of perchloric acid was then added to the sample. The conical flask with its content was gently heated (50-70°C) on a stuart hot plate until the colour changed from brown to colourless. The digest was made up to 100 ml with deionized distilled water. Appropriate dilutions were made for each element. All determinations were carried out in triplicates.

2.4 Proximate Analysis

Proximate analysis involves determination of moisture, ash, crude fibre, crude protein, fat and carbohydrate contents [13]. Ash, moisture fat, fibre and crude protein were determined through methods prescribe by [14]. The percentage available carbohydrate (CHO) was calculated using expression

$$\% \text{ CHO} = 100 - (\% \text{ ash} + \% \text{ crude protein} + \% \text{ crude lipid} + \% \text{ crude fibre})$$

2.5 Phytochemical Screening

The phytochemical screening procedures carried out on the leaves and roots of *S. dulcificum* were adapted from the previous work on medicinal plant analysis [15,16]. Alkaloids, saponins, flavonoids, cardiac glycosides, polyphenols, phlobatannins, anthraquinones, anthranoids, tannins and reducing compounds were screened.

2.6 Quantitative Determination of Some Phytochemicals

Quantitative determination of the following phyto-constituents; alkaloids, saponins, flavonoids, cardiac glycosides, polyphenols, anthraquinones and tannins were determined using methods described by A.O.A.C [14], Sofowora [17], Obadoni and Ochuko [18] and Soladoye and Chukwuma, [1].

2.7 Mineral Element Analysis

The methods of AOAC [14] were used in these determinations. Standard solutions were prepared for each element. Sodium and potassium were determined by flame photometer (Gallen Kamp). Calcium, magnesium, iron, chromium, lead, mercury, nickel, cadmium, cobalt, manganese, zinc and copper, were determined using atomic absorption spectroscopy (AAS).

3. RESULTS AND DISCUSSION

Tables 1 and 2 show the results of proximate compositions and phytochemical screening of the leaves and roots of *S. dulcificum* respectively. Similarly, Tables 3 and 4 show the results of quantitative determination of some phytochemicals and mineral elements compositions of leaves and roots of *S. dulcificum*. The results of proximate analysis of *S. dulcificum* as given in Table 1 shows that the moisture content of the leaves and roots of *S. dulcificum* are (40.30±1.53%) and (29.20±1.06%) respectively. The percentages are slightly lower than the moisture content of *Chrysophyllum africanum* (66.67±0.02)% [4]. The moisture content of food is usually used as a measure of stability and susceptibility to microbial contamination [19]. These compositions indicate that *S. dulcificum* can be stored for a long time without spoilage. The study on the ash content of the leaves and roots of *S. dulcificum* obtained were found to be (6.70±2.00%) and (8.00±1.56%) respectively. These compositions are higher when compared to (2.03%) reported by Edem [20] for *Tetracarpidium conophorum*. Again the ash content of *S. dulcificum* was found to be nearly the same with *Alpinia allighas* (6.40±0.15%) by Landan [21]. The results of the analysis for fibre contents as presented in Table 1 are (17.50±0.50%) and (20.30±1.53%) for leaves and roots respectively. Comparing these values to that reported for *Chrysophyllum africanum* fruits (4.45±0.02%) by Edem and Dosunmu [4]. *S. dulcificum* has a very high percentage of fibre. Emphasis has been placed on the importance of keeping fibre in takes low in the nutrition of infants and pre-school children [22].

The crude lipid contents of the leaves and roots of *S. dulcificum* were found to be (12.0±2.00%) and (7.50±1.40%) respectively. The lipid content of the leaves of *S. dulcificum* is significantly

higher than that reported for *Chrysophyllum africanum* (9.38 ± 0.01) by Edem and Dosunmu [4]. Lipids are monosaturated and considered healthy when consumed on moderation. They are essentials because they provides body with maximum energy [23]. Similarly, the protein contents were found to be ($6.62 \pm 0.02\%$) and ($5.60 \pm 0.07\%$) respectively. Protein is an essential component of the diet needed for survival of animals and humans. Basic function is to supply adequate amount of required amino acids [24]. Protein deficiency causes growth retardation, muscle washing, abnormal swelling

of the belly and collection of fluids in the body [25]. The daily protein intake for children and adult are 23-26 kg and 45-46 g respectively [26]. However, the carbohydrate contents were found to be (57.18 ± 0.01 and $58.60 \pm 0.01\%$) for leaves and roots respectively. These values are higher than (53.20%) reported for *Tetracarpidium conophorum* fruit by Edem [19]. The result obtained in this study indicates that *S. dulcificum* is rich in carbohydrate. If carbohydrate is sufficient in food, it prevents the unnecessary usage of protein and allows it to be used for the body building processes.

Table 1. Proximate composition of leaves and roots of *S. dulcificum*

S/N	Parameters	Leaves (%)	Roots (%)
1	Moisture	40.3 ± 1.53	29.2 ± 1.06
2	Ash	$6.70.0 \pm 2.00$	8.00 ± 1.56
3	Crude lipid content	17.5 ± 0.50	20.3 ± 1.53
4	Crude fat content	12.0 ± 2.00	7.5 ± 1.40
5	Crude protein content	6.62 ± 0.02	5.6 ± 0.07
6	Carbohydrate	57.18 ± 0.01	58.60 ± 0.01

Data are: mean(x) \pm standard deviation of triplicate determinations

Table 2. Phytochemical screening of leaves and roots of *S. dulcificum*

Bio-active compounds	Petroleum ether extract of leaves	Water extract of leaves	Petroleum ether extract of roots	Water extract of roots
Alkaloid	+	-	-	-
Saponnins:	-	+	-	++
Flavonoids	+	-	-	-
Polyphenols	+	++	++	++
phlobatannins	-	-	-	-
Anthranoids:	-	-	-	-
Anthraquinones	-	-	-	+
Cardiac glycosides	+	+	++	+
Tannins	+	-	-	-
Reducing compounds	-	-	-	-

Key: + = present, ++ = present in large quantity, - = not present

Table 3. Quantitative determination of some phytochemicals of leaves and roots of *S. dulcificum*

Bio-active compounds	Leaves %	Roots %
Alkaloid	0.60 ± 0.2	---
Saponnins:	2.80 ± 0.2	0.62 ± 0.02
Flavonoids	2.40 ± 0.2	--
Polyphenols	3.52 ± 0.01	3.44 ± 0.01
Anthraquinones	-	0.02 ± 0.12
Cardiac glycosides	4.40 ± 0.20	4.20 ± 0.20
Tannins	4.00 ± 0.20	-

Data are: Mean (x) \pm standard deviation of triplicate determinations

Table 4. Mineral elements compositions of leaves and roots of *S. dulcificum*

Element	Concentration in Mg/100g	
	(Leaves)	(Roots)
Calcium	0.001±0.00	0.00134±0.01
Magnesium	ND	ND
Iron	0.0029±0.01	0.0025±0.01
Potassium	ND	ND
Sodium	ND	ND
Chromium	0.0006±0.00	0.00073±0.01
Zinc	0.0095±0.00	0.0097±0.01
Manganese	ND	ND
Lead	ND	ND
Copper	0.00082±0.01	0.007±0.01
Mercury	ND	ND
Nickel	ND	ND
Cadmium	ND	ND
Cobalt	ND	ND

Key: ND = not detected, data are: mean (x) ± standard deviation of triplicate determinations

The phytochemical screening of the leaves and roots of *S. dulcificum* show that cardiac glycosides were present in higher concentration in both petroleum ether and water extracts. Quantitatively, the levels of cardiac glycosides were found to (4.40±0.2%) and (4.20±0.2%) respectively. These concentrations are higher when compared to that of *Cissus populnea Guill and Perr. (Vitaceae)* (1.83)% reported by Soladoye and Chukwuma [1], cardiac glycosides have specific characteristics and powerful action exerted on cardiac muscles and therefore is used in congestive heart failure due to diminution of work capacity per unit weight of myocytes tissues, medicinal interest on cardiac glycosides is because of their structure stimulant effect in the heart [27]. Cardolides is a type of cardiac glycosides which is abundant in the asclepiadeceae and apynaceae. Their actions are anti-inflammatory, antiseptic and analgesic and are used in the treatment of rheumatism [28].

The result of phytochemical screening for alkaloids show the presence of alkaloids in petroleum ether extracts. However, quantitative analysis conducted on the leaves showed alkaloids concentration of (0.60±0.2%). This quantity is lower when compared to the alkaloids content of *Allium sativum* (0.12±0.02) reported by Huzaifa [29]. Screening result for saponins showed the presence of saponins in water extract in both leaves and roots. The concentration of saponins in the leaves was

however higher than that of roots. The quantification of the leaves and roots revealed (2.8±0.2%) and (0.6±0.2%). Comparing the levels of saponins in the leaves of *S. dulcificum* to that of *Cissus populnea Guill and Perr. (Vitaceae)*. (2.85±0.35 mg/g) reported by Soladoye [1]. This indicates that the leaves of *S. dulcificum* is very rich in saponins. The amount of saponins in *S. dulcificum* is, however lower than that of *Chrysophyllum africanum* (3.663±0.02 mg/100 g) reported by Edem and Dosunmu [4].

It was found that the petroleum ether extracts of the leaves contain a moderate concentration of flavonoids (Tables 2 and 3). Flavonoids were absent in water extracts of the leaves and roots. Quantitative analysis showed the concentration of (2.40±0.2%) flavonoids. Thus, when compared to that of *Cissus populnea Guill and Perr (Vitaceae)* (0.39±0.03%w/w) reported by Soladoye and Chukwuma [1], revealed that *S. dulcificum* is rich in flavonoids. Flavones are a class of flavonoids and are commonly attached to sugar to form glycosides. They are obtained from the synthesis of O-hydroxyacetophenone. O-hydroxyacetophenone is benzoylated, followed by the base (pyridine/ KOH) treatment of the benzoyl ether to effect an alkyl group migration to produce 1, 3- diketone [30].

Tannins showed a higher concentration in the petroleum ether extract of the leaves, but was however absent in water extract of both roots and leaves.

Quantitative determination of tannins in the leaves showed the levels of (40±0.20%). Sutharsingh [31] reported (8.72±0.44%w/w) for *Naravelia zeylanica* Dc. This is lower than the (40±0.20%) reported for *S. dulcificum* in the present investigation.

Phytochemical screening result of reducing compounds in the leaves and roots of *S. dulcificum* is given in Table 2. Reducing compounds were absent in both petroleum ether and water extracts of the leaf and root samples, even after boiling for 30 minutes in a water bath. The result indicated that leaves and roots of *S. dulcificum* are not rich in reducing compounds compared to the seeds, which is evidence of the taste modifying activity.

Phlobatannins were absent in both petroleum ether and water extracts of the leaf and root

samples. Similarly, the screening for anthranoids showed the absent of anthranoids in both extracts (petroleum ether and water) of the leaf and root samples (Table 2). Phytochemical screening analysis shows that the water extract of the root shows the presence of antraquinones (Table 2) while the quantification of anthraquinones revealed (0.02±0.12%) (Table 3). It was however, absent in the leaves sample. Naturally occurring anthranol are easily oxidized by atmospheric oxygen to anthraquinones. Anthraceae are found as glycosides and aglycone [32]. Anthraquinones and related glycosides are thatatus, which increased smooth muscles tone of the large intestine [33].

The mineral elements analysis of *S. dulcificum* showed the composition of calcium to be (0.001±0.00 mg/100 g) and (0.00134±0.01 mg/100 g) for both leaves and roots respectively. These concentrations are very low when compared to that of calcium obtained from *Aspilla Africana* leaves (1.04±0.03) mg/l [34] and *Achyranthes aspera* Linn roots (849435) ppm [35]. About 200-400 g/day will provide the recommended daily calcium allowance of 360 mg-1200 mg for children and adults especially if combined with other food stuff rich in calcium. Calcium is required in the body for normal growth of bones and teeth [36]. Mineral element analysis result for magnesium is given in Table 4. Magnesium was not detected in both the leaves and root of *S. dulcificum*. The mineral levels of potassium in the leaves and roots of *S. dulcificum* is significantly low as potassium was not detected (Table 4). But this is not so with seed of the same plant the potassium in the seed is 569.500±2.820 mg/100 g [37].

Table 4 shows a significant composition of iron in both leaves and roots of *S. dulcificum*. The iron concentrations were found to be (0.0029±0.01 mg/100 g) and 0.0025±0.01 mg/100 g for the leaves and roots respectively. The obtained results are very low when compared to that reported for *Securinegavirosa* leaves (2.02%) [38]. The recommended intake of iron per day for children and adult is 10-18 mg/day [26]. Iron is required for the formation of blood and its deficiency causes anaemia [39]. Sodium was not detected in both leaves and roots of *S. dulcificum* as presented in Table 4. Meanwhile, chromium composition of the leaves and roots of *S. dulcificum* were found to be (0.0006±0.00 mg/100 g) and (0.00073±0.01 mg/100 g) respectively. For most plants species Cr at 10 mg/kg levels is considered to be toxic [40,41].

Zinc also recorded a low concentration in the leaves and roots of *S. dulcificum* which were found to be (0.0095±0.00 mg/100 g) and (0.0097±0.01 mg/100 g) respectively. The concentrations are very low when compared to that of the seed of the same plant reported to be (2.710±0.009 mg/100 g) [37]. The zinc present in the pancreas may aid in the storage of insulin, zinc in the plants could mean that plants can play essential roles in the management of diabetes, which result from insulin malfunction [42]. The results of the analysis for copper in the leaves and roots of *S. dulcificum* is presented in Table 4. The result obtained showed that leaf contains (0.00082±0.01 mg/100 g) while the roots contain (0.007±0.01 mg/100 g). Comparing the result to that reported for *Averrhoa bilimbil* (0.07±0.01) ppm [43], *S. dulcificum* is not a good sources of copper. Mineral elements composition of the leaves and roots of *S. dulcificum* for manganese is given in Table 4. Manganese was not detected in both leaves and roots. But Jeremiah [37] reported manganese content of the seeds to be (2.280±0.004 mg/100 g).

Similarly, lead, mercury, cadmium, cobalt and Nickel were not detected in the samples but Jeremiahs [37] reported that the seeds of *S. dulcificum* contain a Nickel concentration of (0.240±0.028 mg/100 g).

4. CONCLUSION

The leaves of *S. dulcificum* are rich in carbohydrate and moisture. Similarly the roots are rich in carbohydrate, moisture and fibre. Ash, fat and protein were appreciably present. Phytochemical screening of the extracts (petroleum ether and water) revealed that *S. dulcificum* is highly rich in cardiac glycosides and polyhenols when compared to other detected bioactive compounds. From the quantitative analysis of the phytochemicals, tannins, cardiac glycosides, polyphenols, flavonoids and saponins showed a high levels in the both leaves and roots. Elemental analysis revealed that all the detected mineral elements were present in a moderate concentration in both leaves and roots.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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