

Determination of Morphological and Nutritional Properties of *Thaumatococcus daniellii* and Effect of Harvesting Method on the Plant Growth

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ABSTRACT: *Thaumatococcus daniellii* (Miraculous berry) belonging to the family maranthaceae is one of the sweeteners found in the tropical forest. However in Nigeria, because of the excessive use of this species as wrapping leaf, very little is known about its fruit as a source of sweetener. Although the leaves are sold in the market as wrapping leaf for bean budding (moi-moi) and other cooked foods such as rice. Information on the propagation of this species and utilization of its parts is very scanty making the species one of the threatened forest species. The objectives of this study were therefore to determine; the nutritional properties of the gelatinous layer of the fruit and the effects of various harvesting methods on the plant growth. The gelatinous layer of the fruits of *Thaumatococcus daniellii* is a sweetener known as “Thaumatocin”. Thaumatocin is a sweet-tasting, flavor – enhancing protein. Some of the proteins in the thaumatocin family are natural sweeteners roughly 2000 times more potent. The morphological characteristics of the stands of this species were measured using standard methods. The nutritional and the phytochemical composition of the gelatinous layer extracts of the fruits were analyzed in the laboratory using standard methods, while two harvesting methods were used to determine the effect of harvesting on the plant growth. The mean values of the morphological characteristics of stands of *Thaumatococcus daniellii* were as follows; stem height (154.9cm), stem girth (2.26cm), petiole length (24.86cm), petiole girth (1.69cm), leaf area (1018.93cm²). The phytochemical analysis of the gelatinous layer extracts of the fruit showed that it contains saponin (0.19%), flavonoids (0.41%), phenol (0.06%), HCN (0.81%), Tannin (0.16%), Alkaloid (0.11%), Phytate (0.05%). The nutritional analysis of the gelatinous layer extract of the fruits showed that it contains calcium (6.68mg/100g), magnesium (0.60mg/100g), Ash (5.80%), Fibre (0.41%), Protein (19.01), Nitrogen (3.04%). The result obtained showed that the species are highly nutritious and the sugar content of its fruit can be easily substituted for white sugar and also used in pharmaceutical industries for children syrups. The best harvesting method is the ground level harvesting method. Conservation of this species and should be promoted.

KEYWORDS: *Thaumatococcus daniellii*, Thaumatocin, Morphological properties, Nutritional properties, Harvesting methods.

1 INTRODUCTION

Thaumatococcus daniellii (Miraculous berry) and its products have continued to be of immense benefit to the people in the tropics. A large number of people depend on it for economic purpose. The species are multipurpose perennial herb that offers the widest assortment of uses. It has a soft-jelly like fruit that contain a high amount of protein (sweeter). The plant is of global prominence, consequent upon the discovery of “Thaumatocin”, a non-caloric sweetener derived from the arils of plants which is reportedly about 1600 times sweeter than sucrose (Zemanek and Wasserman, 1995).

It is a member of the maranthaceae family. It has long slender stalk reaching heights of about 2 – 3 metres. The stalk germinates into a single tough, almost round and versatile leaf of varying sizes depending on the age and habitat of the plant (Makinde and Taiwo, 2004). It is a multi-purpose perennial herb that offers wide range of uses with its leaves, fruits, stalks and rhizomes. It has been domesticated in most parts of South-western Nigeria, where it contributes to the economy of the

rural population (Osemeobo, 2005; Arowosoge and popoola, 2006). All parts of the plant are useful, with the stalk used to sweeten foods locally and in the production of Thaumatin (Elemo et. al, 1999), while the leaves are used for thatching roofs and in wrapping foods. The leaves are popularly used as a wrapping material for different categories of food such as unprocessed meat and kola nuts; semi processed foods such as fermented locust beans; processed foods such as cooked rice, beans, maize meal, pounded yam etc. the use of leaves as a food wrapper/preservation material is no more restricted to the local populace resident in the villages and suburbs. It has gained widespread acceptance not only in the towns and cities of South-western Nigeria, but also in some parts of the United States and South Americas, where it is now acceptable for food packaging (wrapping), as not only exotic but also for flavor enhancing (Thorn, 2004).

Depending on location and locality, various types of plant leaves are used as wrappers, example, banana/plantain leaf, i.e. *Musa Sapentium*, *Musa paradisiacal*, *cola nitida* and *Thaumatococcus daniellii* leaves (Adegunloye et. al. 2006), to package and present foods to clientele. The use of these leaves, are very ancient ways (traditions) of the people, the basis of which cannot be easily ascertained, but a cursory look at these leaves reveal that they all have large surface areas i.e. can be used to hold/package/wrap large volumes of food. Quite many populations depend on this plant for economic benefit. The sweetener contained in the seed of *Thaumatococcus daniellii* can be used in pharmaceutical industries, mineral-drink and confectionery industries. The fruit can be used in mitigating the bitterness in quinine or render insipid palm wine palatable. It is also added to palm wine and stale food to improve their taste (Oladele, 1994). There is unprecedented rise in acceptability of the gelatinous layer of the fruit as sweetener used in pulp and paper industries, pharmaceutical industries and confectionery industries (De lucca et. al. 2005).

The most important use of *Thaumatococcus daniellii*, however, is its use as a sweetener or taste modifier. The arils of the fruit contain thaumatin, a mixture of extremely sweet proteins. For that reason, *Thaumatococcus daniellii* is traditionally used for sweetening bread and flavouring palm wine. Since the mid-90s, it is used as sweetener and flavor enhancer by the food and confectionary industry in many countries, substituting synthetic sweeteners (Taiwo, 1995). Integrating *Thaumatococcus daniellii* in cropping systems or plantations seems to be a promising way to lessen these short comings, contributing to both income generation and diversification of crop production by small farmers. The species is a large rhizomatous flowering herb, native to the rain forests of Ghana and surrounding African nations. It is also an introduced species in the rainforests of Northern Australia. It grows 3-4m in height and has a large papery leaves up to 46cm long. It bears pale purple flowers and a soft fruit containing a few shiny black seeds (Gibbs et. al. 1999).

1.1 OBJECTIVES OF THE STUDY

The objectives of this study were to determine the:

1. Morphological characteristics of its stands
2. Interrelationships among the morphological characteristics
3. Effect of harvesting method on subsequent growth
4. Nutritional and phytochemical properties of the gelatinous layer of fruits of *Thaumatococcus daniellii*

1.2 SCOPE OF THE STUDY

This study examined the characteristics of *Thaumatococcus daniellii* stands. It also analyzed nutritional, anti-nutritional phytochemical composition of its fruits in the laboratory using adequate and standard analytical procedures. Effect of harvesting technique on plant regrowth was carried out in Michael Okpara University of Agriculture, Umudike, Abia State – Nigeria, where *Thaumatococcus daniellii* farm has been established.

1.3 THE STUDY AREA

This study was carried out in Michael Okpara University, Umudike, Abia State – Nigeria which lies on a latitude of 5°28.66'N and longitude of 7°32.56'E. It has an altitude of 122m above sea level. Umudike has the following mean annual climate data, 2238mm rainfall, which is biannually distributed between March-July and September-October or early November, with break in August. Minimum and maximum temperature of 23°C and 32°C and relative humidity 65-80% (Agro metrological station, NRCRI, Umudike).

2 MATERIALS AND METHODS

2.1 METHODOLOGY USED IN MORPHOLOGICAL CHARACTERISTICS OF *THAUMATOCOCCUS DANIELLII*

Materials used when determining the morphological characteristics of stands of *Thaumatococcus daniellii* include:

1. Rake: used to rake under the *Thaumatococcus daniellii* stands
2. Measuring tape: used in taking height and length measurements of stem and petiole of randomly selected *Thaumatococcus daniellii* stands
3. Thread: used in taking girth measurement of both stem and petiole
4. Ruler: used in measuring the thread length used in taking measurement of stem and petiole girth
5. Graph book: used in calculating the leaf area of *Thaumatococcus daniellii*
6. Knife: used in cutting stands of *Thaumatococcus daniellii* to determine the effect of harvesting techniques
7. Pen and Pencil: used in taking records of lengths of stands of *Thaumatococcus daniellii*
8. Gum: used in gumming/joining one or two graph sheets together

Methods applied when determining the morphological characteristics of *Thaumatococcus daniellii* include:

1. Racking of *Thaumatococcus daniellii* stands to make it clean
2. 120 stands of *Thaumatococcus daniellii* were randomly selected and tagged numerically
3. The following measurements were taken per plant i.e. (1-120); stem height in centimeters, stem girth in centimeters, petiole length in centimeters, petiole girth in centimeters, and leaf area in centimeters squared (cm²).

2.2 METHODOLOGY USED IN DETERMINING THE EFFECT OF HARVESTING METHODS ON PLANT REGROWTH

Two harvesting techniques were used. Sixty (60) stands of *Thaumatococcus daniellii* were selected for each method, giving a total of 120 plants. The first method involved cutting each stand and 5cm above ground level while the second method involves cutting the stands from the soil surface i.e. at the point of shoot connection to the rhizome.

2.3 METHODOLOGY APPLIED DURING NUTRITIONAL AND PHYTOCHEMICAL ANALYSIS OF THE GELATINOUS LAYER (THAUMATIN) OF *THAUMATOCOCCUS DANIELLII*

The fruits of *Thaumatococcus daniellii* were collected from the plant stands (being subterranean in nature) and analyzed at the central laboratory of the National Root Crops Research Institute, Umudike, Abia State.

3 NUTRITIONAL ANALYSES

3.1 DETERMINATION OF MOISTURE CONTENT

This was determined using the gravimetric method developed by James (1995). Five grams (5g) of each sample was weighed into previously weighted moisture and dried in the oven for 3hours. In the first instance, it was cooled in a desiccator and reweighed. It was then returned to the oven for further drying during which it was weighed at hourly interval until there was no further reduction in weight i.e. a constant weight was obtained. By weight difference, the weight of moisture was determined and expressed as a percentage of the sample weight.

3.2 DETERMINATION OF PROTEIN CONTENT

The kjeldaly method (Pearson 1976; James 1995 and Clang 2003) was used. The total Nitrogen was determine and multiplied with the factor 6.25 to obtain the protein. Procedure: one-half gram (0.5g) of each sample was digested by boiling in 10mls of concentrated H₂SO₄ in the presence of sodium catalyst. The sample was boiled under a fume cupboard until a clear solution was obtained. The digest was diluted to 100mls in a volume flask with distilled water. An adequate (10mls) of the diluted digest was mixed with equal volume of 45% NaOH solution in a Kjeldaly distillation apparatus. The mixture was distilled and the distillate was collected into 10mls of 4% Ban acid solution in which 3 drops of mixed indicator (Mettyl red and bromocresol green) was added. A total of 50ml distillate was collected and filtrated against 0.02 H₂SO₄ solutions from green to a deep red endpoint.

3.3 DETERMINATION OF ASH CONTENT

Ash content was determined gravimetrically following furnace incineration (Ogunwale, 1986; James, 1995). A measured weight of the sample was weighed into a previously weighed porcelain crucible. The sample in the crucible was burnt to ashes in an electric muffle furnace at a temperature of 550°C after 3 mins (when the sample became grey ash), it was carefully put in a desiccator to cool, and then it was reweighed. By weighing, the weight of ash was obtained and passed as a percentage of the sample analyzed.

3.4 DETERMINATION OF FAT CONTENT

This was determined by the continuous solvent extraction gravimetric method (as stated by Min and Boff, 2003). A solvent apparatus was used in the process. 5g of the sample was wrapped in a weighed filter paper and put in a solvent roflare flask. The roflare flask was mounted onto an oil extraction flask containing 300mls of petroleum. The upper end of the roflare flask was connected to a condenser. When heated, the solvent condensed into the roflare flask and covered the wrapped sample. The sample remained in contact with the sample until the roflare flask filled and siphoned over there by carrying extracted oil down to the boiling flask. This process was allowed to go on repeatedly for about 4 hours after which the sample is carefully removed with the aid of a pair of forceps. It was dried in the oven at 100 Celsius for 30mins, cooled in a desiccator and weighed. The weight of fat was less i.e. reduced and expressed as a percentage of the sample weight.

3.5 DETERMINATION OF CRUDE FIBRE

The Weende method by James (1995) was employed. 5g of each sample was defatted (as in fat determination). The defatted sample was boiled under roflare in 150ml of 1.25% of H₂SO₄ solution for 30mins. It was washed in several portions of distilled water using a twofold muslin cloth to retain the particles. The washed sample was boiled again for 30mins in 150mls of 1.25% of NaOH solution. After washing again in several portions of distilled water, it was put in a weighed porcelain crucible and dried in the oven at 105°C for 1 hour. It was cooled in a desiccator and weighed. Following this, the dried weighed sample was burnt to ashes in furnace, cooled again in desiccator and weighed.

3.6 DETERMINATION OF CARBOHYDRATE

Carbohydrate was determined by Cleifeaur as the Nitrogen Free Extract (NFE) as described by James (2003). The formula below was used;

$$\%CHO = 100 - \% (\text{protein} + \text{fat} + \text{fibre} + \text{Ash})$$

4 METHODS OF ANALYSIS FOR MINERAL

Mineral content of the test sample was determined following dry ash acid extraction method (Udoh and Oguhale, 1980; James, 1995). A weighed sample was ashed (as in ash analysis). The resulting ash was dissolved in 10mls of 2M HCl solution and diluted to 100mls in a volume flask. This extract was used for mineral determination using appropriate method for specific minerals as described below.

4.1 DETERMINATION OF CALCIUM AND MAGNESIUM BY COMPLEXIMETRIC TITRIMETRY

Calcium and magnesium in the sample extract were determined by the versanate compleximetric titrimetry (James, 1995). A portion (20mls) of the sample extract was poured into a conical flask and a pinch of a mixed masking agent (Potassium cyanide and potassium ferricyanide) was added to it, shaken to dissolve and then 20mls of ammonia buffer solution (to raise the PH to 10.0). It was filtrated against 0.02N EDTA solution using erichrome black T as indicator. Titration was done from mauve to a permanent blue end point. A reagent blank was also treated as described above and titrated. The above titration was for combined calcium and magnesium (both Ca²⁺ and Mg form complex with EDTA at PH 10.0). A second titration was done for calcium. The same procedure was followed however, sodium cyanide was used in place of ammonia (to raise the PH to 12.0 where only Ca²⁺ form complex with EDTA). Also sodium dark blue was used as indicator in place of Erichrome black – T. The end point colour remained mauve to blue. In each case, a reagent blank was titrated as well.

4.2 DETERMINATION OF PHOSPHORUS

This was determined by the vanadohydrate calorimetric method (James, 1995). A portion of the sample extract was mixed with equal volume of phosphorus reagent (ammonium metavanadate + ammonium molybdate). The mixture was allowed to react for 5mins before it was diluted to 50mls with distilled water. A reagent blank was prepared with 2mls distilled water and treated as described above. Meanwhile, a standard phosphorous solution was prepared and analyzed as described above. The reagent blank was used to bring the instrument at zero before measurement was made.

5 METHOD OF ANALYSIS – PHYTOCHEMICALS

The test plants were screened to determine the presence of specific phytochemical group. The methods described by authors – Harbarne, 1973; Sofowara, 1993 were employed. Aqueous and ethanoic extracts were obtained from the plants and used in the screening test. The procedures are described below:

5.1 TEST FOR TANNING

About 2mls of aqueous extract of each test plant was used with few drops of dilute ferric chloride solution in a test tube. The formation of thick greenish – black precipitation gave a positive result for the presence of tannins in the test sample extract.

5.2 TEST FOR SAPONIN

The presence of saponin in the plant extracts was confirmed following a froth test. About 2mls of the aqueous extract was treated with 5mls of distilled water in a test tube. The mixture was shaken vigorously and observed for the presence of a stable froth (test for positive result for saponin presence in plant extract).

5.3 TEST FOR PHILABOTAMINGS

A portion of the aqueous extract of each plant was mixed with equal volume of 1% aqueous hydrochloric acid solution in a test tube. The mixture was boiled, and the deposition of red precipitation gave a positive result for philabatamins.

5.4 TEST FOR FLAVONOIDS

This was done by the alkaline – acid test. NH_3 solution was mixed with equal volume of the aqueous extract of the sample. The resulting yellow colouration was treated with dilute HCl solution and the disappearance of the yellow colour gave an indication of the presence of flavonoid.

5.5 TEST FOR CYANOGENIC GLYCOSIDE (HCN)

Cyanogenic glycosides are substances which on glycolysis will yield hydrocyanic acid (HCN). The alkaline piorate calorimetric method was used for the test. 1g of the sample (processed) was disposed in 150mls of distilled water in a conical flask and a strip of alkaline piorate paper was suspended over it. After an overnight incubation, the piorate paper was obtained for colour change from yellow to orange indicating the presence of cyanide.

5.6 TEST FOR ALKALOID

The alkaloid extract of the test plant was treated with Meyer's reagent. An orange colouration gave a positive test for the presence of alkaloid.

5.7 TEST FOR PHENOLS

Total phenol was determined using the folin-ciocaltean calorimetric method (AOAC, 1990). A weighed sample (0.2g) was dispersed in 10mls of pure methanol. It was filtered to obtain the extract used in the analysis. 1ml of the extract was mixed with equal volume (1ml) of folin-ciocaltean reagent in 50mls volume. Similarly, 1ml of standard phenol solution was treated

the same way in a separate flask. 2mls of Na_2CO_3 was added to each of the flask and mixed very well. Their respective absorbance was read in a speedrophotometer with a reagent blank at zero.

6 STATISTICAL ANALYSIS

Data collected were analyzed using tables and graphs. The regression analysis carried out showed the relationships between the following:

1. Petiole girth on petiole length
2. Leaf area on stem height
3. Petiole length on stem girth
4. Petiole length on stem height
5. Stem girth on petiole girth
6. Leaf area on stem girth
7. Petiole length on leaf area
8. Leaf area on petiole girth
9. Stem girth on stem height
10. Multiple regression of stem height on leaf area, stem girth, petiole girth and petiole length

7 RESULTS AND DISCUSSION

7.1 MEANS AND RANGE VALUES FOR THE MORPHOLOGICAL CHARACTERISTICS OF *THAUMATOCOCCLUS DANIELLII*

Table 1: Means and range values for the morphological characteristics of Thaumatooccus daniellii

MORPHOLOGICAL CHARACTERISTICS	MEANS	RANGES
Stem Height (cm)	15.9	(73.0 – 226.8)
Stem Girth (cm)	2.26	(1.6 – 2.5)
Petiole Length (cm)	24.86	(11.1 – 16.2)
Petiole Girth (cm)	1.69	(1.3 – 2.0)
Leaf Area (cm ²)	1018.93	(870.11 – 1323.06)

7.2 NUTRITIONAL COMPOSITION OF “THAUMATIN” CONTENT OF *THAUMATOCOCCLUS DANIELLII*

Table 2: Nutritional composition of “thaumatin” content of Thaumatooccus daniellii fruits

NUTRIENT ANALYSIS	MEAN VALUES
Moisture content (%)	83.9
Protein content (%)	19.01
Crude fibre (%)	0.42
Ash (%)	5.80
Fat (%)	3.85
MINERALS	
Calcium (Mg/100g)	6.68
Magnesium (Mg/100g)	0.60

7.3 PHYTOCHEMICAL COMPOSITION OF "THAUMATIN" IN *THAUMATOCOCCUS DANIELLII* FRUITS

Table 3: Phytochemical composition of "thaumatin" in *Thaumatococcus daniellii* fruits

ANALYSIS	MEAN VALUES
Saponin (%)	0.19
Flavonoid (%)	0.14
Phenol (%)	0.06
Tannin (%)	0.16
Alkaloid (%)	0.11
Phytate (%)	0.05
Fat content (%)	3.85
Hydrogen cyanide HCN (Mg/1kg)	0.81

8 DISCUSSIONS

This study of the morphological characteristics of *Thaumatococcus daniellii* stands and fruits through nutritional, phytochemical and regression analysis is an attempt to unravel the scientific benefits derivable from the use of its leaves, fruits and stalks in weaving mats and the seeds extract (thaumatin) as a sweetener used in pharmaceutical and confectionery industries.

The finding of phytochemical analysis reveals the presence of saponin, phytate, fat, alkaloid, tannin, phenol and hydrogen cyanate which means it is edible and from the various contents, it reveals that the fat content is highest while the alkaloid content is the least.

Thaumatococcus daniellii is known to produce secondary metabolites that complement their elites which consider the packaging (wrapping) as not only exotic, but also flavor enhancing (Thorn, 2004). There are about 250,000 – 500,000 species of plants in existence but only very few of these have been screened for their microbial potentials (De Luca et. al., 2005).

8.1 MORPHOLOGICAL CHARACTERISTICS OF *THAUMATOCOCCUS DANIELLII* STANDS

The results of the morphological characteristics on table one (1) show that the plant growth height ranges from at least 73.0cm – 226.8cm), while its average mean growth is about 154.9cm. From the table also, it can be seen that the leaf area ranges from 870.1cm² to 1323.06cm² while its average leaf area is about 1018.93cm². The table 1 also shows that the stem girth ranges from at least 1.6cm to 2.5cm with an average stem girth of about 2.26cm. It also shows the petiole length and petiole girth which ranges from 11.1cm to 16.2cm and 1.3cm to 2.0cm respectively, with an average petiole length of 24.86cm and an average petiole girth of 1.69cm.

8.2 NUTRITIONAL COMPOSITION OF *THAUMATOCOCCUS DANIELLII* FRUITS (THAUMATIN)

From the nutritional analysis of table two (2), thaumatin contains moisture, protein, crude fibre, fat, ash and minerals which include magnesium and calcium. The table shows that the moisture content is highest while the magnesium content is least with 0.60%, the calcium content is also high with about 6.68%. Calcium has the highest mineral content. The protein content is about 19.0%.

8.3 PHYTOCHEMICAL COMPOSITION OF THAUMATIN

The phytochemical analysis showed that the fruit "thaumatin" contains saponin, flavonoid, phenol, tannin, alkaloid and phytate. The table 3 shows that hydrogen cyanide has the highest content with about 0.81 Mg/Kg followed by the flavonoid content with about 0.41%. Saponin content is about 0.19%. Tannin content about 0.16%, alkaloid content is about 0.11%, phytate content is very low with about 0.05% and phenol content about 0.06%.

9 CONCLUSION

In conclusion, there is need to sustainably produce *Thaumatococcus daniellii* in Nigeria. The most economic and realistic means is to improve on the technology of producing sweetener from its "Thaumatocin" in which appear on the interim, the most economic potential of the plant that have attracted global attention. The sweetener which serves as whole food could be substituted for sugar. Developing this technology and marketing will invariably encourage the cultivation of *Thaumatococcus daniellii* not only by the local farmers but also by pharmaceutical and confectionery industries. The phytochemical contents of this species made it a source of raw material for industries. Also, since this species can regenerate itself quickly from the rhizomes as observed in harvesting method involving cutting from the soil surface, it could be introduced to farmers as weed suppressor and its sustainability can be well assured.

10 RECOMMENDATION

1. Awareness (public) should be increased on the local knowledge and practices of developing *Thaumatococcus daniellii*. Also, the importance of *Thaumatococcus daniellii* should be highlighted.
2. It is recommended that "thaumatocin" be used in beverage, confectionery and pharmaceutical industries. It is an internationally traded commodity with a market value of 400 Euros.
3. With the thaumatocin market ripe for expansion increase, pressure is expected on the natural resource, hence; need to sustainably produce *Thaumatococcus daniellii* on a commercial basis.
4. Before working on a *Thaumatococcus daniellii* plot, reconnaissance survey is recommended with relevance to sustainable harvesting and its propagation.
5. From the result of nutritional and phytochemical analysis obtained, it is recommended that people substitute table sugars for this natural sugar as it contains mineral and nutrients including protein.
6. From the result of the morphological characteristics obtained, it is highly recommended that during harvesting, the stand should be cut from the ground surface to facilitate fast regrowth.
7. From the result of the morphological characteristics regarding its growth height, it is recommended that it be grown in areas where sunlight is visible because some stalks are short for easy photosynthesis.
8. Because it contains carbohydrate from the result of nutritional analysis, it is recommended that it be used as source of energy in domestic homes.

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