**PHYTOCHEMICAL SCREENING, PROXIMATE ANALYSIS AND NUTRIENT EVALUATION OF Thaumatoccocus daniellii (SWEET PRAYERS LEAVES) AND Musa accuminata (BANANA LEAVES)**

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**APPROVAL**

This project has been presented to and approved by Godfrey Okoye University, Enugu. In partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.), in Industrial Chemistry from the Department of Chemical Sciences.

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**CERTIFICATION**

This is to certify that everything written in this research work is true to the best of knowledge.

**DEDICATION**

I dedicate this work to Trinity in One God who in His infinite mercy has made the completion of this work possible and my parents, Mr. and Mrs. Simon Ezenwa for the financial support they gave me throughout my first degree.

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 **ABSTRACT**

Thaumatococcus daniellii and Musa accuminata are leaves used for wrapping foods and these herbs have some medicinal value. The affordability, preservation, usage techniques, and seasonal scarcity of the leaves make it difficult for people to use them frequently but they are preferred over plastic bags and aluminum foil in terms of wrapping edible foods like beans pudding (moi-moi), agidi, etc and fresh foods like uncooked meat, vegetables etc. This research work involved phytochemical screening, proximate analysis, and nutrient evaluation of the leave extracts of T. daniellii and M. accuminata .The extraction methods used were, hot / cold water maceration method and soxhlet extraction method on the *Thaumatoccocus daniellii* (sweet prayer leave) and *Musa accuminata* (banana leave). Phytochemical analysis showed the presence of alkaloids (+), tannins (+), glycosides (+), flavonoids (+),and steroids (+) present in cold water , hot water , and methanol extracts on the T. daniellii leaves. For the banana leave, the results obtained were as followed: alkaloids (+), tannin (+), glycosides (+),flavonoid (+), and steroids (-) in cold water extract, hot water extract and methanol extract. The proximate analysis was carried out on the samples evaluating the composition of ash content (20%), crude fibre (0.01%), moisture content (0.596%), crude protein (15.75%) for T. daniellii and M. accuminata, ash content (27%), crude fibre (0.01%), moisture content (00.82%), crude protein (13.13%). Nutrient evaluation was also carried out on the two samples. For T. daniellii, Ca (0.16mg/L), Mg (2.05mg/L), Na (nil), K (0.10mg/L), and Fe (nil).For M. accuminata, it contains Ca (0.19mg/L), Mg (2.45mg/L), Na (0.01mg/L), K (0.81mg/L) and Fe (0.36mg/L).

**CHAPTER ONE**

**1.0 INTRODUCTION**

**1.1 Background of Study**

Anything natural appears as it was made by nature therefore natural products are the starting point of all the synthetic compounds or products. Natural product such as plant extract, either as pure compounds or as standardized extracts help students, scientists, or researchers in discovery of novel products like drugs (for viral, fungi, bacterial and inflammatory and chemotherapeutic agents) and starting or intermediate chemicals (like phenol, benzene, food supplements like vitamins and minerals, fibres etc) for production of goods for human consumption. Twenty first century people are now going back on the natural products by using herbs to cure their diseases and to keep their body system fit.

Phytochemical analysis is a study of natural product compounds known as metabolites e.g alkaloids, steroids, glycosides, tannins, saponins, flavonoids etc. Metabolites are natural chemicals or products that are synthesized by enzymes of plants through photosynthetic process or by the aid of chlorophyll, sunlight, water and carbon dioxide. Proximate analysis is the study of nutrient values present in a specific natural extracts such as leafs, roots, seeds, stem etc of a plants.

**Medicinal plants**

Medicinal plants are plants which have a recognized medical use by test or experiment. They range from plants which are used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparation. Medicinal plants can be found growing in numerous setting all over the world.

Herbal medicines are the finished labeled medicinal product that contains active ingredients, aerial or underground parts of the plant or other plant material or combination (Chakraver et al, 1993, Chaudhari et al, 1996,Ritch, 2000).Herbal preparation constitute valuable natural resource from which chemicals of potential interest for medicine, agriculture, industry and other areas can be identified and isolated (Sneader et al, 1985).Socio-cultural backgrounds of people are more of herbal medicine like Yoruba’s way of life towards medicine. T. daniellii and M. accuminata are among those herbs that might have such medicinal properties.

**Sweet Prayers Leaf (*Thaumatococcus daniellii)***

*Thaumatococcus daniellii* leaves used for this research were purchased from New Market, Enugu (Originally collected from Ihum village in Biast local government area, Calabar in Cross-River State). The plant is a perennial, monocotyledonous herb and it is propagated by its rhizomes. It has longer slender stalks that can grow up to about two meters or more in terms of height. The leaves are broad and some are small depending on the rate of nutrient adsorption of their roots, also greenish colour. It has parallel venation. *T. Daniellii* is herb known as pretty plant to a lay man and some call it sweet prayers. It grows on dry land and swampy area, the dry land herbs do sprout a wine colour leaves on their earlier stage and a pale green leaves on maturity while the other is mainly greenish from its early stage to matured stage. The leaves are used for wrapping some Nigeria foods like moi-moi, agidi jellof, uza aki (Enugu state native food), to give the food taste and hold a particular shape before selling or serving it.

**Musa accuminata**

Banana leaves of the specie Musa accuminata is one of the local wrapping leaves that excel due to its function and necessity in some regions of Nigeria. People especially the old men often use the greenish leaves of it for wrapping of fresh meat. Banana plants are herbaceous perennials. They are mostly foliage, with stems made of rolled leaf layers. The plant leaves, which are up to 9 feet long and 2 feet wide, unfurl from these stalks. Banana plants are a common fruit crop. In some areas, gardeners grow them for ornamental reasons. But banana leaves also offer nutritional and medicinal benefits in addition to having other values. For meals, the wrapped banana leaves that form the plant’s stem contain starch, which is extracted through a fermentation or cooking process. People in some parts of the world use the resulting flour for baking. The starch is also cooked into glue. Both leaves have medicinal values.

**1.2 STATEMENT OF THE PROBLEMS**

* The usefulness of the leaves in herbal medicine to cure diseases.
* The beneficial quality of the two leaves to health.
* Nutritional content each of the leaves possesses as wrapping leaves.

**1.3 HYPOTHESIS**

The wrapping leaves Thaumatococus daniellii and Musa acuminate extracts that were gotten from cold method and soxhlex extraction contain significant amount of alkaloids, flavonoids, taninines, giycosides, and steroids but more concentrated in the Thaumatococus daniellii.

**1.4 AIM / OBJECTIVES**

The aim is to investigate two commercially available leaves (*Thaumatococcus daniellii* and *Musa accuminata*) used for wrapping foods.

The objectives are as follow:

* Determination of phytochemicals present.
* To anayse for the following parameters, ash content, crude fibre, crude protein and moisture content of the leaves.
* To determine the content of Na, Fe, Ca, Mg and K present in the leaves.

**JUSTIFICATION**

This is to justify that all the write ups in this thesis are true to the best of knowledge.

**CHAPTER TWO**

**2.0 LITERATURE REVIEW**

**2.1 HISTORY**

Thaumatococcus daniellii had been existing till the need of wrapping food for sell became a battle neck among human beings of early stage since they can rely on a particular something and they go insearch of alternatives to reduce scarcity, cost and labour. The broadness of the leave and aroma attracts human as they came in touch with it especially when hunting for water in the olden days in the timid areas. But as it stands today, Thaumatococcus daniellii is a tropical economic plant that has gained global popularity and interest owing to its usefulness in food wrapping and packaging nowadays. It is commonly found in the low-land tropical rainforest ecological zone of West Africa. Despite its popularity in food wrapping and packaging, empirical studies targeted at the basis for its savoury characteristics are still sketchy. However, a phytochemical screening of the leaf sample sourced from Calabar, South-south Nigeria revealed that flavonoid, alkaloids, tannins, Glycosides and steroid were significantly. The presence of these phytochemicals lends credence to the medicinal and nutritional benefits that it has been used for in the past years.



The sweetener plant, Thaumatococcus daniellii is a member of the Maranthaceae Family (Makinde and Taiwo 2004). It is particularly found in Southern parts of Ghana, Cote d’Ivoire and Nigeria (Yeboah et al., 2003). It is also known to exist in the Princes Islands, Angola, the Central African Republic, Uganda and Indonesia (Onwueme et al., 1979). The plant forms an undergrowth of trees in its natural habitat. It has long, slender stalks that can grow up to two or three meters high, each bearing a single tough, ovoid shaped leaf of varying sizes depending on the plant’s age and habitat. These leaves are ovate-elliptic rounded, truncate at the base, and shortly acuminate at the apex. The inflorescence of T. daniellii usually arises from the lowest node and may be simple or forked with spikes about 8 to 10 cm in length and bracts, usually umbricate, about 3 to 4 cm in length. The flowers that may be as long as the bracts form in short spikes close to the ground at the base of a swollen petiole. Sepals are broadly linear and about 1 cm in length. Corolla tubes are short and lobes are oblong and about 2.5 to 3 cm long. As many as 10 to 12 purplish-pink flowers may form on each inflorescence, but usually only 2, 3 or 4, rarely more than 4, of these form matured fruits. The plant flowers most of the year but is most prolific from July until late October, followed by fruit formation, maturing and ripening from January until mid-April (Onwueme et al. 1979). Thaumatococcus daniellii, whether cultivated or in wild, contributes to the economy of the rural people in the most parts of the Southern Nigeria through its stalks, leaves, fruits and rhizomes. The plant is of global prominence, consequent upon the discovery of “thaumatin”, a non-caloric sweetener derived from the arils of the plant which is reportedly 1600 times sweeter than sucrose. The use of leaves as a food wrapper/presentation 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, East and Western Nigeria, but also in some parts of the United States and the South Americas, where it is now acceptable and hip to display, buy, and eat foods packaged in such, even among the elites who consider the packaging (wrapping), as not only exotic, but also flavour enhancing. The use of these leaves, are very ancient ways (traditions) of the peoples, 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, and are used to hold/package/wrap large volumes of food and apparently the savoury characteristics which gives it an edge over other leaves with large surface area. It is however against this backdrop that this study tends to complement and validate existing research works on the phytochemistry of Thaumatococcus danielli and the nutritional benefit associated with it. It is both aquatic and teresial plant.It is planted by its rhizome.

**History of Musa acuminate**

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Bananas turned out to have very many benefits and this also applies to banana leaves, the leaf that we know is mainly used as wrapping for food that was sold. But they turned out to have another benefit. The benefits of this banana leaves include as food wrapping, accessories and also as a drug.

In addition to food wraps, turns out banana leaf has a wide range of advantages, the benefits of banana leaves that usually seen is that it is used as plates that are used for eating on. Many people known banana leaf only as food wrapper and only a handful of people know the other benefits of banana leaf that can help carry out a healthy lifestyle.

When you taste banana then you’ll feel its sweetness. There are many types of banana serving, such as fried bananas and banana chips that were processed.

Bananas are one fruit that is very popular with people from young to old, also loved by the monkey. Especially for people who have difficulty in digestion, then there must be a few times where they should be eaten bananas, banana is good for your intestinal tract.

**Health Benefits of Banana Leaves**

Green banana leaf grows on banana trees. As it was explained, the banana leaf has several other benefits in terms of health. Here are the health benefits of banana leaves:

* **Healthy Hair and Overcoming Dandruff:**

If you are having problems of dandruff on your head, then banana leaf is the solution you can use to overcome them. The first step is for banana leaves to be chopped down and crushed till the water is extracted. Then the banana leaf extract is smeared all over the head, let it absorb for a few moments and then rinsed thoroughly with clean water.

The banana leaf extract taken from the leaves will help us get rid of dandruff and make the hair remains perfect black, and if the hair is often itchy due to prickly heat then you should use this leaf because it is proven effective.

Procedure:  Take a fresh banana leaf then blend until it is smooth and then use it as a hair mask.

* **Beauty and Smoother Skin:**

The banana leaf can be used as a substitute ingredient for beauty creams. Not only can make smoothing beautiful skin, but also banana leaves can act as a germ killer that are good to avoid skin irritation problems that are mild or severe.

* **Face Mask:**

A banana mask may not sound strange to us, but what about the banana leaves? Banana leaves are not really used as a face mask, but banana leaves can overcome skin irritation and also kills germs on the skin so the skin can be more clean and clear. So Banana leaves are better for the health of our skin so that it can become a natural remedy.

* **Fever Cure:**

A banana leaf can also be used as a drug to treat fever. Certainly, don’t drink banana leaves, but instead, you use it by attaching a banana leaf that has been cleaned and smeared with coconut oil on the one’s forehead. Over longer term then the fever will be diminished.

* **Traditional Treatments :**

This is especially the traditional treatment that was known, someone can grab the banana leaf and rub with coconut oil and pasted it in several parts of the body that have a high fever, such as the waist and forehead. It is a natural remedy for fever.

* **Treat cuts and open injury:**

Banana leaf health benefits are as a natural wounds treatment. The ways to use it as wounds treatment are by cleaning banana leaves by washing. After that, you need to grind the banana leaves and placed evenly on the wound. This method can only be done for minor injuries. A banana leaf can also be used to help heal with open injuries.

* **As an antioxidant:**

Banana leaf also contains antioxidants that are beneficial to counteract free radicals that exist in the body.

* **The benefits of dried banana leaves:**

Dried banana leaves are rich in protein, hemicellulose, and lignin. Dried banana leaves can be used for mushroom cultivation that can later be eaten. In addition, dried banana leaves can also be used for food packaging.

* **Non-Hazardous:**

When using banana leaves as a base or a replacement dish, then you can avoid the dangers of using the plastic material because the plates that are used for eating sometimes are entirely not washed clean.

* **Appetite:**

 Aromatic scent permeated by banana leaves can increase appetite. Therefore, banana leaf

 used to wrap food can be used as a plate because it can increase appetite.

* **Hygienic:**

The Banana Leaf is a wrapper for food hygiene. To wrap food, better use banana leaves as compared to using a plastic wrap made with polymers that can cause cancer, especially when used to wrap the food that is hot.

* **As Neutralizing poisoning agent against venoms:**

Banana leaf benefits can also be used as a poison neutralizing agent against liquid that was injected into one’s body such as venom. If you are attacked by insect bites such as ants or mosquitoes then you can use banana leaves to neutralize the toxins and it will have no side effects whatsoever. You just need to grind banana leaves until it turns into a powder, then applied to the affected area of insect bites accordingly.

* **For Skin Inflammation Drugs:**

Dermatitis can be handled using a banana leaf which has been transformed into an ointment products. Then can be applied to the affected part of inflammation for treatment.

* **Enhancing Body Endurance:**

Banana leaves can enhance the immune system of a human body that are weak. In addition to enhancing endurance, a banana leaf can also ward off free radicals inducing cancer that are increasingly dangerous for human health. Perhaps this is a strange thing, but the substance that is stored in a banana leaf namely allantoin can be converted into an anti-oxidant producing agent.

* **Packaging food:**

The first benefits of banana leave surely as food wrappers. In the layers contained inside banana leaves have a texture similar to a candle wax. Thus making food wrapped in banana leaves not easily sticky. Additionally, it can add a distinctive aroma to the food wrapped in it. If you are using banana leaves for steaming food, then it will add a unique flavor to the food.

Typical traditional Indonesian food likes to use the banana leaf for its wrappers including for local delicacies. Some many other Indonesian foods are boiled with banana leaves to make the taste much more delicious. Banana leaves can also be used as a tray for the food to be eaten at. So then the tray can become disposable.

* **Food Wrapper:**

During this time banana leaves are mainly used as food wrappers only. Actually, almost all from banana parts has its own benefits, ranging from fruit, banana roots, leaves, and stems of banana. And several usages that can be used or processed into fertilizer, food, medicine and others. Surely, we can find out what are the other benefits of banana leaves other than for wrapping food.

* **Decoration ceremonies:**

Have you ever been to Bali? Then you’ll definitely see a lot of offerings presented to the gods. The offering use banana leaves as a decoration wrapping that is presented to deities. And it also acts as the tray of food from several offerings that are given, so the offering looks charming and interesting.

In the meal that was wrapped in banana leaves, then they have a much more interesting look and can make the meal appear native and hygiene. The advantages are not only constraint for food packaging, but also important to health as well as having another benefit listed below:

* When choosing a banana leaf then you need to check it again to know if cleanliness has been maintained properly or not.
* Because sometimes the wrapping for banana leaves are not that clean so you need to wash it twice before usage.
* But when using it, do not forget to always attempt to preserve the banana leaves in the garden.
* Don’t just consume it but you need to also care for its preservation of nature and the availability of existing banana leaves. As banana leaves have an important role that is beneficial to earth’s population

And many more benefits of banana leaves that can be acquired is by making banana leaves as an art, or can also be used as a canvas for painting or as wall hangings decoration.

It is better to use the banana leave in practice as a natural material for your consumption. The Banana Leaf is one of the natural resources that are very important to create a healthy lifestyle. Because as time goes by, more and more products that contain chemicals that can threaten human health need to be discontinued in usage. And the banana leaf is the solution for the elimination of using chemicals product. It is cultivated by its sucker, also a perennial plant.

**2.2 SITE OF STUDY**

The study was carried-out specifically in Godfrey Okoye University Project Research Laboratory, Thinkers Corner, Enugu, and Eastern Nigeria. The leaves were confirmed by a botanist in afore mentioned university, persevered under a serine environment and were blended with a new electric blender and were sieved twice with different sieving nets till they turn to powder. I stored the samples in air tight containers and labeled accordingly.

**2.3.0 Chemistry of Some Phytochemicals**

**2.3.1 Tannins (or tannoids)** are a class of [astringent](https://en.wikipedia.org/wiki/Astringent), [polyphenolic](https://en.wikipedia.org/wiki/Polyphenol) [biomolecules](https://en.wikipedia.org/wiki/Biomolecule) that bind to and [precipitate](https://en.wikipedia.org/wiki/Precipitation_%28chemistry%29) [proteins](https://en.wikipedia.org/wiki/Protein) and various other organic compounds including [amino acids](https://en.wikipedia.org/wiki/Amino_acid) and [alkaloids](https://en.wikipedia.org/wiki/Alkaloid).

The term tannin (from *tanna*, an [Old High German](https://en.wikipedia.org/wiki/Old_High_German) word for [oak](https://en.wikipedia.org/wiki/Oak) or [fir](https://en.wikipedia.org/wiki/Fir) tree, as in [Tannenbaum](https://en.wiktionary.org/wiki/Tannenbaum)) refers to the use of wood tannins from oak in [tanning](https://en.wikipedia.org/wiki/Tanning_%28leather%29) animal [hides](https://en.wikipedia.org/wiki/Hide_%28skin%29) into [leather](https://en.wikipedia.org/wiki/Leather); hence the words "tan" and "tanning" for the treatment of [leather](https://en.wikipedia.org/wiki/Leather). However, the term "tannin" by extension is widely applied to any large [polyphenolic](https://en.wikipedia.org/wiki/Polyphenol) compound containing sufficient [hydroxyls](https://en.wikipedia.org/wiki/Hydroxyl) and other suitable groups (such as [carboxyls](https://en.wikipedia.org/wiki/Carboxyl)) to form strong complexes with various [macromolecules](https://en.wikipedia.org/wiki/Macromolecule).

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from [predation](https://en.wikipedia.org/wiki/Predation), and perhaps also as [pesticides](https://en.wikipedia.org/wiki/Pesticides), and might help in regulating plant growth.The [astringency](https://en.wikipedia.org/wiki/Astringency) from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit, red wine or tea. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times.

**Structure and classes of tannins**

There are three major classes of tannins: Shown below are the base unit or monomer of the tannin. Particularly in the flavone-derived tannins, the base shown must be (additionally) heavily hydroxylated and polymerized in order to give the high molecular weight [polyphenol](https://en.wikipedia.org/wiki/Polyphenol) motif that characterizes tannins. Typically, tannin molecules require at least 12 hydroxyl groups and at least five phenyl groups to function as protein binders.

|  |  |  |  |
| --- | --- | --- | --- |
| **Base Unit:**  | Gallic acid.svg[Gallic acid](https://en.wikipedia.org/wiki/Gallic_acid) | Phloroglucinol structure.png[Phloroglucinol](https://en.wikipedia.org/wiki/Phloroglucinol) | Flavan-3-ol.svg[Flavan-3-ol](https://en.wikipedia.org/wiki/Flavan-3-ol)'s scaffold |
| **Class/Polymer:**  | [Hydrolyzable tannins](https://en.wikipedia.org/wiki/Hydrolyzable_tannin)  |  [Phlorotannins](https://en.wikipedia.org/wiki/Phlorotannin)  | [Condensed tannins](https://en.wikipedia.org/wiki/Condensed_tannin) and [Phlobatannins](https://en.wikipedia.org/wiki/Phlobatannin) (C-ring isomerized condensed tannins)  |
| **Sources**  | Plants  | [Brown algae](https://en.wikipedia.org/wiki/Brown_algae)  | Plants (former), tree heartwood (latter)  |

[Oligostilbenoids](https://en.wikipedia.org/wiki/Oligostilbenoid) (oligo- or polystilbenes) are oligomeric forms of [stilbenoids](https://en.wikipedia.org/wiki/Stilbenoid) and constitute a class of tannins.

**2.3.2 Alkaloids**

Alkaloids are a class of [naturally occurring](https://en.wikipedia.org/wiki/Natural_product) [chemical compounds](https://en.wikipedia.org/wiki/Chemical_compound) that mostly contain [basic](https://en.wikipedia.org/wiki/Base_%28chemistry%29) [nitrogen](https://en.wikipedia.org/wiki/Nitrogen) atoms. This group also includes some related compounds with neutral and even weakly [acidic](https://en.wikipedia.org/wiki/Acid) properties. Some synthetic compounds of similar structure are also termed alkaloids. In addition to [carbon](https://en.wikipedia.org/wiki/Carbon), [hydrogen](https://en.wikipedia.org/wiki/Hydrogen) and [nitrogen](https://en.wikipedia.org/wiki/Nitrogen), alkaloids may also contain [oxygen](https://en.wikipedia.org/wiki/Oxygen), [sulfur](https://en.wikipedia.org/wiki/Sulfur) and, more rarely, other elements such as [chlorine](https://en.wikipedia.org/wiki/Chlorine), [bromine](https://en.wikipedia.org/wiki/Bromine), and [phosphorus](https://en.wikipedia.org/wiki/Phosphorus).

Alkaloids are produced by a large variety of organisms including [bacteria](https://en.wikipedia.org/wiki/Bacteria), [fungi](https://en.wikipedia.org/wiki/Fungus), [plants](https://en.wikipedia.org/wiki/Plant), and [animals](https://en.wikipedia.org/wiki/Animal). They can be purified from crude extracts of these organisms by [acid-base extraction](https://en.wikipedia.org/wiki/Acid-base_extraction). Alkaloids have a wide range of [pharmacological](https://en.wikipedia.org/wiki/Pharmacology) activities including [antimalarial](https://en.wikipedia.org/wiki/Antimalarial_medication) (*e.g.* [quinine](https://en.wikipedia.org/wiki/Quinine)), [antiasthma](https://en.wikipedia.org/wiki/Asthma) (*e.g.* [ephedrine](https://en.wikipedia.org/wiki/Ephedrine)), [anticancer](https://en.wikipedia.org/wiki/Chemotherapy) (*e.g.* [homoharringtonine](https://en.wikipedia.org/wiki/Omacetaxine_mepesuccinate)), [cholinomimetic](https://en.wikipedia.org/wiki/Cholinomimetic) (*e.g.* [galantamine](https://en.wikipedia.org/wiki/Galantamine)), [vasodilatory](https://en.wikipedia.org/wiki/Vasodilation) (*e.g.* [vincamine](https://en.wikipedia.org/wiki/Vincamine)), [antiarrhythmic](https://en.wikipedia.org/wiki/Antiarrhythmic_agent) (*e.g.* [quinidine](https://en.wikipedia.org/wiki/Quinidine)), [analgesic](https://en.wikipedia.org/wiki/Analgesic) (*e.g.* [morphine](https://en.wikipedia.org/wiki/Morphine)), [antibacterial](https://en.wikipedia.org/wiki/Antibacterial) (*e.g.* [chelerythrine](https://en.wikipedia.org/wiki/Chelerythrine)), and [antihyperglycemic](https://en.wikipedia.org/wiki/Anti-diabetic) activities (*e.g.* [piperine](https://en.wikipedia.org/wiki/Piperine)). Many have found use in [traditional](https://en.wikipedia.org/wiki/Traditional_medicine) or [modern medicine](https://en.wikipedia.org/wiki/Pharmaceutical_drug), or as starting points for [drug discovery](https://en.wikipedia.org/wiki/Drug_discovery). Other alkaloids possess [psychotropic](https://en.wikipedia.org/wiki/Psychoactive_drug) (*e.g.* [psilocin](https://en.wikipedia.org/wiki/Psilocin)) and [stimulant](https://en.wikipedia.org/wiki/Stimulant) activities (*e.g.* [cocaine](https://en.wikipedia.org/wiki/Cocaine), [caffeine](https://en.wikipedia.org/wiki/Caffeine), [nicotine](https://en.wikipedia.org/wiki/Nicotine), [theobromine](https://en.wikipedia.org/wiki/Theobromine)), and have been used in [entheogenic](https://en.wikipedia.org/wiki/Entheogenic) rituals or as [recreational drugs](https://en.wikipedia.org/wiki/Recreational_drug). Alkaloids can be [toxic](https://en.wikipedia.org/wiki/Toxicity) too (*e.g.* [atropine](https://en.wikipedia.org/wiki/Atropine), [tubocurarine](https://en.wikipedia.org/wiki/Tubocurarine)). Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly evoke a [bitter taste](https://en.wikipedia.org/wiki/Bitter_%28taste%29#Bitterness).

The boundary between alkaloids and other nitrogen-containing natural compounds is not clear-cut. Compounds like [amino acid](https://en.wikipedia.org/wiki/Amino_acid) [peptides](https://en.wikipedia.org/wiki/Peptide), [proteins](https://en.wikipedia.org/wiki/Protein), [nucleotides](https://en.wikipedia.org/wiki/Nucleotide), [nucleic acid](https://en.wikipedia.org/wiki/Nucleic_acid), [amines](https://en.wikipedia.org/wiki/Amine), and [antibiotics](https://en.wikipedia.org/wiki/Antibiotics) are usually not called alkaloids. Natural compounds containing nitrogen in the [exocyclic](https://en.wikipedia.org/wiki/Alicyclic_compound) position ([mescaline](https://en.wikipedia.org/wiki/Mescaline), [serotonin](https://en.wikipedia.org/wiki/Serotonin), [dopamine](https://en.wikipedia.org/wiki/Dopamine), etc.) are usually classified as [amines](https://en.wikipedia.org/wiki/Amine) rather than as alkaloids.

 

Bufotenin Nicotine

**2.3.3 Steriods**

A **steroid** is a biologically active [organic compound](https://en.wikipedia.org/wiki/Organic_compound) with four rings arranged in a specific [molecular configuration](https://en.wikipedia.org/wiki/Molecular_configuration). Steroids have two principal biological functions: as important components of [cell membranes](https://en.wikipedia.org/wiki/Cell_membrane) which alter [membrane fluidity](https://en.wikipedia.org/wiki/Membrane_fluidity); and as [signaling molecules](https://en.wikipedia.org/wiki/Signal_transduction). Hundreds of steroids are found in [plants](https://en.wikipedia.org/wiki/Plant), [animals](https://en.wikipedia.org/wiki/Animal) and [fungi](https://en.wikipedia.org/wiki/Fungus). All steroids are manufactured in cells from the sterols [lanosterol](https://en.wikipedia.org/wiki/Lanosterol) (animals and fungi) or [cycloartenol](https://en.wikipedia.org/wiki/Cycloartenol) (plants). Lanosterol and cycloartenol are derived from the [cyclization](https://en.wikipedia.org/wiki/Cyclic_compound) of the [triterpene](https://en.wikipedia.org/wiki/Triterpene) [squalene](https://en.wikipedia.org/wiki/Squalene).

The steroid [core structure](https://en.wikipedia.org/wiki/Parent_structure) is composed of seventeen [carbon](https://en.wikipedia.org/wiki/Carbon) atoms, bonded in four "[fused](https://en.wikipedia.org/wiki/Fused_compound)" rings: three six-member [cyclohexane](https://en.wikipedia.org/wiki/Cyclohexane) rings (rings A, B and C in the first illustration) and one five-member [cyclopentane](https://en.wikipedia.org/wiki/Cyclopentane) ring (the D ring). Steroids vary by the [functional groups](https://en.wikipedia.org/wiki/Functional_groups) attached to this four-ring core and by the [oxidation state](https://en.wikipedia.org/wiki/Oxidation_state) of the rings. [Sterols](https://en.wikipedia.org/wiki/Sterol) are forms of steroids with a [hydroxy group](https://en.wikipedia.org/wiki/Hydroxy_group) at position three and a skeleton derived from [cholestane](https://en.wikipedia.org/wiki/Cholestane). Steroids can also be more radically modified, such as by changes to the ring structure, for example, [cutting](https://en.wikipedia.org/wiki/Bond_cleavage) one of the rings. Cutting Ring B produces [secosteroids](https://en.wikipedia.org/wiki/Secosteroid) one of which is [vitamin D3](https://en.wikipedia.org/wiki/Vitamin_D3)).

Examples include the [lipid](https://en.wikipedia.org/wiki/Lipid) [cholesterol](https://en.wikipedia.org/wiki/Cholesterol), the sex hormones [estradiol](https://en.wikipedia.org/wiki/Estradiol) and [testosterone](https://en.wikipedia.org/wiki/Testosterone) and the [anti-inflammatory](https://en.wikipedia.org/wiki/Anti-inflammatory) drug [dexamethasone](https://en.wikipedia.org/wiki/Dexamethasone).

**STEROID RING SYSTEM**



The parent ABCD steroid ring system (hydrocarbon framework) is shown with [IUPAC](https://en.wikipedia.org/wiki/IUPAC)-approved ring lettering and atom numbering.

**CHAPTER THREE**

**3.0 MATERIALS AND METHODS**

**3.1 REAGENTS AND APPARATUS/ EQUIPMENTS**

Methanol

Distilled Water

Sodium hydroxide

Tetraoxosulphate (VI) acid

Ferric chloride

Fehling solution A

Iodine

Potassium iodide

Nitric acid

Perchloric acid

Hydrochloric acid

CuSO4

Na2SO4

Zinc

Boric acid

Methyl red

NH2SO4

Boiler

Bowl

Thermometer

Hand glove

Filter paper

Aluminum foil

Sample bottle

Masking tape

Towel

Spatula

Glass funnel

Conical flasks

Stirrer

Muslim cloth

Beakers

Soxhlex extractor

Petri dish

Volumetric flask

Test-tube rack

Retort stands and clamps

Fume cupboard

Platinum Crucible

Pipette

pH paper

Thermometer

Measuring cylinder

Weighing balance

Temperature Sleeve

Lab. Oven

Muffle furnace

**AAS**

**3.2 SAMPLE AND SAMPLE COLLECTION**

We have different wrapping leaves all over the whole world but two among others are selected for this research. The selection of plants can affect the research work if care is not taken. There is possibility of different concentrations of phytochemicals on different parts of a particular plant, even on different plants. The two leaves selected have different concentration of both secondary metabolites and proximate analysis. The T.daniellii was bought from New Market and the M. accuminata was gotten from Amorji Nike both places in Enugu State. The leaves were identified by Prof. C. Okezie in the department of Biotechnology and Applied Biology, Godfrey Okoye University, Enugu, Nigeria. To aid taxonomic experts in confirming the field identification and to get a permanent scientific record, the leaves were put in the book of record according to the professor in botany already mentioned. The two leaves were air dried batch by batch and weighed untill I observed constant weight of 1.34g.Then grinding process followed.

**3.3 PROCEDURE**

Two methods were used, namely;

* Maceration Method
* Soxhlet Method

 Maceration method is also called the cold method which involve soaking of fat free samples with perspective solvents in other to get suitable extract for analysis .The samples were soaked for 72 hours per solvent.

The soxhlet method is the use of soxhlet exctractor by the aid of electrical power to get an extract too. The method is more reliable than the cold method and if constant power is maintained, it is faster. The samples were extracted for 14 – 15 hours and they reflux for about 6-8 times.

**3.4. DATA AND SATISTICAL ANALYSIS**

The fat free samples were weighed by using electronic weighing balance and filter paper. When filter paper was placed on the weighing balance, the reading was zeroed to get the accurate measurement. The measured samples were turned into different 1000ml of beakers which were washed, rinsed and oven dried.

50grammes of the two leaves were weighed and poured into the 1000ml empty beakers which were already washed and labeled accordingly with masking tape, 1000ml of cold distilled water was poured gradually inside the beaker containing the individual powdered samples and was stirred gently until the upper layers were mixed the down layers which water and small particles of those samples.

Distilled water was boiled at 100 0C and another 50grammes of the two samples were soaked too. After every 72 hours, the soaked samples were filtered using whatman filter paper. The filtration processes consist of two parts namely; the solid part that is the residue and the liquid part which is the filtrate. The filtrates from the two substances were oven dried till they became highly concentrated and the residues were thrown away.

The same gram mage was used for soxhlet extraction using methanol for both samples. I used muslin cloth to tie the measured sample. The quantity of the sample used, the solvent and the time taken to have a complete extraction were recorded and the table is shown below:

**Table 1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| SamplesBanana leafSweet prayers leaf | Quantity of samples50grammes50 grammes | Time in the Soxhlet (at 650C)18 hrs.31mins15hrs.39 mins | Solvent usedMethanolMethanol | Quantity of the solvent1000ml100ml |

After extraction, the extracts were poured into 1000ml beakers. For sweet prayer sample, it was about 750ml in the beaker and the solvent was evaporated in a water bath at temperature of 500C till the extract reachedone quart (1/4) of the solution and the same procedure was carried out on banana sample. The evaporation was done to concentrate the samples for faster reaction.

**3.4.1 Phytochemical Analysis (Qualitative)**

Phytochemical analysis is the type of analysis that is carried out on food samples, leaves, seeds and so on to discover the medicinal values present in those substances. Phytochemical analysis comprises of quantitative and qualitative analysis but one is carried out on this research. Qualitative phytochemical analysis was carried out evaluating the presence of alkaloids, tannins, glycosides, flavonoids, and steroids using the procedure outlined by Sofowara (1993) and Trease and Evans (2002).

**3.4.2 Test for Alkaloids:**

During the test, 3cm3 of the extracts from the already concentrated filtrates of different solvents of the two samples were pipette into six different test tubes and1cm3 of HCl was added. The mixtures were heated for 20 minutes in a water bath and were shaking while heating. After heating, the extracts were allowed to cool and filtered with filter paper into other test tubes.1ml of the filtrates were added to 0.5cm3 of Wagner’s reagent.

**Observation:**

Creamy colour change observed.

How Wagner’s reagent was Prepared.

1.3g of iodine crystal was weighed into 100ml beaker. I added a pinch of potassium iodide to melt the iodine crystal. About 10ml of distilled water was added to aid the reaction. Then the solution was poured into 50ml volumetric flask and makes it up to the mark.

**3.4.3 Test for Tannins**

In separate test tubes, 2ml of the extracts were boiled gently for 20 minutes and allowed to cool.

Also, 3 drops of ferric chloride solution was added to each test tube.

**Observation:**

Green color was shown on the banana samples and brownish green on sweet prayer samples.

**3.4.4 Test for Glycosides**

In the test tubes were 1ml of aqueous extracts and 10ml of 50% H2SO4 was added, also heated for 15 minutes and allowed to cool. Then, 10ml of fehling solution A was added and boiled again for the same minutes.

**Observation:**

Expect brick red precipitate.

**3.4.5 Test for flavonoid:**

Pipette 3ml of extracts with different solvents into different conical flasks, 10ml of distilled water was added into each and shake very well. Also 1ml of 10% NaOH solution was added to the mixture (Okaka, 2006).

**Observation:**

A yellow coloration appeared.

**3.4.6 Test for Steriods:**

Here 5 drops of concentrated H2SO4 was added to 1ml of the extracts in separate test tubes.

**Observation:**

Redish colour indicates that steroids is present (Okaka, 2006).

**3.5 PROXIMATE ANALYSIS**

The proximate analysis was carried out the samples evaluating the composition of ash content, crude fibre, moisture content and crude protein.

**3.5.1 Ash Content**

Empty platinum crucibles were washed, dried and allowed to cool and weighed.

2grames of grounded samples were weighed accurately into two platinum crucibles and covered separately.

The two covered crucibles were ignited in a muffle furnace at 1000C for 17 minutes. The platinum crucibles and their content were cooled to room temperature in a desicator.

The ash content was obtained by calculation using:

Wt of the filter papers = W2

Wt of the sample = w

Wt of the empty P.C = Wt1

Wt of P.C + sample after burning = Wt2

Ash Content = Wt2 – Wt1

Ash % = Wt2 – Wt1 x 100

 w 1

Sample A = Sweet prayer

Sample B = Banana

For sample A :

Wt1 of the crucible = 23.68g

Wt2  = 24.22g

W = 2g

W2  for Sample A = 1.06g

W2 for Sample B =1.01g

% Ash = (25.68 – 24.22) x 100

 2

 = (1.46 – 1.06)/ 2)\* 100

 = 20%

For sample B:

% Ash = (23.91 – 22.36)/2) \* 100

 = (1.55 – 1.01) /2) \* 100

 = 27%

**3.5.2 Crude Fibre:**

2g of the sample was weighed into 250ml conical flask and soaked in 200ml of H2SO4.It was heated for 30 minutes on a hot plate.

The residue was filtered and washed with hot H2O until it was more acidic, through the use of a pH paper. The residue was resoaked with 200ml of 1.2% of NaOH. It was heated again for another 30 minutes.

It was filtered on a noted weight of filter paper, dried in an oven and weighed again. An empty PC (platinum crucible) was weighed. The residue in the filter paper was transferred into the weighed PC. It was burnt to ashes using muffle furnace. After ashing, it was coled in desicator and weighed again. After which it was then calculated thus;

Weight of sample = W

Weight of the empty filter paper = wt1

Filter paper + sample = wt2

Empty Crucible = wt3

Crucible + Sample after ash = wt4

% Crude fibre = wt2 – wt1/ W x 100/1

Wt2 = 1.06 + 2g = 3.06g

Wt3 = 21.86g

Wt4 = 21.86 + 24.2 = 46.06g

% of sample A = (3.06 – 1.06) / (2 \* 100) = 0.01%

% of sample B = (3.01 – 1.01) / (2\* 100) = 0.01%

**3.5.3 Moisture Content:**

The petri-dish was washed and dried in an oven. 1g of the sample was weighed into the petri-dish. The petri-dish and the sample were weighed first before dry and the weight was noted. It was put in the oven and dried for an hour and the weight was noted. The drying continued until constant weight was obtained and then calculated (Okaka, 2006).

Mn = (Mw –Wd) / Mw \* 100

Mn = moisture content % of the material n

Mw = net weight of the sample and

Wd = weight of the sample after drying.

Sample A weight = 24.17g + 1g

Sample B weight = 23.31 + 1g

Mn for sample A = (25.17 – 25.02) / 25.17) \* 100

 = 0.596%

Mn for sample B = (24.31 – 24.29) / 24.31) \* 100

 = 00.82%

**3.5.4 Crude Protein:**

**Digestive stage**

1g of the sample was placed into Kjelda flasks.

10g of CuSO4 was added with 20ml concentrated H2SO4. The solution was heated with Bunsen burner in a fume cupboard until the solution changed to blue green colour, indicating complete digestion.

Heating was stopped and the solution was allowed to cool for 24 hours. On cooling, the solution solidified and the colour changed from blue to green to white colour (Osasona, 2011)

**Distillation Stage:**

Here, 200 ml of distilled water was added to the solidified sample in the Kjedal flask to dissolve it, giving an exothermic reaction.

After cooling, 40% NaOH was added to it and also 3 pieces of zinc metal, acting as catalyst was added and then transferred to a round bottom flask connected to the distillation apparatus and it was heated. The distillate was collected in a 250ml conical flask containing 4% boric acid (100ml) and 2 drops screened methyl red indicator.

It was observed that a light pink colour occurs when boric acid screen methyl red indicator came in contact. When the distillation was completed, the colour changed to light blue.

**Titration**

The distillate and the collection solution about 200ml, was titrated with 0.1NH2SO4.

The end point was taken when the colour of the solution changed from light blue to pink.

**Calculation:**

% N = (Tv \* 0.0014 \* 100) / Wt of the sample

% protein = % N \* 6.25

N = Nitrogen

Tv = Titration value

Wt = weight

% N for sample A

Tv = 18

(18 \* 0.0014 \* 100) / 1 =2.52

2.52 \* 6.25 = 15.75%

For sample B

Tv = 15

(15 \* 0.0014 \* 100) / 1 = 2.1

2.1 \* 6.25 = 13.12%

**3.6 Nutrient Evaluation**

**Procedure for the samples digestion:**

* 1. Exactly 1 g of those fat free samples were taken in a 250 mL conical flask
	2. Twenty mL of di-acid mixture was added in to, the flasks.
	3. The flasks were stirred to moisten the fat free samples and then the flasks were placed on an electric heat sleeve.
	4. The contents were heated at 30˚ to 65˚C until white fumes were evolved and the solutions become colorless.
	5. Little amount of about 5 mL di-acid mixture was added to the samples in the flasks when the contents become dried or yellowish in color at the end of the digestion.
	6. The flask was removed from the sand bath and was allowed to cool.
	7. Twenty to thirty mL of distilled water was added and was shaken thoroughly.
	8. The solution was filtered through filter paper (Whatman No. 1) into a 100 mL volumetric flask.
	9. The conical flask was washed for several times to ensure that all the minerals are transferred into the volumetric flask.
	10. Volume was made up to the mark with distilled water.
	11. The content transfer to a sample container with proper labeling.

**Precautions:**

1. Plant/crop samples should be weighed exactly and accurately.
2. Care should be taken when di-acid mixture is prepared.
3. The contents should not be dried up during digestion.
4. Conical flask should be washed several times during transfer the solution.

Then the digested samples were labeled accordingly and sent to PRODA for Atomic Adsorption Spectrophotometer analysis.

**CHAPTER FOUR**

**4.0 RESULTS**

**4.1 Result for *Thaumatococcus daniellii***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Solvents used | Hot distilled water | Cold distilled water | Methanol |
| Phytochemicals |  |  |  |  |
| AlkaloidsTanninsGlycosidesFlavonoidsSteriods |   | +++++ | +++++ | +++++ |

**4.2 Phytochemicals result for Musa accuminata**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Solvents used | Hot distilled water | Cold distilled water | Methanol |
| Phytochemicals |  |  |  |  |
| AlkaloidsTanninsGlycosidesFlavonoidsSteriods |   | ++++- | ++++- | ++++- |

Key words:

Present = +

Absent = -

**4.3 Proximate analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples name | % Crude Fibre | % Ash Content | % Moisture Content | % Crude Protein |
| Sweet prayers leaf | 0.01 | 20 | 0.596 | 15.75 |
| Banana leaf | 0.01 | 27 | 00.82 | 13 |

**4.4 Nutrient evaluation (elementary analysis measured in mg/L)**

|  |  |
| --- | --- |
| Samples |  Parameter (mg/L) |
| Ca | Mg | Na | K | Fe |
| Sweet prayers leaf | 0.16 | 2.05 | Nil | o.10 | nil |
| Banana leaf | 0.19 | 2.45 | 0.01 | 0.81 | 0.36 |

**CHAPTER FIVE**

**5.0 DISCUSION**

The phytochemical screening on the two samples *Thaumatococcus daniellii* and *Musa accuminata* showed that presence of alkaloids, tannins, glycosides, and steroids but steroids are not present in Musa accuminata. When the proximate analysis was carried out, the two samples had the same percentage of crude fibre. The proximate analysis was carried out on the samples evaluating the composition of ash content (20%), crude fibre (0.01%), moisture content (0.596%), crude protein (15.75%) for T. daniellii and M. accuminata, ash content (27%), crude fibre (0.01%), moisture content (00.82%), crude protein (13.13%).The evaluation was to partition the compounds into categories based on the chemical properties. Nutrient evaluation was also carried out on the two samples. For T. daniellii, Ca (0.16mg/L), Mg (2.05mg/L), Na (nil), K (0.10mg/L), and Fe (nil).For M. accuminata, it contains Ca (0.19mg/L), Mg (2.45mg/L), Na (0.01mg/L), K (0.81mg/L) and Fe (0.36mg/L) and the evaluation was to determine the elements mainly metals. The results could be used in food sciences and medical aspect if the medicinal compounds should be extracted and properly stored. From the results one could know the importance of using the leaves as food wrappers.

**5.2 CONCLUSION**

Research works have spontaneously increased the standard of living positively. According to the one in question, sweet prayer has more medicinal value than banana while banana has more mineral content than sweet prayer. It is of great advantage to use these wrapping leaves for our foods instead of plastic bags which endanger our lives (causes cancer).

**5.3 RECOMMENDATION**

The analysis in this research work would encourage the new age to sky rocket their research on how to extract in pure state the essential minerals, and phytochemical compounds present in *Thaumatococcus daniellii* and *Musa accuminata* for industrial and home benefits. Research on natural products should be carried out frequently to improve life and economy.

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