



Determination of phytochemicals in *Thaumatococcus daniellii* (sweet prayer leaves) and *Musa paradisiaca* (plantain leaves) as a food packaging material

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Abstract

Leaves from the plants of *Thaumatococcus daniellii* and *Musa paradisiaca* are used for wrapping foods in Ghana. In this study, the types of phytochemicals present in the two leaves were qualitatively determined. Standard qualitative phytochemical tests by Trease and Evans, Sofowora and Harborne were employed in screening for ten selected phytochemicals. The following phytochemicals were present in *T. daniellii* leaf extracts; saponins, polyuronides, phenolic compounds, reducing sugars, alkaloids, flavonoids, cyanogenic glycosides, phytosterols and anthracenosides. However, triterpenes were absent. *M. paradisiaca* also showed the presence of saponins, phenolic compounds, cyanogenic glycosides, polyuronides, reducing sugar, flavonoids and phytosterols. However, alkaloids, triterpenes and anthracenosides were absent. From the results obtained, *T. daniellii* contained more classes of healthful phytochemicals than that of *M. paradisiaca*. Since, the leaves of both *T. daniellii* and *M. paradisiaca* contribute more than just a means of packaging to these local delicacies, their continued use should be highly encouraged and widely promoted.

Keywords: Phytochemicals, ethanolic extraction, plantain leaves, sweet prayer leaves

Introduction

Foods have been packaged for centuries using leaves (Risch., 2009). Using leaves as a traditional packaging material; is a natural, locally-available, cheap and sustainable means of improving the shelf-life of agricultural products (Arowosage and Popoola, 2006). There have been several reports of the medicinal value of leaves used for wrapping foods such as its antimicrobial, anthelmintic and antifungal effects (Hussain et al., 2010; Egbuonu et al., 2016; Hamid et al., 2017). Hence, it is expected that, through the migration of phytochemicals, during preparation or storage of food, beneficial health effects could be imparted to the food from its leaf packaging. Apart from this, the cultivation and trade of these leaves also constitutes a potential resource for economic development through the supply of income and employment to resource-poor farmers in rural communities (Yeboah et al., 2003). Thus, taking advantage of the potential gains of this important commodity can be a means of poverty alleviation through sustainable agriculture (Arowosage and Popoola, 2006; Ezeudu et al., 2020). However, advancement in technology, has led to the replacement of these natural materials with synthetic organic materials such as paper, plastics, rubbers, glass and metal containers (Brody et al., 2008). In spite of this shift, in Ghana, leaves have continued to be used for packaging ready-to-eat cornmeal products and some food vendors continue to use leaves to wrap food for their customers (Mensah et al., 2012). Popular among leaves used as packaging in Ghana, is the leaves of plantain and leaves of the sweet prayer or Katemfe plant (Yeboah et al., 2003; Mensah et al., 2012).

Thaumatococcus daniellii (Benn.) Benth is a perennial plant and monocotyledonous herb. It belongs to the family of Maranthaceae (Makinde and Taiwo, 2004). It grows primarily in West Africa especially in Southern Nigeria, Cote d'Ivoire and Ghana. It is also found in other countries such as Angola, Uganda, the Central African Republic, Australia, the Princes Islands and Indonesia (Yeboah et al., 2003). It produces leaves of varying sizes depending on the plant's age and habitat; which are single tough and ovoid in shape (Yeboah et al., 2003; Makinde and Taiwo, 2004). The economic prospects of its fruits has risen to global significance through its discovery as a natural source of thaumatin, a non-caloric, non-toxic sweetener (Hamid et al., 2017). Ensuring an adequate and a sustainable fruit supply would lead to the generation of abundance of leaves that may just be discarded. So after the fruits are harvested, the leaves could be utilized as packaging material for food as well as investigated for medicinal uses (Ukwubile et al., 2017; Fadahunsi et al., 2021). According to an in vitro study by Ukwubile et al., (2017), the growth of selected pathogenic bacteria was inhibited by the leaves of *Thaumatococcus daniellii*. Hence, they speculated that extracts of *T. daniellii* leaves may be able to cure typhoid fever caused by *Salmonella typhi* and prevent early abortion caused by *Campylobacter jejuni*.

Plantain is a strategic crop for ensuring food security across the globe (FAOSTAT, 2013). The name "plantain" refers to *Musa paradisiaca* L., which requires cooking before consumption. About 32% of its global production comes from West Africa (WA), with 12.4 million metric tons cultivated on 1.7 million hectares in 2011 (FAOSTAT, 2013). Currently, Ghana produces around 4.87 million tonnes of plantain per annum; making it the highest producer of plantain in WA (FAOSTAT, 2019). The consumption and yield of plantain have also been increasing yearly in Ghana (Dzomeku et al., 2011). Plantain is a choice ingredient for many popular Ghanaian delicacies such as "ampesi", "fufu", "ofam", "kaklo", "tatale" and "red red" (Dadzie and Wainwright, 1995, Marimo et al., 2020). There is however, limited utilization of its fresh leaves to feed farm animals and dried leaves for packaging. It is used as containment for ready-to-eat cornmeal products such as sugared "kenkey", "fante" "kenkey", "estew", "fomfom" and "nsihu" (Mensah et al., 2012; Loos et al., 2018). There is, therefore, an existing need to diversify the usage of plantain leaves. The leaves of *Musa paradisiaca* have been found to exhibit antimicrobial, anticancer, hepatoprotection and anthelmintic activity (Ahmad and Beg, 2001; Dikshit et al., 2001; Milder et al., 2005; Alisi et al., 2008; Hussain et al., 2010). Consumers of corn-meal products in Ghana believed that phytochemicals present in the leaves of *M. paradisiaca* can cure fever, abdominal pains and also act as a purgative (Mensah et al., 2012). However, most had no idea about the type of beneficial phytonutrients that can leach from the leaves into the corn-meal products (Mensah et al., 2012).

Moreover, it is necessary to ensure that the foods packaged with these leaves are safe for consumption. To do this requires knowing the physiological and pharmacological effects these leaves impact to the food it is packaged in. This implies screening these leaves to know their phytochemical constituents. Especially, since the phytochemicals in the leaves migrate into the food it is covering, causing changes in its aroma, taste and colour (Ibegbulem et al., 2004; Mensah et al., 2012; Ayodeji et al., 2016). This would help to promote their use and as well inform on which alternative uses may be appropriate, based on the classes of bioactive compounds found in them. This study, was therefore conducted to find out the phytochemicals found in the leaves of *T. daniellii* and *M. paradisiaca*, which are both used as a food package in Ghana.

Materials and Methods

2.1 Sampling Techniques

Purposive sampling was used to obtain the leaves of *Thaumatococcus daniellii* and *Musa paradisiaca* from the market.

2.2 Source of Sample Collection

The two leaf samples of *T. daniellii* and *M. paradisiaca* were collected freshly harvested from the Nima and Koforidua markets, respectively. They were then sun dried and grounded into powder at the Mampong Laboratory Research Institute in the Eastern Region of Ghana.

2.3 Preparation of Samples

The oven (FISHER Isotemp® Oven, SENIOR MODEL-USA) dried (60°C for 20min) leaves were grounded to powder. The powdered samples were then passed through a 0.05mm pore sized sieve. The crude phytochemicals of the samples were then extracted for 3hrs at 60°C of using the different extraction solvents (200ml of 70% Ethanol v/v, 70% Methanol v/v; and 50ml distilled water respectively). Whatman filter paper No.42 (125mm) was then used to filter the extracts. The extracted samples were then used for the tests.

2.4 Procedure for the phytochemical extraction of (*Thaumatococcus daniellii* and *Musa paradisiaca*).

The leaves (*Thaumatococcus daniellii* and *Musa paradisiaca*) were air-dried under shade and good ventilation for 2 weeks. It was then grinded using an electronic blender (Toni®-China) and weighed in a round bottom flask using an electronic balance. The round bottom flask was first weighed and zeroed to get the accurate measurement. Twenty (20) grams of the two different samples were weighed into the flasks. Two hundred (200) ml of 70% ethanol solution, 70% methanol solution and 50ml distilled water were added to the powdered samples in the flasks and stirred gently for a uniform mixture; these samples were labeled accordingly with masking tape. The extraction solvents mixed with the powdered samples of *Thaumatococcus daniellii* and *Musa paradisiaca* in the round bottom flasks were fixed to the reflux apparatus for a period of one (1) hour. Condensation reaction of the sample solution also occur at the same time. After an hour, the boiled samples were collected and whatman filter paper was used to filter it; whereas the residue was discarded. The filtrate was transferred into a petri-dish which was then placed in a water bath for complete evaporation of the extraction solvents. The samples were then allowed to cool before proceeding with their qualitative phytochemical analysis.



Plate 1: Sun drying of *M. paradisiaca*



Plate 2: Sun drying of *T. daniellii*



Plate 3: Powdered sample of *T. daniellii*



Plate 4: Powdered sample of *M. paradisiaca*



Plate 5: Measuring of the powdered samples



Plate 6: Reflux Method



Plate 7: Filtration of the extracted sample using Whatman paper

2.5.0 Phytochemical Screening

The methods used by Harborne (1973), Trease and Evans (1989) and Sofowora (1993) was used to detect the phytochemical constituents of leaves, such as the leaves of *T. daniellii* and *M. paradisiaca*. Qualitative phytochemical screening was carried out on these leaves to evaluate them for the presence of saponins, polyuronides, phenolic compounds, reducing sugar, cyanogenic glycosides, alkaloids, flavonoids, triterpenes, phytosterols and anthracenosides (Ciulei, 1981).

2.5.1 Test for Saponins

1:1 dilution of the sample and the solvent (water) was pipetted into a test tube and shaking vigorously for a period of 15 minutes, a foaming precipitate that persists for 2 minutes indicates the presence of saponins (Adusei et al., 2019).

2.5.2 Test for Reducing Sugar

Water (1-2 ml) was used to dilute each solvent extract (ethanol, water and methanol) (0.5-1ml). After dilution, Fehlings solution (A and B) (0.5 – 1 ml) was added to the extracts and the mixture was then heated. The presence of reducing sugars was attested by the appearance of a brick red precipitate (Adusei et al., 2019).

2.5.3 Test for Polyuronides

2 ml of each solvent extract were added dropwise into a 20ml test tube already containing 10 ml of acetone. The presence of polyuronides is attested by the formation of a thick precipitate (Adusei et al., 2019).

2.5.4 Test for Phenolic Compounds

To 3ml of each solvent extract about two to three drops of 5 % ferric chloride were added. After this, a sudden change in colour is anticipated. The presence of phenolic compounds is attested, if there is a colour change to black, bluish-black or dark green is seen, it shows the presence of phenolic compounds (Adusei et al., 2019).

2.5.5 Test for Alkaloids

The extraction solvents were evaporated to dryness using oven (FISHER Isotemp® Oven, SENIOR MODEL-USA) leaving residues containing alkaloids in a form of organic acid salts. About 5 ml of 10 % HCl was added to the residue converting it into salts of the mineral acid. The alkaloids were precipitated from each solution as bases with the help of ammonia solution (pH=8, 9, 10%). The precipitates were then extracted using non-polar solvent chloroform. An evaporating dish was then used to evaporate to dryness the non-polar solvent solution used. Hydrochloric acid solution (1.5 ml, 2%) was used in the dissolution of the resulting residue. The acidic solution containing the alkaloids in salt form were divided and placed in three separate test tubes. One was used as reference and the other two test tubes for the test. About 2-3 drops of Mayer's reagent were added to the two experiment test-tubes. The presence of alkaloids, is attested by the manifestation of yellowish-white precipitate or an opalescence.

Hydrochloric acid solution (10%, 15ml) was added to 25 ml of each solvent extract by refluxing. During this process of hydrolysis, due to the precipitating aglycones obtained by the division of the glycosides, the solution would turn opalescent. It was then cooled, extracted triplicate using ethyl ether (10-12 ml) the solution with the aid of a separating funnel. Anhydrous sodium sulphate was then added aid in the final dehydrating to obtain a pure extract. This resulted in an ether solution and an extraction solvent solution. By following a series of reactions characteristic of each of the following groups; triterpenes, steroid glycosides, anthracenosides and flavonoids, the ether extract is used to identify each group (Adusei et al.,2019).

2.5.6 Test for Anthracenosides

For this test, the ether extract (4 ml) obtained was concentrated to 2 ml, after which ammonia solution (25%, 1-2 ml) was added by shaking. The presence of aglycones of anthracenosides (Borntrager's reaction) is indicated by a cherish-red colour of the alkaline solution.

2.5.7 Test for Phytosterols and Triterpenes

The ether extract (10ml) was evaporated until dryness. Acetic anhydride (0.5 ml) and chloroform (0.5 ml) were successively added to the resulting residue to dissolve it. These solutions were transferred into another test tube. About 1-2 ml of concentrated sulphuric acid (Liebermann-Burchard's reaction) was added at the bottom. A reddish-brown or violet-brown ring was formed at the separating level of the two liquids. If the superior layer turning green indicates the presences of phytosterols and red or violet for triterpenes.

2.5.8 Test for Flavonoids

About 5 ml of ether extract was evaporated to dryness. The resulting residue was dissolved in methanol (50%, 1-2 ml) with heating. After this, 5-6 drops of concentrated hydrochloric acid and metal magnesium were added to it. Flavonols and flavonones (Shibata's reaction) are present if the solution transforms to a red colour.

2.5.9 Test for Cyanogenic Glycosides

About 1 ml of chloroform was placed in a test tube. A small volume of ether extract was added to it and the test tube was then firmly corked with a yellow picric paper. A water bath was used to heat the mixture for few minutes. If the colour of the yellow picric paper changes either to brown or red it shows the presence of cyanogenic glycosides (Sofowora, 1993).

Results and Discussion

3.1 Qualitative Determination of the Types of Phytochemicals present in the Two Leaves

In this study, the leaves of *Musa paradisiaca* and *Thaumatococcus daniellii* were screened for their phytochemical constituents using three extraction solvents (ethanol, water and methanol).

The presence of phytochemicals in these two leaves are summarized in the tables presented in this section.

Table 1: Phytochemicals Analysis of *Thaumatococcus Daniellii* and *Musa Paradisiaca* Leaves using Ethanol as Extraction Medium.

Phytochemicals	Ethanol Extract	
	<i>Thaumatococcus daniellii</i>	<i>Musa paradisiaca</i>
Saponins	+	+
Reducing Sugar	+	+
Polyuronides	+	+
Phenolics	+	-
Anthracenosides	+	-
Flavonoids	+	+
Cyanogenic glycosides	-	-
Alkaloids	+	-
Triterpenes	-	-
Phytosterols	+	+

Key: Present; +, Absent; -

The qualitative screening of 70% ethanol extract of *T. daniellii* for ten phytochemical constituents showed the presence of saponins, polyuronides, flavonoids, phenolic compounds, alkaloids, reducing sugar, phytosterols and anthracenosides which represents 80% bioactive constituents. However, triterpenes and cyanogenic glycosides were absent (Table 2). For *M. paradisiaca*, the analysis showed 50% of the bioactive constituents present and 50% absent. Saponins, polyuronides, reducing sugar, flavonoids and phytosterols were found to be present whereas phenolic compounds, alkaloids, triterpenes, anthracenosides and cyanogenic glycosides were absent (Table 1).

After the ethanolic extraction, and analysis, the researchers further used methanol (Table 3) and water (Table 2) as extraction media for *Thaumatococcus Daniellii* (Sweet Prayer Leaves) and *Musa Paradisiaca* (Plantain Leaves). Saponins was present in the water extract and methanol extract of both *T. Daniellii* and *M. Paradisiaca*. Anthracenoside, was absent in both the water and methanol extracts of both *T. Daniellii* and *M. Paradisiaca* (Table 2 and 3).

Table 2. Phytochemicals Analysis of *Thaumatococcus Daniellii* and *Musa Paradisiaca* Leaves using Water as Extraction Medium.

Phytochemicals	Water Extract	
	<i>Thaumatococcus daniellii</i>	<i>Musa paradisiaca</i>
Saponins	+	+
Reducing Sugar	+	-
Polyuronides	-	+
Phenolics	-	+
Anthracenosides	-	-
Flavonoids	+	+
Cyanogenic glycosides	+	+
Alkaloids	-	-
Triterpenes	-	-
Phytosterols	-	-

Key: Present; +, Absent; -

The results confirmed that Cyanogenic Glycosides was present in both water and methanol extractions of *M. Paradisiaca*. However, it was not in the methanol extract of *T. Daniellii*. Polyuronides was also confirmed in the water extract of *M. paradisiaca* but absent in water extract of *T. daniellii* and methanol extract of both *M. paradisiaca* and *T. daniellii*.

Phenolics was also confirmed in the water and methanol extract of *M. paradisiaca*, methanol extract of *T. daniellii* but absent in the water extract of *M. paradisiaca*. Flavonoids was also confirmed in the water extract and methanol extract of both *T. Daniellii* and *M. Paradisiaca*. Reducing sugar property was also confirmed in water and methanol extract of *T. daniellii* but absent in the water and methanol extracts of *M. paradisiaca*.

Table 3: Phytochemicals Analysis of *Thaumatococcus Daniellii* and *Musa Paradisiaca* Leaves using Methanol as Extraction Medium.

Phytochemicals	Methanol Extract	
	<i>Thaumatococcus daniellii</i>	<i>Musa paradisiaca</i>
Saponins	+	+
Reducing Sugar	+	-
Polyuronides	-	-
Phenolics	+	+
Anthracenosides	-	-
Flavonoids	+	+
Cyanogenic glycosides	-	+
Alkaloids	-	-
Triterpenes	-	-
Phytosterols	-	-

Key: Present; +, Absent; -

The qualitative phytochemical screening carried out using three different extraction solvents for ten phytochemical constituents of *T. daniellii* showed the presence of saponins, polyuronides, phenolic compounds, reducing sugar, alkaloids, flavonoids, cyanogenic glycosides, phytosterols and anthracenosides which represents 90% bioactive constituents. However, triterpenes were absent. For *M. paradisiaca*, the analysis showed 70% of the bioactive constituents present and 30% absent. Saponins, polyuronides, phenolic compounds, reducing sugar, flavonoids, cyanogenic glycosides and phytosterols were found to be present whereas alkaloids, triterpenes and anthracenosides were absent.

3.2 Health Implications of Determined Phytochemicals in the Leaves as a Packaging Material

3.2.1 Saponins

In the present study, saponins were discovered regardless of the medium used for extraction or the type of leaf been investigated. Saponins are an essential class of beneficial phytochemicals. They are known to contain an incredible compound; glycyrrhizin which is active against the Hepatitis B, the HIV and the SARS (Severe Acute Respiratory Syndrome) viruses (Arase et al., 1997; Cinatl et al., 2003; Harada, 2005). They also possess anti-tumor, immunomodulatory, anti-ulcer, anti-inflammatory, hypoglycemic and anti- neurological activities (Milgate and Roberts, 1995; Attele et al., 1999; He et al., 2001; Friedman, 2002; Matsui et al., 2004). Apart from its medicinal properties, the food industry globally uses it as a sweetening agent (Kitagawa, 2002).

3.2.2 Flavonoids

Flavonoids were also discovered in all the samples studied. They are a large class of phytochemicals with several physiological effects (Triopoli et al., 2007). These include possessing anti-oxidative, anti-inflammatory, anti-carcinogenic and anti-ageing properties (Sharma, 2006).

3.2.3 Alkaloids

Alkaloids in this study, were found only in the ethanolic extract of *T. daniellii*. These compounds have been reported to ensure maximum protection and survival of plants against microorganisms, insects, herbivores and other plants (Molyneux et al., 1996). Alkaloids when consumed as drug or part of the diet have also been reported to function as analgesics, antimalarial, antiasthma, anticancer, antibacterial, antihyperglycemic, anti-inflammatory, immunomodulatory and adaptogenic activities (Hartmann, 1991; Gupta, 1994).

3.2.4 Phytosterols

Phytosterols are plant-derived substances that competes with cholesterol for its binding sites inside the gastrointestinal tract (Oslund, 2002). This displacement function reduces cholesterol absorption and increases biliary excretion (Oslund, 2002; Normen et al., 2000). This reduces circulating LDL-C (Low Density Lipoprotein- cholesterol) (Oslund, 2002; Maki et al., 2013; Katan et al., 2003.). According to the FDA, regularly consuming phytosterol-rich diets may significantly reduce an individual's risk of heart disease (Fed regist, 2010).

3.2.5 Cyanogenic glycosides

According to literature, cyanogenic glycosides are chemical compounds contained in foods that when chewed or digested can pose potential risk to consumers (Kwork, 2008). This compound can cause severe problems to human health (FAO/WHO, 2012) such as Tropical Active Neuropathy (TAN) which is a general term for several neurological syndromes attributed to toxico-nutritional causes, growth retardation in humans and goiter and cretinism, and Konzo (an upper motto neuron disease) which mostly affects children and women of child bearing age (Bull world Org. 1984; Tylleskar et al., 1992; Ernesto et al., 2002). The presence of cyanogenic glycosides, in the both *T. daniellii* and *M. paradisiaca* in the water extract, and in *M. paradisiaca* in the methanol extract means there needs to be further investigations to ascertain if it is at an acceptably safe level.

3.2.6 Phenolic Compounds

Many biological, agricultural and medical studies have focused on understanding the characteristics and functions of phenolic acid compounds. These compounds perform biological activities such as reducing level of cholesterol, increasing bile secretion, and lipid levels in the blood. They also impact therapeutic effects such as anti-inflammatory, antimicrobial, antioxidant, cytotoxic, antiulcer, antitumor, antidepressant and antispasmodic activities (Gryglewski et al., 1987; Silva et al., 2007; Ghasemzadeh et al., 2010).

3.3 General Health Benefits of Phytochemicals in the Two Leaves

The significant thing is that all the plant samples contain some common and abundant secondary metabolites which may be beneficial in biological and pharmacological activities where as some can also be very harmful to human health; like cyanogenic glycoside, a poisonous compound which was recorded present in both plant samples (*T. daniellii* and *M. paradisiaca*). Since the phytochemicals seep into the food, the danger or benefits of these phytochemicals would depend largely on it being present in sufficiently high amounts. This can further promote or render these leaves unsuitable for packaging food, especially ready-to-eat and ready-to-serve meals.

4.1 Conclusion

Phytochemicals impacts therapeutic effects on the body when ingested. Hence, this study aimed at assessing the qualitative phytochemical constituents (secondary metabolites) in three solvents (ethanol, methanol water) extract of *Thaumatococcus daniellii* and *Musa paradisiaca*. The study revealed the presence (+) of saponins, polyuronides, cyanogenic glycosides, phenolic compounds, reducing sugar, alkaloids, flavonoids, phytosterols and anthracenosides for *Thaumatococcus daniellii*, with triterpenes absent (-). Saponins, polyuronides, phenolic compounds, cyanogenic glycosides, reducing sugar, flavonoids and phytosterols were found present (+) for *M. paradisiaca* with alkaloids, triterpenes and anthracenosides being absent (-). The presence of these active phytochemical compounds in *T. daniellii* and *M. paradisiaca* may be responsible for its ethnopharmacological uses in traditional medicine. According to this research, *T. daniellii* appears to be composed of more chemicals of medicinal value than the *M. paradisiaca*. Though plastic bags are more predominately used for food packaging than leaves which is seen as primitive. Leaves offer advantages such as adding to the food's richness, the consumer's health and the environment's protection.

4.2 Recommendations

1. Further studies should be carried out on the leaf extracts of both the *T. daniellii* and *M. paradisiaca*. Studies such as isolation, purification and toxicological studies. This would help to define its exact phytochemical constitution and to determine its suitability in developing novel chemotherapeutic agents.
2. The domestication and cultivation of *T. daniellii* should be highly encouraged and supported for its use in food packaging.

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