

## Phytochemical constituent and antioxidant activity of *Thaumatococcus daniellii* Benn (Benth.) leaves (food wrapper)

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**Keywords:** Antioxidant; Food wrapper; Leaves; Phytochemical; *Thaumatococcus daniellii*

**Abstract.** *Thaumatococcus daniellii* Benn (Benth.) is an organic food wrapper that influences the color and flavour of most foods processed and packaged with it. Hence, this study was designed to evaluate the phytochemical constituent and antioxidant activity of *T. daniellii* leave. Quantitative phytochemical analysis was carried out using standard procedures while antioxidant activity was assayed using 2',2'-diphenylpicrylhydrazine (DPPH) and ferric ion reducing antioxidant potential (FRAP). Results showed that *T. daniellii* leaves contained flavonoids, polyphenols, alkaloids and saponins. Furthermore, aqueous leaf fraction had higher quantity of polyphenols ( $0.41 \pm 0.1$  mg% gallic acid) and flavonoids ( $0.28 \pm 0.1$  mg% quercetin) than polyphenols ( $0.23 \pm 0.3$  mg% gallic acid) and flavonoids ( $0.11 \pm 0.1$  mg% quercetin) in hexane leaf fraction. Investigation of antioxidant activity revealed that 100 - 500 mg/ml aqueous leaf fraction exhibited a significantly ( $P < 0.05$ ) higher DPPH radical scavenging activity than the hexane leaf fraction. In addition, the aqueous fraction of *T. daniellii* leaves exhibited a significantly ( $P < 0.05$ ) higher ferric ion reducing potential than hexane leaf fraction. Thus, data from this study indicated that *T. daniellii* leaves color and flavour enhancing properties could be attributed to the presence of polyphenols and flavonoids. In addition, its use as food wrapper may introduce phyto-antioxidants into foods processed and packaged with *T. daniellii* leaves.

### Introduction

Antioxidants are known to act as first line of defence in the body against free radical-induced oxidative cell damage [1]. Some antioxidants could act independently or as a network to stabilize free radicals, pro-oxidant metals and terminate deleterious chain reactions elicited by free radicals [2]. Phyto-antioxidants have been reported to be present in sundry parts of plant including vegetables and fruits [3].

Dietary antioxidants act by controlling free radical level with a corresponding decrease in the symptoms of oxidative stress [4]. This notion has led to an increased demand for replacement of synthetic additives by food industry with phyto-antioxidants due to its presumed safety and several therapeutic applications [5,6].

*Thaumatococcus daniellii* Benn (Benth.) of Maranthaceae family is commonly known as "sweet prayers plant" [7]. It is a rhizomatous, perennial and monocotyledonous plant located in tropical rain forests and coastal areas of Nigeria, Ghana, Cote d'Ivoire, Princes Island, Uganda and Indonesia [8]. *T. daniellii* has a long and slender stalks that grows up to 2 - 3 m high, each bearing a single tough and ovoid shaped leaf. These leaves vary in sizes depending on the plant's age and habitat [7].

*T. daniellii* leaves, stalk, fruits and rhizomes contribute to the economy of rural people in Southern Nigeria and globally [9]. Furthermore, the global prominence of *T. daniellii* resulted from the discovery of thaumatin [9]. Thaumatin is a non-caloric sweetener and taste modifier that has been reported to be 1600 times sweeter than sucrose [10]. *T. daniellii* leaves which is locally known as katemfe or ewe in Nigeria, is mostly used as food wrapper by local food industry while the petiole is used to weave mats and as building material [11]. In ethnomedical practice, the sap of *T. daniellii* leaf stalk is used as sedative and antidote against venoms, stings and bites while its root sap is used for the treatment of mental retardation [12].

In some part of United States and South America, the use of *T. daniellii* leaves as food wrapper has gained wide acceptance due to its exotic and flavour enhancing property [13]. The leaves have large surface areas to wrap large amount of food [14]. However, most literatures on *T. daniellii* were focused on thaumatin with paucity of scientific validation of *T. daniellii* leaves as food colorant, flavour enhancer and also as phytomedicine. Thus, this study was designed to evaluate the phytochemical constituent and antioxidant activity of *T. daniellii* leaves to proffer a scientific rationale for the use of *T. daniellii* leaves as food wrappers with ethnomedical benefits.

## Materials and methods

### Collection of plant material

Fresh leaves of *Thaumatococcus daniellii* were purchased from a local market at Ilisan Remo, Ogun State, Nigeria in January 2015.

### Extract preparation

*T. daniellii* leaves were washed and oven dried at 35°C for 48h. Dried *T. daniellii* leaves were pulverized using electric blender. Pulverized sample (57.14 g) were soaked in 400 ml 70% methanol for 48 h with intermittent shaking. The suspension was filtered using Whatman No. 1 filter paper and concentrated in a rotary evaporator (Buchi Rotavapor RE, Switzerland) at 30°C. The concentrated extract obtained was reconstituted in distilled water and subsequently partitioned using n-hexane in 1:2 v/v with separating funnel. Hexane fraction and the remaining fraction considered as aqueous fractions were concentrated at 30°C. The two fractions obtained were stored in the refrigerator at 4°C until further use.

## Quantitative phytochemical analysis

### Estimation of total phenolic content

Folin-Ciocalteu colorimetric method as described by Ghasemi et al. [15] was used to determine total polyphenol content. Standard gallic acid solution, hexane and aqueous fractions of *T. daniellii* leaves (0.1- 0.5 ml; 1mg/ml) were pipetted separately into test tubes. Folin-Ciocalteu reagent (5 ml) was added to each test tubes which was allowed to stand for 5 min followed by the addition of 4 ml 1M Na<sub>2</sub>CO<sub>3</sub> and made up to 10 ml with distilled water. All tubes were allowed to stand for 15 min. The absorbance of reaction mixture was measured against reagent blank at 765 nm using UV-visible Shimadzu Spectrophotometer. Polyphenol content of fractions was calculated as mg% gallic acid equivalent using the equation from the standard phenol calibration curve.

### Estimation of total flavonoid content

Complex aluminium chloride method for determination of total flavonoids as described by Ordonez et al. [16] was used to determine total flavonoid content. Standard quercetin solution, hexane and aqueous fractions of *T. daniellii* leaves (0.1 - 0.5 ml; 1 mg/ml) were taken separately into test tubes followed by the addition of 0.5 ml 2% AlCl<sub>3</sub> prepared in ethanol and made up to 5 ml with distilled water. All tubes were allowed to stand for 60 min at room temperature. Absorbance was measured at 420 nm using UV-visible Shimadzu Spectrophotometer. Total flavonoid content was expressed as mg/g quercetin equivalent using the equation from the standard flavonoid calibration curve.

### Determination of saponin content

Saponin content was carried out using the method described by Okwu and Josiah [17]. Pulverized *T. daniellii* sample (5 g) was dispersed in 50 ml 20% v/v ethanol and the suspension heated over a hot water bath for 1 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 50 ml 20% ethanol. Combined extracts were reduced to 20 ml over a hot water bath at 90°C. The concentrate was vigorously mixed with 10 ml diethyl ether in 250 ml separating funnel and aqueous layer was collected while ether layer was discarded. Butan-1-ol (20 ml) was added to the aqueous layer and subsequently washed three times with 10 ml 5% w/v sodium chloride. The mixture was heated to dryness on a hot water bath and further oven dried to a constant weight.

Percentage saponin content was calculated thus:

$$\% \text{ Saponin} = \frac{\text{Weight of final extract}}{\text{Weight of sample}} \times 100$$

### Determination of alkaloid content

Alkaloid content of the plant sample was determined using the method described by Onyilagba and Islam [18]. Pulverized sample (5 g) of *T. daniellii* leaves was weighed into 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and allowed to stand for 4 h. The suspension was filtered and concentrated to one-quarter of its original volume using a hot water bath at 100°C. Concentrated ammonium hydroxide was added drop wise to the filtrate until complete precipitation. The precipitates were washed with dilute ammonium hydroxide and subsequently filtered to obtain residue which was dried and weighed.

Alkaloid content was determined using the formula below:

$$\% \text{ Alkaloid} = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

### Determination of antioxidant activities *in vitro*

#### DPPH free radical scavenging assay

Free radical scavenging activity of hexane and aqueous fractions of *T. daniellii* leaf was carried out according to spectrophotometric method described by Mensor et al. [19]. One ml of a 0.3 mM DPPH methanol solution was added to either 2.5 ml hexane or aqueous fractions (100 - 500 µg/ml) and allowed to react at room temperature for 30 min. Ascorbic acid was used as standard. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage.

Percent antioxidant activity was calculated using the formula:

$$\% \text{ antioxidant activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

#### Ferric reducing antioxidant power

Ferric reducing antioxidant power was determined as described by Hinneburg et al. [20]. One millilitre hexane or aqueous fractions (0.1%) of *T. daniellii* leaf was mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml 1% potassium hexacyanoferrate. After 30 min incubation at 50°C, 2.5 ml 10% trichloroacetic acid was added, and the mixture centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml water and 0.5 ml of aqueous 0.1% FeCl<sub>3</sub>. The absorbance was read at 700 nm. Standard ascorbic acid was used to plot the calibration curve.

## Statistical analysis

Statistical analysis was carried out using SPSS for Windows; SPSS Inc. Chicago Standard Version 17.0. Statistical comparison was performed using Student t test.  $P < 0.05$  was considered to be statistically significant. Data were expressed as mean  $\pm$  SEM

## Results and discussion

*T. daniellii* leaves are used by local food industry in West Africa as an organic wrapper for some foods including rice and bean pudding. Physical observation by consumers had shown that *T. daniellii* leaves influences the color and taste of foods processed and packaged with it.

From this present study, data showed that *T. daniellii* leaves contained flavonoids, polyphenols, alkaloids and saponins (Table 1). This indicated that *T. daniellii* leaves could serve as a source of bioactive compounds with nutraceutical benefits against several degenerative diseases. Phyto-compounds are currently being harnessed by food industry as a source of important food condiments or nutraceutical agents [21,22]. Previous study had shown that bright colors in plants might be attributed to the presence of polyphenols and flavonoids [23]. This could account for the orange-like color that *T. daniellii* leaves impact on some foods.

In addition, flavonoids possesses anti-allergic, anti-inflammatory [24] and anti-cancer properties [25]. Flavonoids and polyphenols have also been reported to serve as potent antioxidant and free radical scavengers capable of protecting the body against oxidative stress-induced cellular damage [26,27]. Investigation of alkaloid bioactivity showed that it possesses antimicrobial, antimalarial and anti-inflammatory properties [28,29]. Saponins are known to reduce glucose uptake and cholesterol at the gut which indicates that it possesses hypocholesterolemic effects [30].

Further study showed that the aqueous fraction of *T. daniellii* leaves contained higher polyphenol and flavonoid contents than the hexane fraction (Table 1). This suggested that majority of the polyphenols and flavonoids present in *T. daniellii* leaves may be structurally polar in nature. Previous study had shown that some polyphenols and flavonoids are structurally polar with antioxidant activity [31,32].

This present study also suggested that the polyphenols and flavonoids could account for the color and flavor introduced into foods wrapped with *T. daniellii* leaves. Perhaps this could be due to processing of some foods like bean pudding which requires aqueous solution [33]. Water can extract polar phytochemicals present in *T. daniellii* leaves into food wrapped with it. However, it has also been reported that the fragrance in food wrapped with *T. daniellii* leaves might be due to the presence of essential oil from *T. daniellii* leaves [14].

Table 1: Phytochemical contents in *T. daniellii* fractions

Phytochemicals	<i>T. daniellii</i> leaves	
	Hexane fraction	Aqueous fraction
Polyphenols (mg% gallic acid)	0.23 $\pm$ 0.3 <sup>a</sup>	0.41 $\pm$ 0.1
Flavonoids (mg % quercetin)	0.11 $\pm$ 0.1 <sup>a</sup>	0.28 $\pm$ 0.1
Saponins (%)	6.5 $\pm$ 0.5	
Alkaloids (%)	49.3 $\pm$ 1.4	

a-indicates statistically significant different from hexane fraction at  $P < 0.05$

Investigation of the antioxidant activity of *T. daniellii* leaves showed that the aqueous fraction exhibited a significantly ( $P < 0.05$ ) higher DPPH radical scavenging activity than the hexane fraction at 100 - 500 mg/ml (Fig. 1). This indicated that the aqueous fraction of *T. daniellii* leaves contained

more phyto-antioxidant compounds than the hexane fraction. This seems to be in consonance with higher phytochemical content in aqueous leaf fraction than the hexane leaf fraction. DPPH radicals had shown that it react with appropriate reducing agent with concomitant stoichiometric color loss depending on the number of electrons taken up [34]. Furthermore, previous study had shown that most antioxidant compounds are structurally polar. This is due to presence of the hydroxyl groups present in the polyphenol and flavonoid ring with antioxidant properties [35].

Further investigation showed that aqueous fraction of *T. danielli* leaves exhibited a significantly ( $P < 0.05$ ) higher ferric ion reducing potential than hexane leaf fraction (Table 2). This observation supported the previous claim that aqueous fraction of *T. danielli* leaves contained more antioxidant compounds than hexane fraction. This might be due to the hydrogen donating potentials of polyphenols and flavonoids as well as the basic structural orientation of the functional groups present. It had also been reported that the ring orientation of compounds determines the extent at which a hydroxyl group donates hydrogen atom to free radicals as well as the capacity of the antioxidants to support an unpaired electron [36].

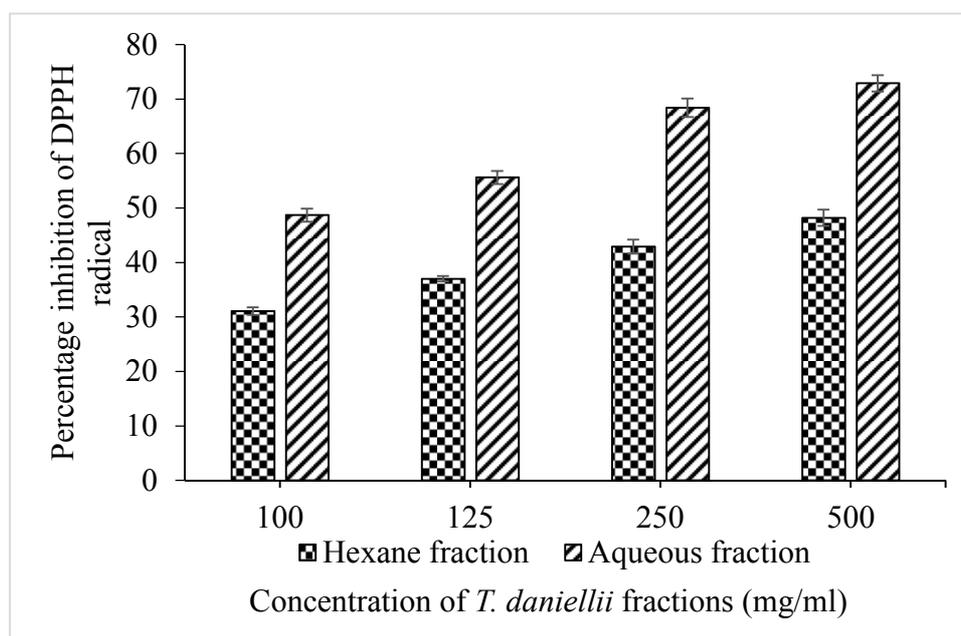


Figure 1: DPPH radical scavenging activities of aqueous and hexane fractions of *Thaumatooccus daniellii* leaves

Table 2: Ferric reducing antioxidant potential (FRAP) of *T. daniellii* leaf fractions

Parameter	<i>T. daniellii</i> leaves	
	Aqueous fraction	Hexane fraction
FRAP (mg% ascorbic acid equivalent)	0.49 ± 0.1 <sup>a</sup>	0.27 ± 0.0

a-indicates statistically significant different from hexane fraction at  $P < 0.05$

### Conclusion

This study indicated that *T. daniellii* leaves possessed phytochemicals with potential antioxidant activity and perhaps the phyto-compounds might have contributed to color and flavour introduced into foods wrapped with it. In addition, data suggested that aqueous fraction of *T. daniellii* leaves contained higher antioxidant compounds than the hexane fraction.

## Acknowledgements

The authors sincerely appreciate Babcock University administration for their support and also Mr. A.J. Ayinde and Mrs. C. Anyasor, Babcock University for the technical assistance they provided during this course of this research and preparation of the manuscript.

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**Volume 2**

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10.18052/www.scipress.com/IJPPE.2.55