Asian Journal of Biotechnology and Genetic Engineering

Volume 6, Issue 2, Page 105-114, 2023; Article no.AJBGE.103608

Nutritional Composition, Phytochemistry, and *In vivo* Potentials of *Thaumatococcus daniellii* (Benn.) Rhizome Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/103608

Original Research Article

Received: 19/05/2023
Accepted: 24/07/2023
Published: 07/08/2023

ABSTRACT

The evidence on the advantages of plant rhizomes remains sparse. Proximate analysis, phytochemical screening (qualitative and quantitative), and lethal dose investigation of the aqueous and ethanolic extracts of the unpeeled and peeled *Thaumatococcus daniellii* (Benn.) rhizome (TdR) and their effects in albino rats are presented in this paper. In order to investigate the extract's effect on albino rats, fifteen animals were divided into five groups (n = 3). Group 1 is the control, and groups 2–5 were orally administered 300 mg/kg body weight of aqueous and ethanolic extracts of unpeeled and peeled TdR for 7 days, respectively. After the experimental period, blood glucose, serum total protein, albumin, globulin, cholesterol, and triglyceride concentrations were determined

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spectrophotometrically. The qualitative analysis carried out revealed the presence of flavonoids, reducing sugar, free anthraquinones, cardiac glycosides, and glycosides in both aqueous and ethanolic extracts of the unpeeled and peeled rhizomes. The quantitative analysis shows that total phenol has the highest percentage of constituents compared to niacin, flavonoids, and tannins. Both qualitative and quantitative analyses revealed the absence of alkaloids and phlobatannins. The blood glucose concentration was significantly ($P \leq .05$) decreased in animals administered with all the crude rhizome extracts, while the total serum albumin, globulin, and protein concentrations were significantly ($P \leq .05$) increased. Likewise, the extracts of the peeled and unpeeled rhizomes caused a significant ($P \leq .05$) increase in serum cholesterol concentration as well as triglycerides compared to the control. The results revealed that the nutritional composition and phytochemistry of both the aqueous and ethanolic extracts of the unpeeled and peeled TdR rhizome and the administration of 300 mg/kg body weight had significant effects on the biochemical parameters of the rat.

Keywords: Glucose; flavonoids; cholesterol; protein; peeled; unpeeled.

1. INTRODUCTION

Thaumatococcus daniellii (Td) (Benn.) is an underutilized rhizomatous plant found in the tropical rain forests and coastal areas of West Africa. In Nigeria, it is called “Ewe-Eran” or “Adundunmitan”, and its stalk, leaves, fruits, and rhizomes contribute to the economy of the rural people. It is commonly referred to as miracle fruit or berry, serendipity berry, sweet prayer plant, soft cane, and katempfe. Td, a perennial monocotyledonous herb, propagates itself by rhizomes and forms an undergrowth of forest trees in its natural habitat [1-9]. The aril of its fruit is a natural source of a sweet protein called thaumatin, which is about 3000 times sweeter than sucrose solution (8-10%) [1,7,8,10]. The removal of thaumatin from the arils of Td fruits leads to the generation of waste (seed, pericarp, and pulp), which has been shown to constitute over 93% of fruit weight and found to have no significant difference in the biological value, net protein utilization, and protein efficiency ratio value between the casein base diet and the Td base diet; therefore, they can be used as livestock feed [7].

In the traditional and folkloric management of some diseases and ailments, the different parts of Td are employed. Several researchers worked on the leaves, fruits, fruit waste (seed, pericarp, and pulp), and stalks of the Td plant [11,12]. It has been found that these parts have medicinal properties such as preservative, antioxidant, antimicrobial, and anti-diabetic, to mention a few [2,7,13-16]. There is a paucity of scientific data on the medicinal properties of the Td rhizome, which is a part of the plant. Therefore, this study is to conduct research on the aqueous and ethanolic extracts of unpeeled and peeled Td rhizomes by investigating their nutritional composition, phytochemistry, lethal dose, and in vivo effects.

2. MATERIALS AND METHODS

2.1 Plant Material and Sample Preparation

The rhizomes of the *Thaumatococcus daniellii* (Td) plant were collected from a farm at Lusada village in Ogun State, Nigeria, and validated by the Department of Botany, Lagos State University, Ojo, Lagos. The rhizomes were rinsed carefully with tap water to remove the dirt and divided into two equal parts. One part was peeled and the other unpeeled (Fig. 1); both parts were cut into pieces for aqueous and ethanolic extraction. Briefly, 100 g of unpeeled and peeled rhizomes of Td were extracted with 70% ethanol for 4 hours using the Soxhlet extractor apparatus. Another 100 g of unpeeled and peeled samples were soaked in 250 cm3 of distilled water for 12 hours and filtered using Whatman filter paper No. 42 (125 mm) to get the aqueous extract. The crude extracts were collected into sterile bottles and stored until further use.

2.2 Proximate Analysis

The Association of Official Analytical Chemists' recommended method was used to determine the TdR's proximate composition (moisture, crude protein, crude fat, and ash) [17]. The difference was used to compute carbohydrate content.

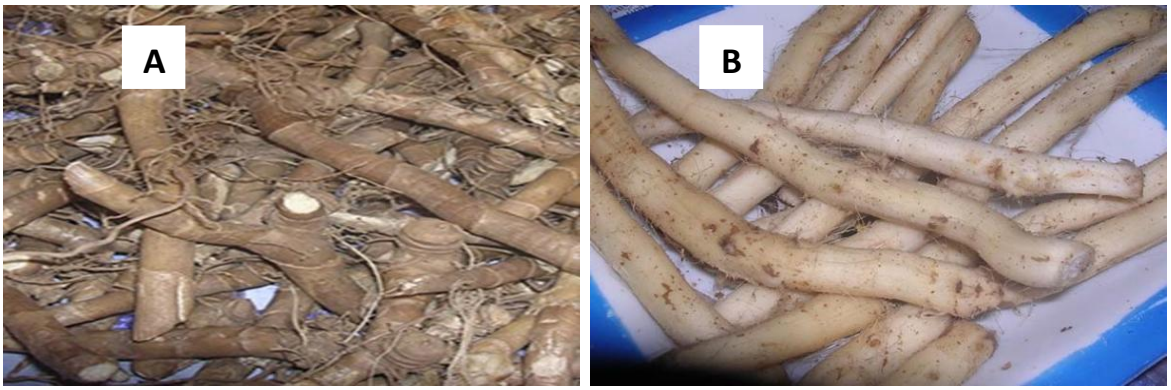

Fig. 1. The unpeeled (A) and peeled (B) rhizomes of *Thaumatococcus daniellii*

2.3 Phytochemical Screening

2.3.1 Qualitative analysis

The determination of tannins, phlobatanins, reducing sugar, glycosides, alkaloids, cardiac glycosides, saponins, flavonoids, bound anthraquinone, anthracyanides, terpenoids, and free anthraquinone in the crude aqueous and ethanolic extracts of unpeeled and peeled TdR was performed according to the methods of Ogunrinola et al. [18] and Kalaichelvi and Dhivya [19].

2.3.2 Quantitative analysis

The estimation of total phenolic content (TPC), total flavonoid content (TFC), niacin content (NC), tannin content (TC), and alkaloid content (AC) was performed in the aqueous and ethanolic extracts of peeled and unpeeled TdR as described by the modified methods of Ogunrinola et al. [18], Ghasemi et al. [20], Iqbal et al. [21], and Chlopicka et al. [22].

2.4 Acute Toxicity Studies (Lethal Dose (LD_{50}))

The modified method of Lorke [23], as modified by the method of Adu et al. [2], was used to estimate the acute toxicity (LD_{50}) of the aqueous and ethanolic extracts of unpeeled and peeled TdR when administered orally. Briefly, fifty-six rats were allotted equally into twenty-eight (n = 2) well-ventilated plastic cages. The first 7 cages were administered with an aqueous extract of unpeeled TdR; the second 7 cages were administered with an aqueous extract of peeled TdR; the third set of cages were administered with an ethanolic extract of unpeeled TdR; and the last 7 cages were given an ethanolic extract of peeled TdR orally at dosage levels of 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, and 350 mg/kg body weight, respectively, for 72 hours.

2.5 Animals and Experimental Procedure

A total of fifteen (15) Wistar male albino rats weighing between 120 - 150 g were used for the experiments. The animals were housed in well ventilated cages to acclimatize for a week and allowed to drink and feed freely. The animals were randomly and equally distributed into five groups (n = 3) as follows: aqueous and ethanolic extracts of TdR.

Group I	Control rats, given distilled water
Group II	Oral administration of 300 mg/kg body weight of unpeeled aqueous extract of TdR
Group III	Oral administration of 300 mg/kg body weight of peeled aqueous extract of TdR
Group IV	Oral administration of 300 mg/kg body weight of unpeeled ethanolic extract of TdR
Group V	Oral administration of 300 mg/kg body weight of peeled ethanolic extract of TdR

After seven days of administration, the animals were fasted overnight and sacrificed under light ketamine anaesthesia. Blood was collected via cardiac puncture and separated into serum, which was stored for analysis.

2.6 Biochemical Analysis

Blood glucose level, serum total protein, albumin, and globulin determination: The blood glucose level was measured before and after treatment by taking a blood sample from the tail. Blood was dripped on the end of the strip, and blood glucose was read on the glucometer after 10 seconds. The serum total protein and albumin were analyzed using the commercial Randox kits, products of Randox Laboratories, U.K. And serum globulin was determined as the difference between serum total protein and albumin.

Lipid profile determination concentrations of total cholesterol and triglycerides in the serum were determined with commercial kits (Spin React S.A., Santa Colona, Sant Esteve de Bas, Spain).

2.7 Statistical Analysis

The results are presented as mean ± standard error of mean (SEM) and were analyzed for statistical significance by one-way analysis of variance (ANOVA). The values with $P \leq .05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

The proximate analysis is used for the estimation of the quantitative properties of food substances, including moisture, crude protein, total fat, ash, total carbohydrate, and dietary fibre [17,24]. Table 1 represents the proximate composition of the unpeeled and peeled TdR. The results revealed that while crude protein content has the lowest percentage of 0.13 and 0.94 in both unpeeled and peeled rhizome samples, the percentage of carbohydrate content is higher (76.75% and 88.53%), respectively. The moisture content of a sample is the amount of water lost during drying and serves as one of the main factors in storage, which may be due to the proliferation of microorganisms [25].

In this study, the percentage moisture content of unpeeled TdR was higher than that of peeled TdR samples, which might be due to the removal of the rhizome bark. The reduced moisture content shows that the peeled TdR samples can withstand microorganism growth and extend the shelf-life of TdR, which means that it has environmental benefits. The ash content is the amount of total mineral (inorganic) residue leftover after the combustion of organic matter in a food sample until it reaches a constant weight [26]. The result revealed a higher percentage of ash content in the peeled TdR than in the unpeeled TdR samples. This enhancement of the ash content might be due to the presence of more fibres and veins in the peeled TdR samples [27].

The percentage crude protein is the amount of total nitrogen (protein nitrogen and a few non-protein nitrogens) multiplied by protein factors [25]. In this study, the TdR samples used a 6.25 protein factor to convert nitrogen to protein and then found that it was higher in the peeled samples. This is similar to the report of Pazhanichamy et al. [28]. Although the observed value is lower than the value, which makes the rhizomes less advantageous as a rich source of plant protein for humans, they can contribute to the formation of hormones that control growth, repair, and maintenance of the plant. Total fat is the amount of fatty acids, fat-soluble vitamins, and steroids in a food sample [25]. Total fat in this study was found to be higher in unpeeled TdR samples, indicating that it can help with the transportation and absorption of fat-soluble vitamins such as vitamins A, D, E, and K and provide energy to the plant. Carbohydrates are the main components of the structural materials (cell walls, cell sap, and protoplasm) in plants.

The carbohydrates produced by the leaves were translocated to the rhizomes, which serve as storage organs [25,29]. In this study, carbohydrate was calculated based on the difference method, and the total carbohydrate of

Table 1. Proximate analysis of unpeeled and peeled *Thaumatococcus daniellii* (Benn.) rhizomes (TdR) (g per 100g)

Rhizome samples	Moisture Content (%)	Ash Content (%)	Crude Protein Content (%)	Crude Fat Content (%)	Carbohydrate Content (%)
unpeeled	1.94	4.78	0.13	16.40	76.75
peeled	1.05	4.88	0.94	4.60	88.53

TdR samples was found to be higher in peeled samples. This is in accordance with the study carried out by Pazhanichamy et al. [28]. This shows that the natural structure (cell walls and protoplasm) of the rhizome is still maintained [29]. These results differ from the report of Oforibika et al. [30] and may be due to the geographical location where the samples were collected and the research protocols.

3.2 Phytochemical Screening

The detection of active principles in medicinal plants plays an important role in regards to their potential pharmacological effects [29]. Appropriate solvents, including organic and/or aqueous solutions, have been reported for extracting active compounds. Because of the polarity differences between water and ethanol, they are often recommended for extract preparation [31-33]. The results of phytochemical constituents in the aqueous and ethanolic extracts of unpeeled and peeled TdR are depicted in Table 2. Qualitative analysis depicts the presence of saponins, flavonoids, reducing sugar, free anthraquinone, cardiac glycosides, and glycosides in the aqueous extract of unpeeled and peeled TdR. And the presence of tannins, flavonoids, reducing sugar, free anthraquinone, bound anthraquinones, anthacyanides, terpenoids, cardiac glycosides, and glycosides in the ethanolic extract of unpeeled and peeled TdR, respectively. This may be due to the high polarity of the ethanol used for the extraction.

In both the unpeeled and peeled aqueous and ethanolic extracts, the quantitative analysis (percentage of crude extract) showed the presence of tannins, niacin, total phenol, and flavonoids but the absence of alkaloids. It was observed that total phenol has the highest percentage, whereas tannins have the lowest percentage, respectively, in all the samples. The results that were observed are comparable to those that Majaw and Moirangthem [29] and Taoheed et al. [33] reported. It has been reported that these bioactive constituents are suggested to be associated with antibacterial, antidiarrheal, antioxidant, and antiviral activity [29,34,35]. The high amount of reducing sugar will help in the central metabolic pathways and in the production of secondary metabolites that enhance the medicinal properties of plants [36,37].

Table 2. Phytochemical constituents of aqueous and ethanolic of unpeeled and peeled *Thaumatococcus daniellii* (Benn.) rhizomes (TdR)

	Groups			
Phytochemicals	**Unpeeled Aqueous Extract of TdR**	**Peeled Aqueous Extract of TdR**	**Unpeeled Ethanolic Extract of TdR**	**Peeled Ethanolic Extract of TdR**
Qualitative Analysis				
Saponins	++	+	nd	nd
Tannins	nd	nd	+	+
Flavonoids	++	+	+	+
Reducing sugar	+	++	+++	++++
Free anthraquinone	++	+	+	+
Bound anthraquinone	nd	nd	+	+
Phlobatannins	nd	nd	nd	nd
Anthacyanides	nd	nd	+	+
Terpenoids	nd	nd	+	+
Cardiac glycosides	+	+	+	+
Glycosides	+	+	++	++
Alkaloids	nd	nd	nd	nd
Quantitative Analysis: Percentage of Crude Extracts				
Tannins	0.08	0.06	0.15	0.28
Niacin	1.13	0.36	0.39	0.69
Total Phenol	5.68	3.65	1.49	3.58
Flavonoid	1.86	0.22	0.67	0.31
Alkaloids	nd	nd	nd	nd

TdR = Thaumatococcus daniellii (Benn.) rhizomes; ++++ = Present in very highly concentration; +++ = Present in very high concentration; ++ = Present in moderately high concentration; + = Present in trace concentration; -nd = Not detected

Table 3. Effects of *Thaumatococcus daniellii* (Benn.) rhizomes (TdR) extracts on blood glucose, serum total protein, albumin and globulin concentrations in the animal

Groups	Blood Glucose (mg/dl)		Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
	Before	After			
I	29.35 ± 2.11[a]	35.35 ± 2.11[a]	7.10 ±0.10[a]	1.94 ± 0.05[a]	5.16 ± 0.04[a]
II	35.20 ± 1.62[b]	31.25 ± 1.72[b]	11.40± 1.20[b]	5.01 ±0.26[b]	6.39 ± 0.98[b]
III	74.75 ± 1.70[c]	73.75 ± 1.72[c]	14.1 ± 0.30[c]	4.42 ± 0.46[c]	9.68 ± 0.66[c]
IV	38.61 ± 1.80[d]	28.63 ± 2.86[d]	10.20 ± 0.20[d]	4.45 ± 0.30[d]	5.75 ± 0.28[d]
V	99.77 ± 2.52[e]	88.75 ± 2.30[e]	13.5 ± 0.30[e]	5.67 ± 0.13[e]	7.83 ± 0.40[e]

I = Control; II = 300 mg/kg body weight of unpeeled aqueous extract; III = 300 mg/kg body weight of peeled aqueous extract; IV = 300 mg/kg body weight of peeled ethanolic extract; V = 300 mg/kg body weight of peeled ethanolic extract. Values are represented as the mean ± S.E.M for 3 rats in each group. Values having different superscripts within a column differ significantly from each other (P≤ .05)

Table 4. Effects of *Thaumatococcus daniellii* (Benn.) rhizomes (TdR) extracts on serum cholesterol and triglyceride concentrations in the animal

Groups	Cholesterol Concentration (mg/dl)	Triglyceride Concentration (mg/dl)
I	69.70 ± 6.71[a]	105.54 ± 4.20[a]
II	86.36 ± 14.05[b]	89.54 ± 9.86[b]
III	77.82 ± 13.64[c]	106.77 ± 6.70[c]
IV	102.12 ± 15.97[d]	117.54 ± 5.73[d]
V	75.76 ± 19.19[e]	99.85 ± 5.20[e]

I = Control; II = 300 mg/kg body weight of unpeeled aqueous extract; III = 300 mg/kg body weight of peeled aqueous extract; IV = 300 mg/kg body weight of peeled ethanolic extract; V = 300 mg/kg body weight of peeled ethanolic extract. Values are represented as the mean ± S.E.M for 3 rats in each group. Values having different superscripts within a column differ significantly from each other (P≤ .05)

3.3 Acute Toxicity Studies (Lethal Dose (LD_{50}))

The results of acute toxicity studies show that there was no mortality within 72 hours after the oral administration of 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, and 350 mg/kg body weight of aqueous and ethanolic extracts of unpeeled and peeled TdR, respectively. Therefore, our research team used 300 mg/kg body weight for the animal study.

3.4 Determination of Blood Glucose, Serum Total Protein, Albumin, Globulin, Cholesterol, and Triglyceride Concentrations

There was a significant ($P\leq .05$) reduction in blood glucose concentrations after the administration of aqueous and ethanolic extracts of unpeeled and peeled TdR compared to the control. The total serum albumin, globulin, and protein concentrations were found to increase significantly ($P\leq .05$) in all groups compared to the control (Table 3). The administration of all the aqueous and ethanolic extracts of unpeeled and peeled TdR caused a significant ($P\leq .05$) increase in serum cholesterol concentration as well as triglycerides compared to control (Table 4). The highest levels of cholesterol and triglycerides increase more with the administration of the peeled ethanolic extract of TdR.

This study observed up- and down-regulation of total protein, albumin, globulin, blood glucose, cholesterol, and triglyceride concentrations in the aqueous and ethanolic extracts of unpeeled and peeled TdR, respectively (Table 4). These alterations are due to the phytochemical constituents present in TdR. Their effects on total protein and globulin may be due to an alternation in the intracellular protein synthesis mechanism and immunoglobulin production. The increased concentration of albumin may be due to high protein formation from the alimentary tract or protein in the liver [34,38,39]. The reduction in the glucose concentration revealed that TdR possesses a glucose-lowering effect, which may be partly due to the action of the flavonoid, thereby modulating some cell signalling like glucose uptake in the intestine [34,40-42].

Likewise, tannins inhibited the activation of α-amylase and α-glucosidase activities to reduce the blood glucose concentration by phosphorylation of the insulin receptor and

translocation of glucose transporter 4, and also inhibited the important genes for adipogenesis [43,44]. Phenols and terpenoids also inhibit the transcription factor associated with the activation of genes involved in the biosynthesis of cholesterol, fatty acids, and triglycerides, thereby increasing glycogenesis and decreasing glycogenolysis, as well as inhibiting aldose reductase [45-48]. The glycosides and cardiac glycosides mediate hypoglycaemic activity by increasing insulin secretion through adenosine monophosphate-activated protein kinase [49,50].

4. CONCLUSION

The present study investigated the phytochemical constituents of TdR, determined its acute toxicity and effects on blood glucose, total protein, albumin, globulin, cholesterol, and triglyceride concentrations. This is the first scientific report on the phytochemical constituents and health benefits of aqueous and ethanolic extracts of unpeeled and peeled TdR. The presence of phytochemicals acts through a number of diverse mechanisms to bring about some potential health benefits of TdR for humans and animals. Further research is needed to investigate the effectiveness of the aqueous and ethanolic extracts of the unpeeled and peeled TdR against degenerative diseases. And there should be awareness of the potential of TdR for animal nutrition and medical purposes.

ETHICAL APPROVAL

All experiments were performed in compliance with the principles for an ethical guide for the care and use of laboratory animals [11], which were approved by the Animal Ethical Committee of the Department and the University.

ACKNOWLEDGEMENTS

The authors received no financial support for the research and publication of this article. They appreciate the support of Y.D. Omiyale, S.O. Faro, T.A. Alabi, T.M. Awolaja, I.O. Agbabiaka, and W.C. Ugbaja, who assisted in the research. Also, the Lagos State University, Ojo management, and technologist in the Department of Biochemistry, for providing the enabling environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/103608