



In Vitro Antimicrobial, Antidiabetic, Antioxidant, and Antiulcerogenic Properties of Methanolic and Aqueous Leaf Extracts of *Thaumatococcus Daniellii*

*Usman, K.M., Olaniyi, S.S., & Ashiyanbi, A.J.

Department of Science Laboratory Technology, School of Applied Science and Technology, Federal Polytechnic, Offa, Kwara State

*Corresponding author email: alfausman@yahoo.com

Abstract

The phytochemical composition, antimicrobial, antidiabetic, antioxidant and antiulcerogenic properties of leaf extracts of *Thaumatococcus daniellii* were investigated. The *invitro* effects of methanolic and aqueous leaf extracts of *T. daniellii* at various concentrations (7.8125-1000 µg/mL) on the activities of α-amylase and α-glucosidase were used to evaluate its anti-diabetic potential. The phytochemical screening of the methanolic and aqueous leaf extracts of *T. daniellii* confirmed the presence of saponins, phenol, alkaloids and tannins. The methanolic leaf extract of *T. daniellii* showed inhibitory activity against *Klebsiella pneumoniae* at 50 mg/ml and 100 mg/ml concentrations. The methanolic and aqueous leaf extracts of *T. daniellii* inhibited α-amylase, α-glucosidase and aldose reductase enzymes in a concentration-dependent manner. The standard oxidant as well as methanolic and aqueous leaf extracts of *T. daniellii* showed increased inhibitory activities of hydrogen peroxide, nitric oxide and hydroxyl radical with increasing concentrations. The total antioxidant activity of the standard antioxidant (Butylated Hydroxytoluene) is higher than those of methanolic and aqueous leaf extracts of *T. daniellii*. The *invitro* assays indicate that methanolic and aqueous leaf extracts of *T. daniellii* is a significant source of natural antidiabetic agent, which might be helpful in preventing and treatment of diabetes. The aqueous and methanolic leaf extracts of *T. daniellii* also showed potent H⁺/K⁺-ATPase inhibitory property; hence, they may be used as potential antiulcerogens.

Keywords: *Thaumatococcus daniellii*. Antidiabetic, Antioxidant, Antiulcerogenic, Antimicrobials

Introduction

Herbal medicines have taken decades in management of several ailments with belief that such therapeutic plants are accompanied with little or no advert effects (Oyeleke et al., 2017). Medicinal plants have demonstrated high therapeutic potentials as antibacterial, antioxidant and anti-inflammatory effects, owing to their proven healing properties and they have been accepted as powerful sources of drugs with some advantages over conventional drugs (Jiang et al., 2019). The creation of novel drugs has been spurred by the potential of medicinal plants for drug development. However, the lack of thorough toxicity data raises questions about their safety and effectiveness. Herbal remedies may be harmful due to a variety of reasons, such as adulteration, plant components or metabolites having poisonous potential and environmental variables. Even while medicinal plants have many advantages, some may pose health risk to users because of side effects that might result from toxicity or overdose. Numerous studies have demonstrated that some therapeutic plants can be harmful to certain organs, and thus, skilled herbalists often provide known hazardous medicinal herbs in small doses (Mensah et al., 2019). In African methods of food packaging, several leaves among which *T. daniellii* belongs are used traditionally in packaging of food products like pudding and local fermented foods, owing to its helpful wrapping qualities such as good aroma, taste, low cost and does not transfer any pigment to the wrapped food. The use of this leaf is very ancient and, the leaf is broad and infective in packaging large volumes of foods (Olorunnisola et al., 2017). Worldwide, the cases of diabetes is alarming, especially the type 2 diabetes (T2D) which poses significant threat among all the types (Hwang et al., 2015). Some commercially available drugs such as acarbose used in the management of diabetes is characterized with various side effects after long time usage (Gong et al., 2020). Based on the International Diabetes Federation (IDF) report, about 536 million people were battling with diabetes in 2021, which may be doubled in few years to come (Ogurtsova et al., 2022). Antioxidants perform an important role in dropping oxidative pressure by maintaining equilibrium between free radical generation and oxidative pressure

(Nawaz et al., 2020). Several researches have proven that plant phytochemicals thwart different oxidative burden-induced diseases due to their antioxidant effects (Vergun et al., 2020).

Among the chronic diseases that occur when there is fracture in the stomach mucosa lining is gastric ulcer. Normally, there is a physiological balance between destructive causes and mucosa defense. When this equilibrium is challenged in support of destructive causes, injury to the gastric mucosa results (Kwon & Kim, 2015). Some of the destructive causes are *Helicobacter pylori*, non-steroidal anticancer drugs, alcohol and hereditary factors. Alcohol has been confirmed as a destructive course of gastric mucosa for its ability to induce gastric ulcer in animal models (Saleem et al., 2016). Reports have shown that alcohol impair the strength of gastric mucosa by supporting acid influx to the subluminal layer of the mucosa and submucosa (Saleh et al., 2016; Ortac et al., 2018). *Thaumatococcus daniellii* is an indigenous African plant, a potential source of thaumatin, a strong sweet protein useful in sweeteners' production (Hamid et al., 2017). *Thaumatococcus daniellii* grows to a height of about three to four meters with large, smooth leaves spanning about 46 centimeters long. It bears pale purple flowers and a soft fruit containing a few shiny black seeds. The leaves are used to wrap food and have been reported to have many traditional medicinal uses (Ukwubile et al., 2017). In traditional medicine, the juice extract of *T. daniellii* leaf is used as an antidote against venoms, stings, and bites. Also, remedies for drowsiness and mental instability are produced from leaf and root juice of *T. daniellii* (Ahmed et al., 2020). Traditionally, *T. daniellii* leaf has folkloric solution against oxidative caused complications. However, no scientific evidence has established the safety of continuous usage. Based on the foregoing, this study aims to evaluate the phytochemical properties of this plant in order to justify full utilization of this plant in the packaging of foods and treatment of diseases and also to determine the antimicrobial, anti-diabetic, antioxidant and anti-ulcerogenic potential of the leaf extracts of *T. daniellii*.

Materials and Methods

Sample Collection and Preparation

Fresh leaves of *T. daniellii* were collected from Ijagbo area, in Offa, Kwara State and authenticated by a botanist in the Department of Biological Sciences in Federal Polytechnic Offa, Kwara State, Nigeria. The collected leaves were washed, dried and grinded into powder.

Preparation of the Extract

Aqueous Extract

The method of Ukwubile et al. (2017) was adopted for the extraction. Three hundred (300) grams of powdered leaves of *T. daniellii* dissolved in 1.5 L of distilled water for 72 hours in a refrigerator. Thereafter, it was filtered with muslin cloth and Whatman No. 1 (320 nm, 4 µm) filtered paper. The filtrate was evaporated to dryness using a water bath (40 °C) to obtain the slurry. The resultant slurry was persevered in a phial, appropriately labeled and kept at 4 °C until needed for use.

Methanol Extract

The method of Ukwubile et al. (2017) was adopted. Three hundred (300) grams of powdered leaves of *T. daniellii* soaked in 1.5 L of methanol for 72 hours, followed by vacuum filtration and the extract was concentrated at low pressure using a rotary evaporator water bath at 40 °C. The concentrate heated over a water bath to obtain a solvent free extract, which was persevered in a phial, appropriately labeled and kept at 4 °C until needed for use.

Test Organisms

The test organisms employed for the antimicrobial assay of aqueous and methanolic leaf extracts of *T. daniellii* included *Klebsiella pneumoniae* and *Enterococcus faecium*. The organisms were obtained from University of Ilorin Teaching Hospital, Ilorin Kwara state Nigeria.

Confirmation and Identification of Organisms

The identities of organisms obtained were confirmed by sub culturing 24 hours of each of the isolate into different selective and differential media such as Eosin methylene, blue agar, macconkey agar, nutrient agar and were further characterized with the use of different biochemical tests such as Gram's stain, catalase, coagulase, oxidase, indole and the resultant characteristics were compared with Bergey's Manual of Determinative Bacteriology (Fawole & Oso, 2007).

Phytochemical Analysis of *Thaumatococcus daniellii* Leaf

Test for Flavonoid

One milliliter (1 ml) of filtrate mixed with 2 ml of 10 % lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 ml of the filtrate mixed with 2 ml of dilute NaOH; flavonoids is confirmed presence by a golden yellow color (AOAC, 2023).

Test for Alkaloids

About 0.2 gram of *T. daniellii* Leaf extract was warmed with 2 % of H₂SO₄ for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the presence of Alkaloids (AOAC, 2023).

Test for Glycosides

About 0.2 g of plant extract was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride solution. 1 ml of concentrated H₂SO₄ was then added. A brown ring obtained at the interface indicated the presence of deoxy-sugar characteristics of cardenolides. A violet ring appeared below the ring while in the acetic layer; a greenish ring formed just above the brown ring and gradually spread throughout this layer (AOAC, 2023).

Test for Steroids

Two milliliters (2 ml) of acetic anhydride added to 0.5 g methanolic extract with 2 ml H₂SO₄. Steroid is confirmed presence by colour changed from violet to blue or green in some samples (AOAC, 2023).

Test for Tannins

One milliliter (1 ml) of the filtrate mixed with 2 ml of FeCl₃. A dark green color indicated a positive test for the tannins (AOAC, 2023).

Standardization of inoculums for antimicrobial assay (0.5 McFarland standard)

The method of Usman et al. (2021) was employed. A 0.1 ml of 1% Barium chloride was added to 9.9 ml of 1% sulphuric acid, reconstituted into 10 ml of sterile distilled water to make 0.5 ml McFarland standard solution. The broth culture of 24 hours test organism was then compared in terms of turbidity to 0.5% McFarland which is equivalent to 10⁵cfu/ml. A loopful of the standardized culture was used for antimicrobial assay.

Antimicrobial activities of *Thaumatococcus daniellii* leaf extract

Agar well diffusion method described by Okunye et al. (2020) was employed. One ml aliquot of 24 hours bacterial culture was poured plated into freshly prepared tempered Muller Hinton agar (MHA) and allowed to gel. Upon setting, wells were made using a sterile cork-borer (6 mm in diameter) into agar plates containing inoculums. A few drops of 50 mg/ml and 100 mg/ml concentrations of aqueous and methanolic extract of *T. daniellii* were poured into agar well containing standardized inoculum. Then, the plates were incubated at 37 °C for 24 hours along with control plates; organisms viability control (OVC). Antimicrobial activity was determined by measuring the zone of inhibition in millimeter.

Determination of minimum inhibitory concentration (MIC) medicinal extract

In the determination of minimum inhibitory concentration (MIC) values, broth dilution method of Okunye et al. (2020) was adopted. Varying concentrations of medicinal plant extract were prepared by accurately measuring 0.05 ml, 0.04 ml, 0.03 ml and 0.02 ml into 1 ml of sterile distilled water to give final concentrations of 50, 40, 30, and 20 (mg/ml) concentrations respectively. Then 1 ml aliquot of 24 hours inoculum of 0.5 McFarland standard equivalent was transferred into each of extract concentration. The tubes containing the culture and the extract were incubated at 37 °C for 24 hours. The results of extract minimum inhibitory concentration (MIC) was checked by comparing the growth on agar culture with that of the control; organism viability control (OVC), where a growth confirmed no activity and no growth confirmed activity of the extract. The least concentration of the extract with no growth confirmed minimum inhibitory concentration.

Determination of Anti-diabetic Activity of *Thaumatococcus daniellii* leaf α -Amylase Inhibitory Assay

The method described by Quan et al. (2019) with slight modification was adopted to determine α -amylase inhibitory activity assay. The reaction mixture contained 250 μ L of different concentration of the sample (100-500 μ g/mL) and 250 μ L of 0.02 M sodium phosphate buffer (pH 6.9) containing α -amylase solution (0.5 mg/mL). The mixture was incubated at 25 °C for 10 minutes after preparation of 250 μ L of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added. This was then incubated at 25 °C for 10 minutes. 500 μ L of dinitrosalicylic acid (DNS) reagent added and the mixture incubated another time for 5 minutes in boiling water.

Upon cooling, 3.750 uL of distilled water was added. Distilled water serves as blank while all reagents without sample and distilled water served as the control and acarbose as standard. The absorbance of the mixture was taken at 540 nm with ultraviolet/visible spectrophotometer model 721N, SearchTech instrument, China against the reagent blank. The inhibitory activities of the samples were calculated as follow:

$$\% \text{ Inhibition} = \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} \times 100$$

α -Glucosidase Inhibitory Assay

Method of Quan et al. (2019) with slight modification was adopted to determine α -glucosidase inhibitory activity assay. The mixture contained 250 uL of different concentration of the sample (7.813–1000 $\mu\text{g/mL}$) and 500 of 0.02 M sodium phosphate buffer (pH 6.9) containing α -glucosidase solution. The mixture was incubated at 25 °C for 10 minutes after which 250 pL of 3.0 mM pNPG in phosphate buffer was added. This was again incubated at room temperature for 20 min after which 1.0 ml of 0.1 M sodium carbonate was added to stop the reaction. Distilled water serves as blank while all reagents without sample and distilled water served as the control and acarbose as standard. The absorbance of the mixture was taken at 405 nm with ultraviolet/visible spectrophotometer model 721N, SearchTech instrument, China against the reagent blank. The inhibitory activities of the samples were calculated as follow:

$$\% \text{ Inhibition} = \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} \times 100$$

Antioxidant Activity of *Thaumatococcus daniellii* Leaf

Hydroxyl Radical (OH.) Scavenging Assay

The method described by Yu et al. (2021) was used to determine scavenging activity of the extract. Two (2) mL of test compounds at 200 to 1000 mg/mL, 0.6 mL of 8 mM ferrous sulfate, 0.5 mL of 20 mM hydrogen peroxide, and 2 mL of 3 mM salicylic acid were mixed and incubated at 37 °C for 30 minutes. Then, 0.9 mL of distilled water was added to each ampoule, centrifuged at 4472 g for 10 minutes and absorbance was read at 510 nm. The percentage OH scavenging activities of the extract were calculated using the following expression:

$$\text{OH Scavenging Activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Nitric Oxide Scavenging Activity

The method described by Yu et al. (2021) was used to determine scavenging activity of the extract. Two (2) ml of the test extracts with varying concentrations (7.813–1000 $\mu\text{g/ml}$) were incubated with 0.5 ml of sodium nitroprusside (5 mM) for 2 hours at 27 °C. Aliquot 1 ml of the incubated solution and mixed with 0.6 ml of Griess reagent (1.0 mL sulfanilic acid reagent (0.33 %) in 20 % glacial acetic acid at room temperature for 5 minutes with 1 ml of naphthyl ethylenediamine dichloride (0.1 %). The absorbance measured immediately at 550 nm and synthetic antioxidant butylated hydroxytoluene was used as positive control. The triplicate of experiment was performed and scavenging activity was expressed as percentage scavenging, using the following formula.

$$\text{Nitric Oxide Scavenging Activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Ferric Reducing Ability of Plasma (FRAP) Assay

Reagents:

The method described by Yu et al. (2021) was adopted. The working ferric reducing ability of plasma reagent was prepared by mixing Acetate buffer, 2, 4, 6-tripyridyl-s-triazine and FeCl_3 in the ratio of 10:1:1 just before testing. Standard was $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.1 - 1.5 mM in methanol. All the reagents were prepared from Merck (Germany) company. Ferric reducing ability of plasma solution (3.6 mL) added to distilled water (0.4 mL) and incubated at 37 °C for 5 minutes. Then this solution mixed with certain concentration of the plant extract (80 mL) and incubated at 37 °C for 10 minutes. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions

Hydrogen Peroxide Radical Scavenging Activity

Hydrogen Peroxide (H_2O_2) radical scavenging activity was determined according to the method of Yu et al. (2021). A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). Briefly, 1 ml of test samples of varying concentrations (7.813–1000 $\mu\text{g/ml}$) were added to the H_2O_2 solution and incubated for 10 minutes. Absorbance was measured at 230 nm against blank solution containing phosphate buffer without H_2O_2 .

Synthetic antioxidant ascorbic acid served as positive control. The experiments were performed in triplicates, and scavenging activity was expressed as percentage scavenging, using the following formula.

Total Antioxidant Capacity

Total antioxidant capacity was determined according to the method of Yu et al. (2021). The total antioxidant capacity reagent was prepared by mixing equal volumes of 0.6 M Sulfuric Acid, 28 mM sodium phosphate and 4 mM Ammonium molybdate. Samples were prepared with the concentration of 100-1000 µg/mL. The reaction solution comprised of 100 µl of the sample and 1000 µl of the total antioxidant capacity reagent. The mixture was incubated in water bath for 1 hour at 95 °C, after which the absorbance was taken with the spectrophotometer at 695 nm upon cooling. Solution of 1000 µl of the reagent and 0.1 ml of methanol served as blank while reagent only without sample and methanol served as control. The antioxidant activity is expressed relative to that of the Butylated Hydroxytoluene.

Anti-ulcerogenic Potential of *Thaumatococcus daniellii* Leaf

Preparation of H⁺, K⁺-ATPase Enzyme

The method described by Hussain et al. (2021) was employed to prepare proton potassium ATPase from mucosal scrapings of goat. Stomach of freshly slaughtered goats was washed gently with tap water. The mucosal layer of fundus was scrapped and homogenized in ice-cold phosphate buffer, pH 7.4. The homogenate was centrifuged for 20 minutes at 18,000 rpm. The supernatant obtained was recentrifuged for 60 minutes at 100,000 rpm. The pellet was resuspended in homogenisation buffer. Ficollsucrose discontinuous density gradient centrifugation was utilized to prepare H⁺K⁺ATPase.

Assessment of H⁺, K⁺-ATPase inhibition

Varying concentrations of the extract 7.813-1000 µg/ml were incubated in the reaction mixture (40 mM Tris-HCl buffer, pH 7.4, containing 2 mM MgCl₂ and 10 µg membrane protein) to make 1 ml volume. Then, 2 mM ATP Tris salt was used to start the reaction; the preparation was incubated for 20 minutes for 37 °C. The reaction was terminated by adding 1 ml of ice-cold trichloroacetic acid (10% v/v). The H⁺-K⁺ ATPase activity was assayed in the presence and the absence of different doses of the extract and omeprazole (Hussain et al., 2021). The amount of inorganic phosphate released from ATP was determined spectrophotometrically at 400 nm.

Statistical Analysis

The data obtained in this study were analyzed using statistical software package SPSS 10.0 for windows. Analysis was undertaken by using student's t-test. P < 0.05 was considered statistically significant.

Results

Table 1: Phytochemical Screening of Aqueous and Methanolic Leaf Extracts of *Thaumatococcus daniellii*

Phytochemical Test	Aqueous Leaf Extract	Methanolic Leaf Extract
Saponins	-	+
Glycoside	-	-
Flavonoids	+	-
Alkaloids	+	+
Terpenoids	-	-
Tannins	-	+
Phenol	-	+

Keys: (+) = Present, (-) = Absent

The phytochemical components of aqueous and methanolic leaf extracts of *T. daniellii* are presented in Table 1. The results of the phytochemical screening of aqueous leaf extracts of *T. daniellii* revealed the presence of flavonoids and alkaloids while saponins, glycoside, phenol and tannins were absent. Also the phytochemical screening of methanolic leaf extracts of *T. daniellii* contained saponins, phenol, alkaloids and tannins while glycoside, terpenoids, glycoside and flavonoids were absent.

Table 2: Antibacterial Activities of Leaf Extracts of *Thaumatococcus daniellii* at 50 and 100 mg/ml concentrations

Test organisms	50 mg/ml		100 mg/ml	
	MEE	AQE	MEE	AQE
<i>Enterococcus faecium</i>	-	-	-	-
<i>Klebsiella pneumonia</i>	5 mm	-	12 mm	-

Key: MEE = Methanol Extract, AQE = Aqueous Extract, mm= millimeter, - = No activity

The methanolic leaf extract of *T. daniellii* inhibited the growth of *Klebsiella pneumoniae* but lacked inhibitory potential against the growth *Enterococcus faecium* at a concentration of 50 and 100 mg/ml concentrations respectively, while the aqueous leaf extracts of *T. daniellii* displayed no inhibitory activity against *K. pneumoniae* and *Enterococcus faecium* at same concentration (Table 2).

Table 3: Minimum inhibitory concentration (MIC) of Methanolic Leaf Extracts (MEE) of *Thaumatococcus daniellii* on test isolate

Microorganism	Concentrations/zone of inhibitions			
	50	40	30	20
<i>Klebsiella pneumonia</i>	5 mm	3 mm	-	-

Key: MEE = Methanol Extract, mm= millimeter, - = No activity

The methanolic leaf extract of *T. daniellii* has minimum inhibitory concentration of 30 mg/ml on *K. pneumoniae* with zone of inhibition of 3 mm (Table 3)

Table 4: Hydrogen Peroxide Scavenging Activities of Leaf Extracts of *T. daniellii*

Concentration (µg/ml)	BHT (%)	AQOH (%)	MEOH (%)
1000	96.64 ± 0.31	62.92 ± 0.31	80.30 ± 0.10
500	88.67 ± 0.36	56.62 ± 0.33	73.15 ± 0.17
250	81.28 ± 0.24	49.71 ± 0.25	66.31 ± 0.4
125	74.32 ± 0.10	43.06 ± 0.06	59.39 ± 0.31
62.5	67.41 ± 0.25	36.88 ± 0.24	50.87 ± 1.99
31.25	59.43 ± 0.10	30.91 ± 0.36	45.20 ± 0.11
15.63	52.29 ± 0.35	24.26 ± 0.10	38.02 ± 0.30
7.81	45.10 ± 0.29	20.59 ± 0.14	30.91 ± 0.24

Data are presented as Mean ± SE (n=2)

Key: BHT: Butylated Hydroxytoluene, AQOH: Methanol, MEOH: Aqueous

The inhibition of hydrogen peroxide radical scavenging activity of various aqueous and methanolic leaf extracts of *T. daniellii* are depicted in Table 4. The results showed that the standard antioxidant (Butylated Hydroxytoluene) recorded the highest hydrogen peroxide radical inhibitory activity, followed by methanolic leaf extracts of *T. daniellii* while aqueous leaf extracts of *T. daniellii* recorded the least hydrogen peroxide radical inhibitory activity across all concentrations.

Table 5: Nitric Oxide (NO) Scavenging Activity of Leaf Extracts of *T. daniellii*

Concentration (µg/ml)	BHT (%)	Aqueous (%)	Methanol (%)
1000	90.90 ± 0.22	61.22 ± 0.22	82.27 ± 0.14
500	82.68 ± 0.60	52.36 ± 0.30	72.04 ± 0.44
250	75.29 ± 0.51	46.57 ± 0.22	59.81 ± 0.30
125	66.61 ± 0.51	36.52 ± 1.01	50.76 ± 0.30
62.5	57.09 ± 0.25	27.60 ± 0.44	43.67 ± 0.44
31.25	50.71 ± 0.38	22.16 ± 0.58	34.81 ± 0.30
15.63	46.99 ± 0.29	18.85 ± 0.30	28.90 ± 0.28
7.81	39.66 ± 0.44	14.07 ± 0.08	22.45 ± 0.22

Data are presented as Mean ± SE (n=2)

Key: BHT: Butylated Hydroxytoluene

The nitric oxide scavenging activity of aqueous and methanolic leaf extracts of *T. daniellii* is shown in Table 5. The percentage nitric oxide scavenging activity of the standard antioxidant (Butylated Hydroxytoluene) was significantly higher than that of aqueous and methanolic leaf extracts of *T. daniellii* at all study concentrations. The standard oxidant as well as aqueous and methanolic leaf extracts of *T. daniellii* exhibits scavenging properties.

Table 6: Hydroxyl (OH) Radical Scavenging Activity of Leaf Extracts of *T. daniellii*

Concentration (µg/ml)	BHT (%)	AQOH (%)	MEOH (%)
1000	89.06 ± 0.51	63.65 ± 0.68	78.60 ± 0.08
500	84.20 ± 0.47	58.79 ± 0.13	68.85 ± 0.33
250	71.70 ± 0.48	50.77 ± 0.09	60.30 ± 0.13
125	61.50 ± 0.48	44.25 ± 0.22	50.77 ± 0.02
62.5	56.32 ± 0.10	35.92 ± 0.13	45.18 ± 0.11
31.25	51.03 ± 0.08	27.36 ± 0.26	35.66 ± 0.24
15.63	46.81 ± 0.17	19.55 ± 0.23	28.05 ± 0.45
7.81	40.65 ± 0.21	14.37 ± 0.10	21.32 ± 0.06

Data are presented as Mean ± SE (n=2)

Key: BHT = Butylated Hydroxytoluene, AQOH = Aqueous, MEOH = Methanol,

The in-vitro hydroxyl radical scavenging activity of aqueous and methanolic leaf extracts of *T. daniellii* shown in Table 6. The results showed that the standard antioxidant (Butylated Hydroxytoluene) recorded the highest hydroxyl radical inhibitory activities when compared to aqueous and methanolic leaf extracts of *T. daniellii* across all concentrations. However, both the standard drug aqueous and methanolic leaf extracts of *T. daniellii* showed increase in hydroxyl radical inhibitory activities with increasing concentrations.

Table 7: Total Antioxidant Activity of *Thaumatococcus daniellii* Leaf

Aqueous	Methanol (%)	Butylated Hydroxytoluene (%)
4.25 ± 0.16	11.18 ± 0.01	14.37 ± 0.10

Data are presented as Mean ± SE (n=2)

The total antioxidant activity of methanolic and aqueous leaf extracts of *T. daniellii* is shown in Table 7. The total antioxidant activity of standard antioxidant (Butylated Hydroxytoluene) is the highest, followed by methanol leaf extracts of *T. daniellii* while and aqueous leaf extracts of *T. daniellii* recorded the least total antioxidant activity.

Table 8: Ferric Reducing Antioxidant Power of Aqueous Leaf Extracts of *T. daniellii*

Aqueous	Methanol (%)	Butylated Hydroxytoluene (%)
1.05 ± 0.00	1.41 ± 0.01	1.97 ± 0.00

Data are presented as Mean ± SE (n=2)

The ferric reducing antioxidant power of methanolic and aqueous leaf extracts of *T. daniellii* are shown in Table 8. The ferric reducing antioxidant power standard antioxidant (Butylated Hydroxytoluene) is significantly higher than that of aqueous leaf extracts of *T. daniellii*.

Table 9: Effect of Leaf Extract of *Thaumatococcus daniellii* on α -amylase Activity

Concentration (μ g/ml)	Acarbose (%)	Methanolic (%)	Aqueous (%)
1000	92.69 ± 0.33	83.04 ± 0.1	65.69 ± 0.51
500	86.82 ± 0.40	75.54 ± 0.70	58.12 ± 0.16
250	79.19 ± 0.49	65.75 ± 0.32	50.23 ± 0.51
125	71.43 ± 0.16	59.36 ± 0.40	42.20 ± 0.33
62.5	61.45 ± 0.42	52.97 ± 0.24	36.07 ± 0.51
31.25	53.42 ± 0.32	47.55 ± 1.15	28.51 ± 0.40
15.63	45.92 ± 0.51	42.14 ± 0.09	24.53 ± 0.40
7.81	40.44 ± 0.24	36.33 ± 0.18	21.98 ± 0.24

Data are presented as Mean ± SE (n=2)

The methanolic and aqueous leaf extracts of *T. daniellii* and acarbose produced concentration-dependent inhibition of α -amylase activities (Table 9). The inhibition of the enzyme tends to be related to the dosage, as the concentration of the methanolic and aqueous leaf extracts of *T. daniellii* clearly influences the amount of enzyme inhibited. The α -amylase inhibitory activity of acarbose was higher than that of methanolic and aqueous leaf extracts of *T. daniellii* at all concentration.

Table 10: Effect of Leaf Extract of *Thaumatococcus daniellii* on α -glucosidase Activity

Concentration (μ g/ml)	Acarbose (%)	Methanol (%)	Aqueous (%)
1000	91.43 ± 0.24	82.67 ± 0.5	51.51 ± 0.44
500	85.31 ± 0.31	74.29 ± 0.31	44.63 ± 0.31
250	78.48 ± 0.41	64.07 ± 0.53	38.98 ± 0.35
125	64.78 ± 5.46	55.64 ± 0.31	33.15 ± 0.35
62.5	58.57 ± 0.18	48.78 ± 0.37	27.58 ± 0.35
31.25	51.88 ± 0.48	41.00 ± 0.48	22.46 ± 0.23
15.63	43.17 ± 0.48	33.24 ± 0.41	17.47 ± 0.29
7.81	39.98 ± 0.31	24.95 ± 0.29	16.20 ± 0.29

Data are presented as Mean ± SE (n=2)

The methanolic and aqueous leaf extracts of *T. daniellii* and Acarbose inhibited α -glucosidase activity in a concentration-dependent manner (Table 10). The inhibition of the enzyme tends to be related to the dosage, as the concentration of the methanolic and aqueous leaf extracts of *T. daniellii* clearly influences the amount of enzyme inhibited. The α -glucosidase inhibitory activity of acarbose was higher than that of methanolic and aqueous leaf extracts of *T. daniellii* at all concentration.

Table 11: H⁺, K⁺-ATPase Inhibition Activity of Leaf Extracts of *T. daniellii*

Concentration (µg/ml)	Aqueous Extract (%)	Omeprazole (%)	Methanolic Extract (%)
1000	64.51 ± 0.16	89.94 ± 0.42	74.38 ± 0.33
500	57.67 ± 0.14	84.76 ± 0.28	65.57 ± 0.42
250	49.28 ± 0.14	78.98 ± 0.21	57.48 ± 0.28
125	40.48 ± 0.32	71.99 ± 0.09	48.22 ± 0.14
62.5	32.46 ± 0.47	65.64 ± 0.19	40.58 ± 0.16
31.25	23.58 ± 0.16	58.42 ± 0.14	33.78 ± 0.52
15.63	18.63 ± 0.19	51.81 ± 0.24	27.96 ± 0.23
7.81	15.76 ± 0.24	43.42 ± 0.37	23.20 ± 0.37

Data are presented as Mean ± SE (n=2)

The H⁺, K⁺-ATPase inhibition activity of methanolic and aqueous leaf extracts of *T. daniellii* and omeprazole were compared (Table 11). The results showed that aqueous and methanolic leaf extracts of *T. daniellii* inhibited the H⁺, K⁺-ATPase with varied potency. Omeprazole exhibited good H⁺, K⁺-ATPase (PPAI) activity followed by aqueous and methanolic leaf extracts of *T. daniellii*.

Discussion

Phytochemical screening of aqueous leaf extracts of *T. daniellii* confirmed the presence of flavonoids and alkaloids. These findings agreed with most literatures on the presence of these phytochemicals in the studied samples (Hamid et al., 2017; Ukwubile et al., 2017). Flavonoids were found in aqueous leaf extracts of *T. daniellii* but absent in methanolic leaf extracts of *T. daniellii*. Research has demonstrated that flavonoids may enhance the body's production of detoxifying enzymes, decrease inflammation, and inhibit the growth of malignancies (Olasupo et al., 2018). According to literatures, tannins have been linked to a variety of physiological and anti-microbial effects, including the triggering of phagocytic cells, host-mediated resistance, and a broad spectrum of anti-infective effects (Sowparthani & Radhika, 2020). Tannins were found in methanolic leaf extracts of *T. daniellii*. This suggests that methanolic leaf extracts of *T. daniellii* are good source of tannins. As a result, consumption of *T. daniellii* leaves may be a source of phytochemical components useful in the management and prevention of tumor in the body (Hamid et al., 2017). Alkaloids were found in aqueous and methanolic leaf extracts of *T. daniellii*. According to Olasupo et al. (2018), alkaloids constitute a significant class of naturally occurring medications that are used to treat congestive heart failure. Because of their extreme toxicity and pronounced bitter taste, alkaloids are employed by plants as a defense mechanism against invertebrate pests, microbial diseases, and herbivores (Udochukwu et al., 2015). Furthermore, according to Sowparthani and Radhika (2020), alkaloids possess antispasmodic, antibacterial, therapeutic, and antimalarial characteristics, making them the most effective phytochemical. This result implies that aqueous and methanolic leaf extracts of *T. daniellii* are good source of alkaloids and its consumption will help in the treatment of some ailments as mentioned above.

More so, bioactive chemicals known as saponins have the ability to reduce the stomach's absorption of glucose and cholesterol (Udochukwu et al., 2015; Audu et al., 2018). Naturally occurring substance called saponins complexes with cholesterol to create holes in bilayers of cell membranes. Consequently, these substances may be employed as agents that lower or inhibit cholesterol (Abdullahi & Santhos, 2018). Saponins were found in methanolic leaf extracts of *T. daniellii*. The presence of saponins in methanolic leaf extracts of *T. daniellii* indicated that it could serve as a major active ingredient in drug production. The antibacterial activity of extracts studied, revealed that methanolic leaf extract of *T. daniellii* showed higher degree of inhibition while aqueous extract showed no activity. *Klebsiella pneumoniae* was highly susceptible to the methanolic leaf extract of *T. daniellii* with zones of inhibition of 5.0 mm and 12.0 mm at concentrations of 50 mg/ml and 100 mg/ml respectively, this agreed with the work of Ukwubile et al. (2017). Therefore, it could be used to cure *K. pneumoniae* associated diseases or used to develop drugs associated with the organisms. However, the aqueous extract of *T. daniellii* on the other hand displayed no activity at all the concentrations used. Thus, the aqueous leaf extract of *T. daniellii* were not effective against *K. pneumoniae* and *Enterococcus faecium* which may be attributed to insolubility of active constituents of the medicinal plant in aqueous solvent. The finding of this study is in agreement with the result of Ukwubile et al. (2017) showing that methanolic extract was more potent than extract of other solvents against microorganisms that were studied. It is clearly seen that methanolic leaf extract of *T. daniellii* has anti-microbial properties. Thus, the methanolic leaf extract of *T. daniellii* can be used as the active constituent of anti-microbial natural products. The results of this study showed that methanolic extracts of

the leaves of *T. daniellii* was effective against *K. pneumonia*. This may be owing to the better solubility of the active components in methanolic solvent (Ukwubile et al., 2017; Hamid et al., 2017).

The results obtained from this study showed that the extract and the standard drug increased their reducing ability with increasing concentration. The reducing power of aqueous and methanolic leaf extracts of *T. daniellii* and the reference compounds increased with increasing concentration. The antioxidant potentials of methanolic leaf extract of *T. daniellii* was estimated from their ability to reduce Fe^{3+} to Fe^{2+} . The reducing value of the plant extracts was significantly lower than that of Butylated Hydroxytoluene (BHT), used as control compounds in this study. There was significant difference in reducing activities of aqueous and methanolic leaf extracts of *T. daniellii* and Butylated Hydroxytoluene. However, reducing power of Butylated Hydroxytoluene was significantly higher than that of aqueous and methanolic leaf extracts of the medicinal plant. Reductones development has been linked with the antioxidant activity, which are removers of free radical chain reactors (Alzandi et al., 2021). The presence of reductants such as antioxidant substances in aqueous and methanolic leaf extracts of *T. daniellii* causes a reduction of the Fe^{3+} to Fe^{2+} form. Therefore, the ability of a compound to transfer electron is an important signal of its potential as an antioxidant (Obboh et al., 2016). This confirmed that the antioxidant compounds are electron donors and could reduce the oxidized intermediate of lipid peroxidation processes; thus acting as primary and secondary antioxidants (Shalom et al., 2018). The antioxidant activities of Butylated Hydroxytoluene were significantly higher than the plant extract. It could be inferred from this study that the reference drug, aqueous and methanolic leaf extracts of *T. daniellii* are electron donor and thus could possibly reduce the oxidized intermediates of lipid peroxidation (Alzandi et al., 2021). This might be attributed to reductines which are known terminators of free radical chain reactors by donating hydrogen atom (Okafor et al., 2019).

The aqueous and methanolic leaf extracts of *T. daniellii* showed strong nitric oxide inhibitory activities. Nitric oxide is a reactive free radical produced from sodium nitroprusside in an aqueous solution at physiological pH and reacts with oxygen in the reaction to form nitrite. The extracts inhibit nitrite formation by directly competing with oxygen in the reaction with nitric oxide and other nitrogen oxides such as NO_3 , and N_2O_3 (Iheagwam et al., 2017). The previous report on the anti-inflammatory role of nitric oxide (Hamid et al., 2017), could support the use of aqueous and methanolic leaf extracts of *T. daniellii* for treatment of inflammation and healing of wound. Therefore, nitric oxide (NO) radical scavenging ability of aqueous and methanolic leaf extracts of the plant may possibly assist to stop the chain reactions instigated by excessive production of nitric oxide and play a role in preventing pathological diseases emerge from oxidative stress. The scavenging ability of aqueous and methanolic leaf extracts of *T. daniellii* (compared to Butylated Hydroxytoluene as standard) on hydrogen peroxide. Butylated Hydroxytoluene were capable of scavenging hydrogen peroxide in a concentration dependent manner and have a stronger hydrogen peroxide scavenging activity when compared to aqueous and methanolic leaf extracts of *T. daniellii*. Hydrogen peroxide itself is not very reactive, but it could be toxic to cells because of its ability to penetrate biological membrane. As a result it may give rise to hydroxyl radical production in the cells (Adedosu et al., 2017). Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2 , thus reducing it to water. There was significantly different hydroxyl radical scavenging activity of the standard antioxidant (Butylated Hydroxytoluene) and aqueous and methanolic leaf extracts of *T. daniellii*. As the aqueous and methanolic leaf extracts of *T. daniellii* concentrations increased, its $\bullet\text{OH}$ radicals' quenching capability also increased. The different concentration of aqueous and methanolic leaf extracts of *T. daniellii* when added to the reaction mixture, scavenged the hydroxyl radicals and prevented the degradation of deoxyribose. All the concentrations of aqueous and methanolic leaf extracts of *T. daniellii* and the standard antioxidant (Butylated Hydroxytoluene) inhibited the production of hydroxyl radicals.

Since oxidative stress is a deep-rooted cause of various disorders, the prevention of reactive oxygen specie and reactive nitrogen specie production in the cell metabolism is of utmost importance. Antioxidant activities exhibited by aqueous and methanolic leaf extracts of *T. daniellii* could be attributed to the availability of various phytochemicals that work synergistically to overcome free radicals (Hamid et al., 2017). Several biological compounds including phenolic compounds; lipids like fatty acids, phytosterols, and fatty acid esters; terpenoids like monoterpene, diterpenes, and triterpenes; alkaloids; and hydrocarbons like alkanes and alkenes were detected (Shalom et al., 2018). This study confirmed the efficacy of aqueous and methanolic leaf extracts of *T. daniellii* as potent inhibitor of α -glucosidase and α -amylase as it was evident from the percentage of inhibition relative to acarbose. From the observation, acarbose used as standard exhibited more α -amylase inhibitory potentials than the aqueous and methanolic leaf extracts of *T. daniellii*. The α -glucosidase inhibitory activity of aqueous and methanolic leaf extracts of *T. daniellii* and acarbose were comparable. Furthermore, the finding from this study indicates that the extract can delay carbohydrate breakdown by inhibiting the α -amylase enzyme. Several studies have documented a positive correlation between phytochemicals, α -amylase and α -glucosidase

inhibition activity (Kalita et al., 2018). The aqueous and methanolic leaf extracts of *T. daniellii* has an excellent potential to inhibit α -glucosidase, and α -amylase enzymes. The overall result in this study suggested that aqueous and methanolic leaf extracts of *T. daniellii* are potential source of drug for the management of diabetes.

H⁺, K⁺-ATPase, which is found in parietal cells, is the enzyme primarily responsible for the acidification and subsequently ulcerations in the stomach. Therefore, the control of acid secretion may be essential for the treatment of the disease. Inhibition of the proton pump was proposed as a mechanism for their gastroprotective activity (Nanda, 2019). In the current study, the tested aqueous and methanolic leaf extracts of *T. daniellii* inhibited the H⁺, K⁺-ATPase with varied potency. As indicated in table 11. Omeprazole exhibited good H⁺, K⁺-ATPase (PPAI) activity followed by aqueous and methanolic leaf extracts of *T. daniellii*. Analysis of in-vitro results indicates that Omeprazole and aqueous and methanolic leaf extracts of *Thaumatococcus daniellii* showed significant proton potassium ATPase (PPA) inhibition activity indicating its presumed anti-ulcer property. The overall result in this study suggested that aqueous and methanolic leaf extracts of *T. daniellii* are potential source of drug for the management of diabetes.

Conclusion

Thaumatococcus daniellii contained flavonoids, alkaloids saponins, phenol, alkaloids and tannins. The antimicrobial analysis showed that methanolic leaf extracts of *T. daniellii* inhibited the growth of *K. pneumoniae*. The methanolic and aqueous leaf extracts of *T. daniellii* demonstrated *invitro* ferric reducing antioxidant power, hydrogen peroxide scavenging and nitric oxide scavenging activity. The methanolic and aqueous leaf extracts of *T. daniellii* possessed α -glucosidase and α -amylase inhibitory potential in comparison with standard acarbose. On the basis of the results obtained in the present study, the methanolic and aqueous leaf extracts of *T. daniellii* is a promising source of potential anti-diabetic compounds that can help prevent diabetes complications through its ability to inhibit α -glucosidase and α -amylase. Also the aqueous and methanolic leaf extracts of *T. daniellii* showed potent H⁺/K⁺-ATPase inhibitory property; hence, they may be used as potential antiulcerogens.

Recommendations

Further studies should be done on other parts of *T. daniellii* plant to establish their potential in drug synthesis and more so characterization of their active components in order to establish their therapeutic efficacy and mechanism of action.

References

- Abdullahi, I. I., & Santhos, I. (2018): Comparative analysis on antioxidant and antibacterial activity of pumpkin wastes. *Journal of Antimicrobial Agents*, 4(3), 180-187.
- Adedosu, O. T., Badmus, J. A., Adeleke, G. O., & Olalere, A. (2017). *Thaumatococcus daniellii* extract modulates glibenclamide activity and ameliorates hematological disorders, oxidative stress and dyslipidemia associated with diabetes mellitus in rats. *British Journal of Pharmacy Research*, 16(5), 1-12.
- Ahmed, M., Qin, P., Gu, Z., Liu, Y., Sikandar, A., Hussain, D., Javeed, A., Jamil, S., Mazher, F., Ran, A., Hongxia, G., Ying, D., Weijing, W., Yumeng, Z., & Mingshan, J. (2020): Insecticidal activity and biochemical composition of *citrullus colocynthis*, *cannabis indica* and *artemisia argyi* extracts against cabbage aphid (*Brevicoryne brassicae* L.). *Science Report*, 10, 522–528.
- Alzandi, A. A., Taher, E. A., Al-Sagheer, N. A., Al-Khulaidi, A. W., & Azizi, M. (2021). Phytochemical components, antioxidant and anticancer activity of 18 major medicinal plants in Albaha region in Saudi Arabia. *Biocatalysis and Agricultural Biotechnology*, 34, 102020.
- Association of Official Analytical Chemists (AOAC) (2023). Official methods of analysis of the association of official's analytical chemists, 22nd Edition. AOAC.
- Audu, I., Shuaibu, S., Alona, L., Murtala, M. M., & Jiya, J. A. (2018): Phytochemical analysis and antimicrobial activity of bitter leaf collected from Lapai, Niger State, Nigeria on some selected pathogenic microorganisms. *Science World Journal*, 13(3), 15-18.
- Fawole, M. O., & Oso, B. A. (2007). *Characterization of bacteria, laboratory manual of microbiology*. Revised Ed. Spectrum Book Limited, p. 24-33.
- Gong, T., Yang, X., Bai, F., Li, D., Zhao, T., Zhang, J. & Guo, Y. (2020). Young apple polyphenols as natural α -glucosidase inhibitors: In-vitro and in-silico studies. *Bioorganic Chemistry*, 96, 103625.
- Hamid, A. A., Aliyu, M. A., Abubakar, L. Z., Mukadam, A. A., Shehu, A., Egharevba, G., Adisa, M. J., Ajibade, S. O., Zubair, A. O., & Fagbohun, E. O. (2017). *Thaumatococcus daniellii* leaves: Its chemical compositions, antioxidant and antimicrobial activities. *Ife Journal of Science*, 19(2), 409-416.

- Hussain, S. A., Srigadi, G. K., Rao, C. S., Hussaini, S. A., Srikanth, A., Vootkuri, J. R., Anuja, K., Rajasri, P. & Sneha, D. (2021). In-vitro anti-ulcer activity of different extracts of *cissus quadrangularis*. *International Journal of Advanced Research*, 9(4), 21-25.
- Hwang, P. A., Hung, Y. L., Tsai, Y. K., Chien, S. Y. & Kong, Z. L. (2015). The brown seaweed *sargassum hemiphyllum* exhibits α -amylase and α -glucosidase inhibitory activity and enhances insulin release in-vitro. *Cytotechnology*; 67, 653-660.
- Iheagwam F. I., Shalom N., Chinedu S.N., Emiloju O. C., & Okenmuo, A. C. (2017). Fruit extract of *Thaumatococcus danielli* Reduces Oxidative Stress in Rats. *FASEB Journal*, 31(1), 779-785.
- Jiang, Y., David, B., Tu, P. & Barbin, Y. (2019). Recent analytical approaches in quality control of traditional chinese medicines. *Analytical Chemistry*, 65(1), 9-18.
- Kalita, D., Holm, D. G., LaBarbera, D. V., Petrash, J. M., & Jayanty, S. S. (2018). Inhibition of α -glucosidase, α -amylase, and aldose reductase by potato polyphenolic compounds. *Plos One*, 13(1), 0191025.
- Kwon, H. W., & Kim, D. J. (2015): Protective effects of BK-1202 on the indomethacin-induced gastric ulcer in rats. *Journal of Korean Medical Science*, 36, 42-55.
- Mensah, M. L., Komlaga, G., Forkuo, A. D., Firemping, C., Anning, A. K., & Dickson, R. A. (2019). Toxicity and safety implications of herbal medicines used in Africa. *Herbal Medicine*, 63, 1992-0849.
- Nanda, B. L. (2019): Antioxidant and proton potassium ATPase inhibitory activity in fruits. *Biological and Pharmaceutical Sciences*, 7(3), 107-115.
- Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian. Journal of Pharmaceutical Sciences*, 56, 17129.
- Oboh, G., Akinyemi, A. J., Oyeleye, I. S., & Williamsnelson, K. (2016). Protective effect of phenolic extracts from two species of miracle berry leaves (*Thaumatococcus daniellii* and *Megaphrynium macrostachyum*) on some pro-oxidant induced oxidative stress in rat pancreas in-vitro. *Journal of Applied Pharmacy Science*, 6(1), 118-124.
- Ogurtsova, K., Guariguata, L., Barengo, N. C., Ruiz, P. L. D., Sacre, J. W., Karuranga, S., & Magliano, D. J. (2022). IDF diabetes atlas: Global estimates of undiagnosed diabetes in adults for 2021. *Diabetes Research and Clinical Practice*; 18(3), 109118.
- Okafor, I. J., Nweke, E. O., & Ewa, O. (2019). Hepatotoxicity and histological evaluation of aqueous and methanolic leaf extracts of *Thaumatococcus daniellii* and *Alchornea cordifolia* in wistar rat models. *Modern Health Science*, 1(2), 42-45.
- Okunye, O. L., Idowu, P. A., & Kolade, T. T. (2020). Antibacterial activity of crude extracts of the leaves of *nauclea latifolia* smith (Rubiaceae) and some selected conventional antibiotics on clinical isolates of *Salmonella typhi*, *Annals of Health Research*, 6(3), 202-210. DOI: <https://doi.org/10.30442/ahr.0603-02-88>
- Olasupo, A. D., Aborisade, A. B., & Olagoke, O. V. (2018). Phytochemical analysis and antibacterial activities of spinach Leaf. *American Journal of Phytomedical Clinical Therapy*, 6(2), 1-4.
- Olorunnisola, O. S., Adetutu, A., Popoola, R. B., Owode, A. O., Adegbola, P., & Adesina, B. T. (2017). Nephroprotective effect of ethanolic leaf extract of *Thaumatococcus daniellii* (Benth.) in streptozotocin induced diabetic rats. *Functional Foods Health Discovery*, 7(12), 923-935.
- Ortac, D., Cemek, M., Karaca, T., Buyukokuroglu, M. E., Ozdemir, Z., & Kocaman, A. T. (2018). In-vivo anti-ulcerogenic effect of okra (*Abelmoschus esculentus*) on ethanol-induced acute gastric mucosal lesions. *Pharmacy Biology*; 56, 165-175.
- Oyeleke, G. O., Adetoro, R. O., Lawal, R. T., & Salam, M. A. (2017). Some aspects of nutrient, anti-nutrient, minerals and sugars contents of *thaumatococcus danielli* (Benn.). *Seeds Advanced Research*, 10(4), 1-7.
- Quan, N. V., Xuan, T. D., Tran, H. D., Thuy, N., Trang, L. T., Huong, C. T., Andriana, Y., & Tuyen, P. T. (2019). Antioxidant, α -amylase and α -glucosidase inhibitory activities and potential constituents of *canarium tramdenum* bark. *Molecules*, 24(3), 605-611.
- Saleem, H., Ahmad, I., Ashraf, M., Gill, M. S. A., Nadeem, M. F., & Shahid, M. N. (2016). In-vitro studies on anti-diabetic and Anti-ulcer Potentials of *Jatropha gossypifolia* (Euphorbiaceae). *Tropical Journal of Pharmacy Research*, 15, 121-125.
- Saleh, M. M., Qader, S. W., & Thaker, A. A. (2016). Gastroprotective activity of *eruca sativa* leaf extract on ethanol-induced gastric mucosal injury in *rattus norvegicus*. *Jordan Journal of Biological Science*, 9, 47-52.
- Shalom, N. C., Franklyn, N. I., Makinde, B. T., Thorpe, B. O., & Emiloju, O. C. (2018). Data on in-vivo antioxidant, hypolipidemic and hepatoprotective potential of *Thaumatococcus daniellii* (Benn.) benth leaves. *Data in Brief*; 20, 364-370.

- Sowparthani, K., & Radhika, M. (2020). Study of phytochemical analysis and antioxidant activity of *spinach oleracea* leaves. *The Pharmacy Innovation Journal*, 9(7), 01-04.
- Udochukwu, U., Omeje, F. I., Uloma, S. & Oseiwe, F. D. (2015). Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *American Journal of Research Communication*, 3(5), 20-23.
- Ukwubile, C. A., Oise, I. E., & Nyiyem, J. T. (2017). Preliminary phytochemical screening and antibacterial activity of *thaumatococcus daniellii* (Benn.) Benth. (Marantaceae) leaf extract. *Journal of Bacteriology Mycology*, 4(2), 1-5.
- Usman, K. M., Arotupin D. J., & Ekundayo, F. O. (2021). Antibiotics resistance pattern of bacteria in untreated hospital wastewaters from Offa Local Government Area, Kwara State, Nigeria. *African Journal of Microbiology Research*, 15(11), 572-582. ISSN 1996-0808. <http://doi.org/10.5897/lajmr2021.9584>
- Vergun, O., Shymanska, O., Rakhmetov, D., Grygorieva, O., Ivanišová, E., & Brindza, J. (2020). Parameters of antioxidant activity of *galega officinalis* L. and *galega orientalis* lam plant raw material. *Slovak Journal of Food Sciences*, 14, 125-134.
- Yu, M., Gouvinhas, I., Rocha, J., & Barros, A. (2021). Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Scientific Reports*, 11(1), 10041.