International Journal of Recent Innovations in Academic Research
E-ISSN: 2635-3040; P-ISSN: 2659-1561
Homepage: https://www.ijriar.com/
Volume-9, Issue-3, July-September-2025: 318-329

Research Article

In Vitro Antioxidant, Hypoglycemic Activities and Phytochemical Profile of Ethanol Leaf Extract of *Tragia benthamii* Baker (Climbing Nettle)

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Received: August 18, 2025 **Accepted:** September 07, 2025 **Published:** September 13, 2025

Abstract
This study aimed to evaluate the in vitro antioxidant, hypoglycemic, and phytochemical profile of *Tragia benthamii* leaves. Ethanol extraction was by maceration and standard analytical methods were employed in the phytochemical analysis. The antioxidant activity of the crude ethanol extract of *T. benthamii* was analyzed using the DPPH (1,1-diphenyl-2-picrylhydrazyl), superoxide anion (O^-), hydrogen peroxide (H_2O_2) radical scavenging, and ferric-reducing antioxidant power (FRAP) assays. The alpha-amylase and alpha-glucosidase inhibition assays were employed to evaluate the in vitro hypoglycemic potential of the extract. The results revealed *T. benthamii* to contain phenols, alkaloids, flavonoids, saponins, glycosides, tannins, terpenoids and sterols. The total flavonoid content of *T. benthamii* was highest in concentration than other phytochemicals identified followed by the total phenol content. A positive correlation observed between the flavonoid content and free radical (DPPH· and O·) scavenging capabilities. Also, the extract exhibited free radical scavenging activities against DPPH, hydroxyl, superoxide anion radical and hydrogen peroxide in a dose dependent manner relative to the standards, ascorbic acid (AA) and butylated hydroxytoluene (BHT). The crude ethanol extract exhibited IC_{50} values of (814.0, 1259.0, 1075.0) µg/mL using DPPH, superoxide anion and hydrogen peroxide respectively, which were significantly different from the standard ascorbic acid and BHT. The ferric reducing power capability (FRAP) also increased with an increase in concentration. The IC_{50} values obtained for the alpha-amylase and alpha-glucosidase inhibition assays were 159.8 and 11.9 µg/mL respectively for *T. benthamii* extract, while the reference drug, acarbose, exhibited IC_{50} values of 575 and 396.5 µg/mL. Conclusively, this study indicates the potential in vitro antioxidant, hypoglycemic properties of *T. benthamii* leaf extract, hence a potential source of natural antioxidant, as well as a potential antidiabetic drug which could be employed as a plant-based pharmaceutical product for several diseases caused by free radicals.
Keywords: *Tragia benthamii*, Hypoglycemic, Phytochemical, Antioxidant.

Introduction

Natural compounds of plant origin have continued to gain prominence as an essential substitute for modern synthetic drug agents. This is due to low toxicity and effective treatment or/ and management of diseases and infections by the former. Oxidative stress owing to the imbalance between reactive oxygen species (ROS) generation and innate antioxidant defense mechanism in our body, plays an imperative role in numerous diseases including cancer (Ezeunala *et al*., 2022), diabetes, cardiovascular diseases such as atherosclerosis and hypertension (Muscolo *et al*., 2024). Medicinal plants are a valuable source of a wide variety of chemical molecules having different structures and functionalities that exhibit important biological activities and are linked to a multitude of beneficial properties, such as antimicrobial, anticancer, antiviral, antioxidant, and enzyme inhibitory, anti-aging, anti-inflammatory, anti-hypertensive, neuroprotective and anti-coagulant effects (Huang *et al*., 2010; Reddy *et al*., 2017; Tafesse *et al*., 2017; Lesellier *et al.,* 2021).

Plant derived products like flavonoids, anthraquinones, carotenoids, tannins and others protect cellular damage due to their significant free radical scavenging property. Antioxidants are substances that prevent and stabilize the damage caused by free radicals by supplying electrons from antioxidants to these damage

cells. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body. Consumption of antioxidant enriched fruits and vegetables is known to lower the risk of several diseases caused by free radicals (Hamid *et al*., 2010). Such health benefits are mainly due to the presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C (Steinmetz and Potter, 1996). Diabetes mellitus (DM) is a syndrome with abnormalities in carbohydrate, lipid and protein metabolism. The central disturbance in DM is a derangement in insulin production or action or both, although other factors can be involved. Hyperglycemia is commonly the end point for all types of diabetes mellitus and is the factor that is measured to evaluate and manage the efficacy of diabetes therapy (Kumar and Clark, 2002). The management of diabetes mellitus has remained a challenge globally (King *et al*., 1998).

T. benthamii is a species of the genus *Tragia*, belonging to the Euphorbiaceae family and commonly known as a "climbing nettle" or "stinging nettle" due to its stinging hairs (Narasimham, 2021). It is an herb native to Botswana, tropical Africa, as well as a traditional medicine used across sub-Saharan Africa, for various ailments including its use as an anti-inflammatory remedy (Attah *et al.,* 2021), as an abortifacient and also the root is used traditionally to alleviate childbirth pains (Schmelzer and Gurib-Fakim*,* 2008). *T. benthamii* and *Graptophyllum pictum* (Acanthaceae) aqueous extracts has been evaluated for their estrogenic properties and their ability to alleviate some menopausal symptoms induced by ovariectomy in Wistar rats (Wanda *et al.,* 2016).

While there is limited information available on the biological activities of *T. benthamii* leaves, related species such as *T. involucrata*, *T. spathulata*, and *T. pungens* have been reported to exhibit antioxidant, anti-urolithiatic, antimalarial, cytotoxic, antidiabetic, and antibacterial properties (Dhara, 2002; Muthuraman *et al.,* 2008; Palani, 2009; Oladosu *et al*., 2013). Also with the increasing cost of orthodox medicine, there may be increased utilization of alternative medicines including herbs in the management of the disease, DM. In the management of diabetes mellitus, a number of natural products including medicinal plants with antihyperglycemic effect have been recognized and utilized (Balogun *et al.,* 2016). The need for novel safer and more effective natural drugs and bridging the knowledge gap regarding the biological activities of *T. benthamii*, forms the basis of this research; thus, the study aims at evaluating the in vitro hypoglycemic, and antioxidant potential of *Tragia benthamii* leaves.

Methodology

Plant Collection

Fresh leaves of *Tragia benthamii* were purchased from the herbal market at Ibadan, Oyo State, Western Nigeria. The plant was identified by a botanist in the Biology Department, Herbarium and Ethnobotany Unit, University of Abuja. The specimen voucher number is HB109853.

Extraction

The leaves were allowed to dry at the temperature of 25°C in a shade. The dried leaves were grounded into fine powder using a blender and 80g of the pulverized sample was then poured into an airtight container. It was macerated using 800ml absolute ethanol for 72 hours at room temperature, with intermittent rigorous shaking. Cold extraction was carried out following the method as adopted by Enefe *et al*., (2024). After 72 hours, it was filtered using muslin cloth and Whatman filter paper number 1; the residue from the filtration was discarded and the filtrate was concentrated using a water bath at 40^{o}C. The concentrated filtrate (the extract) was kept in a fume cupboard to allow the residual ethanol in the extract to evaporate and then air dried to give the crude extract. The extract was kept in a sterile container under refrigerated condition until used. The dried weight of the plant extract was obtained by the solvent evaporation and used to determine concentration in mg/ml. Extraction yield was calculated with the formula:

% Percentage yield = Weight of extract/Weight of pulverized sample x 100……..1

Determination of Qualitative Phytochemical Analysis

This qualitative phytochemical screening was carried out using standard protocols as described by (Trease and Evans, 2002; Sofowora, 2008).

Determination of Quantitative Phytochemical Analysis

Assessment of Total Flavonoids

The total flavonoids of the extract and standards were determined using the aluminium chloride method as adopted by Samantha *et al*., (2012). The concentrations were determined as µg of gallic acid equivalent using an equation from the standard gallic acid graph.

Assessment of Total Phenols
The phenolic content was carried out using the Folin-Ciocalteu method as adopted by Adebiyi *et al.,* (2017). Quercetin was used as the standard for the calibration curve, and the total phenol was calculated using the linear equation from the calibration curve. Assessment of glycoside (Keller-Killiani test), tannin, steroids, and alkaloids were determined by methods as adopted by of Ayoola *et al.,* (2008) and Medini *et al.,* (2014).

Determination of In Vitro Antioxidant Activity of *Tragia benthamii*
The in vitro antioxidant potential of *Tragia benthamii* extract was tested against 1, 1-diphenyl-2-picryl-hydrazyl (DPPH Sigma-Aldrich) radical, by the DPPH spectrophotometric assay as adopted by (Ayoola *et al*., 2017; Jayameena *et al*., 2018). Various concentrations of the extract (62.50, 125.00, 250.00, 500.00, 1000.00 µg/ml) were prepared as well as the standards ascorbic acid (vitamin C) and butylated hydoxytoluene (BHT). All solutions were prepared using ethanol. Two milliliters of each prepared concentration were mixed with 0.5 mL of 1 mM DPPH solution in ethanol test tubes.

The experiments were conducted in triplicates. After 15 minutes of incubation at room temperature, the absorbance at 517 nm was measured with UV spectrophotometer (JP Selecta, SS UV-1100D, Spain). A blank solution containing the same amount of ethanol and DPPH was also measured for absorbance. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The radical scavenging activity was calculated using the formula below.

DPPH scavenging effect (% inhibition) = $(AB_{control} - AB_{sample}) / AB_{control} \times 100$……2

Where ABcontrol is the absorbance of the blank sample and ABsample represents the absorbance of the plant samples and standards respectively.

When a solution of DPPH is mixed with an antioxidant the antioxidant donates a hydrogen atom to the DPPH radical. This process neutralizes the radical and converts it into its reduced, non-radical form, 1,1-diphenyl-2-picryl-hydrazine (DPPH−H). The color change from deep violet to pale yellow is measured using a spectrophotometer. The decrease in absorbance at 517 nm is directly proportional to the concentration of free radicals scavenged by the antioxidant. The higher the scavenging activity of the sample, the greater the decolorization of the DPPH solution and the lower the final absorbance reading.

Quantification
The antioxidant activity is often expressed as the scavenging percentage or as the IC_{50} value (half-maximal inhibitory concentration). It is the concentration of the sample required to scavenge 50% of the DPPH radicals in the solution (calculated from the linear regression equation $y = mx + c$, here, m is the slope, c is the intercept, y is equal to 50 and x is the IC_{50} concentration value. A lower IC_{50} value indicates higher antioxidant potency.

Hydrogen Peroxide Radical Scavenging Ability
The ability of the plant extract to prevent Fe^{2+}:H_2O_2 induced decomposition of deoxyribose was carried out using the method of Mathew and Abraham (2006).

Superoxide Anion Scavenging Assay
The superoxide anion scavenging potential was determined according to the method of Liu *et al*., (1997).

Ferric Reducing Antioxidant Power (FRAP) Assay
This was determined by the method as adopted by Muller *et al*., (2011).

A 1.5 mL of freshly prepared FRAP solution containing 25 mL of 300 mM acetate buffer, 2.5 mL of 10 mM 2,4,6 tripyridyl -5- triazine (TPTZ) in 40 mM HCL and 2.5 ml of 20 mM $Fecl_3.6H_2O$ was mixed with 1mL of extracts and the absorbance read at 593 nm. Calibration was prepared with $FeSO_4.7H_2O$. The results obtained are expressed in µM Fe^{2+}/g of dry plant material and compound with that of a standard drug, ascorbic acid.

When a sample containing an antioxidant is added to the FRAP reagent, the antioxidant donates an electron to the Fe^{3+} in the complex. This reduction reaction converts the yellow Fe^{3+}−TPTZ complex into a vibrant blue-colored ferrous form, Fe^{2+}− TPTZ. The increase in blue color is measured spectrophotometrically at a wavelength of 593 nm. The change in absorbance is directly proportional to the antioxidant capacity of the

sample. A higher absorbance reading indicates a greater ability to reduce ferric ions, and therefore, higher antioxidant power. The results were expressed as the equivalent concentration of a standard antioxidant, such as ferrous sulfate ($FeSO_4$). This allows for the results to be quantified in terms of a known reducing agent (Ayoola *et al*., 2017).

Determination of In Vitro Hypoglycemic Activity of *Tragia benthamii*

Alpha Amylase Inhibition Assay

The α-amylase inhibition assay was conducted following the method as reported by Hedairi *et al*., (2005), with some modifications. 500 µL of the prepared concentrations (62.50, 125.00, 250.00, 500.00, 1000.00 µg/ml) of the samples (ethanol extract, and the standard drug acarbose) were added to 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing porcine pancreatic α-amylase (0.5 mg/mL (Sigma chemical company,) St. Louis, MO, USA). The mixture was then stirred at 32°C for 10 minutes. Subsequently, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each prepared sample concentration.

The reaction mixtures were incubated at 32°C for 10 minutes. Then, 1.0 mL of dinitrosalicylic acid (DNS) color reagent was added, with incubation of the reaction mixtures in test tubes in a boiling water bath for 5 minutes and then cooled to room temperature. The reaction mixture was diluted with 10 mL of distilled water, and the absorbance was measured with UV-(JP Selecta, SS 1100D, Spain) at 540 nm. The experiment was conducted in triplicate, with acarbose serving as the positive control. Acarbose is known to inhibit the enzyme α-amylase, thereby impeding the release of glucose from starch and preventing a rise in glycemic levels.

The inhibition of α-amylase was calculated using equation 3.

Percentage inhibition % = (ABcontrol - ABsample) / ABcontrol × 100……3

Where ABcontrol is the absorbance of blank sample and ABsample represents the absorbance of extract and standard respectively.

Alpha Glucosidase Inhibition Assay

The effect of the extracts on α-glucosidase activity was determined according to the method described by Apostolidis *et al*., (2007) with slight modification. In brief, different concentrations (0.125–1.0 mg/ml) of the extract/ standard were prepared in distilled water. Then, 50 ml from the stock solution was mixed with 100 ml of 0.1 M phosphate buffer (pH 6.9) containing 1.0 M of α-glucosidase solution. The mixtures were then incubated in 96-well plates at 25°C for 10 min. Following this, 50 ml of 5 mM p-Nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals.

The reaction mixtures were incubated at 25 °C for 5 min. The inhibitory effect of the extract/standard on the enzyme activities were determined by measuring the absorbance of the mixtures at 405 nm using a microplate reader (BIO-RAD, model 680, Japan). The control was prepared using the same procedure replacing the extract with distilled water. The experiments were conducted in triplicate and the α-glucosidase inhibitory activity was expressed as % inhibition using the equation 4.

% inhibition = (ABcontrol - ABsample) / ABcontrol × 100……..4

Where ABcontrol is the absorbance of blank sample and ABsample represents the absorbance of extract and standard respectively.

Statistical Analysis

Statistical analysis was performed using a Graph Pad Prism 9.0 statistical package (Graph Pad Software, San Diego, MA, USA). Data were expressed as means of replicate determinations ± SEM (standard error of mean), data was subjected to one-way analysis of variance. Statistical significance was considered at $p< 0.05$.

Results

Extraction Percentage Yield

The percentage yield of the extract was 6.25% w/w. The qualitative and quantitative phytochemical analysis of *T. benthamii* are presented in Table 1 and 2 respectively.

Table 1. Qualitative phytochemical constituents of *T. benthamii*.

S/N	Phytochemicals	*T. benthamii*
1	Alkaloids	+
2	Tannins	+
3	Phenols	+
4	Glycosides	+
5	Saponin	+
6	Flavonoids	+
7	Steroids	-
8	Phlobatannins	-
9	Triterpenes	+
10	Phytosterols	+
11	Fixed oils	-
12	Terpenoids	+
13	Amino acids	-
Note: +: presence; -: absent.		

Table 2. Quantitative phytochemical constituents of *T. benthamii.*

Phytochemicals	Mean ± SEM
Phenol (µg/mL/GAE)	127.09 ± 2.34
Flavonoid (µg/mL/QE)	396.33[a] ± 22.29
Alkaloid (mg/g)	144.26 ± 2.66
Steroid (mg/g)	91.65 ± 0.10
Glycoside (mg/g)	81.73 ± 0.86
Tannin (mg/g)	113.11 ± 0.60
Note: GAE= Gallic acid equivalent; QE= Quercetin equivalent	
The values are expressed as (mean ± SEM) of triplicate determinations [N = 3; p<0.05].	

Table 3. Concentration-dependent antioxidant activity of the *T. benthamii* extract as determined by DPPH, O^-, hydrogen peroxide and FRAP assays.

Concentration (ug/mL)	DPPH (% inhibition)	O^- (% inhibition)	H_2O_2 (% inhibition)	FRAP (µmM Fe) absorbances
62.50	6.65±4.66	4.30±3.37	17.06±2.74	0.07±0.01
125.00	8.88±5.11	9.47±3.61	17.45±2.79	0.07±0.02
250.00	15.20±4.62	14.67±4.18	20.65±3.52	0.02±0.02
500.00	43.35±5.06	20.96±3.92	30.16±3.48	0.04±0.02
1000.00	56.03±5.08	40.58±4.18	48.00±3.52	0.16±0.03
The values are expressed as (mean ± SEM) of triplicate determinations [N = 3; p<0.05].				

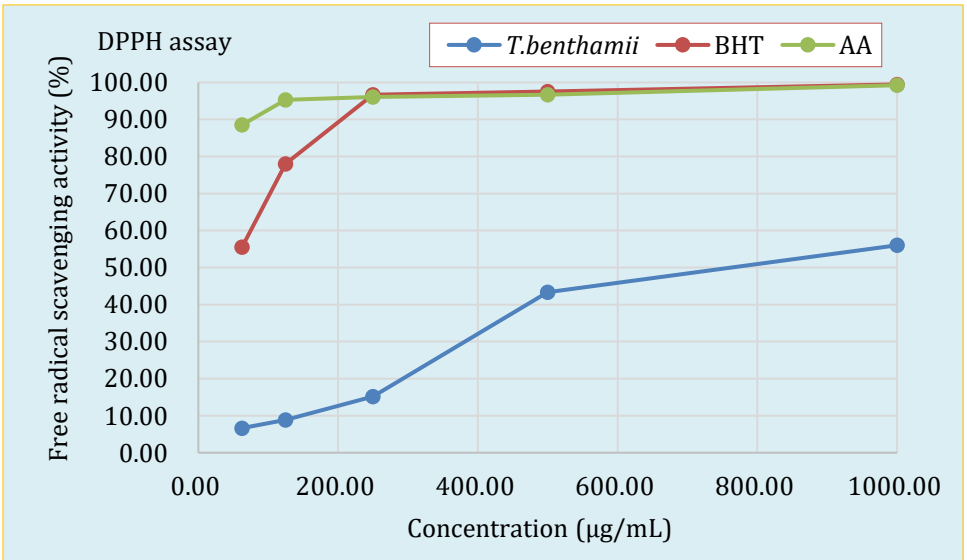

Figure 1. Free radical scavenging activities of *T. benthamii*, BHT and AA by DPPH assay (AA: ascorbic acid, BHT: butylated hydoxytoluene).

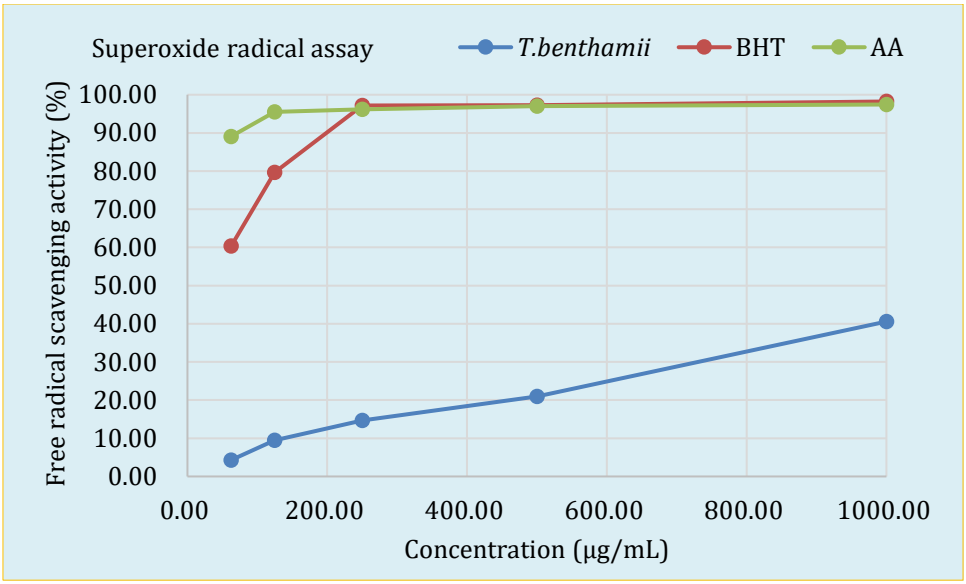

Figure 2. Free radical scavenging activities of *T. benthamii*, BHT and AA by superoxide (O^{-} anion) assay.

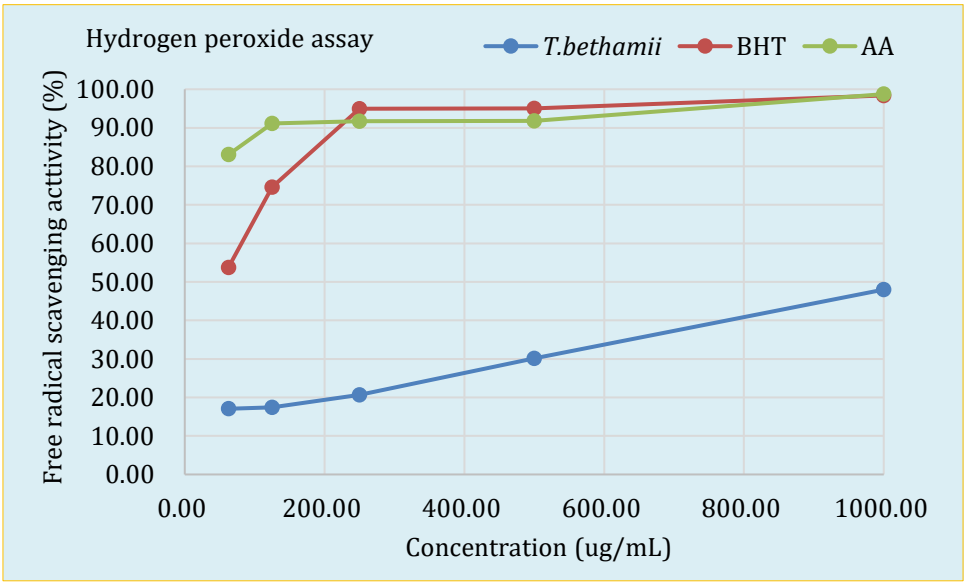

Figure 3. Free radical scavenging activities of *T. benthamii*, BHT and AA by hydrogen peroxide assay.

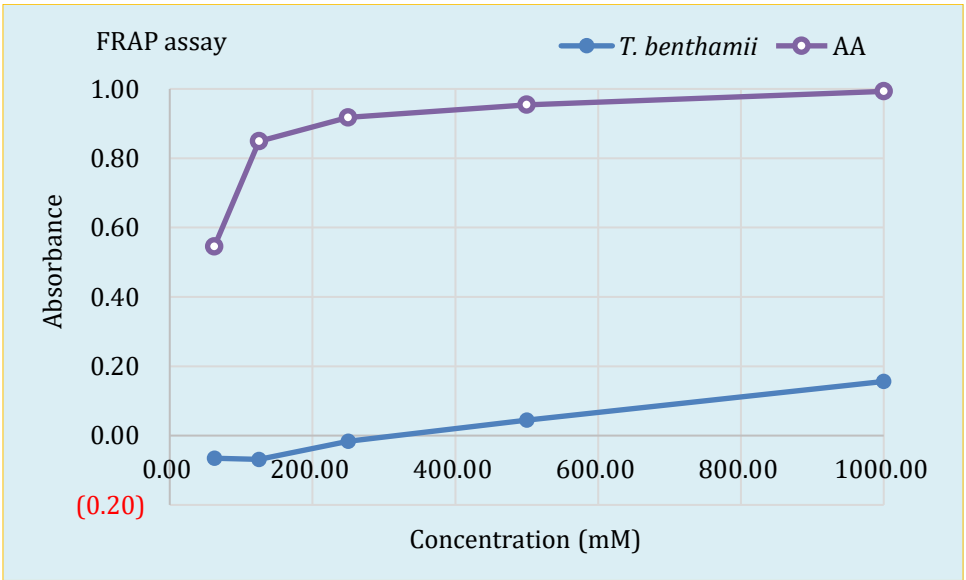

Figure 4. Ferric reducing capabilities of *T. benthamii* and AA.

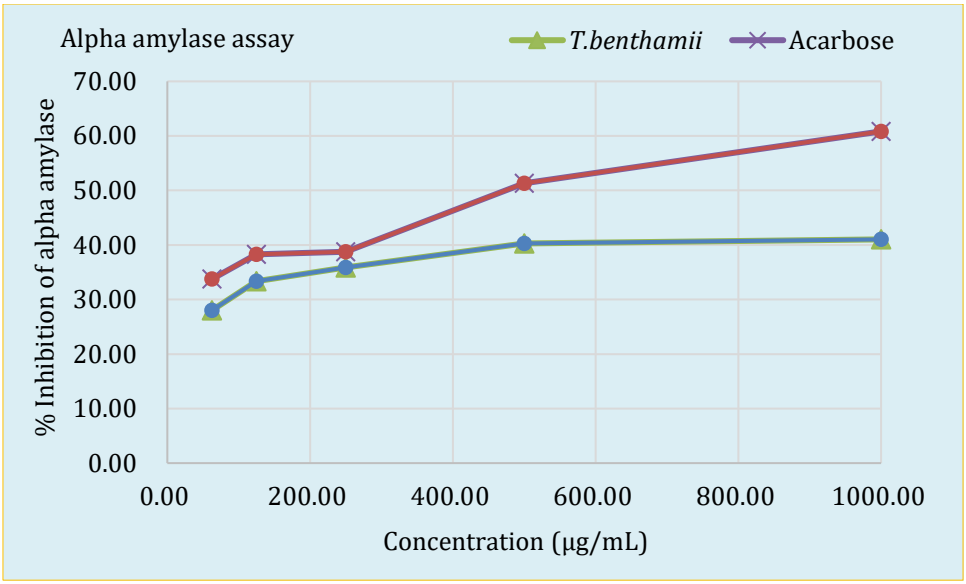

Figure 5. Inhibitory potentials of *T. benthamii* and acarbose on alpha-amylase activity.

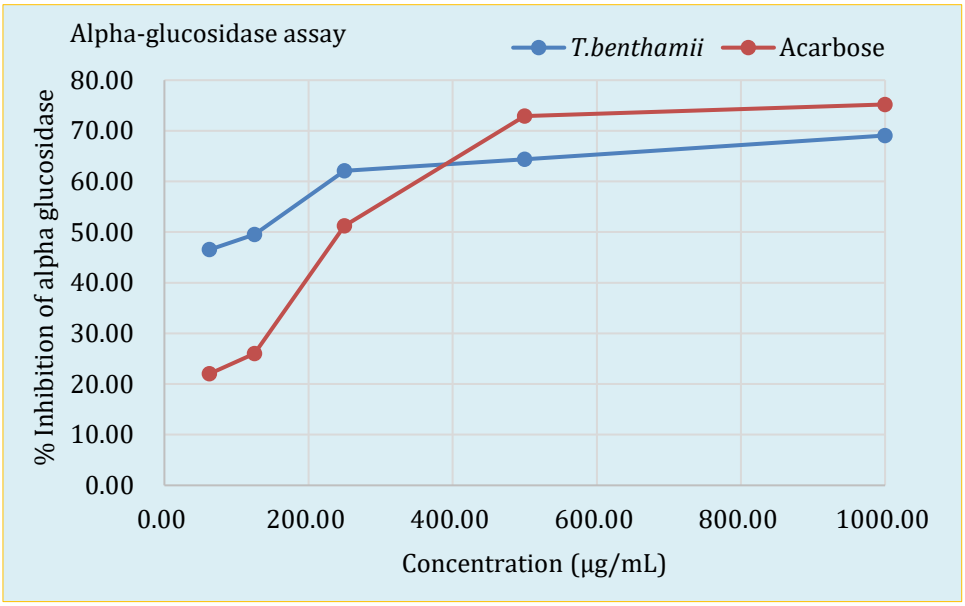

Figure 6. Inhibitory potentials of *T. benthamii* and acarbose on alpha-glucosidase activity.

Table 4. The IC_{50} values of the free radical scavenging/reducing capabilities of *T. benthamii.*

Samples	**IC_{50} µg/mL**			
	DPPH	**O^-**	**H_2O_2**	**FRAP**
T. benthamii	814.0	1259.0	1075.0	2504.23
DPPH-; O^-superoxide anion; H_2O_2-hydrogen peroxide; FRAP-ferric reducing antioxidant power				

Table 5. The IC_{50} values for *T. benthamii* extract and standard acarbose on activities of α-amylase and α-glucosidase enzymes.

Samples	**IC_{50} µg/mL**	
	Alpha-amylase	**Alpha-glucosidase**
T. benthamii	159.8	11.9
Acarbose	575.2	396.5

Discussion

Extraction Yield

The yield of the ethanol extract of *T. benthamii* was found to be 6.25% w/w, this seen to vary in different studies conducted of *Tragia* species, possibly due to the extraction methods, the constituents, the species and geographical location (Bonam *et al.,* 2019; Ayoola *et al.,* 2024).

Phytochemical Constituents

Phytochemicals are natural chemical constituents that can be found in plants and have the ability to positively or negatively impact health. Crude extracts from plants are typically are a mixture of both active and nonactive compounds which exhibit one or more biological activity (Duarte-Casar *et al.,* 2017). This study assessed the phytochemical constituents, in vitro antioxidant and hypoglycemic activities of *T. benthamii.* The phytochemical analysis of the extract revealed the presence of several bioactive compounds (flavonoids, phenols, alkaloids, glycosides, tannins, saponins and sterols) in varying concentrations. Among the constituents identified were total flavonoids which was present in the highest amount with a mean concentration of 396.33±22.29 µg/mL/QE; and this, suggesting a potential for antioxidant and anti-inflammatory activities since flavonoids are well-documented for their protective roles against oxidative stress (Egea *et al.,* 2017).

The result from this study is in agreement with some other studies conducted on *T. benthamii* and other *Tragia* species (Reddy *et al*., 2017; Ayoola *et al.,* 2024). Alkaloids were also identified and quantified with a concentration of 144.26±2.66, mg/g indicating possible pharmacological significance such as analgesic, antimicrobial, or antimalarial effects (Ayoola *et al.,* 2017). Phenols were detected at 127.09±2.34 µg/mL/GAE, further reinforcing the antioxidant potential of the extract, as phenolic compounds are known to neutralize free radicals (Kumar and Goel, 2019). Tannins were moderately present at 113.11±0.60 mg/g, which may contribute to astringent, antimicrobial, and anti-parasitic activities (Tong *et al*., 2022). Steroids were quantified at 91.65±0.10 mg/g, suggesting potential roles in modulating physiological processes while glycosides were the least concentrated at 81.73±0.86 mg/g, though still important for their potential therapeutic and metabolic benefits. Overall, the high concentrations of flavonoids, alkaloids, and phenols from the present study, highlights the extract as a rich source of phytochemicals with promising medicinal and pharmacological relevance.

In Vitro Antioxidant Activity

Concentration-Dependent Antioxidant Activity of the Extract as Determined by DPPH, O^-, Hydrogen Peroxide, Reducing Capacity Via FRAP Assays

Phytochemicals are known to possess varying antioxidant activities (Liu *et al.,* 2015; Egea *et al.,* 2017). Antioxidant activity of a medicinal plant cannot be concluded based on a single antioxidant test model (Egea *et al*., 2017) as such, several in vitro antioxidant tests were conducted on the extracts using ascorbic acid and BHT as standard for all the assays. The antioxidant activities of the extract were evaluated using the various assays, and the results presented in Table 3 and Figure 1-4. The DPPH assay showed a concentration-dependent increase in radical scavenging activity, starting from 6.65±4.66% inhibition at 65.2µg/mL and progressively rising to a maximum of 56.03±5.08% inhibition at 1000 µg/mL. This trend indicates that the extract possesses free radical scavenging potential, although the activity remained relatively low at lower concentrations and became more pronounced only at higher doses.

Phytochemical like polyphenols has the capability to scavenge superoxide and other ROS like hydroxyl and peroxyl radicals (Medini *et al*, 2014). Saponins, triterpenes and phytosterols have also been demonstrated to scavenge superoxide anion (Zhao *et al*., 2013). Flavonoid are currently receiving attention as a potential protector against variety of human disease, major flavonoid has been shown to have neutralizing effect on free radical and ROS like hydroxyl radical, superoxide radical, hydrogen peroxides (Egea *et al*., 2017). Similarly, the FRAP assay demonstrated a gradual increase in ferric reducing capacity with increasing concentrations of the extract, ranging from 0.07±0.01 mM/Fe+ at 65.2 µg/mL to 0.16±0.03 mM/Fe+ at 1000 µg/mL. While the FRAP values did not show a strictly linear progression across all concentrations, the highest reducing ability was also recorded at 1000 µg/mL, suggesting that the antioxidant potential of the extract strengthens with higher concentrations.

The result of the assay showed that ethanol extract of *T. benthamii* exhibited free radical scavenging activity, though much lower when compared to the standards ascorbic acid BHT in the DPPH, O^-, H_2O_2 assays. The highest percentage inhibition was revealed using the DPPH method (Figure 1-4). This generally low percentage inhibition of *T. benthamii*, is contrary to the findings of Bonam *et al*., (2019) in which another species (*T. plukenetii*) of the plant exhibited a strong antioxidant activity. This difference could be attributed to the difference in species, geographical location, genetics and the varying phytochemical constituents.

In Vitro Hypoglycemic Activity of *T. benthamii*

Alpha (α) amylase, a key starch degrading enzyme can rapidly degrade starch into glucose, leading to a sharp increase in blood sugar levels. Phytochemicals in plants can effectively slow down the rate of glucose

production, reduce and delay the absorption of glucose by the intestine, thereby effectively reducing blood sugar levels (Sayem *et al*., 2025). The unregulated hydrolysis of starch by α-amylase and α-glucosidase which catalyze the rate limiting step in the conversion of oligosaccharides and disaccharides into monosaccharide's is responsible for the elevated blood glucose seen in type 2 diabetes mellitus. Therefore, controlling hyperglycemia via inhibition of carbohydrate hydrolysing enzymes is an important strategy in the management of type 2 diabetes mellitus (Mohammed *et al*., 2012). In vitro evaluation of the inhibitory effect of the extracts on α-glucosidase and pancreatic α-amylase enzymes was carried out using acarbose as the standard to determine its percentage inhibition and the IC_{50} value. Mild inhibition of α-amylase and strong inhibition of α-glucosidase enzymes is targeted as a way of reducing post prandial hyperglycemia, and elimination of the unwanted effect like gastrointestinal discomfort flatulence, diarrhoea associated with the use of acarbose (Kazeem *et al*., 2013).

In the present study, ethanol extract of *T. benthamii* strongly inhibited α-amylase and α-glucosidase with their respective IC_{50} values of (159.8 and 11.9) µg/mL which is about 3 times and 33 times much lower and significantly different from those of the standard acarbose (575.2 and 396.5) µg/mL. As shown in Figure 5 and 6, within the concentration range the inhibitory ability of the extract as well as acarbose on alpha amylase increased in a concentration dependent manner ($p<0.05$), thus the result showed that *T. benthamii* has a good inhibition effect on α-amylase and α-glucosidase when compared to the standard acarbose. This is in agreement with another study on a methanol extract of another *Tragia* species (Reddy *et al*., 2017; Ayoola *et al*., 2024).

α-glucosidase is widely used in vitro hypoglycemic experiments. As an important enzyme in the glucose metabolism pathway, it is mainly distributed on the brush edge of the small intestinal epithelial villi and can act on α-glucosidic bonds, hydrolyzing carbohydrate substances into glucose as the final product (Ren *et al*., 2017). As shown in Figure 6, the inhibitory ability of *T. benthamii* on α-glucosidase was much lower than that of acarbose, however this increased significantly in a dose-dependent manner, with the strongest upward trend in the concentration range of 62.5–1000 µg/mL ($p < 0.05$). The IC_{50} of *T. benthamii* on α-glucosidase was 11.9 µg/mL, which was lower than that of standard acarbose, indicating a stronger inhibition ability to inhibit α-glucosidase. Many other *Tragia* species have been shown to have good inhibition effects on α-glucosidase activity, although the inhibition ability was lower than that of acarbose. The results of both α-amylase and α-glucosidase showed that *T. benthamii* have the potential to control blood sugar levels as functional food.

Conclusion

The findings from this research revealed that *Tragia benthamii* leaves exhibited in vitro, antioxidant properties although lower when compared to the standard, ascorbic acid and BHT. The extract exhibited a strong in vitro hypoglycemic activity when compared to the standard drug acarbose; the inhibition was stronger on α-glucosidase than α-amylase. This may be due to the presence of secondary metabolites, especially the high concentration of flavonoids, glycosides and phenols identified in the plant extract. The inhibitory concentrations (IC_{50}) of the extract can provide valuable guidance for traditional medicine practitioners; it may also serve as a potential source of hypoglycemic and antioxidant agents for the prevention and management of free radical induced metabolic diseases.

Recommendation

Further research on isolating bioactive constituents from various parts of the plant as well as in vivo studies to explore the pharmacological potentials.

Declarations

Acknowledgments: We gratefully acknowledge Mr. Najeeb Aliyu of the Department of Biochemistry, Nile University Abuja, and Akinyemi Akinyele Boluwatife of the Faculty of Veterinary Medicine, for their contributions to this paper.

Author Contributions: ENG: Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, manuscript preparation, and manuscript revision. YI: Data collection, data analysis, manuscript preparation; ENG: Review manuscript, submission of article.

Conflict of Interest: The authors declare no conflict of interest.

Consent to Publish: The authors agree to publish the paper in International Journal of Recent Innovations in Academic Research.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Funding: This research received no external funding
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Research Content: The research content of manuscript is original and has not been published elsewhere.

References

1. Adebiyi, O.E., Olayemi, F.O., Ning-Hua, T. and Guang-Zhi, Z. 2017. In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. Beni-Suef University Journal of Basic and Applied Sciences, 6(1): 10-14.
2. Apostolidis, E., Kwon, Y.I. and Shetty, K. 2007. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science and Emerging Technologies, 8(1): 46-54.
3. Attah, A.F., Omobola, A.I., Moody, J.O., Sonibare, M.A., Adebukola, O.M. and Onasanwo, S.A. 2021. Detection of cysteine-rich peptides in *Tragia benthamii* Baker (Euphorbiaceae) and in vivo antiinflammatory effect in a chick model. Physical Sciences Reviews, 8(6): 775-791.
4. Ayoola, G.A., Adeyemi, D.K., Johnson, O.O. and Lawal, L.O. 2017. Evaluation of the phytochemical content, antioxidant and antimicrobial activity of *Citrullus lanatus* (THUNB) seed method extract. Nigerian Journal of Scientific Research, 16(2): 211-216.
5. Ayoola, G.A., Eze, S.O., Johnson, O.O. and Adeyemi, D.K. 2018. Phytochemical screening, antioxidant, antiulcer and toxicity studies on *Desmodium adscendens* (Sw) DC Fabaceae leaf and stem. Tropical Journal of Pharmaceutical Research, 17(7): 1301-1307.
6. Ayoola, G.A., Johnson, O.O., Aderounmu, J., Raji, L., Aremu, R.B. and Bankole, S. 2024. Invitro evaluation of the hypoglycaemic, anti-inflammatory, and antioxidant activities of *Tragia benthamii* Baker (Euphorbiaceae). Tropical Journal of Phytochemistry and Pharmaceutical Sciences, 3(9): 448-452.
7. Balogun, F.O., Tshabalala, N.T. and Ashafa, A.O.T. 2016. Antidiabetic medicinal plants used by the Basotho tribe of Eastern Free State: A review. Journal of Diabetes Research, 2016(1): 4602820.
8. Bonam, S.R., Manoharan, S.K., Pandy, V., Raya, A.R., Nadendla, R.R., Jagadeesan, M. and Babu, A.N. 2019. Phytochemical, in vitro antioxidant and in vivo safety evaluation of leaf extracts of *Tragia plukenetii.* Pharmacognosy Journal, 11(2): 338-345.
9. Dhara, A.K., Pal, S. and Nag Chaudhuri, A.K. 2002. Psychopharmacological studies on *Tragia involucrata* root extract. Phytotherapy Research, 16(4): 326-330.
10. Duarte-Casar, R. and Romero-Benavides, J.C. 2021. *Tragia* L. genus: Ethnopharmacological use, phytochemical composition and biological activity. Plants, 10(12): 2717.
11. Egea, J., Fabregat, I., Frapart, Y.M., Ghezzi, P., Görlach, A., et al. 2017. European contribution to the study of ROS: A summary of the findings and prospects for the future from the COST action BM1203 (EU-ROS). Redox Biology, 13: 94-162.
12. Enefe, N.G., Adetunji, S.A., Ejeh, S.A. and Abalaka, S.E. 2024. Ethanolic leaf extract of parsley (*Petroselinum crispum* (Mill.) Nym. ex A.W. Hill, Apiaceae) effects of oral administration on haematology, hepatic and renal function biomarkers in Wistar albino rats. International Journal of Recent Innovations in Academic Research, 8(5): 44-52.
13. Ezeunala, M.N., Izebe, K., Aboh, M., Ijele, I., Ibekwe, N., et al. 2022. The use of common and exotic teas in managing covid-19 related symptoms. Journal of Phytomedicine and Therapeutics, 21(2): 875-882.
14. Hamid, K., Saha, M.R., Urmi, K.F., Habib, M.R. and Rahman, M.M. 2010. Screening of different parts of the plant *Pandanus odorus* for its antioxidant activity. International Journal of Applied Biology and Pharmaceutical Technology, 1(3): 1364-1368.
15. Heidari, R., Zareae, S. and Heidarizadeh, M. 2005. Extraction, purification, and inhibitory effect of alpha-amylase inhibitor from wheat (*Triticum aestivum* Var. Zarrin). Pakistan Journal of Nutrition, 4(2): 101-105.
16. Huang, W.Y., Cai, Y.Z. and Zhang, Y. 2010. Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. Nutrition and Cancer, 62(1): 1-20.

17. Jayameena, P., Sivakumari, K., Ashok, K. and Rajesh, S. 2018. In vitro antiinflammatory (membrane stabilization) and antioxidant potential of rutin. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences, 4: 265-274.

18. Kazeem, M.I., Adamson, J.O. and Ogunwande, I.A. 2013. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. BioMed Research International, 2013(1): 527570.

19. King, H., Aubert, R.E. and Herman, W.H. 1998. Global burden of diabetes, 1995–2025: Prevalence, numerical estimates, and projections. Diabetes Care, 21(9): 1414-1431.

20. Kumar, N. and Goel, N. 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports, 24: e00370.

21. Kumar, P. and Clark, M. 2002. Diabetes mellitus and other disorders of metabolism. Clinical Medicine, 2: 1069-1071.

22. Lesellier, E., Lefebvre, T. and Destandau, E. 2021. Recent developments for the analysis and the extraction of bioactive compounds from *Rosmarinus officinalis* and medicinal plants of the Lamiaceae family. TrAC Trends in Analytical Chemistry, 135: 116158.

23. Liu, F., Ooi, V.E.C. and Chang, S.T. 1997. Free radical scavenging activities of mushroom polysaccharide extracts. Life Sciences, 60(10): 763-771.

24. Liu, Y., Wang, H. and Cai, X. 2015. Optimization of the extraction of total flavonoids from *Scutellaria baicalensis* Georgi using the response surface methodology. Journal of Food Science and Technology, 52(4): 2336-2343.

25. Mathew, S. and Abraham, T.E. 2006. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food and Chemical Toxicology, 44(2): 198-206.

26. Medini, F., Fellah, H., Ksouri, R. and Abdelly, C. 2014. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. Journal of Taibah University for Science, 8(3): 216-224.

27. Mohamed, E.A.H., Siddiqui, M.J.A., Ang, L.F., Sadikun, A., Chan, S.H., et al. 2012. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. BMC Complementary and Alternative Medicine, 12(1): 176.

28. Müller, L., Fröhlich, K. and Böhm, V. 2011. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chemistry, 129(1): 139-148.

29. Muscolo, A., Mariateresa, O., Giulio, T. and Mariateresa, R. 2024. Oxidative stress: The role of antioxidant phytochemicals in the prevention and treatment of diseases. International Journal of Molecular Sciences, 25(6): 3264.

30. Muthuraman, M.S., Dorairaj, S., Rangarajan, P. and Pemaiah, B. 2008. Antitumor and antioxidant potential of *Tragia plukenetii* R. Smith on Ehrlich ascites carcinoma in mice. African Journal of Biotechnology, 7(20): 3527-30.

31. Narasimhan, S. 2021. Pharmacological potential of the stinging plant *Tragia* species: A review. Pharmacognosy Journal, 13(1): 278-284.

32. Oladosu, I.A., Balogun, S.O. and Ademowo, G.O. 2013. Phytochemical screening, antimalarial and histopathological studies of *Allophylus africanus* and *Tragia benthamii*. Chinese Journal of Natural Medicines, 11(4): 371-376.

33. Palani, S., Kumar, S.N., Gokulan, R., Rajalingam, D. and Kumar, B.S. 2009. Evaluation of nephroprotective and antioxidant potential of *Tragia involucrata*. Drug Invention Today, 1(1): 55-60.

34. Reddy, B.S., Rao, N.R., Vijeepallam, K. and Pandy, V. 2017. Phytochemical, pharmacological and biological profiles of *Tragia* species (family: Euphorbiaceae). African Journal of Traditional, Complementary and Alternative Medicines, 14(3): 105-112.

35. Ren, Y.Y., Zhu, Z.Y., Sun, H.Q. and Chen, L.J. 2017. Structural characterization and inhibition on α-glucosidase activity of acidic polysaccharide from *Annona squamosa*. Carbohydrate Polymer, 174: 1–12.

36. Samatha, T., Shyamsundarachary, R., Srinivas, P. and Swamy, N.R. 2012. Quantification of total phenolic and total flavonoid contents in extracts of *Oroxylum indicum* L. Kurz. Asian Journal of Pharmaceutical and Clinical Research, 5(4): 177-179.

37. Sayem, M.A., Islam, H., Shil, R.C., et al. 2025. Phytochemicals from antidiabetic medicinal plants: A comprehensive review of glycemic control mechanism. World Journal of Advanced Research and Reviews, 26(01): 3576-3590.

38. Schmelzer, G.H. and Gurib-Fakim, A., (Eds.). 2008. Plant resource of tropical Africa 11(1) medicinal plants 1. PROTA Foundation, Wageningen, Netherlands/ Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands, 791.

39. Sofowora, A. 2008. Medicinal plants and traditional medicine in Africa. 3rd Edition. Spectrum Books Ltd Ibadan.

40. Steinmetz, K.A. and Potter, J.D. 1996. Vegetables, fruit, and cancer prevention: A review. Journal of the American Dietetic Association, 96(10): 1027-1039.

41. Tafesse, T.B., Hymete, A., Mekonnen, Y. and Tadesse, M. 2017. Antidiabetic activity and phytochemical screening of extracts of the leaves of *Ajuga remota* Benth on alloxan-induced diabetic mice. BMC Complementary and Alternative Medicine, 17(1): 243.

42. Tong, Z., He, W., Fan, X. and Guo, A. 2022. Biological function of plant tannin and its application in animal health. Frontiers in Veterinary Science, 8: 803657.

43. Trease, G. and Evans, W. 2002. Phytochemicals. In: Pharmacognosy, 15th Edition. Saunders Publishers, London, 42-393p.

44. Wanda, G.J.M.K., Djiogue, S., Njimfo, S.O.D., Awounfack, C.F. and Njamen, D. 2016. Evaluation of the estrogenic properties of aqueous extracts of *Tragia benthamii* Baker (Euphorbiaceae) and *Graptophyllum pictum* (Acanthaceae) and their ability to alleviate some menopausal symptoms induced by ovariectomy in Wistar rats. International Journal of Phytomedicine, 8(3): 366-78.

45. Zhao, J., Xu, F., Huang, H., Gu, Z., Wang, L., et al. 2013. Evaluation on anti-inflammatory, analgesic, antitumor, and antioxidant potential of total saponins from *Nigella glandulifera* seeds. Evidence-Based Complementary and Alternative Medicine, 2013(1): 827230.

Citation: Enefe, N.G. and Yusuf, I. 2025. In Vitro Antioxidant, Hypoglycemic Activities and Phytochemical Profile of Ethanol Leaf Extract of *Tragia benthamii* Baker (Climbing Nettle). International Journal of Recent Innovations in Academic Research, 9(3): 318-329.