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Research Article
Antidiabetic Activity, Phytochemical and Proximate Compositions of Different Extracts of *Tephrosia bracteolata* Leaves

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Abstract

Background and Objective: *Tephrosia bracteolata* is a medicinal plant used traditionally for the treatment of diabetes mellitus. In this study, the anti-diabetic potentials, phytochemical and proximate composition of various extracts of *Tephrosia bracteolata* leaves were investigated. **Materials and Methods:** Extraction of pulverized leaves of *Tephrosia bracteolata* was done using water, ethanol, chloroform/methanol (2:1). The extracts were subjected to phytochemical and proximate analyses and acute toxicity studies using standard methods. The extracts were also studied for anti-diabetic activity using alloxan (150 mg kg^{-1} i.p) model of diabetes. The extracts were administered to diabetic rats at dose levels of 250 and 500 mg kg^{-1} p.o for 28 days during which Fasting Blood Sugar (FBS), food/water intake and bodyweight were monitored as indices of anti-diabetic activity. **Results:** Phenols, alkaloids, saponins, glycosides and flavonoids were present in varying proportions in the extracts. Varying proportions of the proximate constituents were also observed. An acute toxicity study revealed that the extracts were non-toxic up to a dose of 5000 mg kg^{-1}. The extracts produced significant ($p<0.05$) reduction in FBS, water and food intake, but no significant ($p>0.05$) effect on the body weight of the treated rats in comparison with the diabetic control. The ethanol extract produced a significantly ($p<0.05$) higher anti-diabetic effect than the other extracts. **Conclusion:** Ethanol extract of *T. bracteolata* leaves was observed to possess potent anti-diabetic effect compared to other extracts, indicating the relevance of ethanol for the extraction of the anti-diabetic principles in *T. bracteolata* leaves.

Key words: Phytochemical, proximate, anti-diabetic, diabetes mellitus, *Tephrosia bracteolata*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a multifactorial disease that is usually accompanied by hyperglycaemia[1], lipoprotein abnormalities[2], increased basal metabolic rate[3,4], abnormalities in anti-oxidant enzymes and alterations in the metabolism of carbohydrates, lipids and proteins[5]. The hyperglycemia associated with diabetes mellitus usually arises from a complete deficiency in the secretion of insulin (type 1 DM) or a defect in insulin action (type 2 DM) or both[6,7]. If hyperglycemia is left unchecked, it usually leads to the onset and progression of microvascular disorders such as nephropathy, retinopathy and macrovascular complications which include cardiomyopathy, neuropathy, atherosclerosis and myocardial infarction[8,9].

The role of medicinal plants in the regulation and prevention of diabetes cannot be overemphasized, especially in the less-developed countries where there is dearth of modern health facilities thereby making access to conventional antidiabetic drugs nearly impossible. Where these drugs are available, the prices are exorbitant making it difficult for the majority of people who live below poverty line. Also, adverse effects are a major concern with the use of insulin and oral hypoglycemic agents. All these factors have necessitated the search for alternative methods to effectively manage diabetes and its complications[10]. Information from ethno-botanical surveys suggests that about 800 plants from different families may possess anti-diabetic activities. Reports of many cultures and traditional system of medicine across the world lend credence to the beneficial uses of medicinal plants in the management of diabetes. A number of such plants have been used and still in use singly or as an adjuvant to orthodox drugs in managing a number of chronic diseases without any knowledge of their bioactive compounds and pharmacological properties[11].

Recently, efforts made towards the management of diabetes have shifted to medicinal plants and their bioactive compounds[12]. For instance, In Nigeria, many plants have been scientifically proven and reported to be rich in antidiabetic phytochemicals[13-15]. Phytochemicals also known as secondary metabolites are a large variety of chemical substances that occur naturally in plants. These include steroids, terpenoids, glycosides, alkaloids, flavonoids, tannins, saponins and phenolic compounds[16]. These secondary metabolites which usually possess pharmacological properties are the end products of primary metabolites such as, amino acids, carbohydrates, lipids and chlorophyll[17,18]. Proximate composition is also another key criterion to examine the nutritional values and quality of foods. Some nutrients are known to possess protective potentials against certain diseases, hence it is of paramount importance to investigate the proximate composition of herbs.

The plant *Tephrosia bracteolata* is widespread in tropical Africa[19,20]. In Nigeria, it is common in the Northern and Southern parts where it is employed for different purposes among the local people. Information obtained through personal communications in herbal markets around Lokoja and Bassa Local governments of Kogi State, Nigeria confirmed that *T. bracteolata* leaf-preparation is used for the treatment of whitlow, toothache, ear-ache, open wound and diabetes. This study thus aimed at investigating comparatively the antidiabetic potentials, phytochemical and proximate compositions of different extracts of *T. bracteolata* leaves.

MATERIALS AND METHODS

Study area: This study was carried out between May and October, 2017 at Salem University, Lokoja, Kogi State, Nigeria.

Chemicals and drugs: All the chemicals employed in this study were of analytical grade and sourced from Sigma-Aldrich, UK through a local vendor. Alloxan monohydrate (Sigma-Aldrich, UK) was also purchased through a local vendor while metformin® (Aventis) was obtained from Health-Seal Pharmacy Ltd., Lokoja, Kogi State, Nigeria.

Animals: Adult male Wistar rats weighing 120-200 g were used for this study. These animals were acclimatized in stainless steel cages and maintained in standard laboratory conditions. They were given access to clean water and standard rodent feed.

Plant collection and identification: *Tephrosia bracteolata* leaves were collected from their natural habitat along the River Niger Area of Lokoja, Kogi State, Nigeria. The plant was identified by Mr. Gbenga Akanni at the Herbarium Unit of the Department of Botany, Federal University, Lokoja, Kogi State, Nigeria. The plant was assigned a voucher number FULH/0765 and kept for future reference.

Extraction of plant material: The leaves were shade-dried for 15 days and thereafter pulverized using an electric blender. Extraction was subsequently carried out as follows:

Chloroform/methanol extraction: The pulverized leaves (2000 g) were macerated in chloroform-methanol (2:1), stirred vigorously and allow to stand for 72 hrs. The mixture was

filtered with Whatman filter paper (No. 1), after which the filtrate was shaken with distilled water (20% total volume of filtrate) to obtain two layers. The lower layer which represents the chloroform layer was separated from the methanol layer into separate beakers using separating funnel. The extracts were concentrated with a rotary evaporator to obtain the chloroform extract of *T. bracteolata* (CETB) and the methanol extract of *T. bracteolata* (METB), respectively.

Aqueous and ethanol extraction: The pulverized leaves (2000 g) were macerated in distilled water and ethanol separately, shaken vigorously and allowed to stand for 72 hrs after which both were filtered with Whatman filter paper (No. 1). The distilled water filtrate was concentrated using freeze-dryer to obtain the aqueous extract of *T. bracteolata* (AETB), while a rotary evaporator was used to obtain the ethanol extract of *T. bracteolata* (EETB). The percentage yield of the extracts was calculated relative to the starting material.

$$\text{Yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of pulverized leaves (g)}} \times 100$$

Qualitative phytochemical analysis of the extracts: The methods of Harborne[21] and Trease and Evans[22] were used to identify the phytochemical constituents of the extracts of *Tephrosia bracteolata*.

Quantitative phytochemical analysis of the extracts: The quantitative determination of alkaloids, phenols and flavonoids was done according to the method of Harborne[21]. Saponin content was determined according to Obadoni and Ochuko[23], while the cyanogenic glycoside content was determined using the alkaline picrate method[24]. The content of tannins was determined according to the method of Makkar *et al.*[25]. Terpenoid and steroid contents were determined according to the method of Edeoga *et al.*[26].

Proximate analysis of the extracts: The proximate compositions of the various extracts of *T. bracteolata* leaves were determined using the Association of Official Analytical Chemist method[27].

Acute toxicity studies: The oral median lethal dose (LD_{50}) of the extracts was determined in rats following the method of Lorke[28].

Induction of diabetes: Induction was carried out as described by Dunn and Mc Letchie[29].

Experimental design for the comparative anti-diabetic study of the extracts: Sixty-six rats (sixty diabetic and 6 non-diabetic rats) were divided into 11 groups (n = 6) and treated as follows:

Group 1 : Non-diabetic control and received 1 mL kg^{-1} distilled water
Group 2 : Diabetic control and received 1 mL kg^{-1} distilled water
Group 3 : Diabetic and received 150 mg kg^{-1} metformin
Group 4 : Diabetic and received 250 mg kg^{-1} AETB
Group 5 : Diabetic and received 500 mg kg^{-1} AETB
Group 6 : Diabetic and received 250 mg kg^{-1} CETB
Group 7 : Diabetic and received 500 mg kg^{-1} CETB
Group 8 : Diabetic and received 250 mg kg^{-1} METB
Group 9 : Diabetic and received 500 mg kg^{-1} METB
Group 10 : Diabetic and received 250 mg kg^{-1} EETB
Group 11 : Diabetic and received 500 mg kg^{-1} EETB

The extracts and metformin were administered orally to the rats for a period of 28 days. Daily food and water consumption, weekly Fasting Blood Sugar (FBS) and weekly body weight changes were monitored as indices of anti-diabetic action.

Estimation of weekly fasting blood sugar (FBS): FBS was estimated on day 0 (i.e., day 4 post induction) for the hyperglycemic groups and subsequently on days 7, 14, 21 and 28. From each group, blood samples were collected from the tail vein with sterile scissors and tested using the digital glucometer and its corresponding strips.

Estimation of body weight changes: Rats were weighed weekly during the period of treatment and on the day the animals were sacrificed for the determination of changes in body weight.

Estimation of food intake and water intake: The quantity of food and water consumed by the rats was measured daily by subtracting the quantity of feed and water remaining from the quantity supplied after 24 hrs. A weekly average was then determined.

Statistical analysis: Data were expressed as Mean±SEM and statistical differences between means were determined by one-way ANOVA followed by Duncan's *post hoc* test for multiple comparison tests using SPSS version 20. Values were considered significant at $p \leq 0.05$.

RESULTS

Percentage yield of the crude extracts: EETB gave the highest yield followed by CETB, METB and AETB (Table 1).

Qualitative phytochemical analysis of AETB, CETB, METB and EETB leaves: Phenols, flavonoids, saponins, glycosides and alkaloids were present in all the extracts. Tannins were present only in CETB and METB. Steroids were present in CETB and EETB. Terpenes were absent only in CETB (Table 2).

Quantitative phytochemical analysis of AETB, CETB, METB and EETB leaves: METB had the highest concentration of phenol (7.12 mg/100 g) while AETB had the least concentration of phenols (1.18 mg/100 g). CETB had the highest concentration of tannins (5.38 mg/100 g) and alkaloids (3.47 mg/100 g). EETB had the highest concentration of flavonoids (5.69 mg/100 g) while CETB had the least concentration (1.63 mg/100 g) (Table 3).

Proximate composition of AETB, CETB, METB and EETB leaves: Table 4 shows the proximate composition of the crude extracts. CETB had the highest (47.25%) fat content, while EETB contained 21.75% ash which was the highest, among the extracts. METB contained the least (8.70%) moisture content and AETB had the highest percentage (17.10%) of moisture and carbohydrate (26.30%).

Acute toxicity study of AETB, CETB, METB and EETB leaves: In both phases of the experiment, the crude extracts of *Tephrosia bracteolata* leaves did not produce any sign of toxicity or mortality at the doses administered orally. The oral median lethal doses (LD_{50}) of the extracts were therefore estimated to be greater than 5000 mg kg^{-1} b.wt., in rats.

Effect of AETB, CETB, METB and EETB leaves on the Fasting Blood Sugar (FBS) of diabetic Wistar rats: FBS of the diabetic untreated group (Group 2) was significantly ($p<0.05$) increased compared to the normal rats (Group 1). This was significantly ($p<0.05$) reduced from day 14 in Group 11 compared to group 2. Significant ($p<0.05$) reduction in FBS was also observed in Groups 9 and 10 from the 21st day, while Groups 3-8 significantly ($p<0.05$) reduced FBS on the 28th day (Table 5). FBS concentration of Groups 9 and 10 was found to be significantly ($p<0.05$) lowered compared to other treated groups, while FBS concentration of Group 11 was significantly ($p<0.05$) lowered compared to Groups 9 and 10.

Table 1: Yield (%) of the crude extracts

Extract	Weight (g)	Yield (%)
CETB	117	9.75
EETB	179	14.92
METB	98	8.17
AETB	78	6.50

CETB: Chloroform extract of *Tephrosia bracteolata*, EETB: Ethanol extract of *Tephrosia bracteolata*, METB: Methanol extract of *Tephrosia bracteolata*, AETB: Aqueous extract of *Tephrosia bracteolata*

Table 2: Qualitative phytochemical compositions of AETB, CETB, METB and EETB leaves

Phytochemicals	CETB	EETB	METB	AETB
Phenols	+	+++	+++	+
Terpenoids	ND	+	+	+
Saponins	+	++	++	+++
Steroids	+++	+	ND	ND
Glycosides	+	+++	+++	++
Flavonoids	+	+++	+++	++
Tannins	++	ND	+++	ND
Alkaloids	+++	++	++	+

+: Slightly present, ++: Moderately present, +++: Highly present, ND: Not detected, CETB: Chloroform extract of *Tephrosia bracteolata*, EETB: Ethanol extract of *Tephrosia bracteolata*, METB: Methanol extract of *Tephrosia bracteolata*, AETB: Aqueous extract of *Tephrosia bracteolata*

Effect of AETB, CETB, METB and EETB leaves on food consumption of diabetic Wistar rats: Following alloxan administration, there was a statistically significant ($p<0.05$) increase in food consumption of group 2 rats compared to group 1 (Table 6). All the treated groups showed statistically significant ($p<0.05$) reduction in mean food consumption from week 1-4 compared to Group 2 (diabetic control). The reductions in Groups 9, 10 and 11 were found to be statistically significantly ($p<0.05$) higher than other treated groups.

Effect of AETB, CETB, METB and EETB leaves on weekly water intake of diabetic Wistar rats: The mean water intake of all treated groups was significantly ($p<0.05$) reduced from the 1st week after diabetes induction compared to Group 2. The reduction in water intake observed in Groups 10 and 11 were significantly ($p<0.05$) lower compared to other treated groups (Table 7).

Effect of AETB, CETB, METB and EETB leaves on the body weight of diabetic Wistar rats: Table 8 shows significant ($p<0.05$) decrease in the body weight of group 2 rats compared to group 1. However, no significant ($p>0.05$) difference in the body weight of all treated groups was observed compared to Group 2.

Table 3: Quantitative phytochemical composition of AETB, CETB, METB and EETB leaves

Phytochemicals (mg/100 mg)	CETB	EETB	METB	AETB
Phenols	2.61±0.0021	6.38±0.0025	7.12±0.0031	1.18±0.0012
Terpenoids	0.11±0.0031	0.87±0.0024	0.77±0.0023	0.69±0.0019
Saponins	1.01±0.0011	1.58±0.0015	1.56±0.0021	2.05±0.0026
Steroids	1.27±0.0014	0.94±0.0011	0.22±0.0006	0.19±0.0009
Glycosides	0.17±0.0012	1.38±0.0024	1.49±0.0018	0.35±0.0013
Flavonoids	1.63±0.0017	5.69±0.0031	5.21±0.0029	2.52±0.0015
Tannins	5.38±0.0022	1.11±0.0016	4.99±0.0023	0.93±0.0008
Alkaloids	3.47±0.0026	2.64±0.0027	3.02±0.0031	1.21±0.0022

Results are presented as Mean±SD, n = 3, CETB: Chloroform extract of *Tephrosia bracteolata*, EETB: Ethanol extract of *Tephrosia bracteolata,* METB: Methanol extract of *Tephrosia bracteolata*, AETB: Aqueous extract of *Tephrosia bracteolata*

Table 4: Proximate compositions of AETB, CETB, METB and EETB leaves

Parameters (%)	CETB	EETB	METB	AETB
Moisture	10.00±0.28	9.90±1.27	8.70±0.42	17.10±0.99
Ash content	8.05±1.34	21.75±0.35	17.75±1.06	5.75±0.35
Crude fat	47.25±0.35	18.50±1.41	24.25±1.77	14.00±0.71
Crude fibre	27.50±2.12	22.50±2.12	19.00±1.41	27.00±1.41
Crude protein	4.38±0.28	9.65±1.66	8.09±0.55	9.85±0.28
Carbohydrate	2.82±1.76	17.70±0.74	22.21±1.29	26.30±1.76

Results are presented as Mean±SD, n = 3, CETB: Chloroform extract of *Tephrosia bracteolata*, EETB: Ethanol extract of *Tephrosia bracteolata*, METB: Methanol extract of *Tephrosia bracteolata*, AETB: Aqueous extract of *Tephrosia bracteolata*

Table 5: Effect of AETB, CETB, METB and EETB leaves on the Fasting Blood Sugar (FBS) of diabetic Wistar rats

	Post-treatment time (days)				
Groups	0	7	14	21	28
1	74.3±5.72^{a}	76.0±3.58^{a}	73.0±8.65^{a}	71.7±6.15^{a}	73.2±8.38^{a}
2	353.3±100.19^{b}	358.3±100.75^{b}	342.6±100.86^{c}	363.6±104.20^{e}	394.4±97.92^{e}
3	352.5±98.92^{b}	333.4±110.55^{b}	319.2±114.13^{c}	282.6±116.52^{cde}	244.4±118.28^{cd}
4	350.5±94.98^{b}	335.7±95.11^{b}	340.2±107.17^{c}	335.4±131.06^{de}	281.8±102.67^{d}
5	350.0±93.20^{b}	345.3±91.72^{b}	337.2±95.17^{c}	302.3±89.49^{cde}	288.2±83.34^{d}
6	347.0±93.37^{b}	359.5±89.50^{b}	323.0±86.53^{c}	285.8±78.07^{cde}	276.2±72.34^{d}
7	347.3±94.17^{b}	341.7±95.96^{b}	322.3±86.17^{c}	288.7±79.93^{cde}	276.4±87.35^{d}
8	353.5±83.47^{b}	347.2±81.89^{b}	335.2±75.03^{c}	280.7±74.63^{cde}	273.3±75.02^{d}
9	352.5±90.95^{b}	345.5±83.61^{b}	251.7±63.56^{bc}	233.3±56.39^{bcd}	212.0±63.06^{bcd}
10	348.7±87.91^{b}	295.3±89.42^{b}	210.0±74.89^{bc}	195.2±67.28^{bc}	159.7±54.50^{abc}
11	325.3±114.09^{b}	269.5±91.08^{b}	180.7±74.19^{b}	158.3±75.60^{ab}	136.0±42.72^{ab}

Data are presented as mean±SD, (n = 6). Mean values having different lower case alphabets as superscripts are considered significant ($p<0.05$) down the columns. Group 1: Normal control received 1 mL distilled water, Group 2: Diabetic control received 1 mL distilled water, Group 3: Received 150 mg kg^{-1} metformin, Group 4 received 250 mg kg^{-1} AETB, Group 5 received 500 mg kg^{-1} AETB, Group 6 received 250 mg kg^{-1} CETB, Group 7 received 500 mg kg^{-1} CETB, Group 8 received 250 mg kg^{-1} METB, Group 9 received 500 mg kg^{-1} METB, Group 10 received 250 mg kg^{-1} EETB and Group 11 received 500 mg kg^{-1} EETB

Table 6: Effect of AETB, CETB, METB and EETB leaves on weekly food consumption of diabetic Wistar rats

	Food consumption (g)			
Groups	Week 1	Week 2	Week 3	Week 4
1	120.5±6.53^{ab}	121.83±10.15^{ab}	117.0±5.69^{a}	119.0±10.83^{a}
2	173.2±13.99^{g}	192.50±11.29^{e}	208.0±14.46^{e}	219.8±16.46^{c}
3	120.5±4.76^{bc}	142.20±19.63^{bc}	139.7±13.53^{cd}	131.3±11.94^{ab}
4	156.7±11.29^{ef}	166.30±21.53^{d}	147.8±18.69^{d}	133.5±11.00^{b}
5	162.8±6.71^{fg}	136.70±26.06^{abc}	138.3±11.83^{bcd}	122.0±10.70^{ab}
6	152.8±7.51^{ef}	150.20±11.88^{cd}	151.7±20.10^{d}	135.2±7.81^{b}
7	146.7±8.40^{de}	120.20±8.13^{a}	128.7±4.13^{abc}	123.2±7.71^{ab}
8	139.3±12.55^{cd}	135.80±15.21^{abc}	139.8±15.48^{cd}	127.8±8.89^{ab}
9	125.8±9.20^{b}	134.00±24.27^{abc}	119.2±6.88^{a}	118.7±3.20^{a}
10	118.8±5.63^{ab}	131.20±10.53^{abc}	121.2±2.93^{ab}	117.8±9.45^{a}
11	114.5±7.06^{a}	119.80±8.95^{a}	124.7±23.61^{abc}	118.5±8.52^{a}

Data are presented as mean±SD, (n = 6). Mean values having different lower case alphabets as superscripts are considered significant ($p<0.05$) down the columns. Group 1: Normal control received 1 mL distilled water, Group 2: Diabetic control received 1 mL distilled water, Group 3: Received 150 mg kg^{-1} metformin, Group 4 received 250 mg kg^{-1} AETB, Group 5 received 500 mg kg^{-1} AETB, Group 6 received 250 mg kg^{-1} CETB, Group 7 received 500 mg kg^{-1} CETB, Group 8 received 250 mg kg^{-1} METB, Group 9 received 500 mg kg^{-1} METB, Group 10 received 250 mg kg^{-1} EETB and Group 11 received 500 mg kg^{-1} EETB

Table 7: Effect of AETB, CETB, METB and EETB leaves on weekly water intake of diabetic Wistar rats

	Water intake (mL)			
Groups	Week 1	Week 2	Week 3	Week 4
1	138.3±11.69^{a}	141.7±16.02^{a}	145.0±20.73^{a}	130.0±8.94^{ab}
2	313.3±52.03^{g}	383.3±35.59^{g}	421.7±65.23^{e}	366.7±65.23^{d}
3	231.7±56.01^{ef}	245.0±32.71^{ef}	225.0±20.74^{d}	173.3±21.60^{c}
4	250.0±26.83^{f}	276.7±36.70^{f}	208.3±29.94^{cd}	176.7±31.41^{c}
5	213.3±35.03^{def}	221.7±53.45^{bde}	188.3±29.67^{bc}	155.0±20.74^{abc}
6	228.3±37.64^{ef}	230.0±45.61^{de}	198.3±25.65^{cd}	161.7±35.45^{bc}
7	195.0±31.46^{cde}	200.2±34.06^{bcd}	163.3±21.60^{ab}	151.7±16.02^{abc}
8	183.3±28.75^{bcd}	210.0±25.30^{bcde}	185.0±41.35^{bc}	155.0±30.17^{abc}
9	168.3±25.63^{abc}	186.7±19.66^{bc}	155.0±32.71^{ab}	138.3±17.22^{abc}
10	155.0±25.09^{abc}	178.3±24.01^{ab}	150.0±18.97^{a}	141.7±9.83^{abc}
11	146.3±20.22^{ab}	168.3±16.02^{ab}	138.3±9.83^{a}	120.0±6.32^{a}

Data are presented as mean±SD, (n = 6). Mean values having different lower case alphabets as superscripts are considered significant ($p<0.05$) down the columns. Group 1: Normal control received 1 mL distilled water, Group 2: Diabetic control received 1 mL distilled water, Group 3: Received 150 mg kg^{-1} metformin. Group 4 received 250 mg kg^{-1} AETB, Group 5 received 500 mg kg^{-1} AETB, Group 6 received 250 mg kg^{-1} CETB, Group 7 received 500 mg kg^{-1} CETB, Group 8 received 250 mg kg^{-1} METB, Group 9 received 500 mg kg^{-1} METB, Group 10 received 250 mg kg^{-1} EETB and Group 11 received 500 mg kg^{-1} EETB

Table 8: Effect of AETB, CETB, METB and EETB leaves on body weight of diabetic Wistar rats

	Post-treatment time (days)				
Groups	0	7	14	21	28
1	166.0±13.45^{b}	165.5±14.46^{b}	167.7±14.61^{b}	170.3±14.11^{b}	172.5±14.88^{b}
2	147.7±19.17^{a}	145.0±17.82^{a}	142.4±22.09^{a}	139.4±21.59^{a}	139.4±23.09^{a}
3	153.3±23.40^{a}	147.8±26.92^{a}	146.6±30.62^{a}	148.8±29.88^{ab}	150.4±29.63^{ab}
4	148.8±19.16^{a}	145.5±19.25^{a}	142.8±21.28^{a}	139.6±22.89^{a}	137.8±22.40^{a}
5	149.8±20.08^{a}	145.2±21.74^{a}	144.3±24.57^{a}	145.3±24.77^{ab}	146.2±24.38^{ab}
6	143.5±22.68^{a}	139.8±15.92^{a}	138.7±15.88^{a}	140.0±16.31^{a}	141.7±16.06^{a}
7	145.5±7.79^{a}	141.3±9.47^{a}	139.8±8.82^{a}	137.8±12.19^{a}	140.0±11.74^{a}
8	143.7±22.68^{a}	140.5±21.10^{a}	138.5±20.30^{a}	140.7±20.28^{a}	143.0±20.85^{a}
9	149.8±17.23^{a}	144.8±16.02^{a}	144.0±17.65^{a}	146.3±17.53^{ab}	148.7±17.78^{ab}
10	145.8±18.93^{a}	142.7±19.03^{a}	143.8±19.30^{a}	146.2±19.22^{ab}	152.0±22.57^{ab}
11	153.8±27.11^{a}	151.3±28.27^{a}	153.0±27.51^{a}	154.3±27.58^{ab}	157.0±27.55^{ab}

Data are presented as mean±SD, (n = 6). Mean values having different lower case alphabets as superscripts are considered significant ($p<0.05$) down the columns. Group 1: Normal control received 1 mL distilled water, Group 2: Diabetic control received 1 mL distilled water, Group 3: Received 150 mg kg^{-1} metformin, Group 4 received 250 mg kg^{-1} AETB, Group 5 received 500 mg kg^{-1} AETB, Group 6 received 250 mg kg^{-1} CETB, Group 7 received 500 mg kg^{-1} CETB, Group 8 received 250 mg kg^{-1} METB, Group 9 received 500 mg kg^{-1} METB, Group 10 received 250 mg kg^{-1} EETB and Group 11 received 500 mg kg^{-1} EETB

DISCUSSION

This study investigated the antidiabetic potentials, phytochemical and proximate compositions of different extracts of *T. bracteolata*. The acute toxicity studies of the extracts were carried out and the studies revealed no mortality or changes in the appearance, behavior and respiratory rate of rats up to a dose of 5000 mg kg^{-1} of the extract. The oral LD_{50} of the extracts was then taken to greater than 5000 mg kg^{-1} for each extract. Judging from this study, all the extracts are relatively safe.

The phytochemical results showed that the extracts, notably EETB and METB were very rich in phenols, saponins, flavonoids, glycosides and alkaloids. Studies have shown that saponins, flavonoids, glycosides and alkaloids are good antidiabetic metabolites[30-32]. In the past, scientists have successfully isolated a number of alkaloids from plants cutting across families and investigated for their possible antidiabetic activity in different animal models. Such alkaloids through different mechanisms of action exhibit a wide range of anti-diabetic activities[33]. It has been reported that alkaloids like vindoline, vindolinine and catharanthine obtained from *Catharanthus roseus* (L.) G. Don (Apocynaceae) exhibited anti-hyperglycaemic potentials when tested in streptozotocin-induced diabetic rat models[33]. Flavonoids-another group of phytochemicals found in medicinal plants are known to be effective anti-diabetic agents. Another class of phytochemicals present in the extracts is triterpenes. Triterpenes are starting materials for the synthesis of steroids in both plants and animals. Usually, majority of terpenes and sterols exist in a free state. However, some others occur as glycosides or in combination with other compounds. Collectively, triterpenoid and steroidal glycosides are called saponins[34]. These biologically active compounds are known to possess potent

hypoglycemic activity[34]. Apart from the EETB and METB, these phytochemicals were detected in the other extracts but to a lesser extent and this may account for their relatively lower antidiabetic effect.

Proximate analysis of the extracts revealed that the highest ash content was observed in EETB (21.75%). The ash content is an indication of high mineral content in the extract and this is good for metabolic processes which are usually deranged in diabetic conditions. The extracts also contain high percentage of crude fiber, which is a reflection of the amount of indigestible sugar. CETB had the highest fiber content (27.50%) while the lowest content (19%) was observed in METB. When adequate amount of dietary fiber is consumed, it can have beneficial effects on health as it is known to lower serum cholesterol level, blood sugar and reduces the risk of heart diseases[35]. On the flip side, diets that are low in dietary fiber have been linked to conditions such as constipation and colon diseases such as piles, appendicitis and cancer. The fat content was highest in CETB (47.25%) and lowest in AETB (14%). EETB had a moderate fat content and this is necessary for the management of diseases as diets with high fat content can lead to a number of conditions and diseases such as hypercholesterolemia, diabetes, cancer and rapid ageing[36].

Administration of alloxan through the destruction of the pancreatic beta cells produced an increase in the concentration of serum glucose, which is an indication of diabetes mellitus. This observation is in agreement with the findings of Muhammad *et al.*[37]. Results obtained showed that CETB, EETB, METB and AETB produced varying degrees of reduction in blood glucose level in diabetic rats in a dose and time- dependent manner. EETB reduced blood glucose significantly ($p<0.05$) compared to the other extracts. Terpenes such as triterpenes are effective in inhibiting α-glucosidases and α-amylases[38]. This slows down glucose absorption through reduction in carbohydrate catabolism in the intestine[39]. The extract might have acted this way as a result of the high terpene content. This is further buttressed by the ability of *T. bracteolata* to inhibit α-glucosidase activity[40]. Phenolic compounds such as flavonoids serve as natural antioxidants. They have the ability to reduce the oxidative stress found in diabetic states[41]. The extracts due to their high phenolic content might have reduced the blood glucose level through the amelioration of oxidative stress induced by alloxan.

Following injection with alloxan, the Wistar rats also displayed other symptoms of diabetes aside from hyperglycemia, i.e., decrease in body mass gain and increase in food and water consumption (polyphagia and polydipsia) respectively[42]. The observed polydipsia could be as a result of excessive urination and dehydration brought about by plasma glucose levels surpassing the threshold of 180 mg dL^{-1}, therefore creating an osmotic gradient where more liquid is drawn into the renal tubule. Polyphagia on the other hand could have resulted from the cells' inability to utilize glucose in the blood therefore sending signals to the hypothalamus to stimulate hunger[43]. The administration of the extracts and metformin to the diabetic rats caused significant ($p<0.05$) reduction in food and water intakes. With adequate glycaemic control, less quantity of water was probably delivered to the renal tubes producing less urination, hence, less dehydration and ultimately reduced water intake by rats. Also with more glucose uptake by the cells, there might have been a reduced stimulation of hunger by the nervous system leading to the reduced food intake observed in all the diabetic treated groups. The body weights of the rats in the treated diabetic groups were decreased in the first week. This is in agreement with the symptoms of diabetes as stated by American Diabetic Association (ADA)[44] to include unexplained weight loss. This observation could be attributed to the increased conversion of storage fat and proteins to glucose (gluconeogenesis)[45]. However, treatment with the extracts after the first week, possibly, with an increased ability of the cells to take up glucose molecules and the subsequent reversal of gluconeogenesis, increased the body weights of diabetic rats, though with no significant ($p>0.05$) difference throughout the study.

CONCLUSION

The ethanol extract of *Tephrosia bracteolata* leaves appears to be more potent than other extracts judging from its pronounced effect on FBS, food and water intake and body weight of diabetic rats. This study therefore justifies the local use of *Tephrosia bracteolata* leaves in the management of diabetes mellitus. Also, based on the observed potency of the ethanol extract, it is safe to say that ethanol is the best solvent for extracting the anti-diabetic principles in the leaves of the plant.

SIGNIFICANCE STATEMENT

This study discovered the antidiabetic potential of various extracts of *Tephrosia bracteolata* leaves, with the ethanol extract of the leaves being more effective. The findings will therefore serve as basis to isolating the various antidiabetic principles in *Tephrosia bracteolata* leaves and enable researchers to develop novel antidiabetic agents.

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