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Research Article

Phytochemical Constituents, Antioxidant and Antidiabetic Efficacies of the Crude Extract and Fractions of *Ethulia conyzoides* Leaves

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ABSTRACT

Ethulia conyzoides is a plant traditionally used in managing diabetes in Nigeria. Unfortunately, its antidiabetic potential has not been scientifically examined. The research investigated the phytochemical constituents, *in vitro* antioxidant, and antidiabetic efficacies of *E. conyzoides* leaves extract. Seventy percent methanol extraction of *E. conyzoides* leaves was done to obtain its crude extract. It then was partitioned with n-hexane and ethyl acetate to obtain three fractions (n-hexane, ethylacetate and residual aqueous). Then, they were subjected to various phytochemistry investigations: 1,1-diphenyl 2-picryl hydrazyl (DPPH) radical scavenging activity assay and *in vivo* antidiabetic effects in mice. The fasting blood glucose (FBG) levels, weight change, feed and fluid intakes were determined using standard procedures. Phyto-profiles tests for the extract and its fractions revealed the presence of phenolics, tannins, cardiac glycosides, saponins and alkaloids. The result showed that the residual aqueous fraction had the nearest inhibitory concentration (IC_{50}) of 0.011µg/ml to the standard ascorbic acid with 0.010µg/ml compared with other extract and fractions. There was a substantial ($p < 0.05$) rise in fluid and feed intakes and serum levels of FBG in the type 2 diabetic mice. Treatment of the mice using 100 mg/kg b.w. residual aqueous fraction for 21 days significantly ($p < 0.05$) reduced the fluid intake, feed intake and FBG and statistically ($p < 0.05$) improved the body weight. This study revealed that the residual aqueous fraction of *E. conyzoides* has antioxidant and antidiabetic activities against induced type 2 diabetic mice.

Keywords: Antioxidant, Ethulia conyzoides, Fasting blood glucose, Phytochemical, Type 2 diabetes

Introduction

Diabetes is one of the weightiest universal health challenges affecting all genders of humanity in all parts of the world [1, 2]. It is a metabolism disorder with great concern as its prevalence is swiftly upsurging in developing and developed countries [3]. About 19 million adults aged 20 - 79 had diabetes in Africa in 2019. This number is projected to rise by 2045 to about 47 million; in Nigeria, about 2.7 million people (aged 20 – 79) were living with diabetes [1].

There are different kinds of synthetic drugs obtainable for diabetes mellitus management; however, they have a number of adverse effects, such as lactic acidosis, edema, diarrhea and the drugs are expensive even though these antidiabetic drugs offer some hopeful results [4]. This has required technical research into new antidiabetic agents from the wide variety of medicinal herbs for comparatively less lethal natural resources with therapeutic standards as an alternative.

The history of mankind has shown that medicinal plants have been used to treat ailments [5, 6]. These plants synthesize many secondary metabolites (phytochemicals) derived from primary metabolites and become a vital basis for pharmaceutical drugs [5].

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Ethulia conyzoides is known as Ethulia in English, Mashenkuturu in Hausa and Ichonkolo in Idoma. It is a herb that belongs to the Asteraceae family, and grows to a height of about 1.3 meters in riverine areas or wet grassland. Ethulia is found in Asia and some African countries like Cameroon, Nigeria. *E. conyzoides* is used for roundworm treatment (as an anti-helminthic) and for abdominal disorders [7, 8], as a natural antioxidants source [9] and as antihypertensive agents; as a cure to headaches and dysmenorrhea [10].

Today, T2D is the crucial delinquent of diabetes mellitus worldwide. It depreciates human life value, but larger research on diabetes focuses on T1D [11, 12]. This study therefore, seeks to confirm the antidiabetic effect of *E. conyzoides* extract and fractions in a stimulated diet-fed streptozotocin-treated T2D rat model.

Material and Methods

Experimental animals

A total of twenty-four (24) apparently healthy male Wistar mice weighing 22-26 g were purchased (from the Pharmacology and Therapeutics Department's animal house unit) and kept in well-aerated enclosures with *ad libitum* access to water and feed. Ethical approval (Approval Number: ABUCAUC/2019/007) was sought and obtained from the ABU Committee on Animal Use and Care, taking into consideration adherence to international rules and guiding principles for the care and use of laboratory animals.

Plant preparation and extraction

E. conyzoides whole plant was harvested from its natural habitat at Okpokwu L.G.A of Benue State. *E. conyzoides* was identified at the Herbarium unit, Botany Department, in the Life Sciences Faculty, A.B.U Zaria, and voucher number (7098) was obtained. *Ethulia conyzoides* leaves sample was water-cleaned, air dried, and the dried leaves were pulverized using a mortar and pestle. About 1000 g of the ground sample was suspended in 70% methanol (1:10 *w/v*) for 48 hours at room temperature with frequent agitation (cold maceration). This was sieved with a 1 mm mesh sieve and filtered via No. 1 Whatman filter paper. After extraction, the methanol solvent was evaporated completely using a rotary evaporator and then subjected to drying in a water bath (45°C temperature) to obtain the dried extract. The solvent-free crude methanol extract was sealed in a sample bottle and kept at 2-4°C till further needed [13].

Partitioning of the crude methanol extract of E. conyzoides

Eighty-two grams (82g) *E. conyzoides* crude extract was dissolved in 50ml of distilled H_2O; n-hexane was added, turned into a separating funnel, shaken and allowed to stand to separate into two layers. Hence, the n-hexane fraction was carefully poured out, and continuous addition of fresh n-hexane solvent was added severally until it was completely partitioned to obtain the n-hexane fraction. For ethyl acetate solvent, the same above process was repeated. The resulting residue was referred to as the residual aqueous fraction. Each fraction was concentrated to a constant weight at room temperature [13].

Phytochemistry test

E. conyzoides crude extract and its fractions were subjected to phytochemical screening standard protocols by Trease and Evans [14].

Acute toxicity (LD_{50}) determination

The lethal dose (LD_{50}) of the *E. conyzoides* crude extract and its fraction were assessed via the described method by Lorke [15].

Induction and Confirmation of type 2 diabetes

A slight modification was done to the method described by Okoduwa *et al.* [16] to induce type 2 diabetes. After formulation and giving the feeds and drinks to the animals, for six weeks, at the end of the sixth week, the mice fasted overnight, and each mouse was given a single dose of Sigma Aldrich Streptozotocin (STZ) (45mg/kg body weight) intraperitoneally. After induction, 5% glucose solution was given to the mice as drinking water for the first 24 hours.

Ten days after the induction, the confirmation of diabetes was done using blood samples gotten by tail puncture with a glucose strip on the glucometer. After the confirmation, mice with FBG ≥200 mg/dl, HOMA IR > 5 and HOMA - β < 200 were termed to diabetic [17, 18].

Experimental animals grouping

The animals were arranged into six groups of four mice each:

- Group 1: (Normal control) non-induced mice given feed and water only.
- Group 2: (Diabetic control) Diabetic mice

Table 1. Qualitative Phytochemistry Profiles of *E. conyzoides* Samples

Phytochemicals	Test	Methanol	Ethyl Acetate	n-hexane	Residual Aqueous
Carbohydrates	Molish	+	+	+	+
Saponins	Frothing	+	-	+	+
Flavonoids	Shinoda	+	+	-	+
	Sodium Hydroxide	+	+	-	+
	Ferric Chloride	+	+	-	+
Tannins	Lead Sub-acetate	+	+	+	+
Terpernoids/Steriods	Salkowki	+	+	+	+
	Liebermann- burchard	+	+	+	+
Alkaloids	Dragendoffs	+	+	+	+
	Mayer	+	+	+	+
Cardiac Glycosides	Keller-kiliani	+	+	+	+

without treatment.

- Group 3, 4, 5 and 6 diabetic mice were given 100 mg/kg b.w. crude methanol extract, ethyl acetate fraction, n-Hexane fraction and residual aqueous fraction, respectively.

The plant extract and fractions were administered by oral gavage to the diabetic treatment groups on a daily basis for 21 days.

Observation of the animals

The following parameters were measured during the experimental period: daily feed intake, fluid intake, and changes in weekly body weight.

Analysis of Data

Statistical package for the social sciences (SPSS program version 23) computer software was used to conduct data analyses; one-way analysis of variance (ANOVA) followed by the Duncan multiple range tests, which was used to determine the significant level at p-value less than 0.05 ($p<0.05$). Data results were presented as mean ± standard deviation (SD).

Results and Discussion

Medicinal plants constitute part of the basis of lead compounds and drug candidates [19]. Nature is a cradle of medicinal plants for donkey years and it has been a remarkable source of isolating modern drugs [20]. Plants hold an important part in health care, with approximately 60-70 % of people in rural areas mainly depending on folk medicines for their principal wellbeing requirements [21].

Phytochemical constituents of E. conyzoides leaves samples

Qualitative phytochemical constituents (Table 1) of *Ethulia conyzoides* samples showed the presence of tannins, terpenoids/steroids, saponins, flavonoids, alkaloids, and cardiac glycosides in the extracts except for ethyl acetate and n-hexane fractions which had no saponins and flavonoids. The quantitative phytochemistry profiles in the crude and fractions samples of *Ethulia conyzoides* show that the residual aqueous fraction had a statistically ($p<0.05$) higher quantity of flavonoids and phenolics (Figure 1).

Crucial bioactive components embedded in plants have put them at the centre of attention; these phytochemicals in them have been said to be responsible for protecting them [5]. Some of the secondary metabolites are linked with several physiological activities in mammals [22]. These phytochemicals in the tested samples of *Ethulia conyzoides* attest to its medicinal use. The findings on the quantitative phytochemical constituents showed that phenolics had a peak concentration.

Plant phenolics form one of the foremost sets of compounds that act as potent antioxidants with redox properties, thereby performing and partaking in reduction-oxidation (redox) reactions [23, 24]. Phenolic compounds can occur in various chemical natures, such as tocopherols, carotenoids, flavonoids, lignins, phenolics and tannins [25]. Extracts with high phenolic content show high antioxidant activity. Phenolic compounds are fundamental phytochemicals with a great ability to scavenge free radicals and have created huge interest amongst scientists in creating natural antioxidant compounds from plant sources.

Acute toxicity of crude methanol extract and fractions of E. conyzoides

The treated mice showed no symptoms of toxic reactions during the 24 hours of investiga-

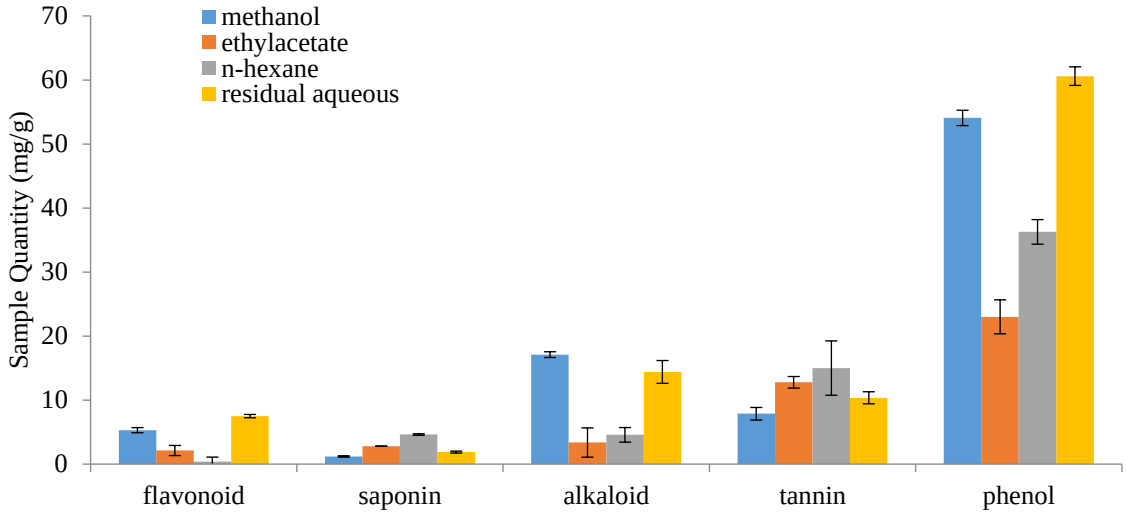

Figure 1. Quantitative phytochemistry profiles of *Ethulia conyzoides* samples

Table 2. DPPH Assay of *E. conyzoides* Samples

Extracts	% Inhibition of DPPH	IC_{50} at 0.1µg/ml
Ascorbic Acid	95.70	0.0096 ± 0.002^{a}
Crude Methanol	81.05	0.0137 ± 0.004^{c}
N-Hexane	50.55	0.0223 ± 0.001^{d}
Ethyl Acetate	68.35	0.0142 ± 0.002^{c}
Residual Aqueous	94.70	0.0108 ± 0.003^{b}

Data are presented as mean ± Standard Deviation; Results down the same column with different superscript are

tion. In addition, no death was recorded at the highest dose of 5000 mg/kg b.w. after completing the 24 hours. Hence their LD_{50} were considered to be above 5000 mg/kg b.w. orally. Therefore, 100mg/kg b.w. dosage was used for the 21 days period of oral administration.

1, 1-Diphenyl-2 Picrylhydrazyl (DPPH) of crude methanol extract and fractions of E. conyzoides

The result of the DPPH assay is presented in Table 2. Ascorbic acid (a well-known antioxidant) showed a greater activity than the crude *E. conyzoides* extract and fractions. The IC_{50} value of the residual aqueous fraction (0.011 µg/ml) was the closest to the standard ascorbic acid with a 0.010 µg/ml IC_{50} value.

One of the utmost active approaches for estimating the concentration of radical-scavenging materials is the DPPH assay [26]. This is due to the fact that it is a steady free radical at ambient temperature and acts as an electron acceptor to become a diamagnetic molecule [27]. Results were reported as $IC_{50,}$ which is the antioxidant concentration required to hunt 50% of DPPH available in the test solution under the experimental conditions [28, 29]. A low IC_{50} value implies the test solution has a high DPPH radical-scavenging capacity [30]. The residual aqueous fraction had the highest DPPH reducing activity suggesting the sample was a free radical scavenger. *E. conyzoides* contain some phytochemicals like phenolics and flavonoids that may be tagged with the scavenging activity [31, 32]. The residual aqueous fraction had the highest amount of total polyphenol, the lowest IC_{50} value and the highest antioxidant activity.

Effect of E. conyzoides administered samples on mean fluid and feed intake of induced type 2 diabetic mice for the 21 days of treatment

The mean fluid and feed intake of each experimental mouse (described in Figure 2 and Figure 3, respectively) showed that, at the induction, the diabetic groups had increased fluid intake compared to the normal group, although it was not significant ($p > 0.05$). In the week in which diabetes was confirmed, the diabetic untreated group had a statistical ($p > 0.05$) rise in fluid intake; also in weeks 2 and 3 when related to all the other groups.

High fluid intake was seen in T2D mice, extract and fractions treatment of *E. conyzoides* sig-

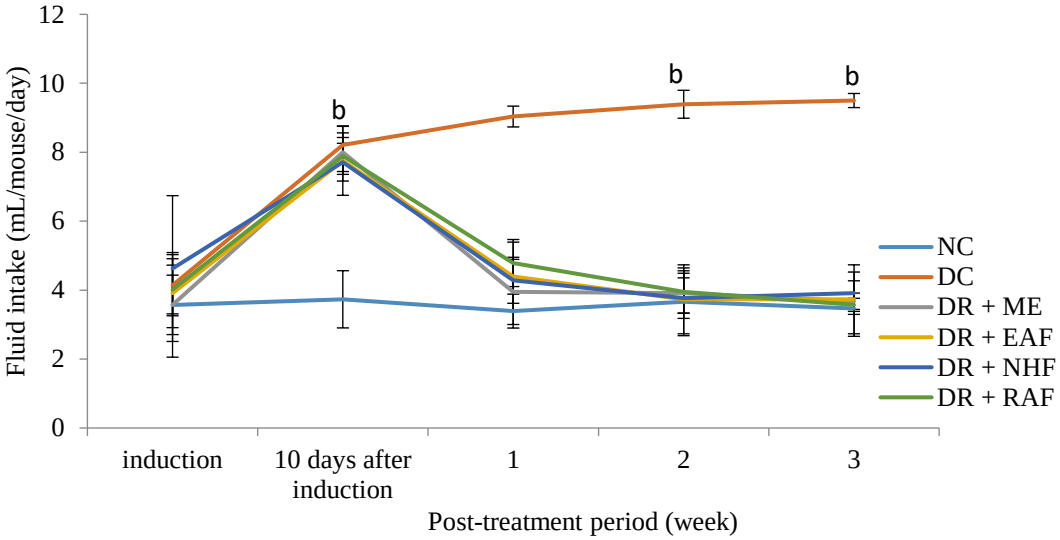

Figure 2. Effect of *E. conyzoides* administered samples on mean fluid intake

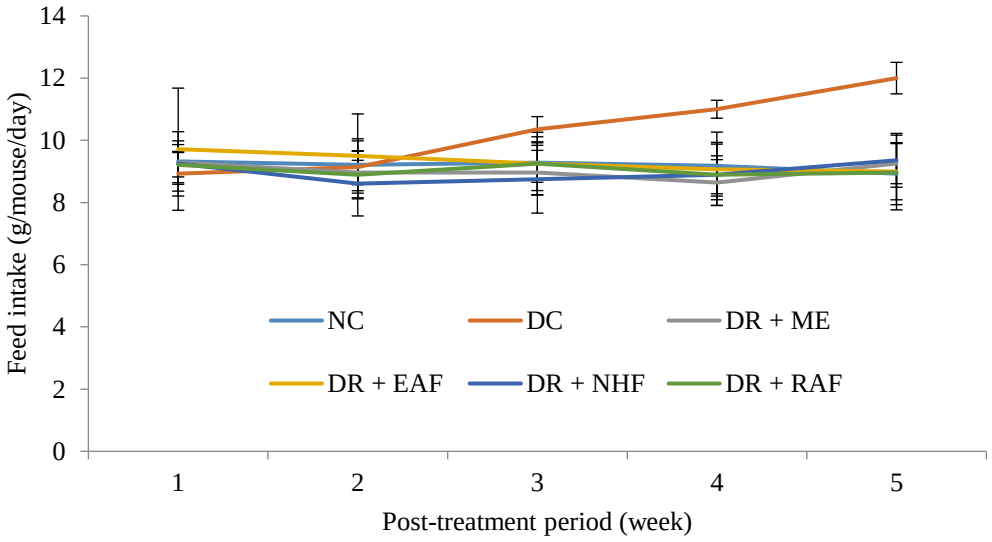

Figure 3. Effect of *E. conyzoides* administered samples on mean feed intake

nificantly ($p<0.05$) lowered the fluid intake, suggesting their possible ameliorative effects against T2D. This could result from increased intracellular water, prompting the osmoreceptor of the brain's thirst centre leading to less water intake [33].

The diabetic groups at induction had a statistical ($p<0.05$) upsurge in feed intake compared to the normal group. The week at which diabetes was confirmed, the group 2 had significant ($p<0.05$) reduction in feed intake. At week 2 and 3, the feed intake was significantly ($p<0.05$) higher in diabetic control compared to all the groups.

The management and or prevention of type 2 diabetes in the obese has been strongly linked with weight reduction [34]. There was a significant ($p<0.05$) decrease in body weight that was observed in the diabetic control group, and this could be due to a decrease in appetite, feed intake or an increase in catabolic effect, which is evident in T2D [35]. Also, a significant ($p<0.05$) reduction was recorded in the feed intake of T2D mice. The extract and fractions *E. conyzoides* increased the feed intake, and any agent that can increase feed intake is said to be a good antidiabetic agent [36].

Effect of crude methanol extract and fractions of E. conyzoides on the body weight of induced type 2 diabetic mice for 21 days of treatment

The result (Figure 4) showed that diabetic untreated mice (i.e. diabetic control mice were the mice that were successfully induced for diabetes

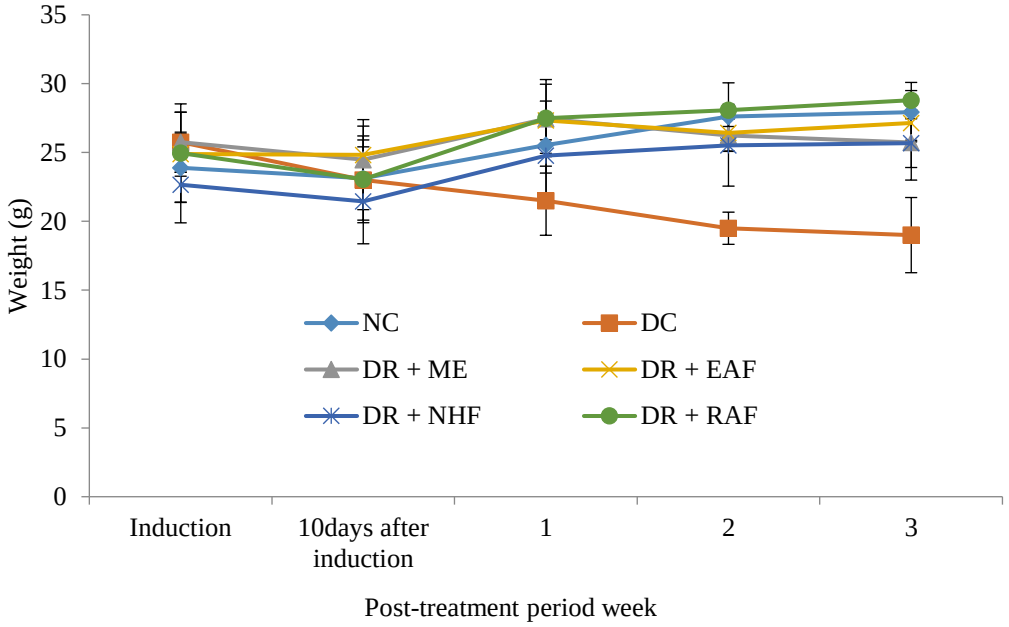

Figure 4. Effect of Crude Methanol Extract and Fractions of *E. conyzoides* on Weekly Body Weight

Table 3. Antidiabetic Activity of *E. conyzoides* Samples on Induced Type 2 Diabetic Mice

Group	Initial FBG (mg/dl)	Final FBG (mg/dl)	% Blood Glucose Change
NC	100.00 ± 0.00	100.50 ± 0.71	0.50 ± 0.71^{b}
DC	216.50 ± 0.71	321.50 ± 0.71	48.50 ± 0.01^{c}
DM + ME	213.50 ± 15.76	181.00 ± 28.81	-17.90 ± 10.9^{b}
DM + EAF	231.00 ± 17.20	235.00 ± 25.90	1.70 ± 21.93^{b}
DM + NHF	217.00 ± 24.45	210.00 ± 40.94	-3.33 ± 27.22^{b}
DM + RAF	232.75 ± 16.50	121.50 ± 15.43	-47.59 ± 13.69^{a}

Data are presented as mean ± Standard Deviation; Results down the same column with different superscript are statistically ($p<0.05$) different. NC: Normal control mice; DC: Diabetic control mice; DM: Diabetic mice; ME: 100mg/kg b.w methanol extract of *E. conyzoides;* EAF: 100mg/kg b.w ethyl acetate fraction of *E. conyzoides*; NHF: 100mg/kg b.w n- Hexane fraction of *E. conyzoides;* RAF: 100mg/kg b.w residual aqueous fraction of *E. conyzoides*

and were not treated) had a significant ($p<0.05$) decrease in body weight compared with normal control (i.e. not infected and not treated mice). Treatment with standard drug (metformin), crude methanol extract and different fractions of *E. conyzoides* significantly ($p<0.05$) increased the body weight.

Antidiabetic activity of crude methanol extract and fractions of E. conyzoides in induced type 2 diabetic mice

The result (Table 3) shows that the initial blood glucose level (mg/dl) of all the diabetic mice was higher when compared to normal control mice. Treatment with *E. conyzoides* extract and fractions significantly ($p<0.05$) lowers the blood glucose level of the diabetic mice.

Of all the treatment groups, the mice group treated with 100 mg /kg body weight (b.w) residual aqueous fraction gave the highest percentage (-47.59%) reduction in blood glucose level, followed by the 100 mg /kg b.w of methanol extract. This result revealed residual aqueous fraction has the highest activity.

This study showed that the level of FBG of the high fat diet/fructose water/streptozotocin-indu-

ced mice increased, indicating T2D. The residual aqueous fraction (100 mg/kg) gave a significant ($p < 0.05$) reduction in the blood glucose level to a normal level. The blood glucose decrease may be attributed to the presence of phytochemicals. Bansal *et al.* [37] reported that certain phytochemicals such as phenolics, alkaloids and flavonoids might be a factor in the decrease in blood glucose and the residual aqueous fraction is very rich in these phytochemicals compared to the other fractions.

Similar research by Tanayen *et al.* [38] also found the residual aqueous fraction to be the most effective antidiabetic fraction of the methanol extract and other fractions of *Spathodea campanulata* used in the treatment of diabetic albino rats. This concurred with earlier speculation that refining a crude extract might enhance the antidiabetic effects of a plant extract [39].

Conclusion

Ethulia conyzoides leaves extract and its fractions investigation showed that they are rich in phytochemicals with good antioxidant capacity; its residual aqueous fraction administered to STZ-induced type 2 diabetic mice showed significant antidiabetic effect after the treatment; hence, its ability to help in reverting the anomalies in the treated animals by the reduced the fluid intake, feed intake and FBG and improved the body weight.

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