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Research Article
Ageratum conyzoides Methanol Leaf Extract: Phytochemicals with Antidiabetic Potential via Antioxidant Activity

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Abstract

Background and Objective: *Ageratum conyzoides* L. is a traditional herbaceous plant that belongs to the family Asteraceae. The folk and traditional uses of the plant include sleeping sickness, anti-inflammatory, insecticidal, etc. The present study aimed at exploring the possible antidiabetic activity of *A. conyzoides* through its antioxidant potential in search of new hit compounds. **Materials and Methods:** The crude extract after maceration was subjected to solvent-solvent fractionation using chloroform, ethyl acetate and ethanol, in increasing order of polarity, followed by quantitative phytochemical estimation. Antioxidant activity was carried out using DPPH radical scavenging assay method. Antidiabetic activities were determined using alloxan-induced diabetic rats, 150 mg of alloxan per kg body weight was given intraperitoneally. All statistical analyses were performed using the SPSS statistical package with data reported as the Mean±SEM. **Results:** Ethyl acetate fraction had the highest value of TPC (46066.87±1350 mg GAE) and TFC (29912.50±6230 mg QuE) per gram dry samples respectively. Similarly, the ethyl acetate fraction had the best relative antioxidant activity (IC_{50} of 0.75 µg mL^{-1}). Both crude and the fractions given in different doses exhibited a good blood-glucose-lowering effect with statistically significant difference ($p<0.05$) between the means of the different groups (and control). The chloroform and ethyl acetate fractions had a better anti-diabetic effect as all administered doses had their average glucose level below the diabetic index (250 mg dL^{-1}). **Conclusion:** This research showed that *A. conyzoides* methanol leaf extract has high flavonoid and phenolic contents. It equally demonstrated good antioxidant and antidiabetic activities, especially the ethyl acetate fraction.

Key words: Antidiabetic, antioxidant, phytochemicals, *Ageratum conyzoides*, maceration, fractionation, flavonoids

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

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by the increased blood sugar level (hyperglycemia) due to low or non-utilization of glucose by body cells resulting from insulin resistance shown by the cells or due to insulin deficiency[1]. It is a growing global health concern that affects all age groups and genders. The debilitating effects of diabetes mellitus include various organ failures, progressive metabolic complications such as retinopathy, nephropathy and/or neuropathy[2]. Diabetes mellitus is usually accompanied by the risk of cardiovascular, peripheral vascular and cerebrovascular diseases. The two main types of diabetes mellitus are type I and type II. Type I diabetes results from the autoimmune-mediated destruction of insulin-secreting beta cells which often leads to ketoacidosis, while type II diabetes (Non-Insulin-Dependent Diabetes Mellitus (NIDDM)), is characterized by persistent hyperglycemia but rarely leads to ketoacidosis.

Treatment and management of diabetes mellitus include the use of drugs that act through different mechanisms to control the accumulation of blood sugar levels and insulin resistance along with changes in diet, as well as a regimen of exercise and a healthy lifestyle. Many a time, combinations of different classes of hypoglycemic drugs are used to increase the efficacy of the treatment and drugs that lower postprandial hyperglycemia could be drugs of choice[3,4]. However, these oral hypoglycemic drugs are not without several side effects, thus the need to beam searchlight on medicinal plants with abundant bioactive compounds for effective and alternative hypoglycemic substances in novel drug discovery and development. Medicinal plants have been known as rich sources of pharmaceutical agents for the prevention and treatment of diseases and ailments such as diabetes mellitus, cancer and cardiovascular diseases due to their antioxidant effects. Exploration of the phytoconstituents of traditional plants and pharmacological screening may provide to researchers in the field of ethnopharmacology the basis for developing the leads/hits for the development and discovery of novel agents of pharmaceutical value[5]. Thus, it is necessary to evaluate plant species for their antioxidant activities and use them against the management of diabetes mellitus. Hence, this research focused on the methanolic leaf extract of *Ageratum conyzoides*.

Ageratum conyzoides L. (Goatweed) is a traditional herbaceous plant with a long history of medicinal uses in several countries of the world which belongs to the family Asteraceae. It is native to tropical America. In Nigeria, *A. conyzoides* has different local names: Igbo-'Imi-esu', Hausa-'Ahenhen' and Yoruba-'Ula or Ujula'. It is traditionally used as purgative, febrifuge, anti-ulcer and wound dressing. According to Koto-te-Nyiwa *et al*.[6], *A. conyzoides* L. is a multipurpose herb (often called "miracle king grass") and will allow researchers to develop drugs of substitution (phytomedicines) at low cost. This plant contains many phytoconstituents which have various benefits in different aspects[7]. The folk and traditional uses of the plant include wound dressing, skin diseases, ophthalmic, colic, ulcers treatment, diarrhoea, dysentery, fever, gynaecological diseases, sleeping sickness, as well as anti-inflammatory and insecticidal, etc[6,8]. Thus, the present study is aimed at exploring the possible antidiabetic activity of *A. conyzoides* through its antioxidant potential in search of new hit compounds.

MATERIALS AND METHODS

Study area: This research work was carried out at Departments of Pharmaceutical and Medicinal Chemistry (Extraction Lab), Pharmacology and Toxicology (Bioactivity Assay Lab) and Pharmacognosy and Drug Development (Quality Control Lab) of Faculty of Pharmaceutical Sciences, Kaduna State University (KASU), Nigeria from September, 2019-August, 2021.

Plant materials

Collection and drying: The plant (*Ageratum conyzoides*) was collected and identified by Dr. Shehu Gallah Umar of Botany Department, Ahmadu Bello University Zaria, Kaduna Nigeria. Voucher Number: 1103. The plant leaves were then detached for onward drying. The detached leaf parts were shade dried under laboratory temperature for two weeks. The dried leaves were then pounded using Pestle and Mortar to obtain fine coarse particles to increase their surface area for solvent extraction. The weight was then weighed using electronic weighing machine.

Reagents/chemicals: All reagents used were of analytical grade. These include methanol, n-hexane, ethyl acetate, ethanol, ascorbic acid, metformin, alloxan hydrate, Folin-Ciocalteu reagent (FCR is a mixture of phosphomolybdate and phosphotungstate), 20% Sodium carbonate solution (Na_2CO_3), Gallic acid, 10% Aluminium chloride, potassium acetate, quercetin, concentrated hydrochloric acid, freshly prepared 4% w/v vanillin in methanol, catechin, chloroform, acetic anhydride, sulfuric acid, ethanol, β-sitosterol, 2,2-diphenyl-picryl-hydrazyl radical (DPPH), phosphate buffer (pH 4.7), 0.2N NaOH, 0.2N HCl, Atropin, extract samples and bromocresol green (BCG).

Extraction procedure: The maceration method was used for this study.

Soaking: The pounded leaf parts were soaked in 5.5 L of 70% methanol. After adding the solvent, they were vigorously stirred using VTCL Excella Mixer for even percolation of the solvent. They were then allowed to soak for 72 hrs.

Filtration: After 72 hrs of soaking, they were filtered using Whatman No 1 filter paper using a filtration funnel. The filtrates were collected and subsequently concentrated in an Electronic Water Bath at 50.1°C to obtain the desired dry extracts. The extract was then weighed and the percentage yield was determined.

Solvent-solvent fractionation of the methanolic crude extract: The crude methanol leaf extract (75 g) was subjected to solvent-solvent fractionation using chloroform, ethyl acetate and ethanol, in increasing order of polarity, after defatting with n-hexane. The extract was dissolved in a small portion of distilled water and poured into a separating funnel. Firstly, n-hexane and distilled were then added in the ratio of 60:40 mL. The separating funnel was then shaking properly and mounted on a retort stand. The n-hexane portion (upper layer) was collected in a petri-dish. The aqueous portion was reconstituted with another 100 mL of n-hexane to collect any remnant of n-hexane soluble constituents. Similar steps were equally followed for other solvents (chloroform, ethyl acetate and ethanol) by adding 100 mL of the respective solvents, in two stepwise circles, on aqueous portion in sequential other. The respective solutions of each solvent were concentrated on a water bath to get a fine fraction of each solvent to be used for the bioactivity study.

Quantitative phytochemical determination: The quantitative phytochemical analysis carried out include the determination of total phenolic, flavonoid, tannin, alkaloid and phytosterol contents.

Estimation of total phenolic content (TPC): The TPC was determined by employing the method involving the use of folin-ciocalteu reagent (FCR) as an oxidizing agent and gallic acid (GA) as the standard[9].

Experimental procedure: About 1 mL of extract sample solution and the various concentration of the standard GA were placed in separate test tubes. About 1 mL of dist.H_2O was added to each test tube. Then 1.5 mL of FCR was added to each content of the tube. The mixture is covered with aluminium foil and allowed to incubate at room temperature for 5 min. Afterwards, 4 mL of 20% (w/w) Na_2CO_3 was added to each mixture. It was then vortexed and placed in a water bath at a temperature of 40°C for 30 min. The test tubes were then removed and placed in ice water to quench the reaction. The absorbances of the test samples and standards were measured at 765 nm using UV/Vis spectrophotometer against blank. The blank contains everything except the standard/sample.

Estimation of total flavonoid content (TFC): The TFC was estimated using the aluminium chloride colorimetric method and quercetin as standard according to Kumar *et al.*[10].

Experimental procedure: About 1 mL of extract sample solution and the various concentration of the standard quercetin were placed in separate test tubes. This was followed by the addition of 1.5 mL of methanol (95%). Then 100 µL of 10% $AlCl_3$ reagent was added to each tube. Afterwards, 100 µL of 1M potassium acetate solution was added and finally, 2.8 mL of dist.H_2O was added to each test tube content. The mixture was then covered with Al foil and allowed to incubate at room temperature for 30 min. Absorbance was thereafter measured at 420 nm using UV/Vis spectrophotometer against the blank. The blank contains everything except the standard/sample.

Standard curve: A standard curve of the absorbance against the concentrations of the standard and used to estimate the quercetin equivalent (QE) of the test sample.

Estimation of total tannin content (TTC): The acidified Vanillin method of evaluation of tannin content was adopted for the estimation of the amount of tannin in the test sample with catechin as standard according to Ahmad *et al.*[11] and Ropiak *et al.*[12].

Experimental procedure: To each separate test tube wrapped with clean Al foil, 0.5 mL of test sample solutions and the various concentration of the standard (catechin) solution were pipetted. The 3 mL of vanillin reagent was added, followed by 1.5 mL conc. HCl in each test tube and mixed thoroughly. The reaction mixture was then allowed to incubate for 15 min at room temperature. Then the absorbances were measured at 500 nm using UV/Vis spectrophotometer against the blank. The blank contains everything except the standard/sample.

Standard curve: A standard curve of the absorbance against the concentrations of the standard and used to estimate the catechin equivalent (CE) of the test samples.

Determination of total alkaloid content (TAC): The TAC of the extracted sample was determined according to the process described by John *et al.*[13] using Atropine as the standard with little modification.

Experimental procedure: Suitable aliquots (20, 40, 60/80 and 100 µL) of the 1000 µL mL^{-1} of standard atropine stock solution and 1ml of sample solution were separately added in test tubes. To each of the test tube content, 1 mL of 0.2N HCl was added and their pH was adjusted to slightly alkaline with 0.2N NaOH. Then 3 mL of phosphate buffer of pH 4.7 was added to each tube. The mixture was vortexed and transferred to different separating funnels. To each funnel, 3 mL of BCG was added. The mixture was shaken vigorously and the complex was extracted or washed with 1-, 2- and 3 mL chloroform. The absorbance was then measured at 470 nm using UV/Vis spectrophotometer against the blank (chloroform).

Standard curve: A standard curve of the absorbance against the concentrations of the standard and used to estimate the atropin equivalent (AE) of the test sample.

Determination of total phytosterols (plant steroid) content: Because of the absence of standard (β-Sitosterol), a standard curve from Toivo *et al.*[14] was adopted.

Test samples preparation and analysis: About 2 mg/2 mL sample solution was suspended in a separating funnel and extracted with 5 mL chloroform (2 times). The chloroform portion (upper layer) was collected and 5 mL was transferred into a test tube. Then 2 mL of Liebermann-Burchard (LB) reagent (20 mL acetic anhydride+5 mL conc. Sulphuric acid in ice) was added. The mixture was vortexed and kept for 5 min before absorbance was measured at 625 nm.

Antioxidant activity of the plant samples: The determination of the antioxidant activity of the extract samples was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method according to Atoui *et al.*[15].

Preparation of standard: About 10 mL of ascorbic acid was weighed into a 10 mL volumetric flask. A small amount of methanol was added and then shaken to dissolution. The volume was then made up to the 10 mL mark with methanol. This gives an ascorbic acid solution of 1 mg mL^{-1} concentration as the stock solution. From this stock solution, 10, 25, 50, 75, 100 and 125 µL were taken and placed in six 10 mL volumetric flasks respectively. Each volume was made up to 10 mL with methanol, shaken and then stored for use.

Preparation of samples: About 10 mg of the crude extract sample was dissolved in 10 mL methanol. From the extract solution, 10, 25, 50, 75, 100 and 125 µL were respectively added into different volumetric flasks and made up to 10 mL capacity. They were shaking and ready for use.

The same preparation was equally carried out for chloroform, ethyl acetate and ethanol fractions from the crude.

Preparation of control: To 1 mL of methanol, 3 mL of 0.1 mM DPPH solution was added. The content was mixed thoroughly and kept in a dark cupboard.

Experimental procedure: About 1 mL each of the sample test concentrations and ascorbic acid standard were separately added inappropriately labelled test tubes. About 3 mL of the 0.1 mM DPPH solution was added to each test tube content and the mixture shook. They were then placed in a dark cupboard at room temperature for 30 min. Afterwards, absorbances were measured at 517 nm with UV/Vis spectrophotometer against blank. Note that the methanol constitutes the blank.

Note: Percentage inhibition (%) was calculated using:

$$\frac{Ac - As}{Ac} \times 100$$

Where:
Ac = Absorbance of the control
As = Absorbance of the sample

The Inhibition (%) was then plotted against the sample concentration. From the plot, IC_{50} was determined for each sample.

***In vivo* antidiabetic activity:** The antidiabetic activities of both crude extract and the partition fractions were determined using Alloxan, $C_4H_2N_2O_6.\ H_2O$ (KEM LIGHT Laboratory Put Ltd, Mumbai, India. CAS No. 2244-11-3) as the diabetogenic agent.

Induction of diabetes: Both male and female (non-pregnant) rats were selected randomly after 2 weeks of laboratory acclimatization. Before induction, the rats were starved for 24 hrs and 150 mg of alloxan per kg body weight was given intraperitoneally. Food and water were reintroduced after 1 hr of induction. After 48 hrs of induction of diabetes, the blood was tested for glucosuria using Accu-chek Glucometer and also after 72 hrs. Rats that showed blood glucose levels greater than 250 mg dL were selected and used for the study.

In vivo antidiabetic activity of the crude methanol leaf extract

Experimental design: Four groups (n = 4) were created and treated accordingly once a day for 28 days except for group one as follows:

- **Group1 (normal healthy control):** Given only vehicle (0.9% normal saline)
- **Group 2 (negative diabetic control):** Given only vehicle (0.9% normal saline)
- **Group 3 (positive diabetic control):** Given standard drug (metformin) only (125 mg kg^{-1} b.wt.)
- **Group 4:** Given crude extract in three divided doses of 50, 100 and 200 mg kg^{-1} b.wt.

In vivo antidiabetic activities of the solvent fractions

Experimental design: Six groups (n = 4) were created and treated accordingly once a day for 28 days except for group one as follows:

- **Group1 (normal healthy control):** Given only vehicle (0.9% normal saline)
- **Group 2 (negative diabetic control):** Given only vehicle (0.9% normal saline)
- **Group 3 (positive diabetic control):** Given standard drug (metformin) only (125 mg kg^{-1} b.wt.)
- **Group 4 (diabetic rats which receive chloroform fraction):** This fraction was given in three divided doses of 50, 100 and 200 mg kg^{-1} b.wt., respectively
- **Group 5 (diabetic rats which receive ethyl acetate fraction):** This fraction was given in three divided doses of 50, 100 and 200 mg kg^{-1} b.wt., respectively
- **Group 6 (diabetic rats which receive ethanol fraction):** This fraction was given in three divided doses of 50, 100 and 200 mg kg^{-1} b.wt., respectively

Statistical analysis: Data were reported as the Mean±SEM for triplicate determinations of each sample. Different samples were analyzed with analysis of variance, followed by the *Post hoc* Turkey test to identify differences between values. A $p<0.05$ was considered to be statistically different. All statistical analyses were performed using the SPSS statistical package, Version 16.0 (SPSS Inc., Chicago, IL).

RESULTS

Quantitative phytochemical determination

Estimation of total phenolic content (TPC): This result (Table 1) showed that the ethyl acetate fraction has the highest GA and TPC followed by chloroform fraction. However, the ethanol fraction had the least GA and TPC value.

Estimation of total flavonoid content (TFC): The ethyl acetate fraction also has the highest QU and TFC contents (Table 2) followed by a crude fraction. Unlike TPC, chloroform fractions had the list of QU and TFC values.

Estimation of total tannin content (TTC): In Table 3, all the fractions showed very low CT and TTC values, indicating that *A. conyzoides* methanol leaf extract has low tannin content.

Estimation of total alkaloid content (TAC): As in tannin content, Table 4 showed that all the fractions have very low AT and TAC values, thus indicating that *A. conyzoides* methanol leaf extract also has low alkaloid content.

Determination of total phytosterols (plant steroid) content (TPSC): All fractions similarly have low phytosterols (Table 5) content as indicated by the negative values for both BS and TPSC.

Table 1: Gallic acid equivalents and TPC of the crude and fractions

Samples	GA (mg mL^{-1})	TPC (mg GA g^{-1})
Crude extract	3.63±0.86	3630.00±860
Chloroform fraction	1.84±0.02	1836.70±20
Ethyl acetate fraction	4.61±1.35	46066.87±1350
Ethanol fraction	-2.74±0.64	-2743.33±640

Absorbance was taken at 765 nm and GA: Gallic acid

Table 2: Quercetin equivalents and TFC of the crude and fractions

Samples	QU (mg mL^{-1})	TFC (mg QU g^{-1})
Crude extract	14.54±4.38	14537.75±4380
Chloroform fraction	-1.75±0.06	-1750.00±60
Ethyl acetate fraction	29.91±6.23	29912.50±6230
Ethanol fraction	2.83±0.71	2833.75±710

Absorbance was taken at 420 nm and Qu: Quercetin

Table 3: Catechin equivalents and TTC of the crude and fractions

Samples	CT (mg mL^{-1})	TTC (mg CT g^{-1})
Crude extract	-7.75±1.68	-7746.60±1680
Chloroform fraction	-7.83±1.32	-7833.34±1320
Ethyl acetate fraction	-7.86±1.51	-7860.00±1510
Ethanol fraction	-7.75±1.08	-7753.40±1080

Absorbance was taken at 500 nm and CT: Catechin

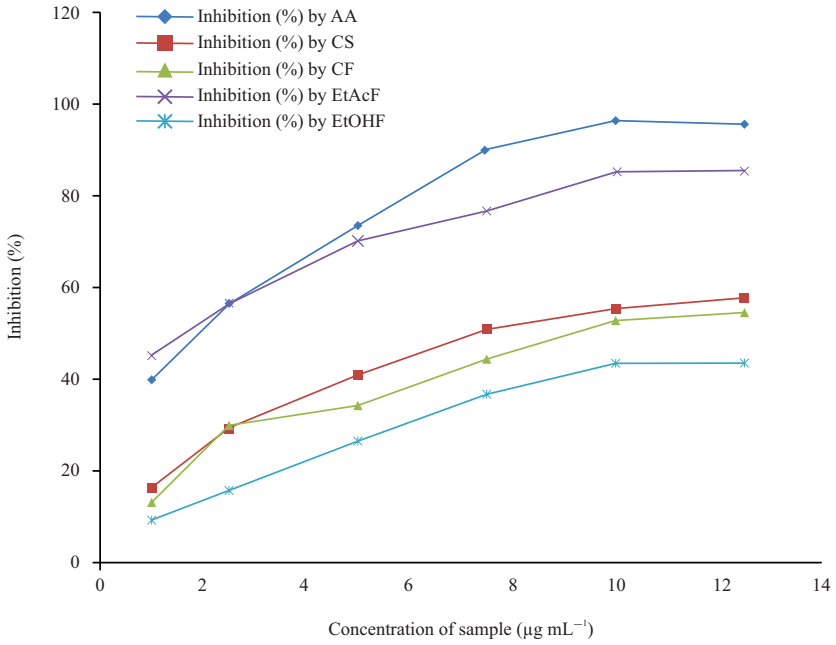

Fig. 1: Inhibition (%) of standard ascorbic acid, crude extract and different fractions

AA: Ascorbic acid, CS: Crude extract, CF: Chloroform fraction, EtAcF: Ethyl acetate fraction, EtOHF: Ethanol fraction, All absorbance was taken at 517 nm and Control absorbance = 0.464

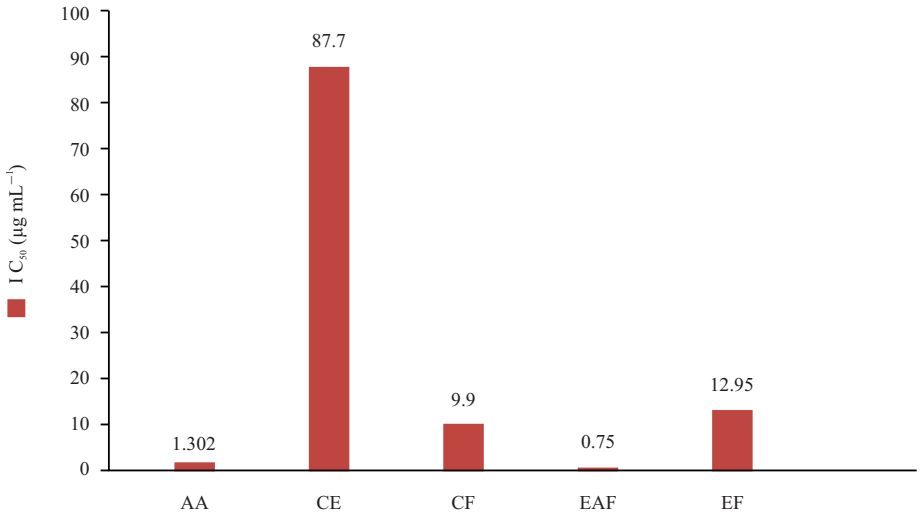

Fig. 2: Graph of IC_{50} of standard antioxidant, crude extract and different fractions

AA: Ascorbic acid, CE: Crude extract, CF: Chloroform fraction, EAF: Ethyl acetate fraction and EF: Ethanol fraction

Antioxidant activity of plant samples: From the antioxidant studies (Fig. 1 and 2), the ethyl acetate fraction had the best antioxidant activity (IC_{50} of 0.75 µg mL^{-1}) even in comparison with the standard antioxidant (ascorbic acid) (with IC_{50} of 1.302 µg mL^{-1}). The least antioxidant activity was recorded from the crude extract (with IC_{50} of 87.70 µg mL^{-1}).

Note: Percentage (%) inhibitions were calculated using:

$$\frac{Ac - As}{Ac} \times 100$$

Where:
Ac = Absorbance of the control
As = Absorbance of the sample

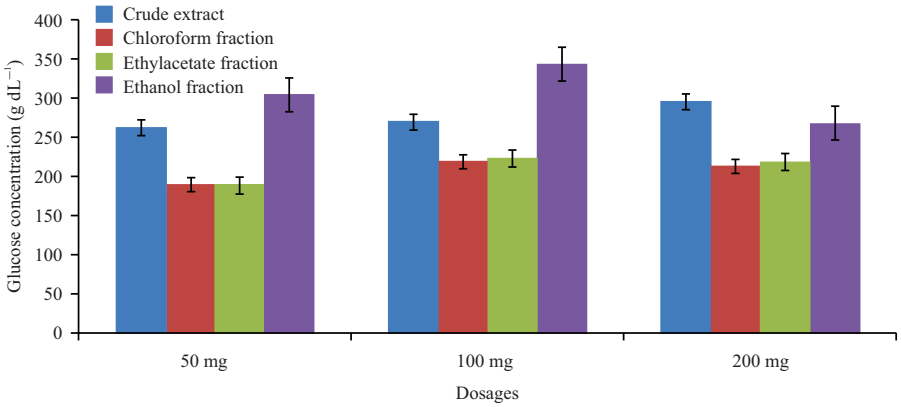

Fig. 3: Graph showing the effect of dosages on the average glucose level (g dL^{-1}) after 28 days of study

Table 4: Atropine equivalents and TAC of the crude and fractions

Samples	AT (mg mL^{-1})	TAC (mg AT g^{-1})
Crude extract	-0.34±0.08	-339.66±80
Chloroform fraction	-0.32±0.07	-319.04±70
Ethyl acetate fraction	-0.37±0.08	-366.66±80
Ethanol fraction	-0.38±0.08	-376.19±80

Absorbance was taken at 470 nm and AT: Atropine

Table 5: β-sitosterol equivalents and TPSC of the crude and fractions

Samples	BS (mg mL^{-1})	TPSC (mg BS g^{-1})
Crude extract	-0.24±0.02	-244.44±20
Chloroform fraction	-0.16±0.01	-155.55±10
Ethyl acetate fraction	-0.29±0.03	-294.45±30
Ethanol fraction	-0.30±0.03	-300.00±30

Absorbance was taken at 625 nm and BS: β-sitosterol

***In vivo* antidiabetic activity of the methanol leaf extracts:** The sugar level (in g dL^{-1}) of each rat was measured at every 4 days interval for 28 days for each of the study groups.

The mean value of glucose level was presented in Table 6 above. The analysis of variance between the crude methanol leaf extract and the control groups showed that the value of F is 4.577, which reaches significance with a p-value of 0.002 (which is less than the 0.05 confidence level). Thus, this means there is a statistically significant difference between the means of the different dosage treated groups and controls. A *Post hoc* Turkey test showed that the healthy control was statistically significant than diabetic negative control ($p = 0.001$) and that of 200 mg crude treatment ($p = 0.033$). However, there were no statistically significant differences between any other groups as all their p-values are above 0.05 (95% confidence value).

From Table 7, comparing the extract fractions and control groups also showed a statistically significant mean difference as demonstrated by the one-way ANOVA [$F(11, 72) = 7.193$, $p = 0.000$]. A *Post hoc* Tukey test showed that 13 pairs were statistically significant. Similarly, by comparing the effect of dosages on glucose level, there was a statistically significant difference between different dosages of the crude and extract fraction treated groups as demonstrated by the one-way ANOVA [$F (11, 72) = 2.339$, $p = 0.016$]. While a Tukey *Post hoc* test showed that only 50 mg ethyl acetate and 100 mg ethanol ($p = 0.016$) pair had statistically significant mean differences.

In Fig. 3, the three different dosages administered indicated that both ethyl acetate and chloroform fractions had lowered the average glucose concentrations below the diabetic index (250 g dL^{-1}) at the end of 28 days study unlike that of crude and ethanol fraction.

DISCUSSION

The result of the quantitative phytochemical analysis showed that both the crude extract and their fractions had high TPC and TFC but a very low TTC, TAC and TPSC. This indicates that the *A. conyzoides* leaf has high flavonoid and total phenolic contents, but very low tannin, alkaloid and phytosterol contents.

From the antioxidant studies, the ethyl acetate fraction had the best antioxidant activity (IC_{50} of 0.75 µg mL^{-1}) even in comparison with the standard antioxidant (ascorbic acid) (with IC_{50} of 1.302 µg mL^{-1}). The least antioxidant activity was recorded from the crude extract (with IC_{50} of 87.70 µg mL^{-1}) (Fig. 2). The high radical scavenging effects of the fractions compared to the crude indicate that as the samples are purified, the biological activity could be improved. These antioxidant activities could also be due to the presence of phytochemicals such as flavonoids and phenol which was high on both the crude extract and the fractions, especially on the ethyl acetate fraction. And flavonoids being modulators of γ-glutamylcysteine synthase could be involved in both cellular antioxidant defenses and detoxification of xenobiotic substances.

Table 6: Glucose (g dL^{-1}) level after treatment with the crude methanol leaf extract

Days	0	4	8	12	16	20	24	28
Group 1								
Mean±SEM (g dL^{-1})	120.33±74.26	106±8.54	102.33±9.94	111.33±12.67	100±3.00	115.33±7.80	116.67±6.44	108.67±5.78
Group 2								
Mean±SEM (g dL^{-1})	473.5±141.00	545.33±43.32	483±32.00	344±35.00	366.5±63.50	357.5±5.50	295±18.00	290.5±15.50
Group 3								
Mean±SEM (g dL^{-1})	513.25±123.00	374±31.21	428±74.22	382±9.07	122.33±4.70	169.33±7.31	173±6.11	200.67±12.81
Group 4 (50 mg)								
Man±SEM (g dL^{-1})	794.25±93.75	418.67±60.99	313±24.06	222.33±9.49	191±11.93	179.67±6.36	181.33±12.17	188±23.13.65
Group 4 (100 mg)								
Mean±SEM (g dL^{-1})	888±0.00	528.67±35.67	280±88.00	229±29.21.00	264.5±28.50	280	250	257
Group 4 (200 mg)								
Mean±SEM (g dL^{-1})	888±0.00	702±43.50	392.5±31.00	298±16.50	272.5±4.00	257±5.6.50	242.5±5.00	236±7.07

Table 7: Glucose (g dL^{-1}) level after treatment with partitioned fractions

Days	0	4	8	12	16	20	24	28
Group 1								
Mean±SEM (g dL^{-1})	120.33±74.26	106±8.54	102.33±9.94	111.33±12.67	100±3.00	115.33±7.80	116.67±6.44	108.67±5.78
Group 2								
Mean±SEM (g dL^{-1})	473.5±141.00	545.33±43.32	483±32.00	344±35.00	366.5±63.50	357.5±5.50	295±18.00	290.5±15.50
Group 3								
Mean±SEM (g dL^{-1})	513.25±123.00	374±31.21	428±74.22	382±9.07	122.33±4.70	169.33±7.31	173±6.11	200.67±12.81
Group 4 (50 mg)								
Mean±SEM (g dL^{-1})	408.5±56.61	165±34.39	194.33±37.56	218.33±27.36	196.33±34.33	225±20.26	201±33.71	127±17.47
Group 4 (100 mg)								
Mean±SEM (g dL^{-1})	382.75±49.40	337.25±31.88	197.33±15.96	207.33±25.91	213±29.16	189.33±16.74	180±9.54	171.67±19.88
Group 4 (200 mg)								
Mean±SEM (g dL^{-1})	420.5±49.75	254.33±23.95	197.33±15.30	211.67±23.38	225.67±8.97	216.33±21.48	190±14.29	180.67±5.24
Group 5 (50 mg)								
Mean±SEM (g dL^{-1})	508.25±134.32	257.5±48.34	199.25±19.74	240±68.07	185.33±20.61	165.33±26.61	160.67±27.83	144±15.31
Group 5 (100 mg)								
Mean±SEM (g dL^{-1})	409.75±42.07	323.75±67.78	295.5±41.38	228±42.19	170.33±20.30	214.33±49.14	198.33±21.31	148±27.30
Group 5 (200 mg)								
Mean±SEM (g dL^{-1})	477.5±143.03	296±83.74	208.67±64.15	245.33±17.89	198.67±28.34	184.67±16.48	172±18.56	151.33±15.39
Group 6 (50 mg)								
Mean±SEM (g dL^{-1})	732.5±90.08	488±41.24	375±11.00	413.5±65.50	305	262	243	236
Group 6 (100 mg)								
Mean±SEM (g dL^{-1})	603±98.56	508±37.07	383±25.00	438±112.00	307	286	263	258
Group 6 (200 mg)								
Mean±SEM (g dL^{-1})	525.25±50.41	385.33±45.00	353±49.49	331.67±58.61	238.5±9.50	180.5±6.50	187±55.00	292.00

Following antidiabetic studies using alloxan-induced diabetic rats, results showed that there is a statistically significant difference between control groups and the crude as demonstrated by one-way ANOVA [F (5,36) = 4.577, p = 0.002] (Table 6). A *Post hoc* Tukey multiple comparison tests also showed that the Healthy Control was statistically significant than Diabetic Negative Control (p = 0.001) and that of 200 mg crude treatment (p = 0.033). However, there were no statistically significant differences between any other groups as all their p-values are above 0.05 (95% confidence value). Similarly, in comparing the control groups and the fractions, there was a statistically significant mean difference as demonstrated by the one-way ANOVA [F (11, 72) = 7.193, p = 0.000] (Table 7). A Post Hoc Tukey test showed that 13 pairs were statistically significant. Also by comparing the effect of dosages on glucose level, there was a statistically significant difference between different dosages of the crude and extract fraction treated groups as demonstrated by the one-way ANOVA [F (11, 72) = 2.339, p = 0.016]. While a *Post hoc* Turkey test showed that only 50 mg ethyl acetate and 100 mg ethanol (p = 0.016) pair had statistically significant mean differences.

Thus, this work demonstrated that crude methanolic leaf extract of *A. conyzoides* as well as the solvent-solvent partition fractions, have a significant antidiabetic effect on alloxan-induced diabetic rats when compared to metformin standard drug (Table 6 and 7). However, comparing the average dosage effect (Fig. 3) of the extracts indicated that the 50 mg dosages of both chloroform and ethyl acetate had the best glucose-lowering effect, followed by the 100 mg and

200 mg dose of the same chloroform and ethyl acetate fractions (Fig. 3). Also, the crude dosage had a better glucose-lowering effect than the ethanol fraction. This could stem from the fact that ethyl acetate extracted more flavonoids and/or phenols which was demonstrated from the high TFC and TPC as well as high DPPH radical scavenging activity (IC_{50} of 0.75 µg mL^{-1} compared to IC_{50} of ascorbic acid, 1.302 µg mL^{-1}) (Fig. 2). Because of this high TPC and TFC as well as high DPPH radical scavenging activity, *A. conyzoides* methanolic leaf extract could be highly effective in the treatment of debilitating diseases such as diabetes mellitus, cancers, CVDs and many free radical aggravating illnesses like ageing.

In support of our findings, other researchers have equally demonstrated the antioxidant as well as the antidiabetic efficacy of this plant. For instance, Koto-te-Nyiwa Ngbolua *et al.*[6] reported that ethanol extract of the plant leaves possesses a significant dose-dependent DPPH free radical scavenging activity with an IC_{50} value of 18.91 µg mL^{-1} compared to ascorbic acid (IC_{50}: 2.937 µg mL^{-1}). In another work, Fatema[16] and Shekhar and Anju[17] reported that the phenol content of *A. conyzoides* had antioxidant potential in diverse models of studies. Their work also evaluated the reducing as well as the proton donating ability of a methanol extract of the stem by DPPH assay which indicated that the IC_{50} value of methanolic extract followed a similar trend with the standard ascorbic acid. Also by using DPPH assay, Hossain *et al.*[18] examined the scavenging effect and activity of the ethanolic extract of *A. conyzoides* leaves and obtained an IC_{50} value of 18.9 µg mL^{-1}, in comparison with ascorbic acid and butylated hydroxyanisole which had an IC_{50} value of 2.937 and 5.10 µg mL^{-1}, respectively. Similarly, Nyunaï *et al.*[19] and Gnagne *et al.*[20] have reported hypoglycemic/glucose-lowering from the aqueous extract of *A. conyzoides* leaves in normoglycaemic and streptozotocin-induced diabetic rats. Agunbiade *et al.*[21] has equally documented that the aqueous leaf extract of *A. conyzoides* had hypoglycemic activity in the tested animals. Distilled water and ethyl acetate leaf extracts of *A. conyzoides* have also been found to have a glucose-lowering effect in Streptozotocin induced diabetic rats[22]. *Ageratum conyzoides* L. also has a positive effect on the redox system when it is exposed to diabetic rats induced by streptozotocin while increasing the glycaemic status of those rats[7].

From this study, *A. conyzoides* methanol leaves extract being, a good source of flavonoids as well as phenols with significant radical scavenging and hypoglycemic activities, should not be seen as a grass weed but its high flavonoid and phenolic contents should be taken advantage of. Also stemming from its abundant in different regions of the world, especially the temperate regions, it will be a good cheap source of starting materials for lead compound isolation for drug discovery and development in search of low-cost pharmacological agents, especially for the management and treatment of chronic diseases such as diabetes, CVDs and other debilitating radical aggravating illnesses. So it is recommended that the ethyl acetate fraction of this methanol leaf extract be subsequently subjected to further purification and isolation processes through HPLC, LC-MS and NMR to isolate and identify the active phenolic compound(s) that could be responsible for the radical scavenging and hypoglycemic activities of the extract. Also, the toxicological studies of the extract should be equally investigated to ascertain the safety of the plant.

CONCLUSION

This research showed that *A. conyzoides* methanolic leave extracts have high flavonoid and phenolic contents. It equally demonstrated good antioxidant and antidiabetic activities. As various researchers have documented its ethnomedicinal potentials, this plant could be a very good low-cost alternative source of phytoconstituents for the development of drugs for the treatment of various debilitating diseases such as diabetes mellitus, cancers, CVDs and many free radical aggravating illnesses like ageing. Thus, the need to isolate and test the flavonoids and/or phenols responsible for the antioxidant and the hypoglycaemic effect of this leaf extract.

SIGNIFICANCE STATEMENT

This study discovered that the more the *A. conyzoides* methanol leaf extract is fractionated, the better for both radical scavenging and antidiabetic activities of the ethyl acetate fraction, unlike most studies which focused mostly on the crude extract. This will certainly help researchers to focus on the ethyl acetate fraction to be able to identify the phenolic compound (s) responsible for the extract activities. Thus, the ethyl acetate fraction could be possibly used in ethnomedicinal practices for the management and treatment of diabetes especially type 2.

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