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Original Article

In-vitro antioxidants activities, alpha-amylase and alpha-glucosidase inhibitory effects of phenolic-rich extract of ageratum conyzoides

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ABSTRACT

The *in-vitro* antioxidants activities, phenolics contents, alpha-amylase and alpha-glucosidase inhibitory effects of phenolic-rich extract of *Ageratum conyzoides* whole plants (PRAC) were determined using standard methods. Phenolics contents are in following order; total phenol > tannins > flavonoids. The maximum inhibitions of PRAC were recorded in 1000.00 µg/mL in both α-glucosidase and α-amylase (75.00 % and 67.37 %) while 100 µg/mL exhibits minimum inhibition (43.18 % and 38.80 %) respectively. The PRAC showed high ferric reducing antioxidant power (FRAP) which showed no significant difference between FRAP of gallic acid when compared with that of PRAC at concentrations 250, 500 and 1000 µg/mL. However, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities of phenolic-rich extract of *A. conyzoides* increased as the concentration increases but not comparable with gallic acid. The phenolic-rich extract of *Ageratum conyzoides* whole plants showed better alpha-amylase and alpha-glucosidase inhibitory effects as well as antioxidants activities. Therefore, the phenolic-rich extract of *Ageratum conyzoides* whole plants could be explored further as antioxidant and antidiabetic agent.

Keywords: Antioxidants, Phenolics, Alpha-amylase, Alpha-glucosidase, *Ageratum conyzoides*

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INTRODUCTION

Diabetes mellitus is one of the non-infectious diseases that is associated with many serious life-threatening complications which result into high health care costs, reduction of quality of life, high morbidity and mortality. The IDF has estimated that 463 million adults live with diabetes worldwide (9.3%) in 2019, with a projected increase to about 800 million (10.9%) by 2045 [1]. Seventy-nine percent (79%) of those with diabetes live in low- and middle-income countries (LMICs). It is projected that diabetes cases will increase by 149% in sub-Saharan African countries in the next two decades, from 19 million in 2019 to 26 million by 2045 [2].

According to International Diabetes Federation (IDF)3.9 million adults lived with diabetes in 2019 and projected to be almost double (6.0 million) by 2045 [3]. Studies, including a national survey, a systematic review and meta-analysis, community-based and other epidemiological reports, showed that the prevalence of diabetes among adults had increased substantially in Nigeria, from ~ 2.2% in 1992 to ~ 10.5% in 2022 [4]. In adult, type II Diabetes mellitus is more common compared to type I Diabetes mellitus and it is expected to reach 366 million cases in the year 2030.

Some of the available drugs for the management of diabetes have limited efficacy, inadequate tolerability and/or significant mechanism-based side effects [5]. One of the therapeutic approaches for treating type 2 diabetes is to reduce postprandial hyperglycaemia through the inhibition of carbohydrate hydrolyzing enzymes such as alpha-glycosidase and alpha-amylase [6]. Amylase inhibitors are called starch blockers because they prevent dietary starches from being hydrolyzed and absorbed by the tissues [7]. The use of phyto-therapy is gaining popularity in the treatment of type 2 diabetes and its complications [8].

Ageratum conyzoides (Plate 1) which is also known as goat weed is a common weed the is widely distributed all over Nigeria. Folklorically, it is being used for the treatment of gynecological diseases, sleeping sickness, mouthwash, anti-inflammatory, insecticides, wound dressing, skin diseases, ophthalmic, colic, ulcers treatment, diarrhea, dysentery and fever [9]. The plant has also been well documented to also have pharmacological activities that includes anti-inflammatory activity, antidiabetic activity, spasmolytic effects, gamma radiation protective effects, anti-cancer activity, analgesic activity, antimicrobial activity, antioxidants activity as well as antimalarial activity [9]. Phenolics are phytochemicals that are known for antioxidants, antimicrobial, antidiabetic, anti-inflammatory, anti-cancers activities [10, 11]. This study is aim at determining the in-vitro antioxidants activities, alpha-amylase and alpha-glucosidase inhibitory effects of phenolic-rich extract of ageratum conyzoides whole plant.

Plate 1: *Ageratum conyzoides*

MATERIALS AND METHODS

Plant collection

The whole plant of *Ageratum conyzoides* were collected within the premises of Bosso Campus, Federal University of Technology Minna, Nigeria in the Month of September, 2025. The plant was authenticated by Dr. O.A.Y Duadu; A Senior Botanist in the Department of Plant Biology, Federal University of Technology, Minna. The voucher number FUT/PLB/ASTE/006 was allotted and the specimen was deposited in the herbarium of the Department for reference.

Chemicals and reagents

The chemicals and reagents used were of analytical grade which were obtained from Sigma-Aldrich Chemical Company St. Louis, USA. The reagents include methanol, acetone, ethanol, quercetin, tannic acid, gallic acid, alpha-amylase, alpha-glucosidase, ferric chloride among others.

Sample preparation

The roots of the whole plants of *Ageratum conyzoides* were cut off, the plants were washed, sliced into pieces and dried under room temperature. The dried plants were milled using electric blender (Binatone BLG 450, United Kingdom). The powdered sample was kept in an air tight plastic container at room temperature (28 $\pm$ 2) °C until further use

Extraction of phenolics from *Ageratum conyzoides*

Method described by Busari et al. [10] was adapted for the extraction of phenolic-rich extract. Fifty grams (50 g) of the powdered sample was placed in a round bottom flask and reflux with 400 mL of 40 % acetone and 60 % methanol mixture at 50°C for 4 hours. Thereafter, the extract was filtered with muslin cloth after which the methanol/acetone solvent were evaporated at low pressure in a rotary vacuum evaporator. The phenolic-rich filtrate of *Ageratum conyzoides* obtained was lyophilized to obtain phenolics rich extract which was stored at -4°C.

Quantification of Phenolic Compounds

Quantification of total phenol

Total phenol content of the extract was quantified as described by Busari *et al*. [10]. Briefly, exact 0.5 mg/mL of either extract was dissolved in methanol and each of the dissolved sample was mixed with exact 2.9 mL of 2 %Na_2CO_3. The mixture was incubated in a laminar flow at room temperature for two minutes followed by addition of 0.1 mL of 10% *F*olin-Ciocalteau reagent. The mixture was then incubated in a laminar flow for another 30 min at 37 °C. The absorbance of the reaction mixture was taken at 750 nm with a Shimadzu UV-Spectrophotometer UV-1800 model (Japan). Exactly 0.5 mg/mL of gallic acid in methanol was used as standard. The phenolics content of both extracts were expressed as milligram gallic acid equivalents (mg gallic acid/g extract). All samples were analyzed in triplicates.

Quantification of flavonoids

The flavonoids content was quantified as described by Busari et al. [10] using spectrophotometric techniques. About 0.5 mg/mL of the extract and quercetin were prepared in absolute methanol and 250 µL

of each of mixture was mixed with deionized water (1.6 mL) and 100 µL of 5% Na_2CO_3**.** The samples were left for 6 min after which 150 µL of 10 % $AlCl_3$ solution was pipetted in to the mixture followed by 5 min incubation at 37 ℃. About 500 µL of 1 M NaOH was added to halt the reaction followed by 15 min incubation at 37 ℃. The absorbance of the resulted mixture was read at 510 nm using a Shimadzu UV-Spectrophotometer against the blank (contained 250 µL of absolute methanol instead of the extract or quercetin). The 0.5 mg/mL of quercetin in methanol was used as standard. The flavonoids content was quantified as milligram quercetin equivalents (mg quercetin/g extract).

Quantification of tannins

The procedure used in determining the tannins contents was described by Busari et al. [10]. Exactly 0.2 g of the extract was weighed into a 50 mL beaker followed by addition of 20 mL of 50 % methanol. The beakers were covered with aluminum foil and placed in a water bath with shaker at 80 ºC for 1 hour. The extract was allowed to cool, filtered with double layered Whatman No.41 filter paper into a 100 mL volumetric flask with addition of 20 mL of water followed by the addition of Folin-Denis reagent (2.5 mL) and 17 %Na_2CO_3 (10mL). Water was added to the mixture to100 mL volume and allowed to stand for 20 mi for colour development after proper mixing; the absorbance was read against blank with spectrophotometer at 760 nm. The same procedure was followed for tannic acid standard. The tannins content was quantified as milligram quercetin equivalents (mg tannic acid/g extract).

Determination of alpha-glucosidase inhibition of phenolic-rich extract of *A. conyzoides*

The method adopted by Khan *et al*. [12] was used to measure the inhibition of alpha-glucosidase. Crude alpha-glucosidase solution was prepared by homogenizing the scraped mucosa of the small intestine of sacrificed rats in cold sodium phosphate buffer (pH 6.8) and centrifuging the solution. Thereafter, 0.1 mL of the extract or acarbose was incubated with 0.1 mL of the mucosa solution (enzyme source) in 1.0 mL of 2 mM phosphate buffer (pH 6.9) for 20 minutes. Afterwards, 0.1 mL of 3 mM para-nitrophenyl glucopyranoside (prepared in 20 mM phosphate buffer of pH 6.9. The mixture was then left for 15 minutes before addition of 0.5 mL of 5.0% sodium carbonate followed by incubation for 90 minutes, and the absorbance was read at 450 nm.

The percentage Inhibition of alpha-glucosidase by the extract was calculated as follow:

Absorbance of the standard-Absorbance of the extract/ Absorbance of the standard x100

Determination of alpha-amylase inhibition of phenolic-rich extract of *A. conyzoides*

The method adopted by Khan *et al*. [12] was used which involves using the Dinitro salicylic acid reagent and measuring absorbance at 540 nm. Precisely 0.1 mL of the extract or acarbose with 0.05 mL of 1% alpha amylase solution in 1.0 mL of 2 mM phosphate buffer (pH 6.9) for 20 minutes. Subsequently, 0.1 mL of a freshly prepared 1.0% starch solution was added, and the

mixture was allowed to stand for 5 minutes. Afterwards, 0.5 mL of Dinitro salicylic acid reagent was added, and the solution was held in boiling water for 5 minutes. Then, the solution was cooled, and the absorbance was read at 540 nm.

The percentage Inhibition of alpha-amylase by the extract was calculated as follow:

Absorbance of the standard-Absorbance of the extract/ Absorbance of the standard x100

Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay of the phenolic-rich extract was determined by spectrometric method as described by Yunusa *et al*. [13]. Briefly, different concentrations of the plant extracts and Gallic acid (250 – 1000 µ/mL) in 1mL of distilled water. The prepared sample were mixed with phosphate buffer (3.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixtures were incubated at 50 ºC for 20 minutes. Then, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, then centrifuged for 10 minutes at 12,000 x g. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was read at 700 nm against a blank with spectrophotometer.

The percentage FRAP was calculated using the following expression;

% Inhibition = $(A_{blank} - A_{sample} / A_{blank})$ x 100

Determination of Scavenging Activity Against 1,1-Diphenyl-2-Picryl Hydrazyl Radical

The DPPH Scavenging activity of the phenolics-rich extract and its fractions was determined by spectrometric method as described by Yunusa *et al*. [13]. Briefly, 1mL of 125 - 1000µ/mL of phenolics-rich extract and gallic acid were prepared in separate test tubes using absolute methanol. Afterwards, 2mL of 0.004% DPPH solution in methanol was added. Each of the test samples were properly mixed and incubated at 25°C for 30 minutes. The absorbances of the test mixtures were read against the reagent blank at 517 nm using a spectrophotometer.

The percentage DPPH scavenging activities was calculated using the following expression;

% Inhibition = $(A_{blank} - A_{sample} / A_{blank})$ x 100

Data analysis

The analysis of variance (ANOVA) followed by *Post hoc* Duncan multiple comparisons test (Statistical Package for Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA) at 95% confidence interval was used on the data obtained with *p*-value less than 0.05 was considered significant difference. The data were expressed as mean $\pm$ standard error mean of five replicates.

RESULTS

Phenolics Contents

Table 1 shows the result of phenolic compounds in the phenolic-rich extract of *A. conyzoides* whole plant. The phenolic compounds present are in the following order total phenol > tannins > flavonoids

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Table 1: Phenolics Contents

Phenolics	mg/g
Total Phenols	94.60 ± 4.20
Flavonoids	36.12 ± 2.60
Tannins	68.80 ± 3.40

Values are Means of triplicates ± Standard Error Mean.

Alpha-glucosidase inhibition of phenolic-rich extract of *A. conyzoides*

The alpha-glucosidase inhibition of phenolic-rich extract of *A. conyzoides* whole plant is represented in Table 2. The maximum inhibition is recorded in 100.00 µg/mL and the inhibition increase as concentration increases while minimum inhibition occurred in 100 µg/mL.

Table 2: Alpha-glucosidase inhibition of phenolic-rich extract of *A. conyzoides* whole plant

Concentration µg/mL	% Inhibition of α-Glucosidase
100.00	43.18 ± 2.27
200.00	50.00 ± 4.55
400.00	56.82 ± 3.10
600.00	63.32 ± 2.84
800.00	69.32 ± 1.14
1000.00	75.00 ± 2.27

Values are Means of four replicates ± Standard Error Mean.

Alpha-amylase inhibition of phenolic-rich extract of *A. conyzoides* whole plant

The alpha-amylase inhibition of phenolic-rich extract of *A. conyzoides* whole plant is shown in Table 3. The maximum inhibition is also recorded in 1000.00 µg/mL just as exhibited in the inhibition of alpha-glucosidase with increase inhibition as concentration increases while the lowest concentration of 100 µg/mL exhibits minimum inhibition.

Table 3: Alpha-amylase inhibition of phenolic-rich extract of *A. conyzoides* whole plant

Concentration µg/mL	% Inhibition of α-Amylase
100.00	38.80 ± 2.13
200.00	42.37 ± 3.47
400.00	51.04 ± 4.02
600.00	56.14 ± 4.12
800.00	61.75 ± 3.47
1000.00	67.37 ± 2.12

Values are Means of four replicates ± Standard Error Mean.

Ferric reducing antioxidant power of phenolic-rich extract of *A. conyzoides* whole plant

The Ferric reducing antioxidant power of phenolic-rich extract of *A. conyzoides* whole plant is presented in Figure 1.

Except for the lowest concentration (125 µg/mL), there is no significant difference between ferric reducing antioxidant power of gallic acid which is the standard antioxidant compared with phenolic-rich extract of *A. conyzoides* whole plant at concentrations 250,500 and 1000 µg/mL.

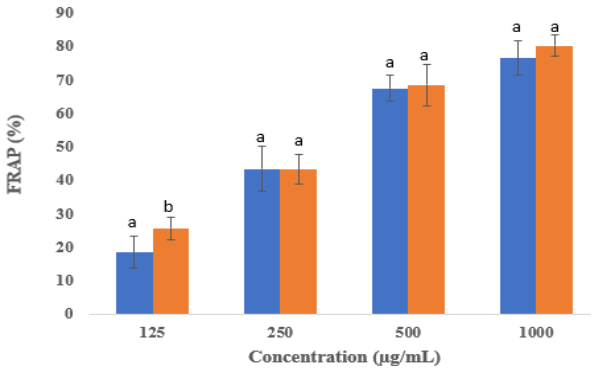

Figure 1: Ferric reducing antioxidant power of phenolic-rich extract of *A. conyzoides* whole plant

The DPPH scavenging activities of phenolic-rich extract of *A. conyzoides* whole plant

The DPPH scavenging activities of phenolic-rich extract of *A. conyzoides* whole plant

is presented in Figure 2. There is significant difference between DPPH scavenging activities of gallic acid when compared with phenolic-rich extract of *A. conyzoides* whole plant at all concentrations. Although, the DPPH scavenging activities of phenolic-rich extract of *A. conyzoides* is quite close to that of gallic acid. However, the DPPH scavenging activities of phenolic-rich extract of *A. conyzoides* increased as the concentration increase.

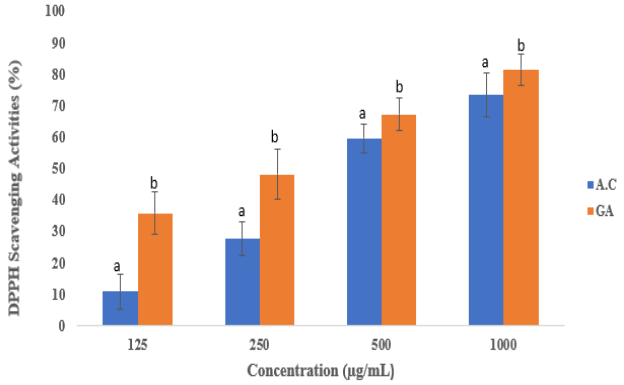

Figure 1: DPPH scavenging activities of phenolic-rich extract of *A. conyzoides* whole plant

DISCUSSION

Phenolic compounds have been reported to have a wide range of medicinal properties [14]. Phenolic compounds such as flavonoids, phenol and tannins found in phenolic-rich extract of *Ageratum conyzoides* might be responsible for inhibitors of α-amylase and α-glucosidase. This could help in slowing down carbohydrate digestion and reduce postprandial blood sugar levels. This result is in tandem with previous report which stated the ability of flavonoids to bind to and block carbohydrate-hydrolyzing enzymes, specifically α-amylase and α-glucosidase [15]. This action of flavonoids could delay the breakdown of complex starches into simple glucose which lead to sharp rises in blood glucose after meal [16].

Similar to other beneficial plant extracts, specific phenolics such as flavonoids, tannins and other isorhamnetin derivatives often show a more pronounced inhibitory action on α-glucosidase than on α-amylase as shown in this study. This pattern of inhibition is ideal because mild α-amylase suppression do assist the severe gastrointestinal side effects such as flatulence and bloating

which constantly caused by synthetic drugs. Similar phenolic and total flavonoid contents of *Perilla frutescens* seed extracted with ethanol also showed potential antioxidant, alpha amylase and alpha glucosidase inhibitors at different concentrations [15]. Likewise, Delgado-Alvarado *et al.* [17] also reported such similar inhibitions α-amylase and α-glucosidase by four foliar extracts from *Pinus* species which include *A. conyzoides.*

The high phenols, tannins and flavonoids content in the extract also provides strong antioxidant properties exhibited by the extract as obtained in results in the DPPH scavenging activities and FRAP. Therefore, the strong antioxidant effects displayed by the extract will make the phenolic-rich extract from *A. conyzoides* a best candidate which could help in combating the oxidative stress that is commonly associated with type 2 diabetes [18]. In addition, other type 2 diabetes complications such as inflammations, retinopathy and others could be corrected by phenolic compounds.

CONCLUSION

Phenolic-rich extract of *Ageratum conyzoides* showed excellent *in-vitro* antioxidants activities, alpha-amylase and alpha-glucosidase inhibitory effects. Therefore, phenolic-rich extract of *Ageratum conyzoides* can be explored further to obtain the no-toxic or less toxic, less expensive and accessible antidiabetic agents.

Authors' contributions

Authors BMB and ANU designed and conceptualized the study; DOAY Identified the plant; **MS,** BMB and HRU participated in the extraction, data collections and data analysis; **MMI, OA A, ON, ADA, OSO** performed the laboratory analysis; BMB and ANU interpreted the data; BMB, prepared the first draft of the manuscript, reviewed by ANU, DOAY and HRU. All authors contributed to the development of the final manuscript and approved its submission.

Conflict of interest

None

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