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ORIGINAL ARTICLE

Identification of Antidiabetic Compounds from Polyphenolic-rich Fractions of *Bulbine abyssinica* A. Rich Leaves

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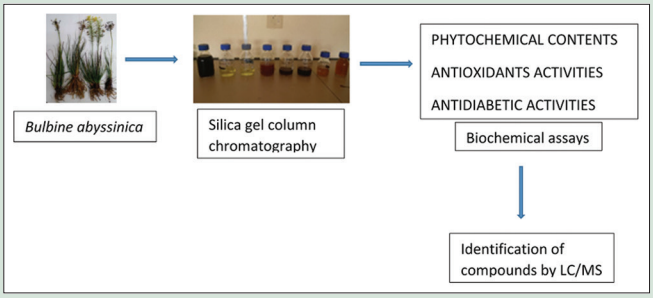
ABSTRACT

Background: *Bulbine abyssinica* has been reported to possess a variety of pharmacological activities traditionally. Previous work suggested its antidiabetic properties, but information on the antidiabetic compounds is still lacking. **Objective:** The present research exertion was aimed to isolate and identify biologically active polyphenols from *B. abyssinica* leaves and to evaluate their efficacy on carbohydrate digesting enzymes. **Materials and Methods:** Fractionation of the polyphenolic contents from the methanolic extract of *B. abyssinica* leaves was executed by the silica gel column chromatography to yield different fractions. The antioxidant activities of these fractions were carried out against 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl radicals, and ferric ion-reducing antioxidant power (FRAP). *In vitro* antidiabetic experimentation was performed by evaluating the α-amylase and α-glucosidase inhibitory capacity. The isolated polyphenols were then identified using liquid chromatography and mass spectroscopy (LC/MS). **Results:** Out of the eight polyphenolic fractions (BAL 1–8), BAL-4 has the highest inhibitory activity against ABTS radicals whereas BAL-6 showed potent ferric ion-reducing capacity. BAL-5 was the most effective fraction with antidiabetic activity with IC_{50} of 140.0 and 68.58 ± 3.2 μg/ml for α-amylase and α-glucosidase inhibitory activities, respectively. All the fractions competitively inhibited α-amylase, BAL-5 and BAL-6 also inhibited α-glucosidase competitively, while BAL-4 and BAL-1 exhibited noncompetitive and near competitive inhibitions against α-glucosidase, respectively. The LC/MS analysis revealed the presence of carvone in all the fractions. **Conclusions:** The present study demonstrates the antioxidant and antidiabetic activities of the isolated polyphenols from *B. abyssinica*.

Key words: Antidiabetics, antioxidants, *Bulbine abyssinica*, liquid chromatography and mass spectroscopy, medicinal plants, polyphenolics

SUMMARY

- Polyphenols were successfully isolated and identified from *Bulbine abyssinica* leaves
- The isolated polyphenols are biologically active with high antioxidant as well as inhibitor of carbohydrate-digesting enzymes
- *B. abyssinica* can be a good source of amylase and glucosidase inhibitors
- *B. abyssinica* can be used as complementary or alternative therapeutic agents especially for the treatment of diabetes
- Carvone, quercetin, and psoralen could be the compounds responsible for the α-amylase and α-glucosidase inhibitory activities.



Abbreviations Used: ABTS: 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric ion-reducing antioxidant power, LC/MS: Liquid chromatography and mass spectroscopy, AGEs: Advanced glycation end products, TLC: Thin-layer chromatography, MeOH: Methanol, PNP-G: ρ-Nitrophenyl-α-D-Glucoside, R^2: Coefficient of determination, mgQE: Milligram quercetin equivalent, mgTAE: Milligram tannic acid equivalent, mgCE: Milligram catechin equivalent, g: Gram

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INTRODUCTION

Plants produce a variety of secondary products having one or more hydroxyl functional groups on an aromatic ring called phenol.[1] These plant phenolics play important role in the plant defense mechanism against diseases and pests.[2] Phenolic acids can be classified into derivatives of benzoic acid and caffeic acid. Example of the benzoic derivative is the gallic acid while examples of the caffeic acid are coumaric and ferulic acids.[3] However, flavonoids, flavonols, tannins, lignins, and stilbenes are examples of plant phenolics. Plant phenolics have been used as food additives due to their distinctive odor and as ingredients in perfume. Recently, much attention has been focused on the potent antioxidative properties of polyphenolics and conversely their effects in the prevention of various oxidative stress-associated diseases. Free radicals are produced during oxidation processes and have been implicated in the cause of many human diseases such as cancer, diabetes, atherosclerosis, inflammation, cardiac, Alzheimer's, kidney, liver diseases, and aging.[3-5] The prevention of oxidative stress by plant polyphenols and their use in treatment of diseases is of increasing importance from the epidemiological data as well as *in vitro* and *in vivo* studies.[6] Asteraceae have been reported to be the largest family of flowering plants with many of its members shown to have pharmacological activities which contained important phytochemicals including polyphenolics and diterpenoids.[7,8] *Bulbine abyssinica* A. rich belongs to this family and has been previously described to be rich in polyphenolics.[9]

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Further studies on the acetone and water extracts from the whole plant evaluated *in vitro* assays showed antioxidant potential, hypoglycemic, and advanced glycation end-products inhibition capacity.[9] In this study, antioxidant and antidiabetic properties of different polyphenol fractions were investigated through bioassay-guided isolation from the leaves of *B. abyssinica*.

MATERIALS AND METHODS

The plant materials

B. abyssinica were collected from lower Ncera location in Nkonkobe Municipality of the Eastern Cape Province and authenticated by Prof. A Maroyi from the Department of Botany, University of Fort Hare, where the voucher specimen (KibMed 2014/01) was deposited in the Giffen's Herbarium, University of Fort Hare, South Africa.

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid and Butylated hydroxytoluene, and Folin–Ciocalteu reagent were obtained from Merck Limited (South Africa). Alpha-amylase, alpha-glucosidase, and p-nitrophenyl-α-D-glucoside solution (pNPG) were products of Sigma-Adrich Co., St. Louis, USA, while starch soluble (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade, and water used was glass distilled.

Preparation of plant extracts and fractionation

The dried *B. abyssinica* leaves were washed, dried, and finely chopped using a grinder and then about 1 kg was macerated with methanol for 5 days and the extract evaporated under reduced pressure at 40°C, affording 200 g of the crude extract. This was then loaded into a glass column containing silica gel 60 (0.063–0.200 nm) that has been previously washed with methanol and then eluted with methanol/ethyl acetate (2:8), ethyl acetate/hexane (7:3), methanol/ethyl acetate (8:2), and methanol to yield 8 fractions (BAL-1 to BAL-8). The elutions were examined by thin-layer chromatography and each one of them was dried, weighed, and reconstituted with methanol to prepare a stock solution at concentration of 1 mg/ml for preliminary screening of total phenolic contents, flavonoids, proanthocyanidins, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and DPPH scavenging activity. The fractions having the best polyphenolic contents and antioxidant activity were selected for further experiments; these fractions are BAL-4, BAL-6, BAL-5, BAL-8, BAL-7, and BAL-1 in the order of their activity.

Determination of the phytochemical contents

Phenolic content

The phenolic contents of all the fractions were determined in a 96-well plate as previously described with slight modification.[10] Briefly, a freshly prepared Folin–Ciocalteu reagent (1N) was used with different concentrations (2–10 μg/ml) of tannic acid in distilled water for the preparation of the standard calibration curve. The absorbance was measured at 725 nm using a spectrophotometer. The phenolic constituents were expressed in grams of tannic acid equivalents (TAE)/100 g of the extract using the equation: $Y = 0.0043x$, $R^2 = 0.9919$, where Y is the absorbance and x is TAE (μg/ml). The total phenolic constituent was calculated as TAE using the following equation:

$TP = C \times V_T/W$

Where TP = total phenolic constituent in μg/g of the extracts as TAE, C = tannic acid concentration derived from the calibration curve (μg/ml), V_T = volume of the extract in the reacting solution (ml), and W = weight of the extract (g).

Flavonoid content

The flavonoid contents in all the fractions were determined in a 96-well plate as previously described.[11] Briefly, 0.5 ml of 2% $AlCl_3$ in MeOH was mixed with 0.5 ml of the sample (500 μg/ml) or standard (0–100 μg/ml). The absorption was taken after 10 min at 415 nm against a sample without aluminum trichloride using the ultraviolet-visible spectrophotometer. The total flavonoid content was expressed mean ± standard deviation (SD)] as grams of quercetin equivalent (QE)/100 g of the extract using the equation $Y = 0.0025x$ ($R^2 = 0.9743$) where Y = absorbance and x = catechin equivalent (CE) (μg/g).

The total flavonoid content was calculated as QE/100 g by the following equation:

$TF = C \times V_T/W$

Where TF is the flavonoid constituent in mg/g of the extracts as QAE, C = the concentration of quercetin established from the calibration curve (μg/g), V_T = the volume of the extract in the reacting solution (ml), and W = the weight of the extract (g).

Proanthocyanidin

The proanthocyanidins content in all the fractions were determined in a 96-well plate as described previously.[12] Briefly, an aliquot of 0.5 ml of the extracts was mixed with 3 ml of 4% vanillin in MeOH and 1.5 ml of HCL. The mixture was then vortexed and allowed to stand at room temperature for 15 min and absorbance read at 500 nm. Total proanthocyanidin content was expressed as CE (μg/g) using the calibration curve equation $Y = 0.0023x$; $R^2 = 0.9951$, where Y was the absorbance and x is the CE (μg/g). The content of proanthocyanidins in extracts was calculated as CE/100 g by the following equation:

$TP = C \times V_T/W$

Where TP is the proanthocyanidins content (μg/g) of the extracts as CE, C is the concentration of quercetin established from the calibration curve (μg/g), V_T is the volume of the extract in the reacting solution (ml), and W is the weight of the extract (g).

Determination of the antioxidant activities

The antioxidant activities in all the fractions were analyzed using DPPH, ABTS, and ferric ion-reducing antioxidant power (FRAP) assays.

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The modified method of Carmona-Jiménez *et al.*[13] using microtiter plate was used for the determination of DPPH-free radical scavenging potential. Briefly, 100 μl of methanol was added into all the wells except second (B) and third (C) rows. Then, 100 ml of different concentrations of the plant extracts (0.05 mg/ml) or standards prepared in MeOH was then added in triplicates from the third row (C) to the seventh row. Different concentrations of the plant extracts and standards from 0.05 to 0.01 mg/ml were prepared. A solution of 0.135 mM DPPH radical in methanol was prepared. One hundred microliters of this solution was then added into all the wells. The reaction mixture was then vortexed and left in the dark for 30 min at room temperature. The absorbance of the mixture was measured using a spectrophotometer at 517 nm. The actual decrease in absorbance was measured against that of the control. The scavenging ability of the plant extract was then calculated using the following equation:

DPPH scavenging activity (%) = $([\{A_{control}\text{-}A_{sample}\}]/A_{control}) \times 100$

Where $A_{control}$ is the absorbance of DPPH + methanol and A_{sample} is the absorbance of DPPH + sample (or standard).

2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging assay

The method described by Thaipong *et al.*[14] was used for the determination of ABTS scavenging activity. Briefly, the reaction stock solutions including 7 mM ABTS and 2.4 mM potassium persulfate solutions were prepared. The working solution was then prepared by mixing (1:1 v/v) the two stock solutions and allowed to react for 12 h at room temperature in the dark. Then, the solution was diluted by mixing 60 ml of methanol with 1 mL ABTS + solution and measured using the spectrophotometer to obtain an absorbance of 0.708 ± 0.001 units at 734 nm. The plant extracts (1 ml) and their controls were reacted with 1 ml of the ABTS radical solution and the absorbance measured using the spectrophotometer at 734 nm after 7 min. The ABTS + scavenging capacity of the extract was then compared with that of the standards. The percentage inhibition was then calculated as follows:

ABTS scavenging activity (%) = $(1\text{-}A_{sample}/A_{control}) \times 100$

Where A_{sample} is the absorbance of ABTS radical + sample (extract/standard) $A_{control}$ is the absorbance of ABTS radical + methanol.

Ferric ion-reducing antioxidant power

The modified method of Rabeta and Nur Faraniza[15] was used to determine the FRAP of the samples. A freshly prepared ferric ion-reducing antioxidant power (FRAP) reagent contained the mixture of 20 mL glacial acetate buffer (300 mM, pH 3.6), 2 mL 2,4,6-Tripyridyl-s-triazine (TPTZ) solution, and 2 ml $FeCl_3.6H_2O$. The solution is kept in a water bath at 37°C for 10 min prior to use. An amount of 5 µl of the samples was mixed with 45 µl of deionized water in a 96-well plate followed by the addition of 100 µl of freshly prepared FRAP reagent. Sample blanks were also prepared. The 96-well plate was incubated in the dark at room temperature for 30 min and the absorbance was measured at 593 nm. Different concentrations of $FeSO_4.7H_2O$ dissolved in deionized water at 50, 100, 200, 400, 800, and 1000 µM (R^2 = 0.9798) were used for the standard curve.

Determination of antidiabetic activity

The fractions with the best antioxidants activities were selected for the antidiabetic investigations.

Determination of α-amylase inhibition

About 5 µl of α-amylase solution was pipetted into a 96-well plate and 15 µl of the sample that was previously diluted in a phosphate buffer was added. This was then incubated for 10 min at 37°C. The reaction was initiated by adding 20 µl of 2% starch solution and incubated for exactly 30 min. To stop the reaction, 10 µl of HCl (1M) was added followed by 75 µl of iodine reagent. A blank containing phosphate buffer (pH 6.9) instead of the sample and a positive control containing acarbose (20 mM) were prepared. No enzyme control was included for each test sample. The absorbance was read at 580 nm and the percentage inhibition was calculated using the equation:

% inhibition = ([A-B]/A) × 100

Where A = the absorbance of the reaction mixture without the enzyme and B = the absorbance of the reaction mixture with the enzyme.

A standard curve for starch was prepared with different concentrations of starch (0–80 µg/ml). The concentration of starch was determined using the equation Y = 0.033x + 0.0841; R^2 = 0.9996. The concentrations of the samples that resulted in the 50% inhibition of α-amylase activity (IC_{50}) were determined graphically using GraphPad Prism version 6.01 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

Determination α-glucosidase inhibition

Five microliters of the fractions was added to 20 µl of 50 µg/ml α-glucosidase enzyme inside a 96-well plate. Sixty microliters of 67 mM potassium phosphate buffer (pH 6.8) was then added before being incubated at 37°C. After 5 min, 10 µl of 10 mM pNPG was then added. This was incubated for 20 min at 37°C. After incubation, 100 µl of 100 mM sodium carbonate solution was added and the absorbance read at 405 nm. A blank and sample blank were also prepared by adding 5 µl of deionized water instead of plant extracts and 20 µl of deionized water instead of enzyme, respectively. The percentage inhibition was calculated using the equation:

% inhibition = ([A-B]/A) × 100

Where A = the absorbance of blank solution and B = the absorbance of the extract solutions.

The concentrations of the samples that resulted in 50% inhibition of α-glucosidase activity (IC_{50}) were determined graphically using GraphPad Prism software.

Mode of inhibition

The mode of inhibition was carried out on selected fractions that exhibited well α-amylase and α-glucosidase inhibition.

Mode of α-amylase inhibition

The mode of inhibition of α-amylase by the samples was carried out according to the modified method described by Kazeem *et al.*[16] Briefly, 100 µl of the sample (5 mg/ml) was preincubated with 100 µl of α-amylase solution for 10 min at 25°C in a 96-well plate. In another 96-well plate, 100 µl of α-amylase was preincubated with 100 µl of phosphate buffer (pH 6.9). One hundred microliters of starch solution at increasing concentrations (0.30–5.0 mg/mL) was added to both sets of reaction mixtures to start the reaction and was then incubated for 30 min at 25°C. Fifty microliters of each concentration was picked at 0, 5, 10, 20, and 30 min intervals and then 40 µl of DNS was added. The experiment was carried out in triplicate and the amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double-reciprocal plot (1/V vs. 1/[S] where V is reaction velocity and [S] is substrate concentration) was plotted. The mode of inhibition of each sample was determined by analysis of the double-reciprocal (Lineweaver–Burk) plot using GraphPad Prism 5 (GraphPad Inc.,) using Michaelis–Menten kinetics.

Mode of α-glucosidase inhibition

The mode of inhibition of α-glucosidase by the samples was determined using the modified method described by Kazeem *et al.*[16] Briefly, 50 µl of the (5 mg/ml) extract was preincubated with 100 µl of α-glucosidase solution for 10 min at 25°C in one set of tubes. In another set of tubes, α-glucosidase was preincubated with 50 µl of phosphate buffer (pH 6.9). Fifty microliters of PNPG at increasing concentrations (0.63–2.0 mg/ml) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25°C, and 500 µl of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a paranitrophenol standard curve and converted to reaction velocities. A double-reciprocal plot (1/V versus 1/[S] where V is reaction velocity and [S] is substrate concentration) was plotted. The type (mode) of inhibition of the crude extract on α-glucosidase activity was determined by analysis of the double-reciprocal (Lineweaver–Burk) plot using Michaelis–Menten kinetics.

Liquid chromatography and mass spectroscopy analysis and identification of components

Agilent liquid chromatography and mass spectroscopy (LC/MS) system (ABSCIEX Triple TOF, 5600) with the analyzer and electron

spray ionization source was used for the LC/MS analysis. The source parameters that were optimized to provide high sensitivity are negative mode, gas temperature 600°C, drying gas flow rate 0.5 mi/min, nebulizer pressure 50 psi, capillary voltage 5500 V; separation was carried out by ultra-high-pressure LC using Shimadzu UFLCXR with a phenomenex Kinetex 2.6 µm (100 × 2.1), column mobile phase used was A (water; 5 Mm ammonium formate +0.5% formic acid) and B (acetonitrile). The gradient program was as follows: 5% B for starting condition was increased up to 95% B in 20 min and decrease 5% B in 21–30 min at the final step. Injection volume was 50 µl. The standardized collision energy was 35V with collision energy of ±15V. The compounds were identified with computer-assisted evaluation of the resulting data by searching against the spectral library.

Statistical analysis

The results were analyzed using Minitab 12.1 1. The data were expressed as mean ± SD of three replicates and were subjected to analysis of variance; $P < 0.01$ was considered statistically significant.

RESULTS

The preliminary phytochemical screening of the different fractions of *B. abyssinica* methanolic leaf extract is shown in Figure 1. Fractions of BAL-2 and BAL-3 have the least polyphenolic contents for total phenol, flavonoids, and proanthocyanidins. BAL-6 has the highest total phenol (506.5 ± 11.02 mgTAE/g extract) and flavonoid contents (181.04 ± 1.12 mgQE/g extract) whereas BAL-1 has the highest proanthocyanidin content (752.08 ± 44.18 mgCE/g extract). BAL-1 appeared to be the potent DPPH scavenger with 94.59 ± 2.2% inhibition whereas BAL-4 appeared to be the potent ABTS scavenger with 93.9 ± 2.1% inhibition [Figure 2]. However, BAL-6 revealed the highest FRAP with 5936 ± 612.4 µM of $FeSO_4$ followed by BAL-4. BAL-1 and BAL-5 were not significantly different from each other with 1642.67 ± 376.9 µM of FeSO4 and 1272.67 ± 282.9 µM of $FeSO_4$, respectively [Figure 3].

The result of the α-amylase inhibition is shown in Table 1. Out of the eight fractions, only four were selected for the antidiabetic assays based on their phytochemical and antioxidant results. BAL-6 has higher percentage inhibition compared with acarbose but was not significantly different at $P < 0.01$. BAL-4 and BAL-5 also showed good α-amylase inhibitory activities while BAL-1 has the least percentage inhibition. The extrapolation of the different fractions from the dose-response curve indicated that BAL-5 as the most effective against α-amylase with the least IC_{50} of 140 µg/ml followed by BAL-1 with 227.9 µg/ml and BAL-4 with 231.7 µg/ml. All the samples showed lower IC_{50} compared with acarbose except BAL-6 with 387.1 µg/ml [Table 2]. The mode of inhibition of the different fractions on amylase activity was determined using the Lineweaver–Burk plot which showed that all the fractions displayed a near competitive inhibition of the enzyme activity. Also, for the α-glucosidase inhibition, BAL-6 showed the highest inhibitory activity compared with the other fractions while BAL-1 has the least with 4.11 ± 1.5%. In contrast, the extrapolation of the different fractions from the dose-response curve against α-glucosidase indicated BAL-5 as the most effective with the least IC_{50} of 159.1 µg/ml followed by BAL-1 with 164.3 µg/ml [Table 2]. The mode of inhibition of all the fractions against the α-amylase and α-glucosidase evaluated with different concentrations of the starch and pNPG, respectively, using the Lineweaver–Burk plot is shown in Figures 4 and 5 while the kinetic properties are summarized in Table 3. All the fractions increased the Michaelis–Menten constant (Km) for α-amylase while the Vmax for BAL-1 remained constant; other fractions had the Vmax reduced. For the α-glucosidase, the Michaelis–Menten constant (Km) of BAL-1 and BAL-4 remained constant while BAL-5 and BAL-6 showed increased Km. However, the Vmax was reduced for all the fractions [Table 3]. The mode of inhibition of the different fractions on α-glucosidase displayed mixed noncompetitive inhibition of the enzyme [Figure 5]. BAL-1 displayed near competitive inhibition, BAL-4 displayed non-competitive inhibition, and BAL-5 and BAL-6 displayed competitive inhibitions of the enzyme.

The LC/MS profile revealed the presence of a large group of compounds and were identified by comparison of their m/z values

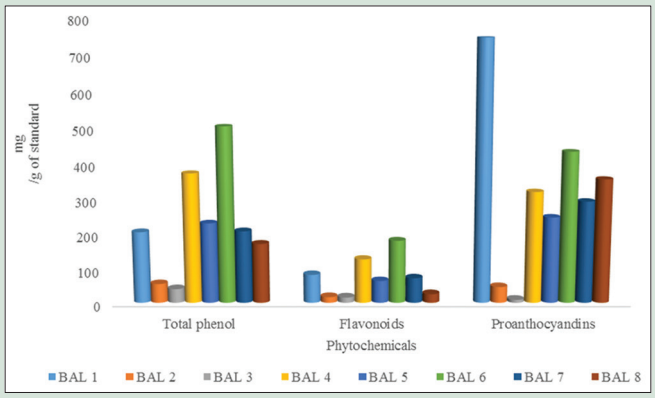

Figure 1: Polyphenolic contents of the *Bulbine abyssinica* fractions

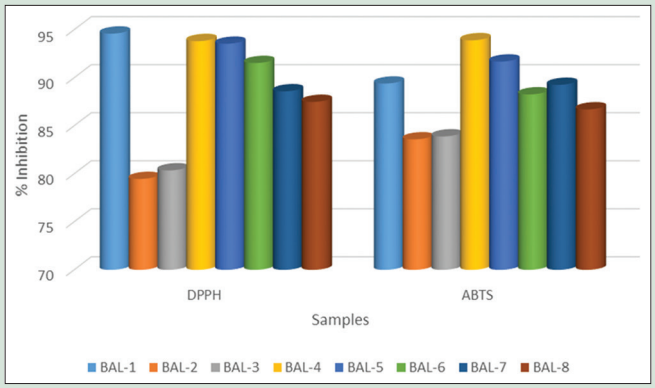

Figure 2: 2,2-diphenyl-1-picrylhydrazyl and 2, 2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) antioxidant activities of the *Bulbine abyssinica* fractions

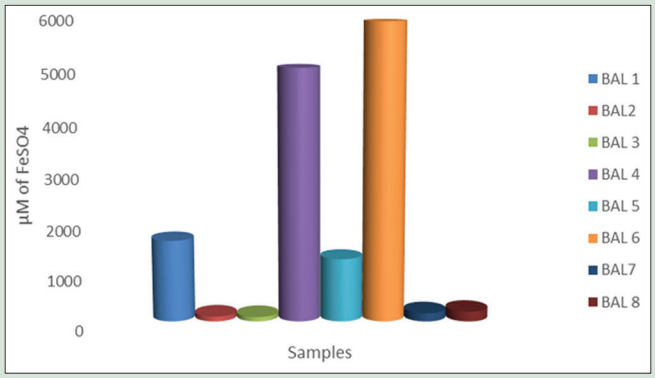

Figure 3: Ferric ion-reducing antioxidant power antioxidant activity of *Bulbine abyssinica* fractions

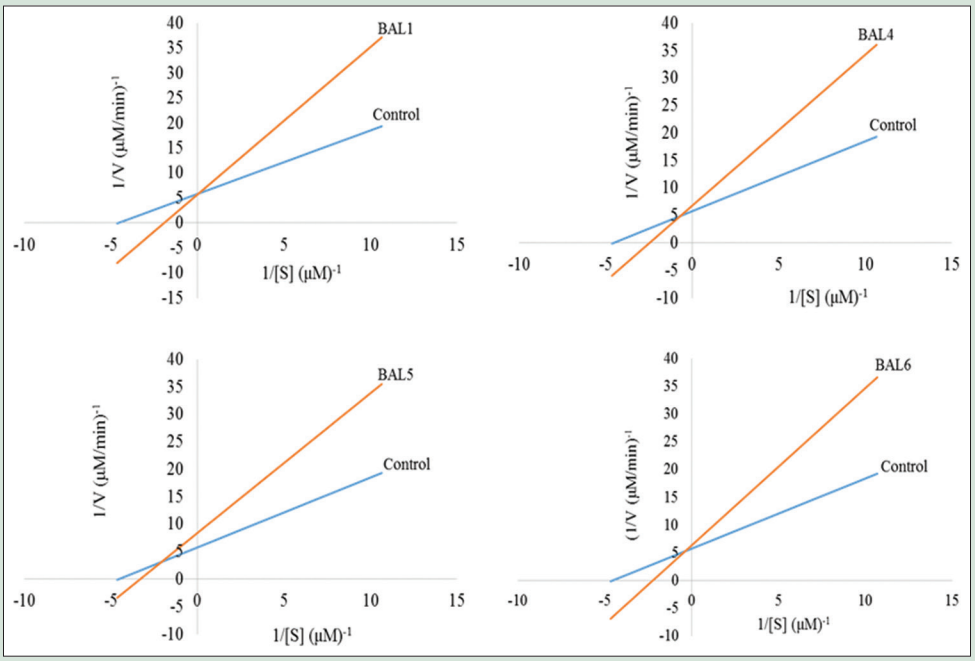

Figure 4: Lineweaver–Burk double-reciprocal plots for kinetic analysis of the reaction of α-amylase with starch (mM) used as substrate in the absence and presence of sample

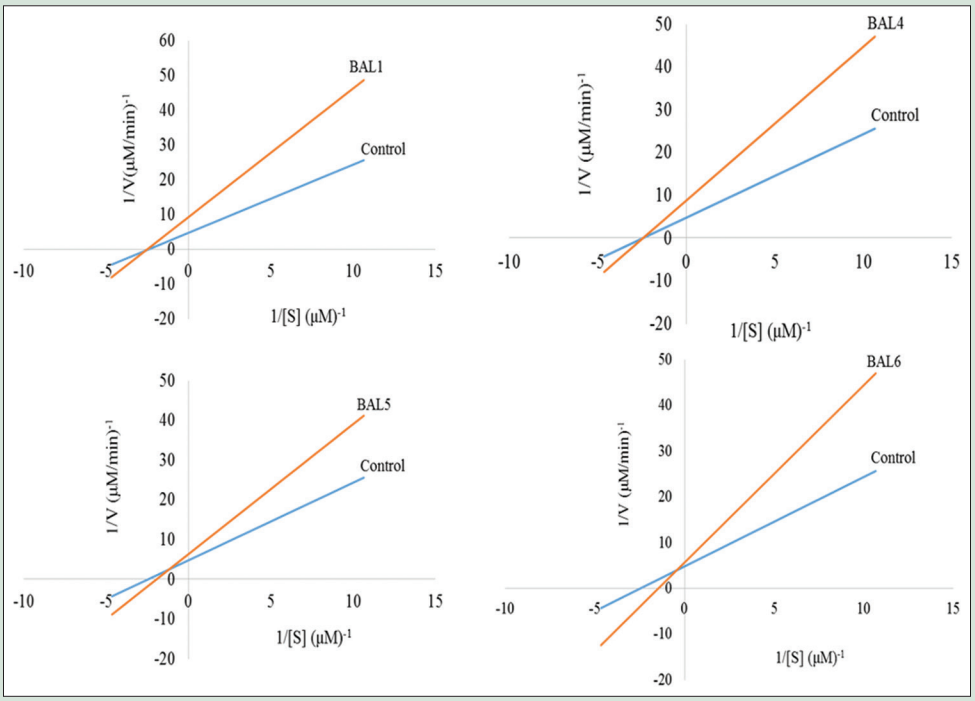

Figure 5: Lineweaver–Burk double-reciprocal plots for kinetic analysis of the reaction of α-glucosidase with starch (mM) used as substrate in the absence and presence of sample

and retention time with those of the selected compounds described in literature [Table 4] and/or by matching their chemical formula and MS/MS spectra with those reported in public repositories such as MassBank, PubChem compound, Chemspider, National Center for Biotechnology information, and Google Scholar. Fourteen polyphenolics were identified in all the fractions and are listed in Table 4. Among the compounds, only three were identified in all the samples. Among these compounds common to all the fractions (carvone, D-erythro-imidazole-glycerol-phosphate and D-myo-inositol-1,3,4-triphosphate), only carvone is a terpenoid. The chromatogram of carvone corresponding to the protonated molecular ions of different polyphenols [Figures 6-9]. Other polyphenolics

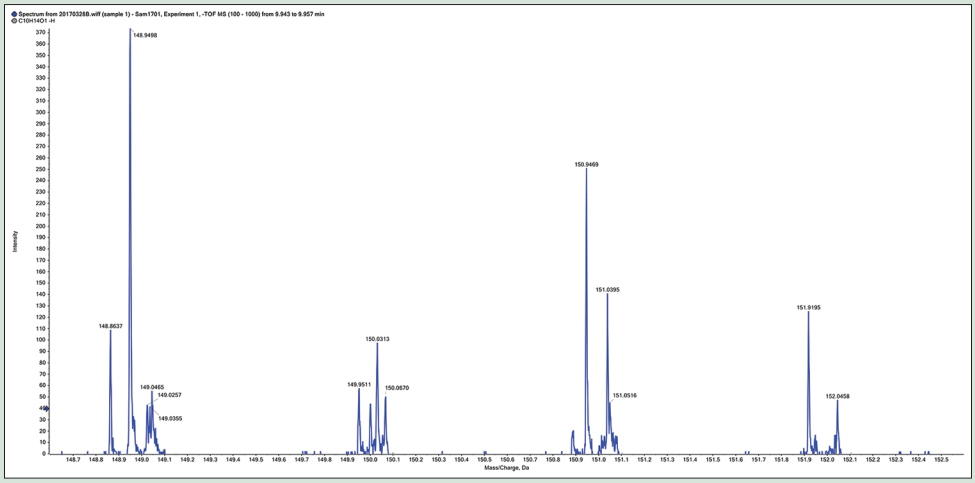

Figure 6: Chromatogram of carvone in BAL-1

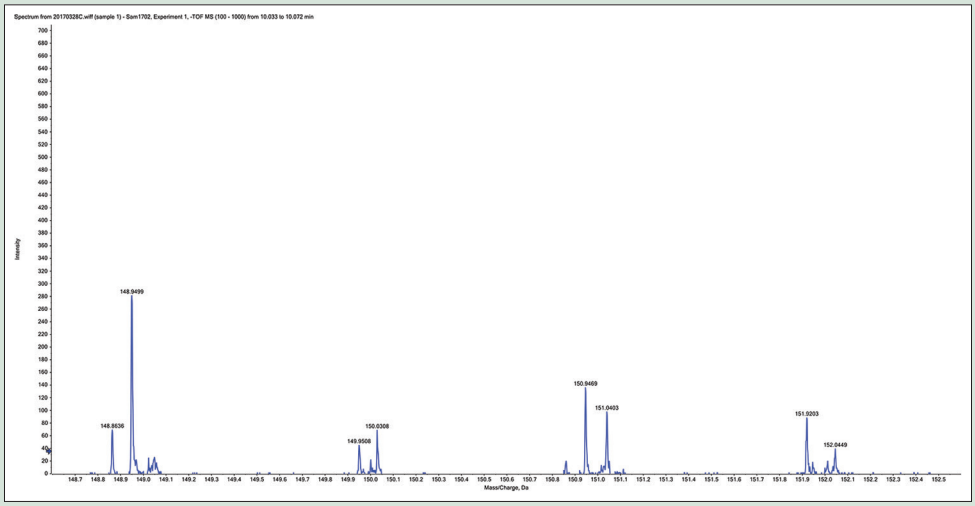

Figure 7: Chromatogram of carvone in BAL-4

Table 1: Inhibitory activity of the *Bulbine abyssinica* fractions on carbohydrate digesting enzymes (%)

Samples	BAL-1	BAL-4	BAL-5	BAL-6	Acarbose
α-amylase	4.98±0.1	69.32±1.91	74.74±2.05	83.33±0.62	81.30±1.66
α-glucosidase	4.11±1.5	56.32±1.6	68.58±3.2	71.10±0.9	77.18±2.3

BAL: Bulbine abyssinica leaf fractions 1 - 6

Table 2: IC_{50} value (μg/ml) of the *Bulbine abyssinica* fractions on carbohydrate digesting enzymes

Samples	BAL-1	BAL-4	BAL-5	BAL-6	Acarbose
α-amylase	227.9±13.1	231.7±12.3	140.0±21.2	387.1±9.82	376.0±10.64
α-glucosidase	164.3±0.1	255.63±0.1	159.1±0.1	325.5±0.1	290.8±0.1

Data were reported as mean±SD. SD: Standard deviation; BAL: Bulbine abyssinica leaf fractions 1 - 6

identified are antirrhinin, astilbin, eriodictyol, galangin, kaempferol 3-O-rutinoside, petunidin 3-rutinoside, pratensein O-methylated isoflavone, psi-tectorigenin, psoralen, quercetin, quercetin 7-glucoside 3-(6'-malonylglucoside), rutin, and variabiloside A [Table 4].

DISCUSSION

Antioxidant-guided isolation of compounds from medicinal plants is one of the systemic approaches of discovering novel and potent compounds that may contribute to the evidence-based traditional medicines.[17] The methanolic extracts of *B. abyssinica* leaves were fractioned. These fractions identified as BAL 1–8 were provisionally considered to be polyphenolics since the preliminary photochemical screening indicated the presence of polyphenolic-rich fractions. This is similar to previous reports on the isolation of polyphenols that have been said to be abundantly present in methanolic extracts.[17-19] Furthermore, reports have suggested the antioxidant and antidiabetic properties of polyphenolics.[5,17,19-24] Therefore, the methanolic leaves extract of *B. abyssinica* was fractioned based on their polyphenolic contents and antioxidant properties, so that the fractions with good polyphenolic content together with good antioxidant activities were chosen for the antidiabetic assays. The antioxidant assays chosen for the antiradical-guided isolation of the compounds of interest were based on previous report that suggests that the interaction of bioactive compounds with antioxidant constituents of DPPH assay is entirely

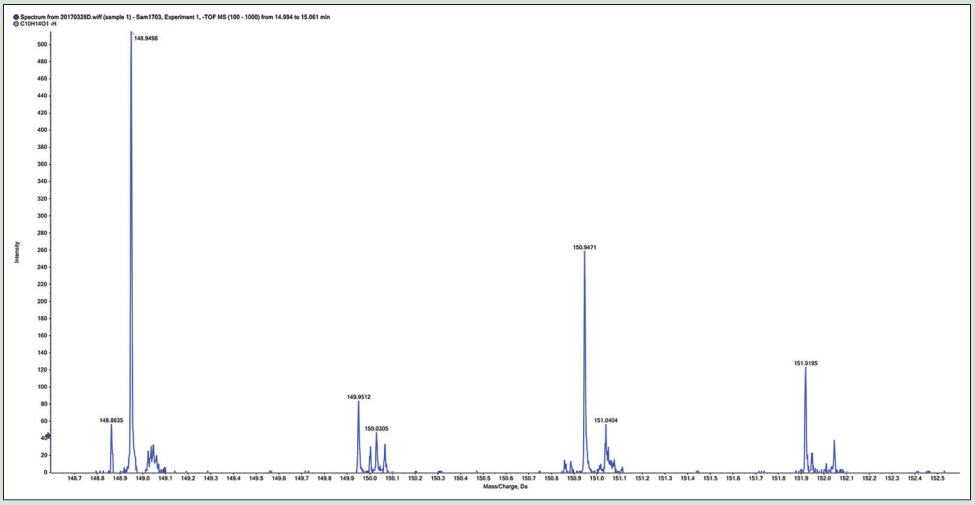

Figure 8: Chromatogram of carvone in BAL-5

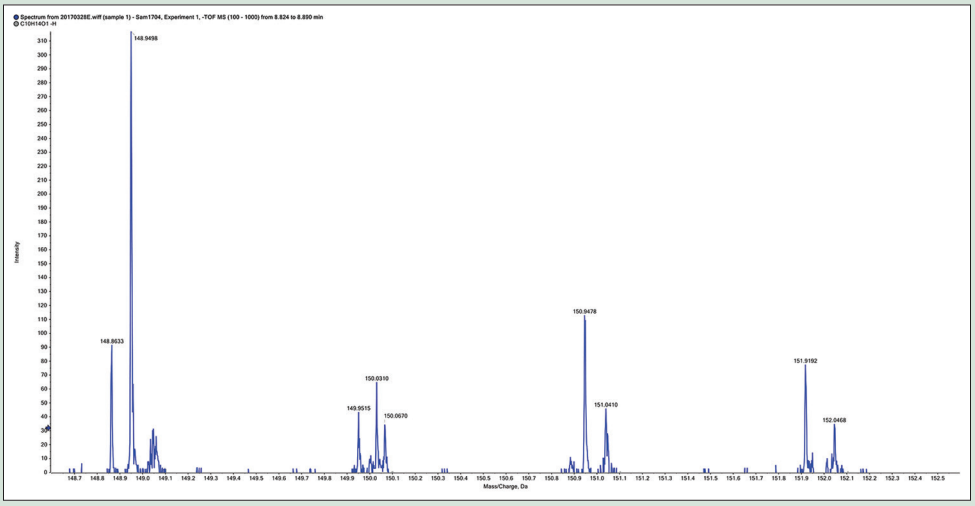

Figure 9: Chromatogram of carvone in BAL-6

Table 3: Kinetic properties of the *Bulbine abyssinica* leaves fractions on α-amylase and α-glucosidase

	Control	BAL-1	BAL-4	BAL-5	BAL-6
α-amylase					
Km	0.2188	0.5102	0.4062	0.2997	0.4495
V_{max}	0.173	0.1733	0.1483	0.1183	0.1582
α-glucosidase					
Km	0.4097	0.4027	0.4073	0.5104	0.6836
V_{max}	0.2103	0.1088	0.1135	0.1568	0.1768

BAL: Bulbine abyssinica leaf fractions 1 - 6

dependent on structural conformation.[25] All the fractions except 2 and 3 showed weak antiradical activities against DPPH and ABTS. This was also observed with FRAP indicating the absence of polyphenolics of interest from these fractions. Therefore, the result from this study indicated that the fractions with good antioxidant activities seemed to contain some antioxidative phenolic compounds. Likewise, BAL 7 and 8 with least ferric-reducing antioxidant potential also suggest weak antioxidant activity of these fractions. Previous screening has suggested the antidiabetic property of *B. abyssinica*, but the identification of the active compound from this plant is still lacking.[9] The α-amylase inhibitory study showed that the polyphenolic fractions of *B. abyssinica* had significant inhibitory potentials. The IC_{50} values of BAL-1 (227.9 ± 13.10 μg/ml), BAL-4 (231 ± 12.30 μg/ml), and BAL-5 (140.0 ± 21.20 μg/ml) were lower than that of acarbose (376.0 ± 10.64 μg/ml), a widely used antidiabetic drug. The α-amylase and α-glucosidase inhibitory activities of these fractions suggest the antidiabetic properties of the isolated compounds. Therefore, BAL-5 is likely to contain the potent α-amylase inhibitor. Almost all polyphenols have been suggested to inhibit glucose absorption in the intestine by inhibiting amylase and glycosidase activity.[26] The Lineweaver–Burk plot revealed that all the fractions inhibit α-amylase competitively, suggesting that the compounds compete with the substrate for binding to the active site of α-amylase. Furthermore, the Lineweaver–Burk plot for fraction BAL-4 revealed noncompetitive inhibition for α-glucosidase, suggesting that the compounds in BAL-4 bind to another site other than the active site of the enzyme, while fractions BAL-1 revealed near competitive inhibition and

Table 4: Retention time and qualitative confirmation of compounds occurring in *Bulbine abyssinica* fractions by liquid chromatography/mass spectroscopy

Polyphenolic compounds	BAL-1	BAL-4	BAL-5	BAL-6
	Retention time			
Antirrhinin	-	-	-	8.66
Astilbin	-	8.24	-	-
Carvone	9.95	10.06	15.04	8.86
Eriodictyol	-	9.9	-	9.91
Galangin	-	8.03	-	-
Kaempferol 3-O-rutinoside	-	-	-	8.66
Petunidin 3-rutinoside	-	8.07	-	-
Pratensein O-methylated isoflavone	-	11.41	-	11.43
psi-Tectorigenin	-	11.41	-	-
Psoralen	-	-	14.73	-
Quercetin	-	8.26	8.26	8.26
Quercetin 7-glucoside 3-(6'-malonylglucoside)	-	-	12.45	-
Rutin	-	8.26	-	-
Variabiloside A	-	8.26	-	-

BAL-5 and BAL-6 revealed competitive inhibitions, suggesting that the compounds in BAL-4, BAL-5, and BAL-6 compete with the p-nitrophenyl glucopyranoside (pNPG) for binding to the active site of α-glucosidase. The qualitative analysis using LC/MS confirmed the presence of polyphenolics in all the fractions. The presence of carvone in all the fractions may suggest that this compound is responsible for the antidiabetic activities observed in all these fractions. Carvone has been previously reported to have antidiabetic activity.[27-30] Carvone has also been reported to possess antioxidant and antiproliferative properties.[29,31,32] The most effective fraction (BAL-5) contained carvone, quercetin, and psoralen compounds that have been reported to possess antidiabetic activities.[22,33-36] This may account for the effectiveness observed for this fraction against α-amylase and α-glycosidase. Other polyphenolics implicated in the inhibitory of carbohydrate-digesting enzymes that are found in the other fractions are acacentin,[36] quercetin,[35,36] and rutinoside;[37] these compounds may account for the inhibitory activities observed in these fractions. Some compounds that were also found such as quinine, lactucopicrin, cannabichromene, 3-methylxanthine, and artemisinin have been implicated with analgesic and anti-inflammatory activities.[38,39]

CONCLUSIONS

Based on the results of this study, it can be concluded that the polyphenolics isolated from *B. abyssinica* have significant antioxidant activities and inhibitory properties against α-amylase and α-glucosidase enzymes. The antidiabetic activities could be as a result of the carvone, quercetin, and psoralen contents of the plant. Therefore, *B. abyssinica* is an easily accessible source of natural antioxidants and antidiabetic compounds. This also confirms the folkloric usage of the plant for the treatment of diabetes. However, further studies need to be carried out on the isolation, purification, and elucidation of the structural properties of these compounds.

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Conflicts of interest

There are no conflicts of interest.

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