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An *in vitro* evaluation of the antioxidant and antidiabetic potential of *Sutherlandia montana* E. Phillips & R.A. Dyer leaf extracts

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ABSTRACT

Objective: To evaluate the antioxidant and antidiabetic activities of *Sutherlandia montana* E. Phillips & R.A. Dyer leaf extracts using the *in vitro* model.

Methods: The antioxidant activities of aqueous, decoction, ethanol and hydro-ethanol extracts of the plant were determined using seven different assays; the antidiabetic potential was evaluated through the inhibition of key carbohydrate hydrolysing enzymes (α-amylase and α-glucosidase), while the modes of the enzymes inhibition were assessed using enzyme kinetic analysis.

Results: The ethanol extract exhibited the best scavenging activity (IC_{50}: 0.47, 0.36, 0.20, 0.29 and 0.01 mg/mL) against the tested radicals like 1,1-diphenyl-2-picrylhydrazyl, 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), nitric oxide, hydroxyl and superoxide anion, respectively. It also showed the best reducing power efficiency when compared with the standard (silymarin), while the decoction extract displayed the strongest metal chelating potential (IC_{50}: 0.71 mg/mL). The ethanol (IC_{50}: 5.52 mg/mL) and decoction (IC_{50}: 0.05 mg/mL) extracts exhibited mild and strong inhibitory effects on the specific activities of α-amylase and α-glucosidase respectively, through an uncompetitive and non-competitive mode of action.

Conclusions: The observed properties might be linked to the presence of active principles as shown by the results of the phytochemical analyses of the extracts. This research has validated the folkloric application of *Sutherlandia montana* as a potential antidiabetic agent, which is evident from the inhibition of specific activities of key enzymes involved in carbohydrate metabolism.

1. Introduction

Diabetes mellitus (DM) encompasses a group of metabolic diseases that has seriously threatened the human health and quality of life, due to its inherent complications [1]. Alterations in carbohydrate, fat and protein metabolism are the major contributing factors to DM, with consequential elevated blood glucose (hyperglycemia), resulting from defective insulin metabolism [2,3]. Recent reports have shown that DM is undoubtedly a rising global challenge, constituting major health risk in most countries [4,5]. Boyle *et al.* [6] reported that about 143 million people are estimated to be diabetic worldwide; and this number is projected to double by the year 2030. Precisely, type 2 diabetes mellitus (T2DM), is the most encountered form of DM associated with postprandial hyperglycemia [7], which accounts for about 80% of reported diabetic cases [8,9]. A disproportion between reactive oxygen species (ROS) production and antioxidant scavenging capacity induces oxidative stress, which ultimately leads to cellular and tissue damage in diabetic individuals [10]. These effects can be mitigated when oxidants are neutralized or scavenged by increased antioxidant supplementation [11]. The maintenance of a moderate blood glucose level in T2 diabetic patients is largely achieved through the use of oral hypoglycemic agents

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and insulin. However, these treatment options are expensive and have limited efficacy with significant adverse effects [12,13]. Thus, continuous research with natural extracts from plants with hypoglycemic effect is imperative. Such research will definitely offer new and more efficacious therapeutic approaches for the treatment of diabetes and its inherent complications [12,14].

The genus *Sutherlandia* (L.) R.Br. (Family: Fabaceae) is one of the most treasured South African medicinal plant species. The montane species, *Sutherlandia montana* (*S. montana*) is an attractive soft-wooded shrub, about 0.5–1.0 m high with fine silvery green foliage, large red flowers and attractive bladdery pods [15]. Traditionally, infusion of *S. Montana* leaves is ethnopharmacologically valued by the Basotho people of South Africa in curing cardiac ailments and as a sedative [16]. Similarly, findings from our systematic ethnobotanical survey of plants with medicinal value within the local communities in the montane regions of Qwaqwa, Phuthaditjhaba, South Africa, also revealed that different formulations (decoction and infusion) of *S. montana* leaves are widely used in the management and/or control of DM. Unlike *Sutherlandia frutescens* (L.) R.Br., whose traditional usage as an hypoglycaemic agent has been scientifically validated [17,18], the therapeutic attributes of *S. montana* remains largely unexplored. In view of this, coupled with no known scientific reports on its antioxidant and antidiabetic potentials, the present study was designed to analyse the phytochemical constituents, as well as evaluate the antioxidant and antidiabetic activities of *S. montana* leaf extracts via the *in vitro* models.

2. Materials and methods

2.1. Plant collection, identification and authentication

The fresh aerial parts of *S. montana* were collected in June 2016 in Kestell (28° 18.786′ S and 28° 40.498′ E; altitude 10 245 m), Free State Province, South Africa. The plant's name was checked at http://www.theplantlist.org (assessed on 12th, April, 2017). Proper identification and authentication were done by Dr. Sieben EJJ of the Department of Plant Sciences, University of the Free State (QwaQwa campus). A voucher specimen with reference number AliMed/01/2016/QHb was subsequently deposited at the Herbarium of the University.

2.2. Extracts' preparation

The freshly collected stems were rinsed with distilled water to remove foliar contaminants after which the leaves were separated from the twigs and stems. The leaf components were then air-dried at room temperature and subsequently ground into fine powder materials. Thereafter, exactly 20 g each of the powdered sample was extracted in 200 mL ethanol, hydro-ethanol (50: 50), decoction, and distilled water. The flasks were placed on Labcon Platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h to allow for proper agitation. All extracts were filtered using Whatman No. 1 filter paper. The ethanol extract was concentrated under reduced pressure at 40 °C using rotary evaporator (Cole-Parmer, SB 1100, Shanghai, China), while water, decoction and hydro-ethanol extracts were freeze-dried using lyophilizer (Virtis Bench Top, SP Scientific Series, USA). This yielded 14.1%, 22.5%, 35.3% and 32% of ethanol, hydro-ethanol, decoction and aqueous extracts, respectively. All extracts were kept air-tight and refrigerated (4 °C) prior to further experimental analysis.

2.3. Chemicals and reagents

Porcine pancreatic α-amylase, rat intestinal α-glucosidase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, acarbose, and para-nitrophenyl-glucopyranoside (pNPG) were obtained from Sigma–Aldrich Co. St Louis, USA. Starch soluble (extra pure) was a product of J.T. Baker Inc., Phillipsburg, USA, while other chemicals and reagents used were of analytical grade. The water used was glass-distilled.

2.4. Qualitative phytochemical analysis

The extracts of *S. montana* were analysed for the detection of different phytonutrients using standard methods of [19,20]. Nine chemical groups were tested.

2.4.1. Test for alkaloids

S. montana extracts were dissolved in dilute hydrochloric acid and filtered. Thereafter, Wagner's reagent (2 g iodine and 6 g potassium iodide in 100 mL water) was added to the filtrate. Formation of brown/reddish precipitate revealed the presence of alkaloids.

2.4.2. Test for anthraquinones

Exactly 2 mL of chloroform was added to 0.2 g of the extracts. The resulting mixture was vigorously shaken for 5 min and filtered, after which 10% ammonia solution was thoroughly mixed with filtrate obtained. A bright pink colouration formed in the aqueous layer of the mixture confirmed the presence of anthraquinones.

2.4.3. Test for glycosides

For this test, 10% aqueous hydrochloric acid was used to hydrolyse the extracts and thereafter treated with 2% sodium nitroprusside in pyridine and 20% sodium hydroxide. The pink to blood red colour observed showed that cardiac glycoside is present in the extracts.

2.4.4. Test for flavonoids

Few drops of 10% sodium hydroxide solution were added to 0.5 g of the extracts. An intense yellow colour was obtained, which turned colourless on the addition of dilute acid, indicated the presence of flavonoids.

2.4.5. Test for phenols

In this test, 3–4 drops of 10% ferric chloride solution was added to 0.5 g of *S. montana* extracts. Formation of bluish black colour suggested the presence of phenols.

2.4.6. Test for saponins

Exactly 2.0 g of extracts powder was boiled in 20 mL distilled water for 5 min and filtered. Thereafter, 5 mL distilled water was mixed with 10 mL filtrate in a graduated cylinder, shaken vigorously and left to stand for 15 min for persistent frothing. Following this, 3–4 drops of olive oil was mixed with

the froth and shaken again. An emulsion layer was observed which signified the presence of saponins.

2.4.7. Test for tannins

To test for tannins, 1% gelatin solution containing 10% sodium chloride was added to 0.5 g of the extracts. Formation of white precipitate revealed the presence of tannins.

2.4.8. Test for triterpenes

Chloroform was added to *S. montana* extracts and filtered. Then the filtrate was subjected to a few drops of concentrated sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicated the presence of triterpenes.

2.4.9. Test for phytosterols

A part of the extracts was mixed with chloroform and filtered. The filtrate obtained was then treated with few drops of acetic anhydride, boiled and cooled. Formation of a brown ring at the layer junction following addition of concentrated sulphuric acid, suggested the presence of phytosterols.

2.5. Quantitative phytochemical analysis

2.5.1. Determination of total phenolic content

Adopting the method described by Wolfe *et al.* [21], the total phenolic content in the plant extracts was determined. Briefly, 1 mL aliquot of the extracts was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and left to stand for 30 min at 40 °C for colour change. Absorbance was taken at 765 nm using a spectrophotometer (Biochrom WPA Biowave II, Cambridge, England). The equation obtained from the calibration curve of gallic acid was used to extrapolate the total phenolic content and expressed as mg/g gallic acid equivalent.

2.5.2. Determination of total flavonoids content

The total flavonoids contained in the extracts were estimated following the procedure of Ordon-ez *et al.* [22]. Briefly, 0.5 mL of 2% $AlCl_3$ ethanolic solution was added to 0.5 mL of the extracts, left for 1 h at 25 °C; after which the absorbance was measured at 420 nm. The appearance of yellow colour suggested the presence of flavonoids. Extracts sample was evaluated at final concentration of 1 mg/mL. Total flavonoid content was expressed as quercetin equivalent (mg/g) using the equation obtained from the calibration curve.

2.5.3. Determination of total flavonols content

Total flavonols content was determined using the method reported by Kumaran and Karunakaran [23]. Exactly 20 g of $AlCl_3$ and 50 g of sodium acetate anhydrous powder were separately dissolved in a little amount of distilled water and then made up to 1 L with distilled water respectively. Following this, rutin calibration curve was got by mixing 2 mL of varying concentrations of rutin (0.2–1.0 mg/mL) with 2 mL (20 g/L) $AlCl_3$ and 6 mL (50 g/L) sodium acetate. After 2.5 h at 20 °C, the absorbance was taken at 440 nm. Similar method was used for 2 mL of *S. montana* extracts (0.1/1.0 mg/mL) in place of rutin solution. The flavonol content was obtained from rutin calibration curve and expressed as rutin equivalents (mg/g).

2.6. In vitro antioxidant assays

2.6.1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the various extracts of *S. montana* was determined by measuring their ability to decolorize the purple-coloured methanol solution of DPPH, as described by Turkoglu *et al.* [24]. In brief, 1 mL of a 0.2 mM DPPH methanol solution was added to 1 mL of various concentrations (0.2–1.0 mg/mL) of the extracts and incubated at 25 °C for 30 min. The absorbance of the resulting mixture was measured against blank at 516 nm using a microplate reader (BIO RAD, Model 680, Japan). The percentage inhibition rate (I%) on the DPPH radical was calculated using the formula:

$$\text{Percentage inhibition (I\%)} = [(A_{control} - A_{extract})/A_{control}] \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of the control, $A_{extract}$ is the absorbance of the extract. The concentration of *S. montana* extracts causing 50% inhibition (IC_{50}) of DPPH radical was calculated from the standard calibration curve.

2.6.2. Nitric oxide scavenging ability

The capacity of *S. montana* extracts to scavenge nitric oxide radical was evaluated using the procedure reported by Garrat [25]. Two mL of 10 M sodium nitroprusside was prepared in 0.5 mL phosphate buffer saline (pH 7.4) and mixed with 0.5 mL of different concentrations of the extracts (0.2–1.0 mg/mL) in a 96-wells plate. The mixture was then incubated at 25 °C for 2 h, after which 0.5 mL was taken from the incubated mixtures and added to 1 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid). The mixture was further incubated at 25 °C for 5 min. Thereafter, 1 mL naphthyl ethylenediamine dihydrochloride (0.1% w/v) was added to the mixtures and the resulting solution was incubated at 25 °C for 30 min. The absorbance was read at 540 nm and the IC_{50} was then estimated from calibration curve following estimation of percentage nitric oxide radical scavenging capacity of *S. montana* extracts using the expression above [Eq. (1)].

2.6.3. Metal chelating ability of the extracts

The metal chelation ability of *S. montana* extracts was assayed following the procedure of Dinis *et al.* [26]. Summarily, 0.1 mL of the extract (0.2–1.0 mg/mL) was added to 0.5 mL of 0.2 mM ferrous chloride solution. The reaction was started by adding 0.2 mL of ferrozine (5 mM) and incubating at 25 °C for 10 min. The absorbance was read at 562 nm in a microplate reader (BIO RAD, Model 680, Japan). Citrate was used as control and the chelating potential of the extracts that competed with ferrozine for the ferrous ions was revealed from the colour reduction. IC_{50} value was extrapolated from the calibration curve.

2.6.4. Reducing power property

The reduction property of the extracts was assessed according to the method of Oyaizu [27]. Different concentrations (0.2–1.0 mg/mL) of *S. montana* extracts were added to 1 mL of distilled water and then mixed with 2.5 mL of 0.2 m phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated at 50 °C for 20 min before the addition of 2.5 mL of trichloroacetic acid. The

resulting mixture was centrifuged at 3 000 rpm for 10 min. After this, 2.5 mL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% $FeCl_3$. The colour change of the resulting solution was then taken at 700 nm.

2.6.5. 2, 2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging ability

This test was conducted using the procedure of Re *et al.* [28]. The $ABTS^+$ was generated by reacting 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 16 h and adjusting the pH to 0.7 with ethanol. Exactly 0.2 mL of the various dilutions of extracts (0.2–1.0 mg/mL) was added to 2.0 mL $ABTS^+$ solution and the absorbance was measured at 734 nm after 15 min. The silymarin equivalent antioxidant capacity was thereafter determined.

2.6.6. Hydroxyl radical inhibitory potential

The ability of the various extracts of *S. montana* to stop Fe_2^+/H_2O_2 induced decomposition of deoxyribose was assayed using the modified method of Oboh and Rocha [29]. In brief, 40 μL of the freshly prepared extracts (0.2–1.0 mg/mL) was added to a reaction mixture containing 20 μL 20 mM deoxyribose, 80 μL of 0.1 M phosphate buffer, 10 μL of 500 μM $FeSO_4$, and the volume was made up to 200 μL with distilled water. The reaction mixture was initiated at 37 °C for 30 min, and stopped by adding 50 μL of 2.8% TCA (trichloroacetic acid). This was followed by the addition of 50 μL of 0.6% thiobarbituric acid solution. The mixture was then incubated in boiling water for 20 min and absorbance was read at 532 nm in a microplate reader (BIO RAD, Model 680, Japan). The same procedure was done for a standard antioxidant by replacing the extracts with silymarin (0.2–1.0 mg/mL), and the IC_{50} value was then calculated from the calibration curve.

2.6.7. Superoxide anion radical scavenging capacity

Determination of superoxide anion scavenging effect of the various extracts of *S. montana* was conducted using the procedure of Liu *et al.* [30]. Superoxide radicals was generated in 50 μL of Tris–HCl buffer (16 mM, pH 8.0) containing 50 μL of 50 mM nitroblue tetrazolium solution, 50 μL of 78 mM nicotinamide adenine dinucleotide and varying concentrations of the extracts (0.2–1.0 mg/mL). The reaction was initiated by adding 1 mL of 10 mM phenazine methosulphate solution to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance measured at 560 nm. Superoxide anion radical scavenging ability of a standard antioxidant was also tested by replacing the extracts with silymarin (0.2–1.0 mg/mL). Results were estimated from the calibration curve and expressed as IC_{50} value.

2.7. In vitro antidiabetic assays

2.7.1. α-amylase inhibitory assay and kinetics

The α-amylase inhibitory activity and kinetics of inhibition were assessed using methods described by Elsnoussi *et al.* [31] and Kazeem *et al.* [32] respectively. Briefly, 500 μL of each of the varying extracts' dilutions (0.2–1.0 mg/mL) was mixed with 500 μL of 0.02 M sodium phosphate buffer (pH 6.9) containing 0.5 mg/mL of α-amylase solution. The mixture was pre-incubated in test tubes at 25 °C for 10 min. Thereafter, 500 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each test tube at timed intervals. The reaction mixtures were incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid colour reagent. The tubes were incubated in a boiling water bath for 5 min and left to cool at 25 °C. Then 15 mL of distilled water was used to dilute the reaction mixtures, and the absorbance was measured at 504 nm using a spectrophotometer (Biochrom WPA Biowave II, Cambridge, England). Similar procedure was repeated for acarbose which serves as the positive control by preparing it in distilled water at same concentrations (0.2–1.0 mg/mL) as extracts. The values were compared with those of acarbose used as control. The result of the triplicate determinations of α-amylase inhibitory activity was expressed as % inhibition [Eq. (1)]. The concentration of the extract causing 50% inhibition (IC_{50}) of α-amylase activity was calculated from its standard calibration curve.

For the kinetics of α-amylase inhibition, 250 μL of the mild inhibitor of α-amylase (ethanol extract; IC_{50}: 5.52 mg/mL) was incubated with 250 μL of α-amylase and 250 μL of varying concentration (0.3–5 mg/mL) of starch (substrate). Thereafter, the reaction followed the same procedure as stated above. The quantity of reducing sugars released was determined spectrophotometrically using maltose standard curve and converted to reaction velocities (v). Lineweaver–Burk double reciprocal plot (1/v versus 1/[S]) was drawn and the mode of α-amylase inhibition by the extract was evaluated [33].

2.7.2. α-glucosidase inhibitory assay and mode of inhibition

The α-glucosidase inhibitory potential was conducted adopting the method of Elsnoussi *et al.* [31]. In brief, 50 μL of varying concentrations (0.2–1.0 mg/mL) of *S. montana* extracts were mixed with 100 μL of 0.1 M phosphate buffer (pH 6.9) containing 1.0 M of the α-glucosidase solution. The mixtures were incubated in 96-well plates at 25 °C for 10 min. Thereafter, 50 μL of 5 mM p-NPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min and stopped with 50 μL of 0.1 M Na_2CO_3. The absorbance was read at 405 nm using a microplate reader (BIO RAD, Model 680, Japan) and the values were compared with acarbose were used as control. The α-glucosidase inhibitory activity of triplicate experiments was expressed as % inhibition using the formula:

$$\%\text{Inhibition} = [(\Delta A_{control} - \Delta A_{extract})/\Delta A_{control}] \times 100 \quad (2)$$

where $\Delta A_{control}$ and $\Delta A_{extract}$ are the absorbance changes of the control and extracts respectively. The concentration of *S. montana* extracts causing 50% inhibition (IC_{50}) of α-glucosidase activity was determined using standard calibration curve.

The kinetics of α-glucosidase inhibitory activity of *S. montana* was assayed following the modified method of Dnyaneshwar and Archana [34]. In brief, 50 μL of decoction extract (strongest α-glucosidase inhibitor) was pre-incubated 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 pre-incubated with 50 μL of phosphate buffer (pH 6.9). And 50 μL of p-NPG at concentrations (0.63–2.0 mg/mL) was added to

both sets of test tubes to initiate the reaction. The mixture was thereafter incubated for 10 min at 25 °C, and 500 µL of 0.1 M Na_2CO_3 was added to halt the reaction. The amount of reducing sugars released was determined spectrophotometrically using a para-nitrophenol standard curve. Reaction velocities (v) were then determined and double reciprocal plot of enzyme kinetics was constructed according to Lineweaver and Burk method to establish the type of inhibition. K_m and V_{max} values were also calculated from the Lineweaver–Burk plot (1/v versus 1/[S]) [34].

2.8. Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5 statistical package (GraphPad Software, USA). One-way analysis of variance (ANOVA) was used to analyse the data followed by Bonferroni test. Results were expressed as mean ± standard error of mean (SEM) of triplicate determinations. Statistical significance of the mean values was considered at $P < 0.05$.

3. Results

3.1. Phytochemicals

The qualitative phytochemical screening conducted on the different extracts of *S. montana* showed the presence of a diverse class of chemical groups, including alkaloid, cardiac glycosides, flavonoids, phenols, saponin, triterpene, and phytosterols (Table 1) in all the extracts. Triterpene was detected in aqueous and decoction extracts but absent in ethanol and hydro-ethanol extracts. Presence of phytosterols was detected in aqueous, decoction and hydro-ethanol extracts but absent in ethanol extract. However, anthraquinones, tannins, and sterols were absent in all the extracts (Table 1).

Table 1
Qualitative phytochemical constituents of *S. montana* leaf extracts.

Phytochemicals	Aqueous	Decoction	Ethanol	H. Ethanol
Alkaloid	+	+	+	+
Anthraquinones	−	−	−	−
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Saponin	+	+	+	+
Tannin	−	−	−	−
Triterpene	+	+	−	−
Phytosterols	+	+	−	+
Sterols	−	−	−	−

+: Detected; −: Not Detected; H. Ethanol: Hydro-Ethanol.

Table 2 presents the total phenolics, flavonoids and flavonols composition of *S. montana* extracts. Although marginally different, the total phenolic contents of the ethanol (12.48 mg gallic acid/g), decoction (12.09 mg gallic acid/g) and hydro-ethanol (10.81 mg gallic acid/g) extracts were not significantly different ($P > 0.05$), but significantly higher ($P < 0.05$) than that of aqueous extract (6.19 mg/g). The decoction extract had the highest quantity of flavonoids (68.20 mg/g), which was significantly greater ($P < 0.05$) than those of other extracts. Just as for the other phenolics, the aqueous extract had the least flavonol constituent (3.52 mg/g) while ethanol extract had the highest (12.19 mg/g) which was significantly higher ($P < 0.05$) than those of decoction and hydro-ethanol extracts.

3.2. Antioxidant activities of S. montana extracts

The result of the *in vitro* antioxidant activities of *S. montana* extracts is shown below (Table 3). Judging by the IC_{50} values, ethanol extract elicited the most potent antioxidant capacity in scavenging DPPH, NO, ABTS, O^{2-} and OH radicals. As the extract (ethanol) compared favourably well with silymarin in annihilating ABTS and O^{2-} radicals, it is noteworthy that it had significantly ($P < 0.05$) better effect than silymarin against DPPH, NO and OH radicals.

3.2.1. Reducing power effect of S. montana extracts

Ethanol extract significantly ($P < 0.05$) exhibited the best reducing power efficiency on ferric ion in a concentration dependent manner; with the highest dose (1.0 mg/mL) displaying the strongest effect (Table 4). This effect was even superior when compared with the standard (silymarin).

3.3. Antidiabetic potential of S. montana extracts

The IC_{50} values for the inhibitory efficiency of *S. montana* extracts on the specific activities of α-amylase and α-glucosidase are presented in Table 5. There was a significant difference ($P < 0.05$) in the inhibitory potential of the standard (acarbose) and all extracts tested on α-amylase activities. Aqueous extract displayed the best inhibition against α-amylase (IC_{50}: 0.13 mg/mL), and the IC_{50} value was significantly lower ($P < 0.05$) than those of other extracts and acarbose. However, decoction extract significantly ($P < 0.05$) exhibited the greatest inhibitory effect on the activities of α-glucosidase (0.05 mg/mL), when compared with other extracts and standard.

Table 2
Total phenolics, flavonoids, and flavonols contents of *S. montana* leaf extracts.

Phytochemicals	Aqueous	Decoction	Ethanol	H. Ethanol
Total phenolics	6.19 ± 0.02^a	12.09 ± 0.02^b	12.48 ± 0.01^b	10.81 ± 0.02^b
Total flavonoids	8.90 ± 0.10^a	68.20 ± 0.02^b	54.90 ± 0.15^c	15.90 ± 0.15^d
Total flavonols	3.52 ± 0.02^a	8.83 ± 0.02^b	12.19 ± 0.01^c	5.31 ± 0.11^d

Values are presented as means ± SEM of triplicate determinations and expressed per g of plant extracts. Means not sharing a common letter are significantly different ($P < 0.05$) with each other. Unit for phenolics: mg gallic acid/g; Unit for flavonoids: mg quercetin/g; Unit for flavonols: mg rutin/g; H. Ethanol: Hydro-Ethanol.

Table 3

IC_{50} (mg/mL) values for the antioxidant properties of *S. montana* leaf extracts.

Assay	IC_{50} (mg/mL)					
	Silymarin	Aqueous	Decoction	Ethanol	Hydro-ethanol	Citrate
DPPH	2.74 ± 0.01[a]	5.11 ± 0.01[b]	1.94 ± 0.01[c]	0.47 ± 0.01[d]	1.93 ± 0.01[c]	ND
Nitric oxide	0.47 ± 0.01[a]	0.65 ± 0.01[b]	0.35 ± 0.01[c]	0.20 ± 0.01[d]	0.28 ± 0.01[d]	ND
ABTS	0.39 ± 0.01[a]	0.50 ± 0.01[b]	0.67 ± 0.01[c]	0.36 ± 0.02[a]	0.50 ± 0.01[b]	ND
Superoxide	0.02 ± 0.01[a]	0.63 ± 0.01[b]	0.61 ± 0.01[b]	0.01 ± 0.01[a]	0.30 ± 0.01[c]	ND
Hydroxyl	0.77 ± 0.01[a]	0.85 ± 0.01[b]	1.41 ± 0.01[c]	0.29 ± 0.01[d]	1.02 ± 0.01[e]	ND
Metal chelating	ND	0.87 ± 0.01[a]	0.71 ± 0.01[b]	0.96 ± 0.01[c]	0.76 ± 0.01[c]	0.59 ± 0.02[d]

The values are presented as mean ± SEM of triplicate determinations. Means along the same row not sharing a common superscript for each parameter are significantly different ($P < 0.05$). ND: Not determined.

Table 4

Reducing power potential of *S. montana* extracts.

Conc. (mg/mL)	Absorbance (nm)				
	Silymarin	Aqueous	Decoction	Ethanol	Hydro-ethanol
0.2	0.32 ± 0.01[a]	0.39 ± 0.01[a]	0.42 ± 0.02[a]	0.67 ± 0.02[b]	0.31 ± 0.01[a]
0.4	0.53 ± 0.01[a]	0.52 ± 0.01[a]	0.59 ± 0.01[a]	0.88 ± 0.03[b]	0.55 ± 0.01[a]
0.6	0.64 ± 0.02[a]	0.65 ± 0.01[a]	0.63 ± 0.01[a]	0.95 ± 0.03[b]	0.60 ± 0.01[a]
0.8	1.10 ± 0.01[a]	0.96 ± 0.02[a]	0.82 ± 0.01[a]	1.83 ± 0.02[b]	0.99 ± 0.02[a]
1.0	1.67 ± 0.02[a]	1.55 ± 0.02[a]	1.24 ± 0.01[a]	2.79 ± 0.01[b]	1.40 ± 0.01[a]

Values presented as mean ± SEM of triplicate determinations. Means with different superscript along the same row are significantly different ($P < 0.05$).

Table 5

IC_{50}(mg/mL) values for the inhibitory potentials of *S. montana* leaf extracts on the specific activities of α-amylase and α-glucosidase.

Samples	IC_{50} (mg/mL)	
	α-amylase	α-glucosidase
Aqueous	0.13 ± 0.01[a]	0.29 ± 0.02[a]
Decoction	1.48 ± 0.02[b]	0.05 ± 0.01[b]
Ethanol	5.52 ± 0.03[c]	0.43 ± 0.01[c]
Hydro-ethanol	2.27 ± 0.01[d]	0.24 ± 0.01[a]
Acarbose	0.24 ± 0.01[e]	0.31 ± 0.02[a]

The values are expressed as mean ± SEM of triplicate determinations. Means along vertical columns not sharing a common superscript are significantly different ($P < 0.05$) from each other. Acarbose is the standard α-amylase and α-glucosidase inhibitor.

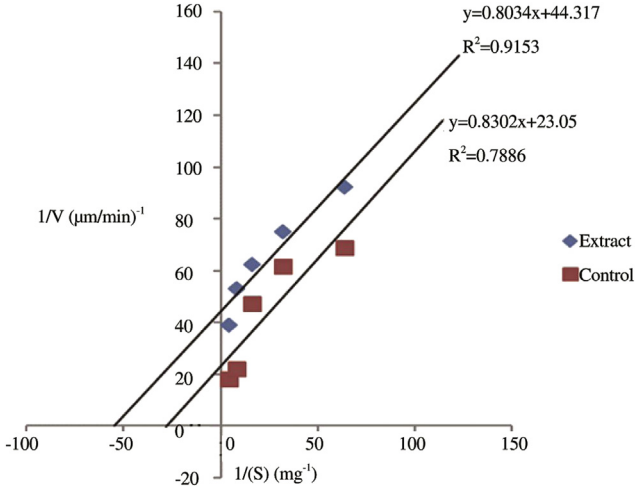

Figure 1. Lineweaver–Burk plot of *S. montana* ethanol extract eliciting uncompetitive inhibition on α-amylase activity.
Results were expressed as mean ± SEM; $n = 3$; $P < 0.05$ is considered to be significant.

3.4. In vitro kinetics of α-amylase and α-glucosidase enzymes

The modes of inhibition of α-amylase and α-glucosidase enzymes are shown in Figures 1 and 2, respectively. It revealed that ethanol extract uncompetitively inhibited α-amylase activities. This is obvious from the double reciprocal plot, showing a decrease in both V_{max} and K_m values. The relative V_{max} and K_m values of the control were 0.04 μM/min and 0.03 mg respectively, which decreased to 0.02 μM/min and 0.01 mg for the extract (Figure 1). Conversely, decoction extract exhibited non-competitive inhibition against α-glucosidase activities, as apparent in the reduction in V_{max} value of the control from 0.05 μM/min to 0.02 μM/min for the extract, and constant K_m values of 0.003 mg for both the control and extract (Figure 2).

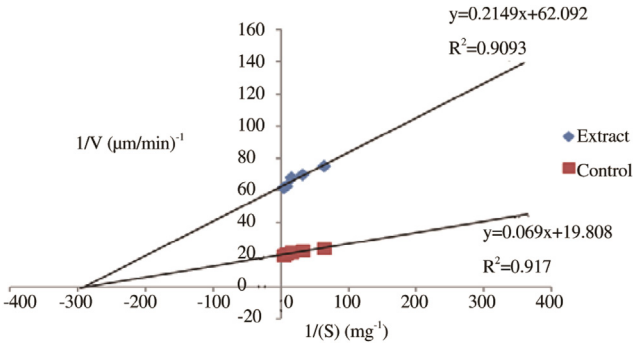

Figure 2. Lineweaver–Burk plot of *S. montana* decoction extract exhibiting non-competitive inhibition on α-glucosidase activity.
Results were expressed as mean ± SEM; $n = 3$; $P < 0.05$ is considered to be significant.

4. Discussion

The value of medicinal plants to mankind is well established, as numerous discoveries have shown that plants' extracts contain not only minerals and primary metabolites, but also a wide range of secondary metabolites with great therapeutic efficiencies [35,36]. The presence of phytochemicals like alkaloid, glycosides, flavonoids, phenols, saponin, triterpenes and phytosterols detected in various extracts of *S. montana* have been reported to support bioactive activities in medicinal plants and may therefore justify the pharmacological effects of the extracts. For instance, phenols are well known antioxidants and cellular event modifiers [37], while flavonoids are powerful free radical scavengers with strong anticancer activity [38,39]. In the same vein, the protective functions of alkaloids are well documented [35], just like plant saponins are reported to possess various biological activities, including antioxidant, anti-cancer, anti-viral and hepatoprotective actions [40,41]. Similarly, phytosterols have been proven to have direct immunemodulatory activity on human lymphocytes [42]. The presence and synergistic relationship among most of these general phytochemicals might be responsible for the overall medicinal effects of *S. montana.* The antioxidant efficiency of medicinal plants has generally been ascribed to their high phenolic contents [43,44]. In the present study, the occurrence of phenolic compounds (phenols, flavonoids, and flavonols) in various extracts of *S. montana,* is suggestive of the plant as a potent antioxidant. Similarly, the strongest scavenging activity and metal chelating effect displayed by ethanol and decoction extracts respectively (judging by their IC_{50} values), may not be unconnected with the active constituents present in the phenolics of the extracts. This submission agrees with previous findings [45–47], where polyphenolic compounds in plants were therapeutically valued as antioxidants.

The inhibition of the specific activities of key enzymes involved in the absorption of dietary carbohydrates is a unique pharmacologically approach towards diabetes management [48,49]. In this study, aqueous extract displayed the strongest inhibition of α-amylase activity (IC_{50} 0.13 mg/mL), showing more potency than the standard (IC_{50} 0.24 mg/mL). However, strong inhibition of α-amylase activity is a one of the factors that results in the common downside connected with the use of synthetic antidiabetic drugs; hence a mild inhibitor becomes a better alternative [32]. Going by this reason, ethanol extract (IC_{50} 5.52 mg/mL) was chosen in the present study as a mild inhibitor of α-amylase activity, as it exhibited a remarkably higher IC_{50} than that of acarbose. Conversely, decoction extract demonstrated the strongest inhibition against the specific activity of α-glucosidase (IC_{50}: 0.05 μg/mL) when compared with other extracts and acarbose. Therefore, the respective moderate and strong inhibition of α-amylase and α-glucosidase by both extracts (ethanol and decoction) is expected to delay starch hydrolysis in the gastro-intestinal tract, thus decreasing the amount of glucose released into the blood stream, and ultimately suppressing post-prandial hyperglycemia. These findings corroborate previous studies that reported extracts with strong inhibitory potential against α-glucosidase as an ideal antidiabetic agent both *in vitro* [11,50,51] and *in vivo* [52–54]. The uncompetitive mode of α-amylase inhibition displayed by ethanol extract infers that some bioactive components in the extract bind to the enzyme–substrate complex and possibly interferes with the formation of the product. On the other hand, non-competitive inhibition of α-glucosidase exhibited by decoction extract implies that its phytoconstituents bind to another site (allosteric site) rather than the enzyme's active site. This results in conformational change in the enzyme structure, hence slowing down the hydrolysis of starch to glucose. Generally, the diverse group of phytochemicals present in *S. montana* extracts could have acted synergistically to produce the observed potential hypoglycemic effect observed in this study. This potency may also be related to the high antioxidant activity of the plant's extracts, thereby mopping up free radicals that could be generated under hyperglycaemic condition.

Findings from this study revealed that *S. montana* is rich in phytochemicals with proven antioxidant activities. Specifically, this study has substantiated and scientifically appraised the folkloric use of the plant as a potential antidiabetic agent. Further studies are necessary to determine the exact nature of the bioactive principle(s) in the plant extracts that are responsible for its hypoglycemic effect. This can promote their use as natural products with the prospects of increasing the quality of life of diabetic sufferers.

Conflict of interest statement

The authors declare no conflict of interests.

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