Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.350178

Impact Factor: 1.51

Senna petersiana inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury

Kolawole A. Olofinsan[1], Ochuko L. Erukainure[2], Nontokozo Z. Msomi[1], Md. Shahidul Islam[1]✉

[1]*Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa*

[2]*Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa*

ABSTRACT

Objective: To evaluate the effect of *Senna petersiana* leaf extracts on key digestive enzymes and $FeSO_4$-induced oxidative injury.

Methods: Dried *Senna petersiana* leaf powder (60 g) was defatted in *n*-hexane and then extracted sequentially at room temperature with dichloromethane, methanol, and distilled water. The total phytochemical content of the extracts was estimated using established methods. The *in vitro* antioxidant, anti-lipase, and antidiabetic activities and the effect of the extracts on intestinal glucose absorption and $FeSO_4$-induced pancreatic oxidative injury were determined using different protocols. Moreover, GC-MS analysis was performed to identify the main compounds of the plant extract. Molecular docking analysis was also carried out to evaluate the binding energy of compounds with digestive enzymes.

Results: *Senna petersiana* leaf extracts showed significant antioxidant activities in FRAP, DPPH, and hydroxyl radical scavenging assays. They also inhibited pancreatic lipase and lowered intestinal glucose absorption by suppressing activities of α-amylase and α-glucosidase. Treatment with the extracts also lowered lipid peroxidation (malondialdehyde), nitric oxide level, acetylcholinesterase, and ATPase activities with simultaneous improvement of antioxidant (catalase, superoxide dismutase, glutathione) capacity in the type 2 diabetes model of oxidative pancreatic injury. GC-MS characterization of the extracts revealed the presence of stilbenoids, alkaloids, and other compounds. Molecular docking screening assay indicated the extract phytochemicals showed strong interaction with the active site amino acids of the targeted digestive enzymes. Among the *Senna petersiana* compounds, veratramine had the highest affinity for α-amylase and lipase, whereas dihydrostilbestrol was most attracted to α-glucosidase.

Conclusions: *Senna petersiana* inhibits carbohydrate digestive enzymes, reduces intestinal glucose absorption, and exerts ameliorative effects on $FeSO_4$-induced oxidative pancreatic injury with significant antioxidant capabilities. Detailed *in vivo* studies are underway to understand the plant's therapeutic potential in diabetes management.

KEYWORDS: *Senna petersiana*; Antioxidant; Digestive enzymes; Oxidative pancreatic injury; Type 2 diabetes

Significance

Senna petersiana, a deciduous medicinal shrub native to the Tropical African region, is mainly utilized for managing indigestion, intestinal worm infestation, skin infection, and many other traditional uses. Despite the indigenous and pharmacological relevance attributed to this plant, its biological activities in diabetes management are rarely studied. In the current study, the plant leaf extracts inhibit carbohydrate digestive enzymes, lower intestinal glucose absorption, display antioxidant properties, and modulate dysregulated enzyme activities in the oxidative pancreatic injury model of type 2 diabetes. These findings suggest *Senna petersiana* is a natural source of lead chemical agents for the treatment and management of diabetes and its complications.

✉To whom correspondence may be addressed. E-mail: islamd@ukzn.ac.za

How to cite this article: Olofinsan KA, Erukainure OL, Msomi NZ, Islam MS. *Senna petersiana* inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury. Asian Pac J Trop Biomed 2022; 12(7): 300-311.

Article history: Received 25 March 2022; Revision 12 May 2022; Accepted 13 June 2022; Available online 20 July 2022

1. Introduction

Oxidative stress has been implicated as one of the underlying mechanisms in the onset and progression of many pathological conditions. Among these diseases is diabetes mellitus, which is estimated worldwide to affect about 537 million people aged 20-79 years[1]. Moreover, the International Diabetes Federation[1] further projected that by 2030, the incidence rate of this disorder would increase to around 783 million, with people in the African region accounting for 7%. Diabetes is a multifaceted metabolic pathology that interferes with the normal metabolism of carbohydrates, fatty acids, and proteins, resulting in persistent hyperglycemia. Interestingly, this biochemical dysfunction has been linked significantly with the total loss of pancreatic β-cell insulin secretion or insufficient insulin production/insensitivity of body cells to the regulatory hormone signals. While type 1 diabetes is associated with the former condition, type 2 diabetes (T2D) which accounts for 90%[1] of all reported diabetes cases is related to the latter situations.

Oxidative stress induced by chronic hyperglycemia has been reported as a major factor leading to cellular insulin insensitivity and β-cell dysfunction in T2D[2]. Findings suggest that chronic hyperglycemia results in elevated production of free radicals *via* biochemical processes that include increased activation of protein kinase C isoforms, enhanced production of advanced glycation end products, activation of the hexosamine pathway, and upregulation of the polyol pathway[3]. Consequently, high cellular levels of free radicals overwhelm the body's antioxidant defense system and thus result in redox imbalance that mediates the progression of T2D into several micro- and macrovascular-complications[4].

The pancreas plays a crucial role in regulating circulating blood glucose concentration. Apart from producing insulin for glucose utilization by body cells, the organ also has exocrine functions with which it secretes digestive enzymes for the hydrolysis of dietary food substances. According to Cooley *et al*.[5], pancreatic acinar cells synthesize proenzymes and enzymes such as amylase and lipase in carbohydrate and lipid digestion. Surprisingly, inhibition of these enzymes has been implicated in the management of diabetes and its associated diseases[6,7]. Despite its crucial functions in energy metabolism, the pancreas is more easily susceptible to oxidative attack than other body tissues. The studies by Lenzen *et al*.[8] showed lower gene expression levels of catalase, superoxide dismutase (SOD), and glutathione peroxidase in the pancreas relative organs such as the liver, kidney, muscles, *etc*. Considering the pancreas' low intrinsic antioxidant capacity and its involvement in diabetes, food materials with the dual abilities to protect the organ against oxidative damage and modulate its enzyme activities could present an excellent treatment regimen for managing T2D.

Senna petersiana (*S. petersiana*) (Bolle) Lock, also called *Cassia petersiana* Bolle var is a deciduous shrub widely distributed in many African countries. It belongs to the Fabaceae family of the plant and is commonly called earner senna or monkey pod in English. *S. petersiana* plant is characterized by 12 opposite pinnate leaflets with brightly colored yellow flowers. At maturity, it annually produces hairy compressed pods consumed fresh or cooked as gruel locally[9]. Different parts of the plant have been used for traditional therapeutic purposes. The leaves are utilized to manage malaria and typhoid fever, whereas the roots are employed as medicine for coughs, stomachaches, and sexually transmitted diseases such as syphilis[9,10]. Interestingly, pharmacological evidence has also described the bioactivity of *S. petersiana* plant products. An *in vitro* study revealed that methanol and aqueous extracts of the root reduced red blood cell hemolysis and coagulation better than salicylic acid[11]. Moreover, the plant leaves were reported to show antifungal and antibacterial activities in addition to significant cyclooxygenase-1 inhibition[12,13]. However, there is scarce information on antidiabetic and tissue-protective antioxidant activities of *S. petersiana*.

Therefore, the present study was conducted to evaluate antidiabetic and anti-lipase activities of *S. petersiana* leaf and its protective antioxidant effects on Fe^{2+}-induced oxidative pancreatic injury. Bioactive chemical compounds in the aerial part were also identified and screened *in silico via* molecular docking analysis for binding affinities with primary carbohydrate and lipid digestive enzymes.

2. Materials and methods

2.1. Chemicals and reagents

Acetylcholine iodide, 6-hydroxydopamine, *p*-nitrophenyl butyrate, and 4-nitrophenyl-*D*-glucopyranoside were obtained from Sigma-Aldrich *via* Merck Chemicals, Durban, South Africa. Diethylenetriaminepentaacetic, ethylenediaminetetraacetic acid, iron (III) chloride, trichloroacetic acid, thiobarbituric acid, potassium ferricyanide, calcium chloride ($CaCl_2$), ammonium molybdate and aluminium chloride were procured from United Scientific, Durban, South Africa. Dichloromethane (DCM), methanol, magnesium sulphate, hydrogen peroxide, sodium carbonate, and ferrous sulphate were purchased from Shalom Laboratory Supplies, Durban, South Africa.

2.2. Plant collection

The fresh leaves of *S. petersiana* (Bolle) were obtained from shrubs growing at Westville Campus, University of KwaZulu-Natal, Durban, South Africa, in January 2020. The leaf materials were identified by the curator of the Ward Herbarium, School of Life Sciences, University of KwaZulu-Natal (Dr. Syd Ramdhani). A voucher of the leaf specimen (K. Olofinsan 7) was deposited at the Ward Herbarium (UDW), Biology building, School of Life Sciences. Then, the leaves were washed with running tap water and air-dried to constant weight under shade. After drying, the plant material was pulverized with a miller (Wiley, Philadelphia, USA).

2.3. Preparation of extracts

About 60 g of the *S. petersiana* dried leaf powder was transferred into a 1 L beaker containing 500 mL *n*-hexane and kept at room temperature (25 ℃) for 24 h. After defatting with *n*-hexane, the plant material was extracted sequentially under a similar condition with DCM, methanol, and distilled water. The filtrates obtained from the DCM and methanol extracts were concentrated in a rotatory evaporator at 45 ℃, while the aqueous extract was concentrated over a boiling water bath (100 ℃). After the different extraction steps, the dried extracts were refrigerated at 4 ℃ before *in vitro* analysis.

2.4. Animals

Six Sprague-Dawley male rats, weighing (217.00±21.14) g, were obtained from the Biomedical Resource Unit, University of KwaZulu-Natal, Westville Campus, Durban, South Africa. A day before sacrifice, the animals were subjected to 12 h overnight fasting, during which they were denied access to food but given water. Then, the rats were euthanized in an isoform saturated gas chamber, and their pancreas and intestines were harvested after dissection. Biochemical studies with the tissues were carried out within 2 h after being excised.

2.5. Estimation of phytochemical content

2.5.1. Total flavonoids

The total flavonoid content of the *S. petersiana* extracts was determined using the aluminum chloride colorimetric method of Chang *et al*.[14], with slight modifications. Briefly, 200 µL of each extract solution (1 mg/mL) was added to 150 µL of aluminum chloride (10%) and 150 µL potassium acetate (1 M). Then 2 700 µL of distilled water was added, and the mixture was incubated at 25 ℃ for 30 min. The absorbance of the resulting mixture was measured at 415 nm in a spectrophotometer. The flavonoid content of the extracts was evaluated from a standard curve prepared with known concentrations of quercetin.

2.5.2. Total phenolics

The total phenolics in each extract were estimated spectrophotometrically with Folin Ciocalteau reagent by adopting the method of McDonald *et al*.[15] with minor modifications. A total of 800 µL sodium carbonate (700 mM) and 900 µL of 10 times diluted Folin Ciocalteau reagent were added to 200 µL solutions of each extract (1 mg/mL). The mixtures were incubated at room temperature (25 ℃) for 30 min, and the absorbance was read at 765 nm. The total phenolic content of the extract was estimated as gallic acid equivalent (GAE) using a gallic acid standard curve.

2.6. Determination of antioxidant properties

2.6.1. Ferric (Fe^{3+}) reducing antioxidant power (FRAP)

The reducing power of the *S. petersiana* extracts was evaluated using a modified protocol of Oyaizu[16]. Briefly, 1 mL of the extracts or ascorbic acid at 20-320 µg/mL concentrations was added to 500 µL of 0.2 M phosphate buffer (pH 6.6) and 250 µL potassium ferricyanide (1%) in a test tube. The mixture was incubated at 50 ℃ for 30 min before acidification with 500 µL of 10% trichloroacetic acid. Then 500 µL aliquot of distilled water and iron (III) chloride (0.1%) was added to the solution in sequence. The absorbance of the reaction mixture was read at 700 nm, and the reducing power of extracts was expressed as:

$$\%\ \text{FRAP} = \frac{\text{Absorbance of sample}}{\text{Absorbance of gallic acid (320 µg/mL)}} \times 100$$

2.6.2. Hydroxyl radical ($OH^{\cdot}$) scavenging activity

The hydroxyl radical ($OH^{\cdot}$) scavenging activity of the extracts was determined by evaluating their ability to suppress Fe^{2+} mediated attack on deoxyribose in the presence of hydrogen peroxide using the protocol of Halliwell and Gutteridge[17] with minor modifications. Briefly, 0.15 mL deoxyribose (20 mM), 0.25 mL phosphate-buffered saline, 0.1 mL ferrous sulphate (500 µM) and 0.1 mL of 1% hydrogen peroxide (H_2O_2) were added to 0.1 mL extract (20-320 µg/mL) in sequence. The reaction mixture was incubated at 37 ℃ for 30 min. Then 0.2 mL of 10% trichloroacetic acid and 0.6 mL of 0.25% thiobarbituric acid were added. The mixture was boiled at 100 ℃ for 20 min and later cooled to room temperature. The absorbance of the pink solution formed was read at 532 nm, and hydroxyl radical scavenging activity was calculated with the formula of Olofinsan *et al*.[18]:

$$\%\ OH^{\cdot}\ \text{scavenging activity} = \frac{AB-(AM-AS)}{AB} \times 100$$

Where AB = absorbance of the mixture without sample; AM = Absorbance of mixture with sample; AS = absorbance of the sample only.

2.6.3. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH mopping activity of the extracts was measured by their capacity to change DPPH purple color using a modified method previously described by Ak and Gülçin[19]. Briefly, 100 µL of 0.3 mM DPPH solution (prepared in methanol) was added to 100 µL of the extracts or gallic acid at 20-320 µg/mL in a 96-well microplate. The solution was kept in the dark at room temperature (25 ℃) for 30 min before the absorbance was measured at 517 nm. The % DPPH radical scavenging activity was calculated against a blank solution containing distilled water using the equation:

$$\%\ \text{DPPH scavenging activity} = \frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}} \times 100$$

2.7. Carbohydrate digestive enzyme and intestinal glucose absorption inhibitory activities

2.7.1. α–Amylase assay

The effect of *S. petersiana* extracts on α-amylase activity was determined using the method of Ibitoye *et al*.[6] with slight modifications. Briefly, 0.2 mL extract at 20-320 µg/mL was added to 0.3 mL solution of 3 mg/mL porcine pancreatic α-amylase dissolved in 0.2 M of pH 6.9 sodium phosphate buffer. The solution was incubated for 15 min at 37℃ before adding 0.2 mL of 1% starch solution. The mixture was incubated at 37℃ for an additional 30 min. Then 0.9 mL of dinitrosalicylic acid reagent was added, and the mixture was boiled at 100℃ for 15 min. After cooling to room temperature, 1 mL distilled water was added to the resulting solution and absorbance was read at 540 nm. The % inhibition of the extracts was calculated using the expression:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

2.7.2. α–Glucosidase assay

The effect of the extracts on α-glucosidase activity was determined using the protocol of Ademiluyi and Oboh[20] with minor modifications. In brief, 0.2 mL of each extract or acarbose at 20-320 µg/mL was transferred into Eppendorf tubes containing 0.4 mL of 1 U/mL yeast α-glucosidase in 0.1 M phosphate buffer (pH 6.8). Then, the mixture was incubated at 37℃ for 15 min, followed by adding 0.2 mL of 5 mM 4-nitrophenyl-*D*-glucopyranoside in the same buffer solution. The resulting mixture was incubated further for 20 min before the absorbance was read at 405 nm. The enzyme inhibitory activity of the extracts was calculated as:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

2.7.3. Glucose absorption in isolate rat jejunum

The effect of *S. petersiana* aerial extracts on glucose absorption in freshly harvested rat jejunum was determined according to the method of Chukwuma and Islam[21]. The rat jejunum measuring about 20 cm from the pylorus was severed into 5 cm segments. The inner part of each segment was flushed with Krebs buffer containing 25 mM sodium bicarbonate, 5 mM potassium chloride (KCl), 1.2 mM potassium dihydrogen phosphate, 1.328 mM calcium chloride ($CaCl_2$), 118 mM sodium chloride, and 1.2 mM magnesium sulphate. Then the jejunum pieces were inverted to expose the inner session before each was transferred into an 8 mL solution with varying concentrations of the extract (20-320 µg/mL) in Krebs buffer and 11.1 mM glucose. The reaction solution was incubated in a Steri-Cult incubator (Labotec, South Africa) at 37℃ and 5% CO_2 for 2 h. A solution with glucose and the buffer only served as the control and a 2 mL aliquot of reaction solution taken before and after the incubation period was used for intestinal glucose absorption estimation *via* the following formula:

$$\text{Intestinal glucose absorption} = \frac{\text{C1} - \text{C2}}{\text{Length of jejunum (cm)}}$$

Where C1 = Glucose concentration (mg/dL) before 2 h incubation; C2 = Glucose concentration (mg/dL) after 2 h incubation.

2.8. Pancreatic lipase assay

The activity of the leaf extracts on pancreatic lipase was determined according to the protocol described by Kim *et al*.[22] with orlistat as a reference standard. Briefly, 2 mg/mL porcine pancreatic lipase was prepared in a buffer solution containing 0.10 M morpholine propane sulphonic acid and 0.001 M ethylenediaminetetraacetic acid, pH 6.8. Then, the enzyme solution (0.02 mL) was incubated with 0.17 mL of Tris buffer (100 mM Trizma hydrochloride and 5 mM $CaCl_2$, pH 7.0) and 0.02 mL of different concentrations of each extract for 30 min at 37℃. Five µL of *p*-nitrophenyl butyrate (prepared in 20 mM dimethylformamide) was added to the mixtures in a 96-well microplate, and the reaction mixture was incubated at 37℃ for an additional 5 min. The absorbance of 2, 4-dinitrophenol released from the reaction was read at 405 nm, and the enzyme inhibitory activity was expressed as:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

2.9. Tissue ex vivo studies

2.9.1. Preparation of pancreatic tissue homogenate

About 1.5 g of pancreatic tissue was homogenized in a 15 mL solution of 10% Triton X and sodium phosphate buffer (50 mM, pH 7.5). The resulting homogenate was centrifuged at 20000 ×*g* for 15 min in a refrigerated centrifuge at 4℃. Afterward, the supernatant obtained was preserved at –80℃ before biochemical analysis.

2.9.2. Induction of oxidative pancreatic injury

The induction of oxidative injury in pancreatic tissue homogenate was done according to Oboh *et al*.[23]. Briefly, 120 µL ferrous sulphate (0.1 mM) was added to a reaction mixture containing 300 µL tissue homogenate and 300 µL of increasing concentrations (20-320 µg/mL) of the extracts or gallic acid. The treatments were incubated for 30 min at 37℃, and after that, samples were taken for enzyme assay. The normal treatment contains only the tissue homogenate, while the untreated treatment contains the homogenate and $FeSO_4$ pro-oxidant solution.

2.9.3. Estimation of reduced glutathione (GSH) concentration

The GSH concentration of tissue homogenate was measured using the procedure of Ellman[24]. A total of 100 µL of the homogenates was deproteinized with 300 µL of 10% trichloroacetic acid. The solution was centrifuged at 5000 ×*g* for 5 min. Then, 100 µL of the

resulting supernatant was added to 50 μL Ellman's reagent (0.5 mM) prepared in 0.2 M sodium phosphate buffer (pH 7.8). The mixture was incubated at 25℃ for 30 min, and the concentration of 5′-thio-2-nitrobenzoic acid produced from the reaction between GSH and 5,5′-dithiobis (2-nitrobenzoic acid) was read at 415 nm. The amount of GSH in the sample was extrapolated from a standard curve with known GSH concentrations.

2.9.4. SOD assay

The SOD activity in homogenate samples was estimated according to the method of Kakkar *et al*.[25] with slight modifications. A total of 0.17 mL of 0.1 mM diethylenetriaminepentaacetic acid was added to 0.015 mL of homogenate in 96-well microplates. Then, 0.015 mL of 1.6 mM 6-hydroxydopamine was added to the mixture. Immediately, the absorbance of the reaction solution was read thrice at 1 min interval by a spectrophotometer set at 492 nm.

2.9.5. Catalase assay

The catalase activity in pancreatic tissue homogenate was determined using the method by Hadwan and Abed[26]. Briefly, 200 μL of homogenate sample was added to 2 mL of H_2O_2 (65 μM) prepared in 6 mM sodium phosphate buffer (pH 7.4). The mixture was incubated for 2 min at 37℃, and the reaction terminated with 0.4 mL of 32.4 mM ammonium molybdate. The colored molybdate/H_2O_2 complex formed was measured at 347 nm against a blank solution with only H_2O_2.

2.9.6. Estimation of malondialdehyde (MDA) concentration

The MDA concentration in the pancreatic homogenate was determined using a slightly modified method by Fraga *et al*.[27]. Briefly, 0.1 mL of homogenate was transferred to a mixture containing 0.1 mL sodium dodecyl sulfate solution (8.1%), 0.375 mL glacial acetic acid (20%), 1 mL thiobarbituric acid solution (0.25%) and 425 μL Milli-Q water. The mixture was boiled in a water bath for 1 h and then cooled to room temperature. The absorbance of 200 μL of each sample was read in 96-well microplates at 532 nm, and MDA concentration was extrapolated from a standard curve with known MDA concentrations.

2.9.7. Estimation of nitric oxide (NO) concentration

The level of NO in the homogenate samples was estimated according to the method of Erukainure *et al*.[28] and Tsikas[29] using Griess reagent. Briefly, 150 μL of pancreatic homogenate was added to 150 μL of Griess reagent in 96-well microplates. The mixture was maintained in a dark chamber at room temperature for 30 min, and absorbance was measured at 546 nm against a blank containing distilled water instead of the homogenate.

2.9.8. Acetylcholinesterase (ACTh) activity assay

The pancreatic ACTh activity was determined using the protocol described by Ellman *et al*.[30]. Briefly, 100 μL of treatment supernatant was added to 50 μL of 3.3 mM Ellman's reagent (pH 7.0) and 250 μL of 100 mM phosphate buffer (pH 8). The reaction solution was incubated at room temperature (25℃) for 20 min. Then 50 μL of 50 mM acetylcholine iodide was added to the resulting mixture before absorbance was measured immediately at 412 nm at 3 min intervals.

2.9.9. ATPase activity assay

The activity of the ATPase enzyme in treatment samples was estimated according to the previously modified method of Erukainure *et al*.[31]. Briefly, 0.2 mL of tissue homogenate was added to a mixture containing 0.2 mL of 5 mM KCl, 1.3 mL of 0.1 M Trizma hydrochloride (Tris-HCl) buffer, and 40 μL of 50 mM ATP (adenosine 5′-triphosphate disodium salt). Then, the resulting mixture was kept on a shaker for 30 min at 37℃ before the reaction was terminated with 1 mL of ammonium molybdate solution. A 1 mL solution of freshly prepared ascorbic acid (9%) was added to the mixture and then incubated further for 30 min at room temperature (25℃). The absorbance of the final solution was measured at 660 nm, and the activity of the ATPase enzyme was expressed as the amount of inorganic phosphate (μmol Pi) liberated/mg of tissue sample.

2.10. Gas chromatography–mass spectrometric (GC–MS) analysis

The GC-MS characterization of the *S. petersiana* extracts was done with a Shimadzu gas chromatograph (series AOC-20i) coupled Mass Spectrophotometer (GCMS-QP2010 SE). Before the analysis, the methanol and aqueous extracts were derivatized, according to the method of Olofinsan *et al*.[18]. Then, the DCM extract, methanol, and aqueous silylated derivatives were analyzed using an Ultra-pure helium carrier gas at a flow rate of 1.03 mL/min and linear velocity of 37 cm/s. The injector temperature was programmed at 250℃, and the oven temperature was set to 280℃ from 60℃ at the rate of 10℃/min, with 3 min hold time. Then, 1 μL of the samples were injected in a splitless mode of a 20∶1 split ratio. The mass spectrometer was operated in an electron ionization mode of 70 eV and electron multiplier voltage of 1 859 V. Additionally, the ion source temperature was 230℃ and the quadrupole temperature was 150℃. The solvent cut time was maintained at 3 min while the scan range was kept between 50-700 amu. The compounds present in the extracts were identified by directly comparing retention times and mass spectral data results with those stored in the NIST library.

2.11. Molecular docking analysis

The free binding energy (ΔG) of compounds identified in the extracts with digestive enzymes was calculated *via* molecular docking simulation. The SDF format of 3D chimeric structures of the compounds was obtained from PubChem and was prepared using the dock prep tool of UCFS Chimera V. 1.14. This software

removed co-crystallized water molecules and then added hydrogen atoms and gasteiger charges. 3D crystallographic structures of α-amylase, α-glucosidase, and pancreatic lipase with 1B2Y, 3CTT, and 1LPB access codes were retrieved from the protein data bank. The chimeric coordinates of the catalytic site were obtained using the CASTp automated online server. All the compounds identified in the leaf extracts were docked separately with each protein using the Lamarckian genetic algorithm of Autodock Vina software. The complex of the protein-ligand pose with the lowest ΔG was saved in PDB format and then visualized in 2D and 3D with the BIOVIA Discovery Studio software suite.

2.12. Statistical analysis

Data collected from this study were expressed as mean±SEM of three independent experiments (n=3). The data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's HSD *post hoc* test. P<0.05 was considered significantly different. All statistical analyses in this study were computed with windows IBM SPPS version 26 (IBM Corp., Armonk, NY, USA).

2.13. Ethical statement

Animal handling, maintenance, and processing of harvested tissue samples were carried out as per the approved guidelines of the Animal Research Ethics Committee (AREC), University of KwaZulu-Natal, Durban, South Africa (protocol number: AREC/00002325/2021).

3. Results

3.1. Phytochemical content of S. petersiana

The total phenolic and flavonoid content of *S. petersiana* leaf extracts were estimated as gallic acid and quercetin equivalents, respectively, as indicated in Supplementary Figure 1. While the DCM extract had a significantly higher flavonoid concentration (P<0.05) (Supplementary Figure 1A) compared with other extracts, its total phenolic content was significantly lower than those of the methanol and aqueous extracts (Supplementary Figure 1B).

3.2. S. petersiana in vitro antioxidant properties

In Figure 1A, the DCM extract showed significantly higher reducing power at 160 µg/mL and 320 µg/mL compared with other extracts (P<0.05). However, its higher IC_{50} value [(51.60±2.82) µg/mL] suggests a lesser ferric reducing antioxidant activity compared with

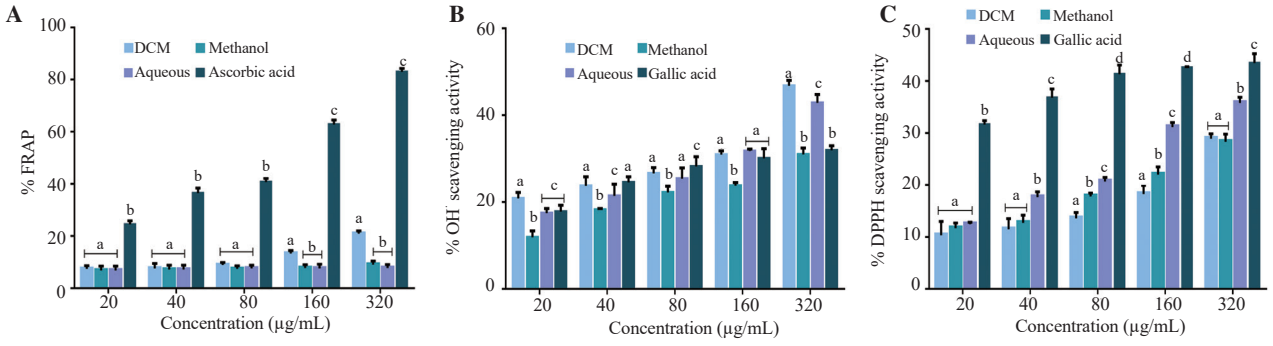


Figure 1. (A) Reducing power, (B) OH radical, and (C) DPPH radical scavenging activities of *Senna petersiana* leaf extracts. Values are presented as mean±SEM (n=3). Bars with different alphabets (a-d) for each concentration are significantly different from each other (P<0.05). DCM, dichloromethane.

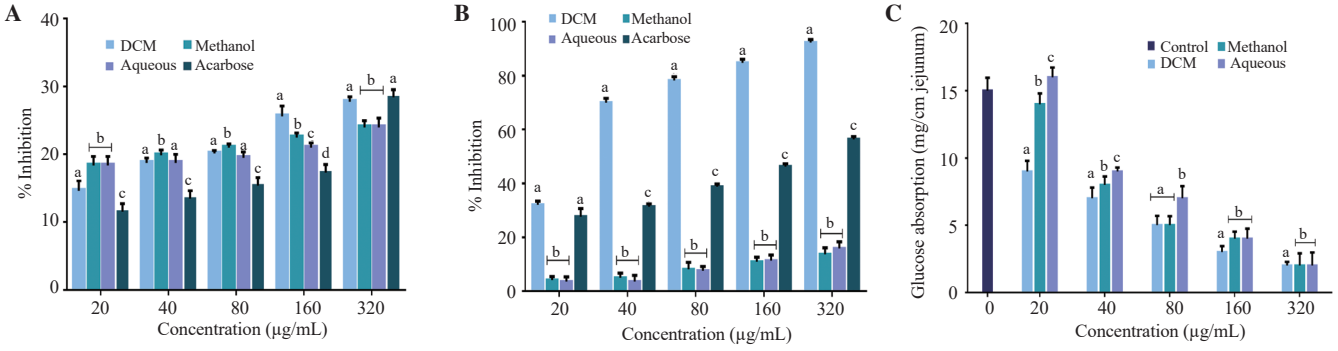


Figure 2. Effect of *Senna petersiana* leaf extracts on (A) α-amylase, (B) α-glucosidase, and (C) intestinal glucose absorption. Values are presented as mean±SEM (n=3). Bars with different alphabets (a-d) for each concentration are significantly different from each other (P<0.05).

ascorbic acid [(11.10±1.78) μg/mL] as the standard compound in Supplementary Table 1. Moreover, in Figure 1B, all the extracts showed OH· scavenging ability at the test concentrations. The DCM extract [IC_{50}=(44.70±0.76) μg/mL] demonstrated significant hydroxyl radical (OH·) scavenging ability compared with methanol and aqueous extracts (Supplementary Table 1), which was comparable to that of gallic acid [IC_{50}=(44.90±0.49) μg/mL]. In Figure 1C, the extracts showed dose-dependent DPPH mopping activities. Notably, the aqueous sample had significantly (P<0.05) higher free radical scavenging activity (40-320 μg/mL) with an IC_{50} value of (37.30±1.83) μg/mL, which, however, suggests lower potency compared with that of the standard compound [IC_{50}=(23.20±0.76) μg/mL] (Supplementary Table 1).

3.3. Effect of S. petersiana on carbohydrate digestive enzyme activities and intestinal glucose absorption

Figures 2A and 2B display inhibitory effects of *S. petersiana* extract on α-amylase and α-glucosidase digestive enzymes. The DCM extract showed superior α-amylase inhibition with significantly higher activities at 160-320 μg/mL (Figure 2A). The significantly low IC_{50} value of the extract was observed compared with other extracts [(50.40±1.36) μg/mL], as shown in Supplementary Table 1. Similarly, in Figure 2B, the DCM extract showed a remarkable dose-dependent α-glucosidase inhibitory effect (P<0.05) [IC_{50}= (8.10±1.50) μg/mL] compared with the other extracts and acarbose. Although all leaf extracts showed the ability to lower intestinal glucose absorption with increasing concentrations, the DCM extract had better potency than others (P<0.05) (Figure 2C).

3.4. Effect of S. petersiana on lipid digestive enzyme

Figure 3 displays the *in vitro* pancreatic lipase inhibitory activity of the extracts. The activities of methanol and aqueous extracts were significantly higher than those of DCM extract and orlistat at 80-320 μg/mL (P<0.05). However, as shown in Supplementary Table 1, there were no significant differences between the lipase inhibitory capacities of the methanol and aqueous extracts of *S. petersiana* [IC_{50}: (42.50±0.72) μg/mL and (42.00±1.36) μg/mL].

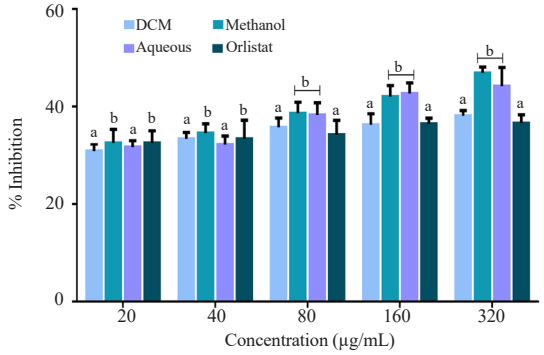


Figure 3. Pancreatic lipase inhibitory activity of *Senna petersiana* leaf extracts. Values are presented as mean±SEM (n=3). Bars with different alphabets (a-b) for a given concentration are significantly different from each other (P<0.05).

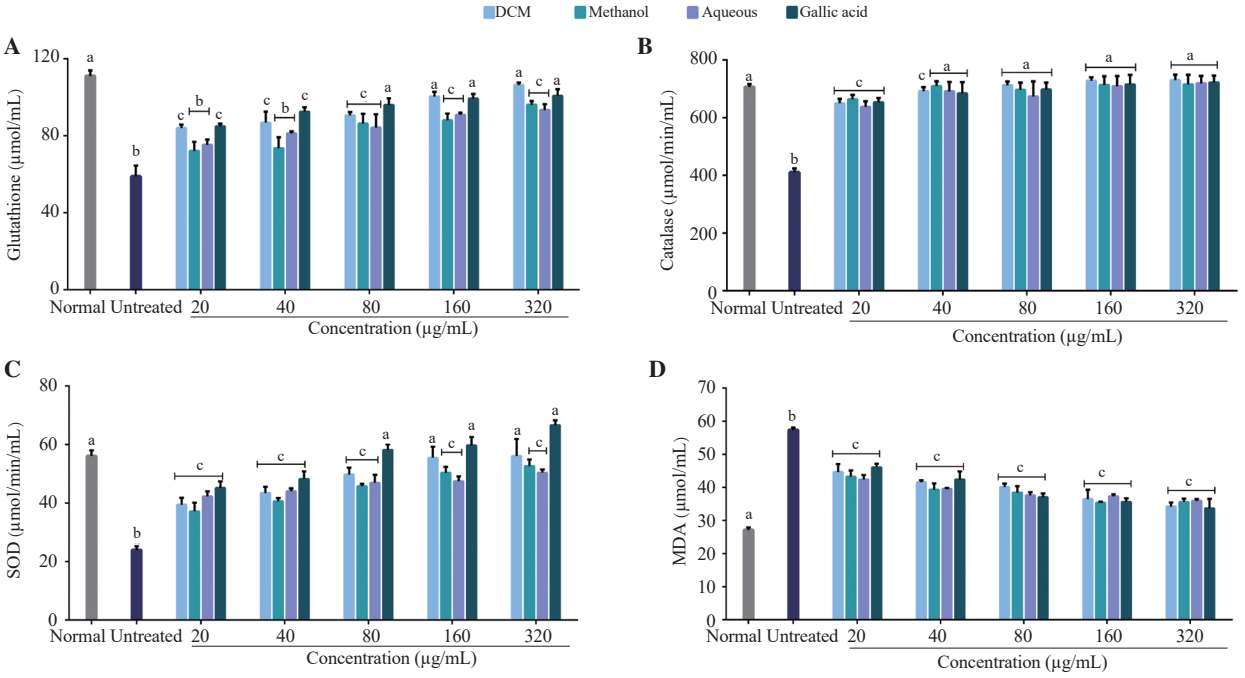


Figure 4. Effect of *Senna petersiana* leaf extracts on (A) glutathione, (B) catalase, (C) superoxide dismutase (SOD), and (D) malondialdehyde (MDA) concentration in oxidative pancreatic injury. Values are presented as mean±SEM (n=3). Bars with different alphabets (a-c) are significantly (P<0.05) different from each other.

3.5. Effect of S. petersiana on oxidative stress biomarkers in $FeSO_4$ induced pancreatic injury

Incubation of pancreas homogenate with $FeSO_4$ led to a reduction in the tissue GSH concentration (Figure 4A) with decreased SOD (Figure 4C) and catalase (Figure 4B) activities. Additionally, Figure 4D revealed a significant increase in MDA concentration in the untreated sample after oxidative imbalance induction. Treatment with the *S. petersiana* extracts, especially the DCM extract at 160-320 µg/mL, increased GSH levels (Figure 4A). All the extracts caused a dose-dependent elevation of SOD activities (Figure 4C), but only the DCM extract and gallic acid had similar SOD activities to the normal group. In addition, all treatments increased catalase activities compared with the untreated group (Figure 4B).

3.6. Effect of S. petersiana on NO concentration in oxidative pancreatic injury

Figure 5 shows that $FeSO_4$ significantly elevated NO level (P<0.05). Treatment with plant extracts and gallic acid at 160-320 µg/mL lowered NO to a normal level.

3.7. Effect of S. petersiana effect on ACTh activity in oxidative pancreatic injury

In Figure 6, ACTh activity was increased significantly (P<0.05) after oxidative pancreatic injury induction. The plant extracts dose-dependently decreased ACTh activity. At 160-320 µg/mL, all the extracts had similar ACTh activity to the normal group.

3.8. Effect of S. petersiana on ATPase activity in oxidative pancreatic injury

$FeSO_4$ treatment elevated ATPase activity (Figure 7). Treatment with the extracts resulted in a dose-dependent reduction in ATPase activity. The DCM and methanol extracts suppressed the enzyme activities better than the aqueous extract.

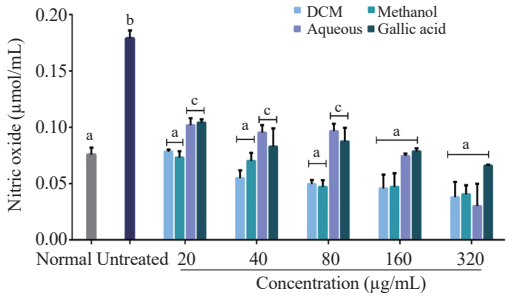


Figure 5. Effect of *Senna petersiana* leaf extracts on nitric oxide concentration in oxidative pancreatic injury. Values are presented as mean±SEM (n=3). Bars with different alphabets (a-c) are significantly different from each other (P<0.05).

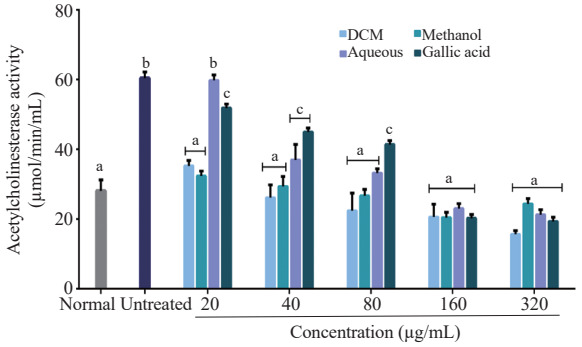


Figure 6. Effect of *Senna petersiana* leaf extracts on acetylcholinesterase activity in oxidative pancreatic injury. Values are presented as mean±SEM (n=3). Bars with different alphabets (a-c) are significantly different from each other (P<0.05).

Table 1. Chemical compounds identified in *Senna petersiana* leaf extracts by GC-MS analysis.

Compound	Retention time (min)	Relative abundance (%)
DCM extract		
Dihydroactinidiolide	12.444	0.31
Dihydrostilbestrol	14.913	1.34
n-Nonadecanol-1	15.239	7.50
Phytol, acetate	15.693	1.68
Methyl palmitate	16.604	0.23
Pentadecanoic acid	17.049	0.66
Phytol	18.433	5.33
1-Heptacosanol	19.191	5.40
Squalene	24.925	18.44
Methanol extract		
Hexanoic acid	5.128	0.37
p-Vinylguaiacol	9.495	0.40
Phytol, acetate	15.676	1.22
Pentadecanoic acid	17.108	6.85
n-Nonadecanol-1	17.279	1.64
Phytol	18.431	3.63
Octadecanoic acid	18.991	2.23
Veratramine	20.785	0.32
Cassine	20.929	17.90
1-Heptacosanol	22.534	0.34
Squalene	24.806	12.06
Aqueous extract		
Methyl alpha-*D*-mannopyranoside	15.449	49.32
Phytol, acetate	15.671	2.48
Trans-geranylgeraniol	19.329	1.25
Cassine	20.994	22.88
Alpha-tocopherol	24.405	1.67

3.9. GC–MS chemical analysis of S. petersiana

Table 1 shows bioactive phytochemicals identified in *S. petersiana* leaf extracts. Compounds such as dihydroactinidiolide and dihydrostilbestrol were mainly found in the DCM extract, while *p*-vinylguaiacol and veratramine were in the methanol extract. Additionally, GC-MS analysis shows the presence of methyl alpha-*D*-mannopyranoside, alpha-tocopherol, and *trans*-geranylgeraniol in the aqueous extract. The structures of other vital compounds like cassine, squalene, and phytol acetate found in different leaf extracts are displayed in Supplementary Figure 2.

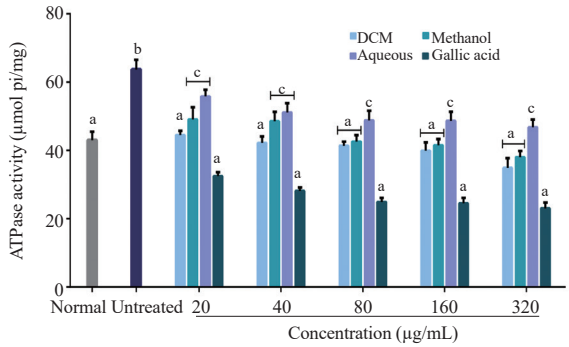


Figure 7. Effect of *Senna petersiana* leaf extracts on ATPase enzyme activity in oxidative pancreatic injury. Values are presented as mean±SEM (n=3). Bars with different alphabets (a-c) are significantly different from each other (P<0.05).

3.10. Molecular docking with digestive enzymes

In silico molecular screening revealed that all compounds in the plant extracts have negative binding energies with carbohydrate and lipid digestive enzymes. Supplementary Table 2 indicates that the alkaloid veratramine had the highest affinity with α-amylase and lipase with calculated binding energies of −10.5 kcal/mol and −10.4 kcal/mol, respectively. Amongst the compounds, dihydrostilbestrol bonded more strongly to α-glucosidase (−6.9 kcal/mol). The 2D inspection of the molecular forces responsible for stabilizing the protein-ligand complex of these compounds indicates the presence of hydrogen bonds, Van der Waals forces, pi-pi stacked bonds, and pi-sigma bonds, with other different interactions as presented in Supplementary Figure 3.

4. Discussion

Several studies have described oxidative stress as a mechanism that underpins chronic hyperglycemia-mediated pancreatic cell dysfunction in diabetic conditions[3]. Moreover, this organ's low intrinsic antioxidant gene encoding capacity makes it vulnerable to free radical attack[8]. Interestingly, plant products have emerged as excellent sources of antioxidants with the ability to protect cellular macromolecules against oxidative damage, most especially under disease conditions[18,28,32]. The antioxidant protective properties of plants are associated with their bioactive chemical composition. The total flavonoid and phenol contents of *S. petersiana* leaf extract revealed that the plant has potential antioxidant bioactive molecules, some of which were also identified *via* GC-MS chemical analysis. Nevertheless, the higher total phenolics in the methanol and aqueous extracts may suggest the presence of more polar constituents. This observation is similar to the findings of Widyawati *et al*.[33] where polar leaf extract of a traditional herbal plant had higher total phytochemical content relative to non-polar extracts.

In an *in vitro* analysis of chemical compounds, antioxidant activities are usually determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} and scavenge reactive free radicals such as superoxide anion, hydrogen peroxide, NO, *etc*. In this study, the antioxidant potential of *S. petersiana* extracts is evidenced by significant ferric reducing property, as well as DPPH and hydroxyl radical scavenging activities. Interestingly, the antioxidant activities of this plant may be attributed to the intrinsic chemical compounds found in its extracts, which need to be verified by future studies. The antioxidant potency of compounds such as alpha-tocopherol, cassine, and veratramine found in *S. petersiana* leaf extracts has also been described in previous studies[34,35].

Glucose molecules required for energy generation in the human system are obtained mainly for dietary carbohydrates. This process is facilitated by catabolic digestive enzymes, which hydrolyze complex carbohydrate polymers into smaller monosaccharide units. Amongst these enzymes are α-amylase and α-glucosidase, which function in the digestive tract to enhance glucose released into the general blood circulation *via* intestinal absorption. Consequently, inhibition of these enzymes' activities has been explored as a promising target in managing diabetes and its complications. In this study, the leaf extracts showed effective α-amylase and α-glucosidase inhibitory effects comparable with the standard antidiabetic agent acarbose. More importantly, the DCM extract displayed pronounced suppression of α-glucosidase activity, which was consistent with its significant reduction of intestinal glucose absorption. The low binding energy of dihydrostilbestrol and veratramine found in the extracts may suggest their role in the anti-diabetic properties of the *S. petersiana* leaf. Moreover, the formation of strong molecular forces between the compounds and the amino acid residues of the enzymes may further indicate better modulation. In support of the current study, the use of plant alkaloids and stilbenoid derivatives similar to those in leaf extracts in managing diabetes-associated physiological alterations has been well documented[36,37].

While obesity is highly associated with T2D, one common approach to managing the former metabolic disorder is decreasing excessive energy intake *via* reducing intestinal absorption of dietary fat[22]. In this regard, phytochemicals that inhibit pancreatic lipase activity have been a primary therapeutic target, more importantly, due to their lesser side effects when compared with synthetic drugs. In the present study, *S. petersiana* extracts demonstrated anti-lipase activities with a lower IC_{50} value comparable to the orlistat drug. The negative binding energies of veratramine and other compounds present in the leaf with chimeric 3D structures of pancreatic lipase may indicate the involvement of these compounds in providing the plant with this biological activity. Consistent with our study, the anti-obesogenic activity of some senna species has been described

in other studies where the plants inhibited pancreatic lipase and cholesterol esterase enzymes[38].

In normal physiological conditions, specific metabolic processes in the body generate non-lethal levels of some reactive radical species. However, the body's antioxidant defense system components prevent these chemical entities from damaging cellular DNA, lipids, and protein components. One of such protective proteins is GSH present in all body cells. When this protein interacts with electron-deficient free radicals, it quenches their capacities to activate reaction cascades that may offset cellular redox homeostasis[2]. Similarly, SOD and CAT enzymes also perform antioxidant functions. When molecular oxygen accepts an electron, it forms a superoxide anion, which SOD transforms into H_2O_2, a substrate that CAT converts subsequently into water and oxygen molecules[39]. Fe^{2+} reaction with hydrogen peroxide in the Fenton reaction produced $OH^{\cdot}$ radicals, which can attack membrane unsaturated fatty acids and thus triggers lipid peroxidation cascade[40]. Hence, the decrease in GSH level, SOD and CAT activities in the untreated group may suggest the depletion of cellular antioxidant reserves due to $FeSO_4$ treatment. The extracts' ability to increase CAT, GSH, and SOD activities may demonstrate the protective effects of the plant on redox imbalance in T2D models as similar to those of other plants reported in previous studies[7,28].

Scientific findings have associated NO with vital physiological roles such as microvascular blood flow regulation, exocrine enzyme secretion, and endocrine insulin production in pancreatic tissues[41]. Despite these functions, excessive levels of the pro-inflammatory molecule have been described as one of the markers of redox imbalance in the pathology of acute pancreatitis[42]. Consequently, the ability of the leaf extracts to reduce NO levels may further credence the plant's antioxidant properties *via* suppressing oxidative free radicals, as observed previously in the study. Similar to this study, an *in vitro* investigation by Djemgou *et al*.[10] revealed that chemical compounds isolated from *S. petersiana* leaf showed anti-inflammatory properties in tissue culture.

Cholinergic signaling involving β-cells muscarinic acetylcholine receptors has been described in the pancreas's metabolic functions and glucose homeostasis regulation. However, increased cholinesterase gene expression and activity have been implicated in apoptosome formation leading to programmed pancreatic β-cell death in chronic diabetic conditions[43]. The high ACTh activity after induction of oxidative pancreatic injury represents the possible adverse outcome of redox imbalance due to chronic hyperglycemia in T2D. The ability of the extracts to significantly suppress the cholinergic enzyme activity may suggest the cholinesterase inhibitory property and hence its possible use in the therapy of diabetic neuropathies linked with the enzyme. Interestingly, cohort studies with human subjects have revealed that cholinesterase inhibitors lowered mortality in patients with diabetes-mediated dementia[44].

Purine enzyme signaling plays an integral role in insulin release from the pancreas. According to Owada *et al*.[45], the membrane depolarization resulting from β-cells Na^+ K^+-ATPase inhibition was described as a mechanism involved in glucose-stimulated insulin secretion. The high ATPase activity in the untreated group in this study may undermine effective membrane depolarization processes. Consequently, the reduction in enzyme activities after treatment with the extracts may demonstrate the leaf's ability to improve insulin release in diabetic conditions, which needs further verification.

In conclusion, this study demonstrates that the *S. petersiana* leaf extracts possess antihyperglycemic and antioxidant activities, inhibit carbohydrate digestive enzymes and improve redox imbalance-induced biochemical alteration in oxidative pancreatic injury. However, further *in vivo* studies are underway to properly understand the plant's potential clinical benefits in diabetes management.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

Acknowledgments

The first author acknowledges the financial support received from the National Research Foundation-The World Academy of Science (NRF-TWAS), South Africa (Grant no. 116093).

Funding

This study was supported by the Research Office, University of KwaZulu-Natal, 439 Durban, South Africa.

Authors' contributions

KAO was responsible for the study conceptualization, experimental analysis, and manuscript writing. OLE reviewed the original draft, NZM edited the manuscript, and MSI supervised and made all provisions for the project.

References

[1] IDF. *International Diabetes Federation Atlas*. 10th ed. Belgium: International Diabetes Federation Brussels; 2021.

[2] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, et al. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular complications: Avenues for a mechanistic-based therapeutic approach. *Curr Diabetes Rev* 2011; **7**(5): 313-324.

[3] Pitocco D, Tesauro M, Alessandro R, Ghirlanda G, Cardillo C. Oxidative stress in diabetes: Implications for vascular and other complications. *Int J Mol Sci* 2013; **14**(11): 21525-21550.

[4] Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm J* 2016; **24**(5): 547-553.

[5] Cooley MM, Jones EK, Gorelick FS, Groblewski GE. Pancreatic acinar cell protein synthesis, intracellular transport, and export. Pancreapedia: The Exocrine Pancreas Knowledge Base [Online] 2020. Doi: 10.3998/panc.2020.15. [Accessed on June 9, 2022].

[6] Ibitoye O, Olofinsan K, Teralı K, Ghali U, Ajiboye T. Bioactivity-guided isolation of antidiabetic principles from the methanolic leaf extract of *Bryophyllum pinnatum*. *J Food Biochem* 2018; **42**(5): e12627.

[7] Olofinsan KA, Erukainure OL, Brian BK, Islam MS. *Harpephyllum caffrum* stimulates glucose uptake, abates redox imbalance and modulates purinergic and glucogenic enzyme activities in oxidative hepatic injury. *Pac J Trop Biomed* 2022; **12**(1): 9.

[8] Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med* 1996; **20**(3): 463-466.

[9] Gatsing D, Djemgou P, Garba I, Aliyu R, Tchuendem M, Tane P, et al. Dihydronaphtalenone and chromone from *Cassia petersiana* Bolle and the antisalmonellal activity of its crude extract. *Res J Phytochem* 2010; **4**(3): 201-206.

[10]Djemgou PC, Gatsing D, Tchuendem M, Ngadjui BT, Tane P, Ahmed AA, et al. Antitumor and immunostimulatory activity of two chromones and other constituents from *Cassia petersiana*. *Nat Prod Commun* 2006; **1**(11): 961-968.

[11]Cordier W, Cromarty AD, Botha E, Steenkamp V. Effects of selected South African plant extracts on haemolysis and coagulation. *Hum Exp Toxicol* 2012; **31**(3): 250-257.

[12]Aremu A, Ndhlala A, Fawole O, Light M, Finnie J, Van Staden J. *In vitro* pharmacological evaluation and phenolic content of ten South African medicinal plants used as anthelmintics. *S Afr J Bot* 2010; **76**(3): 558-566.

[13]Tshikalange T, Meyer J, Hussein A. Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. *J Ethnopharmacol* 2005; **96**(3): 515-519.

[14]Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002; **10**(3): 178-182.

[15]McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem* 2001; **73**(1): 73-84.

[16]Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr Diet* 1986; **44**(6): 307-315.

[17]Halliwell B, Gutteridge JM. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. *FEBS Lett* 1981; **128**(2): 347-352.

[18]Olofinsan KA, Salau VF, Erukainure OL, Islam MS. *Ocimum tenuiflorum* mitigates iron-induced testicular toxicity *via* modulation of redox imbalance, cholinergic and purinergic dysfunctions, and glucose metabolizing enzymes activities. *Andrologia* 2021; **53**(9): e14179.

[19]Ak T, Gülçin İ. Antioxidant and radical scavenging properties of curcumin. *Chem Biol Interact* 2008; **174**(1): 27-37.

[20]Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*. *Exp Toxicol Pathol* 2013; **65**(3): 305-309.

[21]Chukwuma CI, Islam MS. Effects of xylitol on carbohydrate digesting enzymes activity, intestinal glucose absorption and muscle glucose uptake: A multi-mode study. *Food Funct* 2015; **6**(3): 955-962.

[22]Kim GN, Shin MR, Shin SH, Lee AR, Lee JY, Seo BI, et al. Study of antiobesity effect through inhibition of pancreatic lipase activity of *Diospyros kaki* fruit and *Citrus unshiu* peel. *Biomed Res Int* 2016; **2016**. Doi: 10.1155/2016/1723042.

[23]Oboh G, Akinyemi AJ, Ademiluyi AO. Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. Rubra) and white ginger (*Zingiber officinale* Roscoe) on Fe^{2+} induced lipid peroxidation in rat brain *in vitro*. *Exp Toxicol Pathol* 2012; **64**(1-2): 31-36.

[24]Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82**(1): 70-77.

[25]Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 1984; **21**(2): 130-132.

[26]Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. *Data Br* 2016; **6**: 194-199.

[27]Fraga CG, Leibovitz BE, Tappel AL. Lipid peroxidation measured as thiobarbituric acid-reactive substances in tissue slices: Characterization and comparison with homogenates and microsomes. *Free Radic Biol Med* 1988; **4**(3): 155-161.

[28]Erukainure OL, Reddy R, Islam MS. Raffia palm (*Raphia hookeri*) wine extenuates redox imbalance and modulates activities of glycolytic and cholinergic enzymes in hyperglycemia-induced testicular injury in type 2 diabetic rats. *J Food Biochem* 2019; **43**(3): e12764.

[29]Tsikas D. Review Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. *Free Radic Res* 2005; **39**(8): 797-815.

[30]Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; **7**(2): 88-95.

[31]Erukainure OL, Mopuri R, Oyebode OA, Koorbanally NA, Islam MS. *Dacryodes edulis* enhances antioxidant activities, suppresses DNA fragmentation in oxidative pancreatic and hepatic injuries; and inhibits carbohydrate digestive enzymes linked to type 2 diabetes. *Biomed Pharmacother* 2017; **96**: 37-47.

[32]Ibitoye O, Olofinsan K, Teralı K, Ghali U, Ajiboye T. Bioactivity-guided

isolation of antidiabetic principles from the methanolic leaf extract of *Bryophyllum pinnatum*. *J Food Biochem* 2018; **42**(5): e12627.

[33]Widyawati PS, Budianta TDW, Kusuma FA, Wijaya EL. Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* less leaves extracts. *Int J Pharmacogn Phytochem* 2014; **6**(4): 850-855.

[34]Rehman S, Khan H. Advances in antioxidant potential of natural alkaloids. *Curr Bioact Compd* 2017; **13**(2): 101-108.

[35]Burcova Z, Kreps F, Schmidt S, Strizincova P, Jablonsky M, Kyselka J, et al. Antioxidant activity and the tocopherol and phenol contents of grape residues. *BioResources* 2019; **14**(2): 4146-4156.

[36]Ajebli M, Khan H, Eddouks M. Natural alkaloids and diabetes mellitus: A review. *Endocr Metab Immune Disord* 2021; **21**(1): 111-130.

[37]Akinwumi BC, Bordun KAM, Anderson HD. Biological activities of stilbenoids. *Int J Mol Sci* 2018; **19**(3): 792.

[38]Yuniarto A, Sukandar EY, Fidrianny I, Setiawan F, Ketut I. Antiobesity, antidiabetic and antioxidant activities of Senna (*Senna alexandrina* Mill.) and Pomegranate (*Punica granatum* L.) leaves extracts and its fractions. *Int J Pharm Phytopharmacolog Res* 2018; **8**(3): 18-24.

[39]Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem* 2015; **30**(1): 11-26.

[40]Repetto M, Semprine J, Boveris A. Lipid peroxidation: Chemical mechanism, biological implications and analytical determination. In: Catala A (ed). *Lipid peroxidation*. London: IntechOpen; 2012, p. 3-30.

[41]Buchwalow I, Schnekenburger J, Samoilova V, Boecker W, Neumann J, Tiemann K. New insight into the role of nitric oxide pathways in pancreas. *Acta Histochem Cytochem* 2018; **51**(6): 167-172.

[42]Xue M, Han L, Qian W, Li J, Qin T, Xiao Y, et al. Nitric oxide stimulates acute pancreatitis pain *via* activating the NF-κB signaling pathway and inhibiting the kappa opioid receptor. *Oxid Med Cell Longev* 2020; **2020**: 9230958.

[43]Zhang B, Yang L, Yu L, Lin B, Hou Y, Wu J, et al. Acetylcholinesterase is associated with apoptosis in β cells and contributes to insulin-dependent diabetes mellitus pathogenesis. *Acta Biochim Biophys Sin* 2012; **44**(3): 207-216.

[44]Secnik J, Schwertner E, Alvarsson M, Hammar N, Fastbom J, Winblad B, et al. Cholinesterase inhibitors in patients with diabetes mellitus and dementia: An open-cohort study of ~23 000 patients from the Swedish Dementia Registry. *BMJ Open Diabetes Res Care* 2020; **8**(1): e000833.

[45]Owada S, Larsson O, Arkhammar P, Katz AI, Chibalin AV, Berggren PO, et al. Glucose decreases Na^+, K^+-ATPase activity in pancreatic beta-cells. An effect mediated *via* Ca^{2+}-independent phospholipase A_2 and protein kinase C-dependent phosphorylation of the alpha-subunit. *J Biol Chem* 1999; **274**(4): 2000-2008.

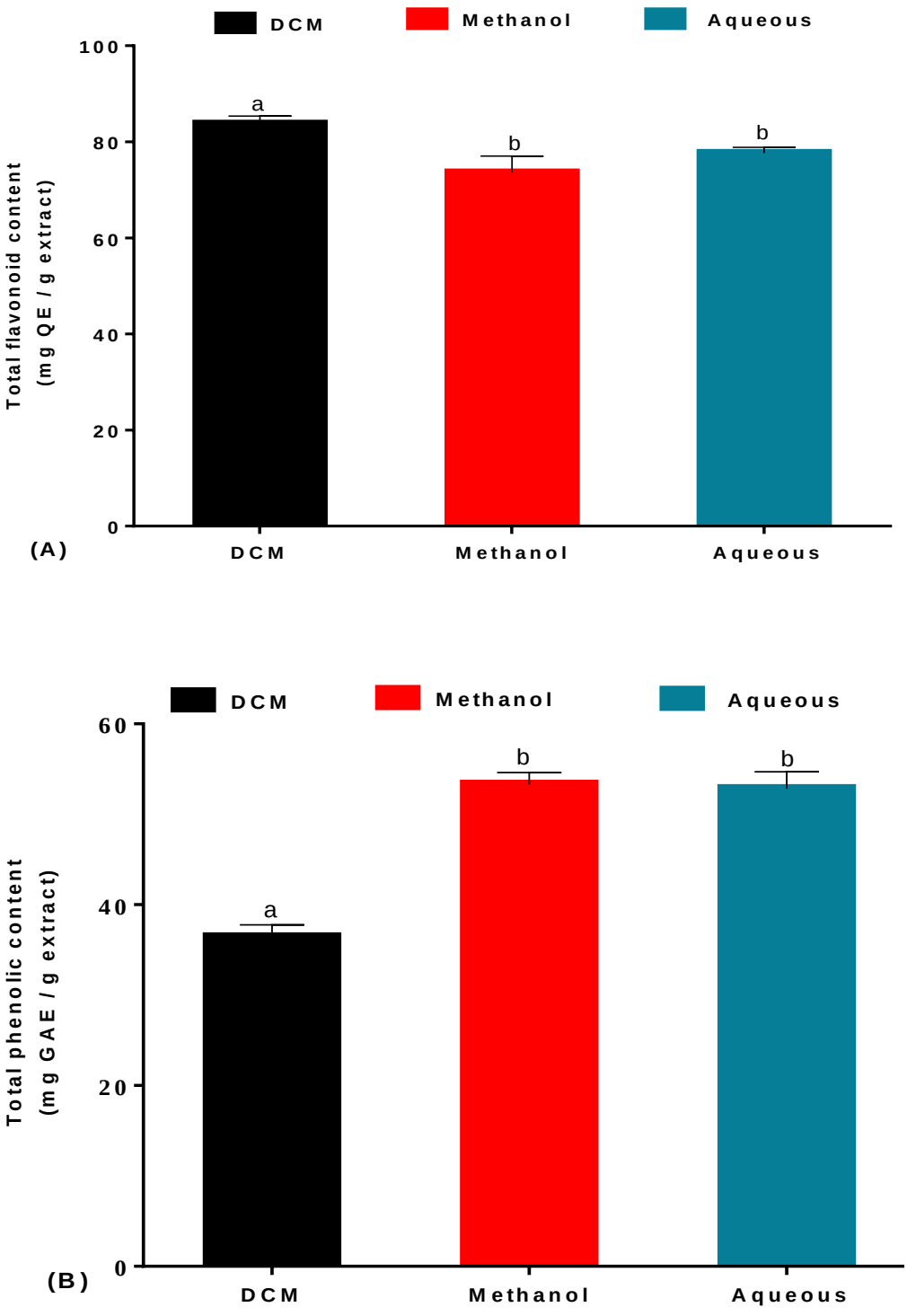
Senna petersiana inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury

Running title: Antidiabetic activities of *Senna petersiana*

Kolawole A. Olofinsan[1], Ochuko L. Erukainure[2], Nontokozo Z. Msomi[1], Md. Shahidul Islam[1]*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa
2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

*** Corresponding author**: islamd@ukzn.ac.za



Supplementary Figure 1. (A) Total flavonoid and (B) total phenolic contents of ***Senna petersiana*** leaf extracts. Values are presented as mean ± SEM (n = 3). Bars with different alphabets

(a-b) are significantly different from each other ($P < 0.05$).

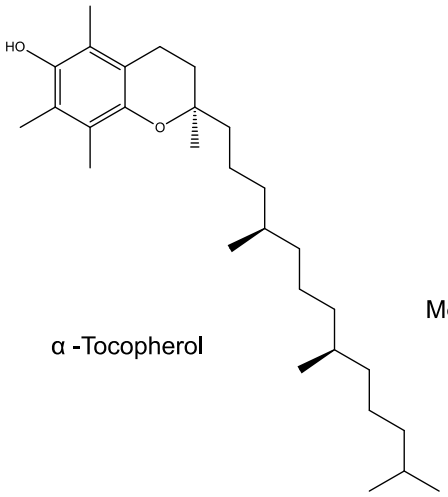
Senna petersiana inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury

Running title: Antidiabetic activities of *Senna petersiana*

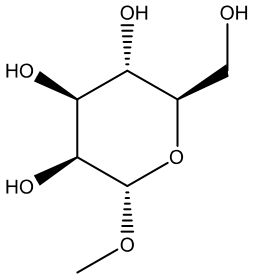
Kolawole A. Olofinsan[1], Ochuko L. Erukainure[2], Nontokozo Z. Msomi[1], Md. Shahidul Islam[1]*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa
2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

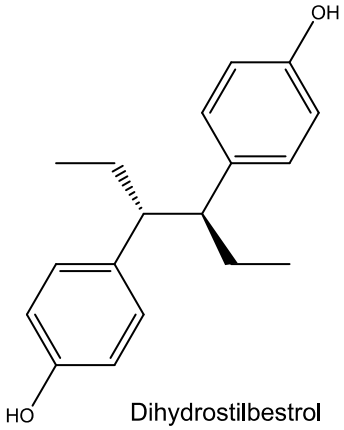
*** Corresponding author**: islamd@ukzn.ac.za



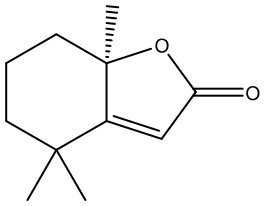
α -Tocopherol



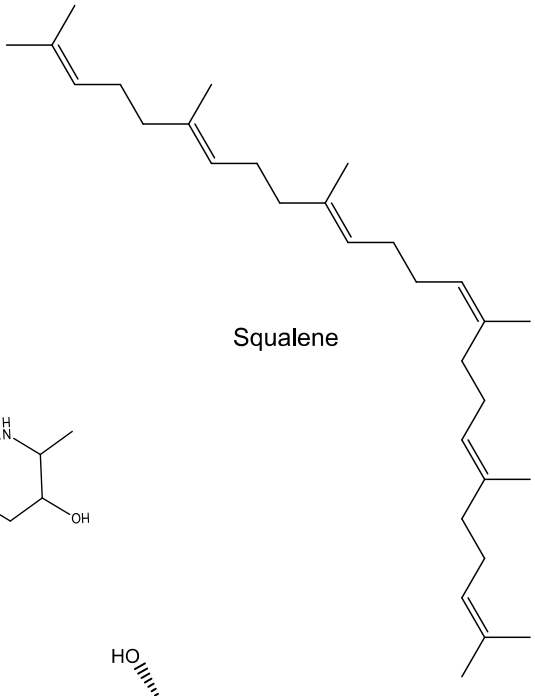
Methyl alpha-D-mannopyranoside



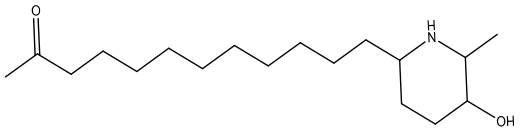
Dihydrostilbestrol



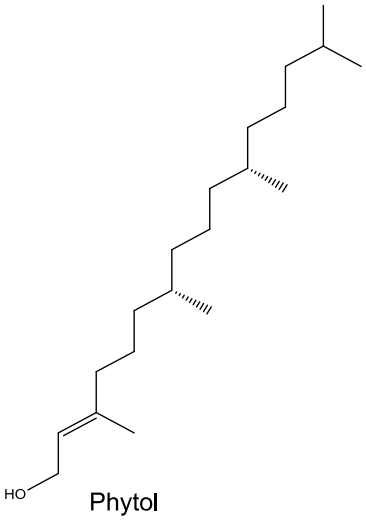
Dihydroactinidiolide



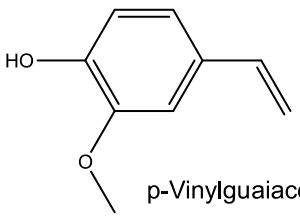
Squalene



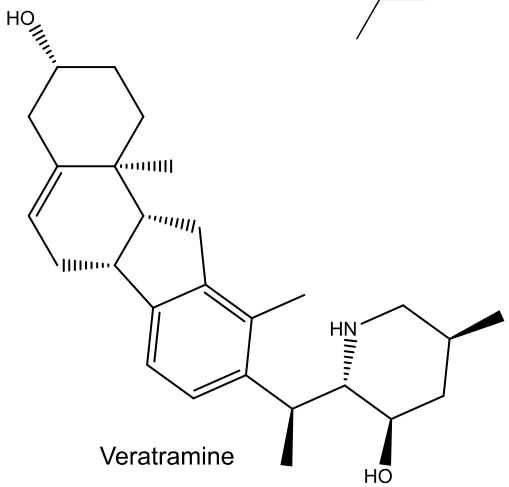
Cassine



Phytol



p-Vinylguaiacol



Veratramine

Supplementary Figure 2. Structures of chemical compounds of ***Senna petersiana*** leaf extracts.

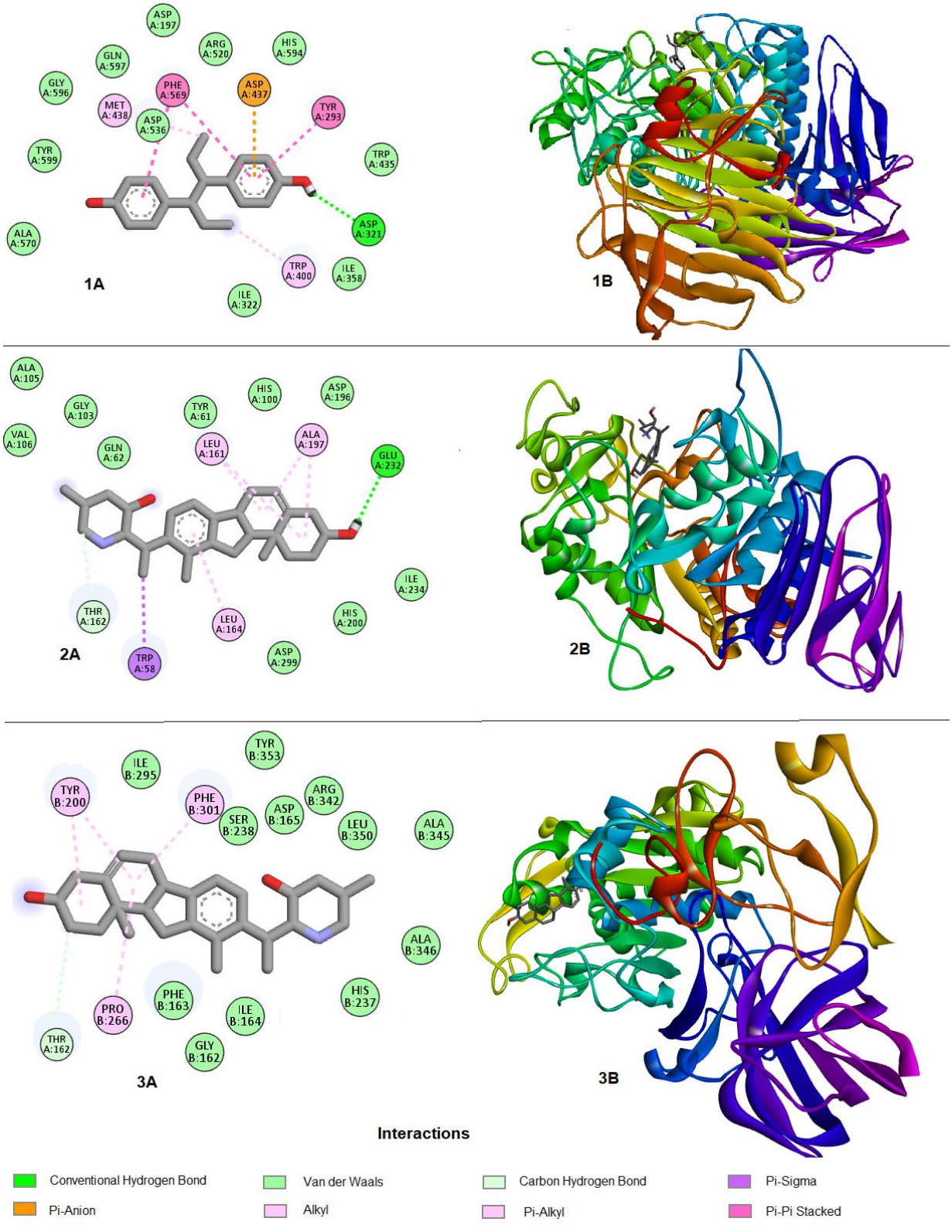
Senna petersiana inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury

Running title: Antidiabetic activities of *Senna petersiana*

Kolawole A. Olofinsan[1], Ochuko L. Erukainure[2], Nontokozo Z. Msomi[1], Md. Shahidul Islam[1]*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa
2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

*** Corresponding author**: islamd@ukzn.ac.za



Supplementary Figure 3. 2D and 3D molecular interaction images of ***Senna petersiana***

bioactive compounds that have highest binding affinities with catalytic site amino acid residues of carbohydrate and lipid digestive enzymes. 1 (A-B) Dihydrostilbestrol-α-glucosidase, 2 (A-B) veratramine-α-amylase and 3 (A-B) veratramine-lipase.

Senna petersiana inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury

Running title: Antidiabetic activities of *Senna petersiana*

Kolawole A. Olofinsan[1], Ochuko L. Erukainure[2], Nontokozo Z. Msomi[1], Md. Shahidul Islam[1]*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa
2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

*** Corresponding author**: islamd@ukzn.ac.za

Supplementary Table 1. IC_{50} values of *in vitro* antioxidant and antidiabetic activities of ***Senna petersiana*** extracts (µg/mL).

	DCM	Methanol	Aqueous	Ascorbic acid	Gallic acid	Acarbose	Orlistat
FRAP	51.60 ± 2.82^{b}	75.10 ± 2.21^{c}	75.60 ± 1.62^{c}	11.10 ± 1.78^{a}	ND	ND	ND
$OH^{\cdot}$ scavenging	44.70 ± 0.76^{a}	57.60 ± 2.88^{c}	45.40 ± 3.19^{b}	ND	44.90 ± 0.49^{a}	ND	ND
DPPH scavenging	60.60 ± 1.64^{d}	49.80 ± 1.60^{c}	37.30 ± 1.83^{b}	ND	23.20 ± 0.76^{a}	ND	ND
α-Amylase	50.40 ± 1.36^{a}	52.80 ± 2.72^{b}	56.40 ± 0.97^{b}	ND	ND	71.30 ± 0.77^{c}	ND
α-Glucosidase	8.10 ± 1.50^{a}	70.60 ± 1.49^{c}	71.60 ± 1.16^{c}	ND	ND	15.30 ± 2.73^{b}	ND
Lipase	52.30 ± 1.52^{b}	42.50 ± 0.72^{a}	42.00 ± 1.36^{a}	ND	ND	ND	53.50 ± 1.08^{b}

ND = Not determined. DCM: dichloromethane. Values represent mean ± SEM of three independent experiments ($n = 3$). Different alphabets a-d in each row indicate significantly difference from each other ($P < 0.05$).

Senna petersiana inhibits key digestive enzymes and modulates dysfunctional enzyme activities in oxidative pancreatic injury

Running title: Antidiabetic activities of *Senna petersiana*

Kolawole A. Olofinsan[1], Ochuko L. Erukainure[2], Nontokozo Z. Msomi[1], Md. Shahidul Islam[1]*

1. Department of Biochemistry, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa
2. Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

*** Corresponding author**: islamd@ukzn.ac.za

Supplementary Table 2. ***Senna petersiana*** bioactive compounds show high binding energies (ΔG) with digestive enzymes **(kcal/mol)**.

Compound	α-Glucosidase	α-Amylase	Lipase
Dihydrostilbestrol	-6.9	-7.4	-7.9
Squalene	-4.1	7.6	-7.7
Veratramine	-4.7	-10.5	-10.4
Cassine	-6.4	-6.4	-7.2
Trans-geranylgeraniol	-6.3	-6.1	-7.6
Alpha-tocopherol	-4.9	-8.0	-8.1