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Phytochemical Screening, Free Radical Mitigation and Antidiabetic Potentials of *Pentanisia prunelloides* (Klotzsch ex Eckl. & Zeyh.) Walp. Root Extracts

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Abstract Diabetes mellitus is attributed as one of the major health problems globally. This study evaluated the antioxidative and antidiabetic potentials of *P. prunelloides* in an *in-vitro* model and also screened for the presence of phytochemicals in the extracts. The antioxidant activity of the extracts (water, ethanol, aqueous-ethanol and hexane) was determined by superoxide anion, hydroxyl, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays and iron chelation method while the antidiabetic potential was assessed by determining the inhibitory effects of the extracts on the activities of α-amylase, α-glucosidase, maltase and sucrase enzymes. The hexane extract displayed significantly higher ($p < 0.05$) inhibition of α-amylase (0.48 µg/mL) and α-glucosidase (18.08 µg/mL) respectively. Additionally, water extract demonstrated strong inhibition of sucrase (3.85 µg/mL), and aqueous-ethanol extract (26.03 µg/mL) on maltase activity when compared with other extracts and control. The Lineweaver Burke plot revealed the non-competitive inhibition of α-amylase and α- glucosidase by the ethanol extract. While hexane extract demonstrated significant ($p < 0.05$) scavenging activities against superoxide anion (0.33 µg/mL) and hydroxyl radical (0.51 µg/mL), water (75.42 µg/mL) and aqueous-ethanol (4.24 µg/mL) extracts exhibited the strongest DPPH activity and iron chelation effect respectively. The phytochemical analysis of the extract revealed the presence of tannins, terpenoids, alkaloids, saponins, flavonoids and cardiac glycosides while quantification of phytochemicals revealed total flavonoids with 15.40 mg quercetin equivalent (QE)/g from hexane extract, highest tannin content with 45.60 mg gallic acid equivalent (GAE)/g (aqueous-ethanol extract) and total phenol from water and aqueous-extracts with 0.07 mg GAE/g while alkaloids and saponins contents were found to be low in the roots of *P. prunelloides*, at 0.6 and 13.9% respectively. It can therefore be concluded that *P. prunelloides* extracts possessed antioxidant and antidiabetic activities *in vitro* and could thus be suggested that its mechanism of antidiabetic action is through the inhibition of diabetes-related enzymes.

Keywords: *antidiabetic, antioxidants, P. prunelloides, phytochemicals, quantification*

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1. Introduction

Diabetes mellitus (DM), a fast growing menace, is a chronic endocrine disorder that affects the metabolism of carbohydrates, fats, proteins, water and electrolytes [1]. It is considered as one of the non-curable illnesses controlled through the monitoring of the blood sugar level over healthy diet, exercise and medication [2]. There are several classes of DM but type II diabetes also known as non-insulin dependent diabetes mellitus (NIDDM) is regarded as the most common type affecting about 90% of the people worldwide [3]. Diabetes-induced metabolic dysfunction may be caused by the action of reactive oxygen species (ROS) produced in the mitochondrial electron transport chain [4]. The excessive levels of ROS such as the superoxide anion radical (O_2^-) and hydroxyl radicals (OH^-) have been linked to the onset of many diseases like cancer, stroke and diabetes [5,6]. Diabetes management can be through the use of drugs (oral hypoglycaemic agents, OHAs) but a very few of these recognized pharmaceutical drugs namely acarbose, voglibose and miglitol etc. have shown potentials in controlling hyperglycaemia. These drugs despite wide usage are often reported to cause several side effects including cramping, abdominal distention, flatulence and diarrhoea [7,8,9]. Therefore, the search for the discovery of antioxidant and

antidiabetic agents from plant sources is an important strategy required to combat the widespread nature of this condition. Alpha-amylase and alpha-glucosidase are prominent enzymes that plays important roles in carbohydrate metabolism. They serves as one of the therapeutic approaches for decreasing postprandial hyperglycemia by slowly or delaying the digestion of carbohydrates by the inhibition of these enzymes in the digestive tract [10].

Pentanisia prunelloides, a perennial herb belonging to the Rubiaceae family is commonly called wild verbena (English), setimamollo (Sotho) translated as fire extinguisher, icimamlilo (Zulu) putting out the fire and sooibrandbossie (Afrikaans) little heartburn bush [11,12]. The roots and leaves of this plant are mostly used for treating a variety of ailments such as heartburn, fever, tuberculosis, blood impurities, haemorrhoids, toothache, chest pains, sore joints, swellings and snake bites [13,14]. The plant had been reported to be effective during pregnancy to ease childbirth; the leaf against retained placenta [15] and diarrhea as reported by Madikizela et al. [16] while the roots are used for managing gynaecological complaint such dysmenorrhoea [17]. Several studies have established that boiled grated dried bulb taken orally stops vomiting [18], the decoction of the bruised and boiled root mixed with sour milk are taken orally [19] for swelling of the stomach and the root extract for treating aches and pains [20]. The antibacterial compound, palmitic acid had been isolated from this plant by Yff et al. [21] and Madikizela et al. [22] also reported the antibacterial activity of some *P. prunelloides* extracts. The plant was also found to possess anti-inflammatory, antiviral and antioxidant activities by inhibiting cyclooxygenase-1 (COX-1), viral replication of the influenza A virus [21,23] and DPPH radicals through hydrogen transferring reactions [24]. Despite the several reports on the antioxidants and phytochemicals effectiveness of this plant, there exist insufficient information on the antioxidant with no report on antidiabetic potentials. Therefore, this study was carried out to evaluate the antioxidant and antidiabetic potentials of different extracts in an *in-vitro* model.

2. Material and Methods

2.1. Plant Materials Collection and Authentication

The plant material (roots) was collected naturally from a multi population in the eastern part of Free State near Golden Gate at Beste Farm in October 2015. The location was found at a mountainous area with the following coordinates: latitude 28º 23' 55.60"S and longitude 28º 47' 18.83" E. The plant was further authenticated by Dr. EJJ Sieben of Plant Sciences Department at the University of the Free State, Qwaqwa campus. The voucher specimen (MakMed 03/ 2015/ QHB) was prepared and deposited at the University herbarium.

2.2. Extract Preparation

The fresh root materials were separately rinsed under running water to remove debris and then dried in an Ecotherm oven (Laboratory Consumables Pty, RSA) at a temperature of 45°C until constant weight was reached. The dried root material was ground using an electric blender (Nanning Mainline Food Machinery Company Ltd, China) to a powdered form and weighed. Exactly 20 g of the powdered root materials was separately extracted in four solvents (200 mL each) of different polarities namely hexane, ethanol, aqueous-ethanol (1:1) and distilled water (Aqueous). The flasks were placed on a Labcon platform shaker (Laboratory consumables, PTY, Durban, South Africa) at 115 rpm for 24 h. The extracted mixtures were filtered using Whatman No.1 (Whatman, UK) filter paper to obtain a homogenous mixture. The filtrates of the hexane, ethanol and aqueous-ethanol solvents were concentrated in a rotary evaporator at 45°C while the aqueous filtrate was placed in water bath at 45°C to obtain a dried extract. Extracts were dissolved in dimethysulphoxide (DMSO) to give stock solutions of 1.0 mg/mL and different concentrations (6.25, 12.50, 25.00, 50.00 and 100.00 µg/mL) of the extracts were prepared using a serial dilution method with distilled water. The leftover extracts were stored at 4°C until further use.

2.3. Chemicals and Reagents

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

2.4. Evaluation of Antioxidant Activities

2.4.1. Superoxide Anion Radical Scavenging Ability

The measurement of superoxide anion scavenging activity of the various extracts was based on the method described by Liu et al. [25]. Superoxide radicals were generated in 50 µL of Tris-HCl buffer (16 mM, pH 8.0) containing 50 µL of NBT (50 mM) solution, 50 µL NADH (78 mM) solution and different concentrations (6.25 - 100 µg/mL) of *P. prunelloides* extracts (100 µL). The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance was measured at 560 nm. Superoxide anion radical scavenging ability of a standard antioxidant was also tested by replacing the extract with gallic acid (6.25-100 µg/mL). The percentage inhibition of the radical by the extract was calculated following the expression below:

$$\%\text{Inhibition} = \frac{Acontrol - Asample}{Acontrol} \times 100 \qquad (1)$$

Where Acontrol is the absorbance measurement of the control and Asample is the absorbance of the test sample (extract/standard).

2.4.2. Hydroxyl Radical Scavenging Ability

The ability of the root extracts of *P. prunelloides* to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose

was carried out using the modified method described by Oboh and Rocha [26]. Briefly, 40 µL freshly prepared extracts (6.25-100 µg/mL) was added to the mixture containing 20 µL (20 mM) deoxyribose, 80 µL (0.1 M) phosphate buffer, 10 µL (20 mM) hydrogen peroxide and 10 µL (500 mM) $FeSO_4$, afterwards, the volume was made up to 200 µL with distilled water. The reaction mixture was incubate at 37°C for 30 min and stopped by the addition of 50 µL of 2.8% TCA (trichloroacetic acid), followed by the addition of 50 µL of 0.6% TBA solution. The mixtures were subsequently incubated for 20 min, transferred to a 96-well microtiter plate before measuring the absorbance at 532 nm in a microplate reader (Model 680, BIO-RAD). Hydroxyl radical scavenging ability of a standard antioxidant was also tested by replacing the extract with gallic acid (6.25-100 µg/mL). Percentage inhibition determined following equation shown 1 above.

2.4.3. DPPH Free Radical Scavenging Ability

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical was evaluated by a modified method of Saha et al. [27]. Different concentrations (6.25-100 µg/mL) of the extracts (150 µL) were mixed with 150 µL of 0.4 mmol/L methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability of each extract was subsequently calculated with respect to the reference (which contains all the reagents without the test sample). DPPH free radical scavenging ability of a standard antioxidant was also tested by replacing the extract with ascorbic acid (6.25-100 µg/mL) and determined as percentage inhibition based on equation 1 above.

2.4.4. Iron Chelation Assay

The chelation of ferrous ions by the root extracts of *P. prunelloides* was determined by the modified method of Dorman et al. [28]. Briefly, 200 µL $FeCl_2$ (0.2 mM) was added to 40 µL aliquots of extracts (6.25-100 mg/mL). The reaction was initiated by the addition of 5 mM ferrozine (80 µL) in a 96-well plate, the mixture vigorously shaken and left to stand at room temperature for 10 min. The absorbance was then measured at 562 nm in a microplate reader (Model 680, BIO-RAD, USA). Iron chelating potential of a standard antioxidant was also tested by replacing the extract with gallic acid (6.25-100 µg/mL) while calculating the percentage inhibition as above (equation 1).

2.5. Determination of Antidiabetic Potentials

2.5.1. Alpha-glucosidase Inhibitory Activity

The effect of the plant extract on α-glucosidase activity was determined according to the method described by Adisakwattana et al. [29] with modifications. The substrate solution p-nitrophenyl glucopyranoside (pNPG), sucrose (50 mM) and maltose (25 mM) were prepared in 0.02 M phosphate buffer (pH 6.9), 100 µL of glucosidase (0.5 mg/mL) was pre-incubated with 50 µL of the different concentrations (6.25-100 µg/mL) of the extracts for 10 min. Then 50 µL of 5 mM (pNPG), maltose and sucrose in 0.02 M phosphate buffer (pH 6.9) was individually added to start the reaction. The reaction mixture was incubated at 37 ˚C for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The enzyme activities (alpha-glucosidase, sucrase and maltase) were determined by measuring the yellow coloured para-nitrophenol released from pNPG and absorbance at 405 nm (α-glucosidase) or 540 nm (maltase and sucrase) using micro plate titre reader (Model 680, BIO-RAD). The control was prepared using the same procedure replacing the extract with distilled water while the activity of the standard was evaluated by replacing the extract with acarbose (6.25 -100 µg/mL). Percentage inhibition was calculated, thus;

$$\% \text{ Inhibition} = \frac{\Delta Acontrol - \Delta Asample\ test}{\Delta Acontrol} \times 100 \quad (2)$$

Where ΔAcontrol = Acontrol –Ablank and ΔAsample = Asample – Ablank. The sample test is extract and the concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.5.2. Mode of α-glucosidase Inhibition

The mode of inhibition of α-glucosidase by the extract was determined using the extract with the most potent hypoglycaemic result according to the modified method described by Ali et al. [30]. Ethanol extract was selected based on its mild inhibition against alpha-amylase (highest IC_{50} value) and strongest alpha-glucosidase inhibition (lowest IC_{50} value) as compared to the other extracts. Briefly, 50 µL of the (5 mg/mL) ethanol extract was pre-incubated with 100 µL of α-glucosidase solution for 10 min at 25°C in one set of five test tubes. In another set of tubes, α-glucosidase was pre-incubated with 50 µL of phosphate buffer (pH 6.9). 50 µL of pNPG at increasing concentrations (0.20 - 1.0 µg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25°C and 500 µL of Na_2CO_3 was added to stop the reaction.

The amount of reducing sugars released was determined spectrophotometrically using a para-nitrophenol standard curve and converted to reaction velocities. A double reciprocal (Lineweaver–Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted to determine the mode of inhibition.

2.5.3. Alpha-amylase Inhibitory Activity

The assay was carried-out using the modified procedure of McCue and Shetty [31]. Briefly, 50 µL extract was placed in a tube and 50 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was pre-incubated at 25°C for 10 min, after which 50 µL (1%) starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and then further incubated at 25°C for 10 min. The reaction was terminated by adding 200 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 10 min and cooled at room temperature. The reaction mixture was diluted with 300 µL of distilled water and the absorbance was measured at 540 nm using micro plate titre reader (Model

680, BIO- RAD). A control was prepared using the same procedure replacing the sample test with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition following the expression shown in equation 2.

2.5.4. Mode of α-amylase Inhibition

The mode of inhibition of α-amylase by ethanol extract of the plant was conducted according to the modified method described by Ali et al. [30]. Briefly, 250 µL of the (5 mg/mL) extract was pre-incubated with 250 µL of α-amylase solution for 10 min at 25˚C in one set of tubes. In another set of tubes, α-amylase (250 µL) was pre-incubated with 250 µL of phosphate buffer (pH 6.9). 250 µL of starch solution (1%) at increasing concentrations (0.20 – 1.0 µg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25˚C, boiled for 5 min after addition of 500 µL of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using maltose standard curve converted to reaction velocities. A double reciprocal (Lineweaver–Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted to determine the mode of inhibition.

2.6. Qualitative Analysis of Secondary Metabolites

Phytochemical composition of the plant extracts were determined by the methods described by Trease and Evans [32] and Sofowora [33].

2.6.1. Test for Tannins

To 0.5 mL of the extract solution, 1 mL of distilled water and one to two drops of ferric chloride solution were added and observed for brownish green or a blue colouration confirming the presence of tannins

2.6.2. Test for Terpenoids

A 5 mL of extract was mixed with 2 mL of chloroform in a test tube. Thereafter, 3 mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface reddish brown colouration was formed indicating the presence of terpenoids

2.6.3. Test for Alkaloids

A 1 mL of 1% HCl was added to 3 mL of extract in a test tube and was treated with few drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids

2.6.4. Test for Saponins

A 5 mL of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with three drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

2.6.5. Test for Flavonoids

A few drops of 1% NH_3 solution was added to the extract in a test tube. A yellow colouration was observed for the presence of flavonoids.

2.6.6. Test for Cardiac Glycosides

A 5 mL of extract was mixed with glacial acetic acid containing one drop of ferric chloride. The above mixture was carefully added to the 1 mL of concentrated H_2SO_4. The presence of cardiac glycosides was detected by the formation of brown ring.

2.6.7. Test for Phlobotannins

About 10 mL of extract was boiled with 1% HCl in boiling tube. Deposition of red precipitate indicated the presence of phlobatannins.

2.7. Quantitative Analysis of Secondary Metabolites

2.7.1. Quantitative Analysis of Secondary Metabolites

To a 250 mL beaker, 5 g of sample was mixed with 200 mL of 10% acetic acid in ethanol and the mixture was allowed to stand for 4 h. The filtrates were collected and concentrated on a water bath to one-quarter of the original volume. Afterwards, concentrated ammonium hydroxide was added drop wise until precipitation was complete. The solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, alkaloid, were dried through evaporation of solvent and weighed [34]. Percentage yield of the extract is defined as the total dry mass after evaporation and was calculated using equation:

$$\%\ \text{Yield} = \frac{Mass\ of\ dry\ weight(g)}{Total\ mass\ of\ dry\ powdered\ sample(g)} \times 100 \quad (3)$$

2.7.2. Determination of Total Saponins Content

Ten grams of the powdered material was extracted in 100 mL of 20% aqueous-ethanol.

The mixture was heated over a hot water bath for 4 h with continuous stirring at about 55°C, this was followed by filtration. The residue was re-extracted with 100 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90 °C. The concentrate was transferred into a 250 mL separation funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated two times until 60 mL of n- butanol was then added to the extract. The n-butanol extracts was washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution after washing was heated in a water bath [35]. Following evaporation, the samples were dried in an oven to a constant weight and the saponin content was then calculated as illustrated above (equation 3).

2.7.3. Determination of Total Tannins Content

The total tannins content of the roots extracts of *P. prunelloides* was determined by Folin-Ciocalteu method as described by Miean and Mohamed [36]. In 10 mL volumetric flask, 0.1 mL of extract was mixed with 7.5 mL of distilled water followed by 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35% Na_2CO_3

solution further diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared. Absorbance for test and standard solutions were measured against the blank at 725 nm with Biowave II (Biochrom, United Kingdom) spectrophotometer. The tannin content was expressed in terms of mg of gallic acid equivalent (GAE) /g of extract. A standard curve of absorbance against gallic acid concentration was prepared and results were expressed as percentage w/w i.e. tannin content (% w/w) = GAE×V×D×10–3×100 where GAE - gallic acid equivalent (µg/mL), V - total volume of sample (mL), D -dilution factor

2.7.4. Determination of Total Flavonoids

Ten milligrams of quercetin was dissolved in 100 mL of methanol (100 µg/mL) and then further diluted to 10, 20, 40, 80 or 160 µg/mL [37]. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of methanol, 0.1 mL of aluminium chloride ($AlCl_3$) (10%), 0.1 mL of 1 M potassium acetate and 2.8 mL of diluted water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm using a Biowave II (Biochrom, UK) spectrophotometer. The amount of $AlCl_3$ (10%) was substituted by the same amount of distilled water in blank. Quantification was done based on the standard curve of quercetin.

A standard curve of absorbance against quercetin concentration was prepared and results were expressed as percentage w/w i.e. flavonoids content (% w/w) = QE×V×D×10–3×100 where QE is quercetin equivalent, V is sample total volume and D is the dilution factor.

2.7.5. Determination of Total Phenolic Content

Total phenol content in the plant extract was determined by the method of Singleton et al. [38] with modification of Folin-Ciocalteu by Wolfe et al. [39]. 50 µL of aliquot extract (1 mg/mL) was mixed with 50 µL of Folin-Ciocalteu reagent (10%) and 50 µL of water. 150 µL of 7.5% Na_2CO_3 was added to neutralize the reaction. The tubes were mixed thoroughly and incubated for 40 min at 45°C. Absorbance was then measured at 765 nm using a Biowave II (Biochrom, UK) spectrophotometer. The total phenolic content was expressed as mg/mL gallic acid adopting the equation obtained from calibration curve (using 0.05 mg/mL - 0.5 mg/mL of gallic acid).

A standard curve of absorbance against gallic acid concentration was prepared and results were expressed as percentage w/w i.e. phenolic content (% w/w) = GAE×V×D×10–3×100 where GAE – gallic acid equivalent (µg/mL), V - total volume of sample (mL), D – dilution factor.

2.8. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (Graph Pad Software, USA). The data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as mean ± standard error of mean (SEM) of triplicate determinations.

3. Results

3.1. Antioxidant Assays

The radical scavenging abilities of the root extracts of *P. prunelloides* are shown in Table 1 and Figure 1. It was observed that going by half-maximal inhibitory concentration, hexane extract reveal the best significant ($p < 0.05$) activities in superoxide anion (0.33 µg/mL) and hydroxyl (0.51 µg/mL) when compared with other extracts and the control (55.50 and 65.70 µg/mL respectively). The DPPH evaluation revealed water extract (75.42 µg/mL) showing the best effect ($p < 0.05$) while aqueous-ethanol (4.24 µg/mL) was the best chelator of metal ions. These activities are dose dependent for all the extracts and control (gallic acid) although at the highest concentration of 100 µg/mL, gallic acid reflected the highest peak inhibition of superoxide and hydroxyl radicals as well as ascorbic acid against DPPH (Figure 1).

3.2. Antidiabetic Assays

Table 2 established the strongest inhibition of alpha-amylase (0.48 µg/mL) and alpha-glucosidase (18.08 µg/mL) by hexane extract while aqueous (3.85 µg/mL) and aqueous- ethanol (26.03 µg/mL) extracts best-inhibited ($p < 0.05$) the activities of sucrase and maltase respectively in comparison with other extracts and acarbose. As witnessed with radical scavenging evaluations, the inhibition of the extracts and control are dose- dependent (Figure 2). The result of mode inhibition of the enzymes as obtained from Lineweaver Burke plot (Figure 3) by the ethanol extract depicts a non-competitive inhibition of alpha-glucosidase and a mixed non-competitive inhibition of alpha-amylase.

Table 1. IC_{50} values for the diabetes-related enzymes' inhibitory potentials of different extracts of *P. prunelloides*

Samples	IC_{50} (µg/mL)			
	α-glucosidase	Sucrase	Maltase	α-amylase
Water	36.01 ± 6.21^a	3.85 ± 2.11^a	90.49 ± 2.90^a	8.82 ± 0.53^a
Ethanol	19.73 ± 1.53^b	51.48 ± 5.31^b	44.84 ± 1.88^c	18.51 ± 1.78^c
Aqueous-ethanol	40.33 ± 0.49^a	64.36 ± 0.84^c	26.03 ± 0.33^b	9.03 ± 2.08^a
Hexane	18.08 ± 0.03^b	61.66 ± 1.43^c	31.59 ± 2.81^b	0.48 ± 0.35^b
Acarbose	129.4 ± 5.11^c	30.65 ± 1.13^d	122.1 ± 4.64^d	9.87 ± 1.61^a

The values are expressed as mean ± standard error of mean (SEM) of triplicate determinations (n=3). Means not sharing a common superscript for each enzyme are significantly different ($p < 0.05$). Acarbose is the standard for α-glucosidase and α-amylase inhibitor.

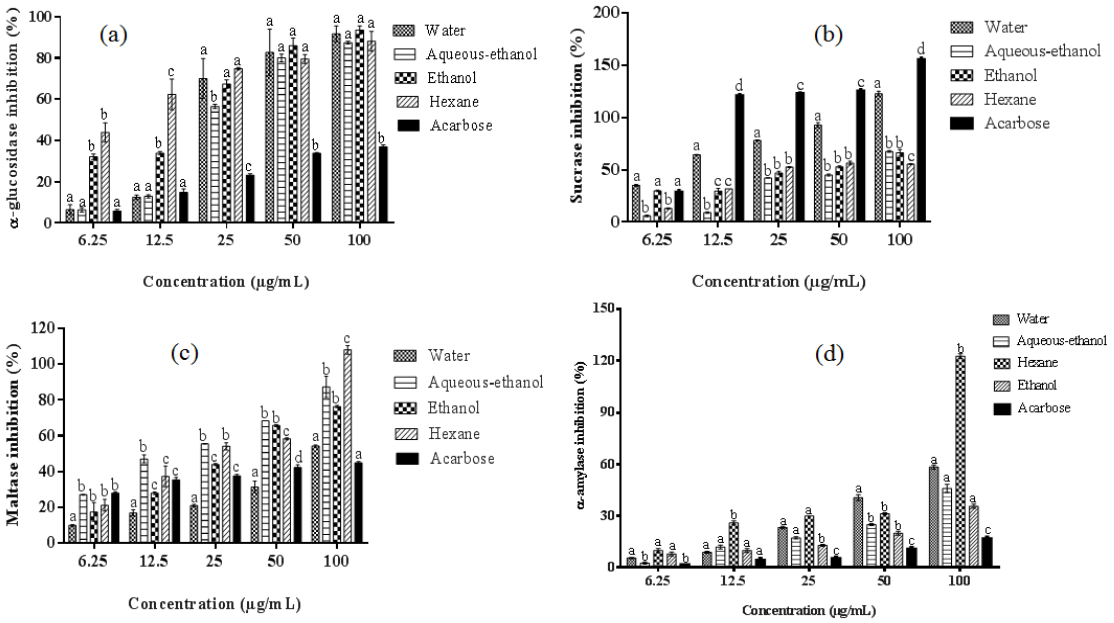

Figure 1. Inhibitory effects of different extracts of *P. prunelloides* on the activities of (a) α-glucosidase, (b) sucrase, (c) maltase and (d) α-amylase. Values are expressed as mean ± standard error of mean (SEM) of triplicate determinations. Means not sharing a common letter at the same concentration

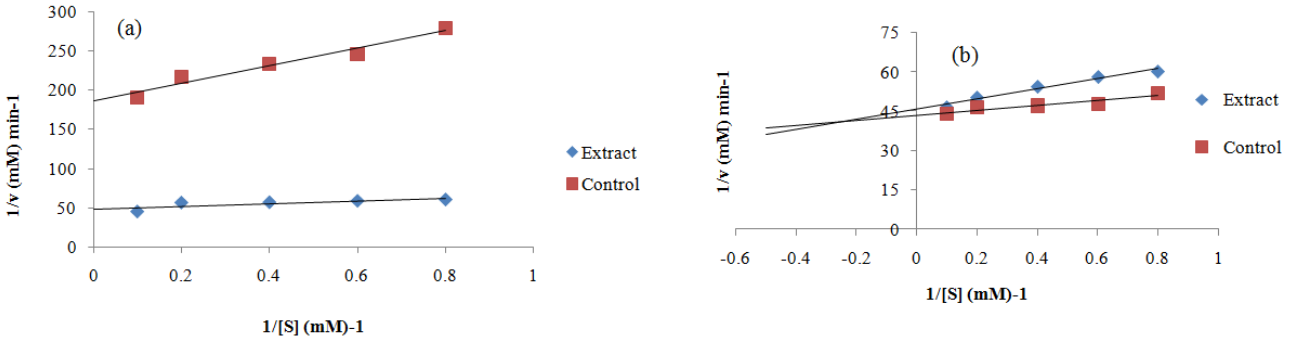

Figure 2. Lineweaver-Burk plot of *P. prunelloides* ethanol extract showing (a) non-competitive and (b) mixed non-competitive inhibition against α-glucosidase and α-amylase respectively

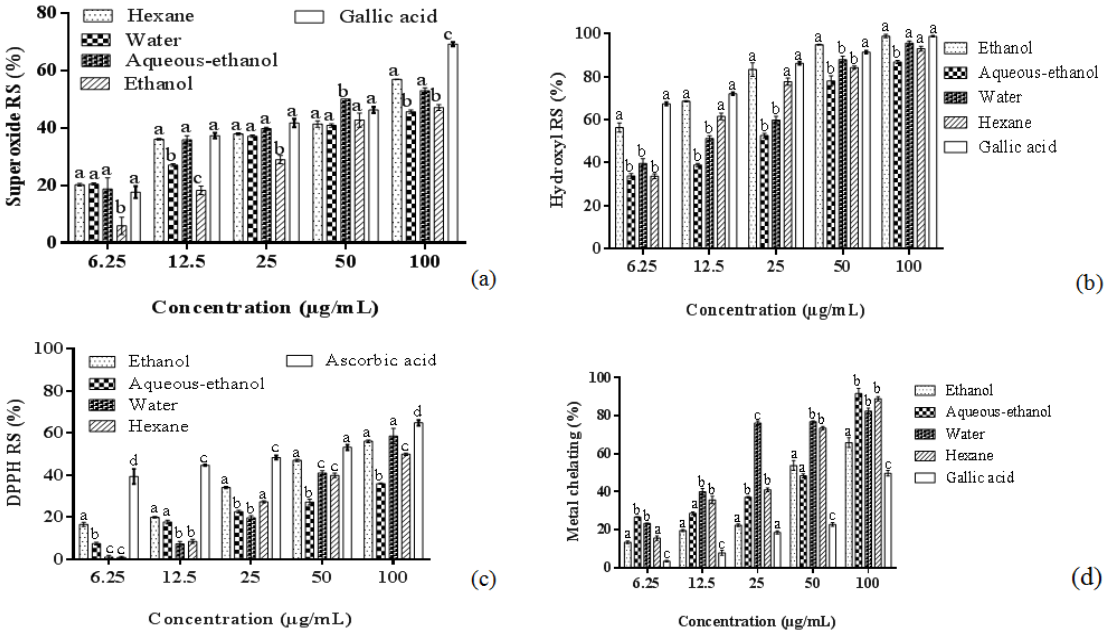

Figure 3. Scavenging abilities of different extracts of *P. prunelloides* against (a) superoxide, (b) hydroxyl, (c) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical anion and (d) metal chelation. Values are expressed as standard error of mean (SEM) of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p < 0.05$)

Table 2. IC_{50} values for the free radical scavenging abilities of different extracts of *Pentanisia prunelloides*

Samples	IC_{50} (µg/mL)			
	Superoxide	Hydroxyl	DPPH	Iron Chelation
Ethanol	92.98 ± 1.20[a]	42.99 ± 1.50[a]	77.06 ± 0.22[a]	64.86 ± 3.20[a]
Aqueous-ethanol	74.72 ± 1.94[b]	24.78 ± 0.72[b]	142.7 ± 0.47[b]	4.24 ± 0.03[b]
Water	118.7 ± 0.66[c]	12.28 ± 0.51[c]	75.42 ± 2.28[a]	20.30 ± 1.56[c]
Hexane	0.33 ± 0.02[d]	0.51 ± 0.43[d]	88.67 ± 1.57[c]	37.47 ± 0.19[d]
Gallic Acid	55.50 ± 0.12[e]	65.70 ± 1.48[e]	34.03 ± 1.56[d]	101.7 ± 2.26[e]

The values are expressed as mean ± SEM of triplicate determinations (n=3). Means not sharing a common superscript are significantly different ($p < 0.05$).

Table 3. The phytochemical constituents of extracts of *P. prunelloides* roots

Phytochemical type	Water	Ethanol	Hexane	Aqueous-ethanol
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	+	-	-	-
Saponins	+	+	+	+
Flavonoids	-	+	-	-
Cardiac glycosides	-	-	-	+
Phlobatannins	-	-	-	-

(+) detected, (-) not detected.

Table 4. The quantitative analysis of phytochemicals of extracts of *P. prunelloides* roots

Phytochemical type	Mass extracted (g)	% yield
Alkaloids	0.03	0.6
Saponins	1.39	13.9

Table 5. Total flavonoids, total tannins and total phenols of the root extracts of *P. prunelloides*

Extracts	Total flavonoids (expressed in mg/g quercetin)	Tannins (expressed in mg/g gallic acid)	Total phenols (expressed in mg/g gallic acid)
Water	14.70 ± 0.06[a]	27.54 ± 0.03[a]	0.07 ± 0.002[a]
Ethanol	13.21 ± 0.14[b]	34.87 ± 0.04[a]	0.04 ± 0.003[b]
Aqueous-ethanol	13.74 ± 0.01[b]	45.60 ± 0.05[b]	0.07 ± 0.002[a]
Hexane	15.40 ±0.17[a]	30.21 ± 0.08[a]	0.04 ± 0.000[b]

Values are presented as mean ± standard error of mean (SEM) (n=3). Values with different superscript in the same column for each parameter are significantly different ($p < 0.05$) to each other.

3.3. Phytochemical Screening

The results of the chemical groups' detection are presented in Table 3. It was observed that tannins, terpenoids and saponins were present while phlobatannins was absent in all the extracts. Alkaloids, flavonoids and cardiac glycosides were only detected in water, ethanol and aqueous-ethanol extracts respectively. The results of the quantitative determination of saponins and alkaloids are presented in Table 4 while those of the total flavonoids, tannins and phenolics are shown in Table 5. The roots of *P. prunelloides* were found to be very low in alkaloids (0.6), moderate with saponins (13.9) percentage yields.

The highest total flavonoids, tannins and phenols contents in all the extracts were significantly ($p < 0.05$) witnessed with hexane (15.40 mg quercetin/g), aqueous-ethanol (mg/g GAE) and aqueous (mg/g GAE) extracts respectively.

4. Discussion

One of the important strategies for the management of diabetes mellitus is to maintain a near normal blood glucose levels in fasting and postprandial conditions [40,41]. According to Rhabasa-Lhoret and Chiasson [42], inhibition of the activity of α-amylase and α-glucosidase delays the degradation of carbohydrate, which in turn decreases the absorption of glucose into tissues, thus, leading to reduction in postprandial blood glucose level. Acarbose and miglitol are α-glucosidase inhibitors which act competitively by modulating the postprandial digestion and absorption of glucose [43,44]. However, the problem with these inhibitors is the resultant gastrointestinal discomfort such as flatulence and diarrhoea [9].

All the extracts (water, ethanol, aqueous-ethanol and hexane) investigated in this study demonstrated significant potency in inhibiting the activities of α-amylase and α- glucosidase. The strongest inhibitory activities of the extracts (particularly hexane) on the α-amylase is in agreement with previous reports by Balogun and Ashafa [45] which indicated that excessive inhibition of pancreatic α-amylase could result in the abnormal bacterial fermentation of undigested carbohydrates in the colon [46,47]. Although α-amylase inhibitory activity had positive effects on prevention of hyperglycemia linked to type II diabetes mellitus, however, mild inhibitory activity is desirable. Hence the ethanol extract was found to be the most suitable of the extracts showing mild/moderate activity in inhibiting this enzyme thus, the reason for being selected in order to study the kinetics of inhibition of the enzyme and this is similar to the report from Balogun and Ashafa [45] for *D. anomala* roots. The mode

of inhibition of the enzyme indicated a mixed non-competitive type of inhibition suggesting that the active components in the extract did not compete with the substrate at binding at the active site of the enzyme. However, binds to a separate site to retard the conversion of oligosaccharides to disaccharides [48].

Alpha-glucosidases are intestinal enzymes catalysing the conversion of disaccharides to monosaccharides. Maltase and sucrase are forms of α-glucosidases which catalyse the hydrolysis of maltose and sucrose to their constituent monosaccharides respectively [49]. The best activity of hexane and ethanol extracts (lower IC_{50} values) against α-glucosidase (Table 2) is suggestive of the hypoglycaemic effect of the plant and this could be attributed to the presence of the phytochemicals (phenolic compounds) present in the extracts (Table 3 and Table 5). In fact, a number of studies had submitted the antidiabetic effect of medicinal plants to be linked to the presence of polyphenolic compounds such as flavonoids [50-53], tannins [54,55,56], etc. Interestingly, the strong inhibition witnessed by these extracts hexane, water, aqueous-ethanol against these enzymes alpha-glucosidases, sucrase and maltase respectively favours both polar and non-polar solvents thus, suggest inhibition by both polar and non-polar antihyperglycaemic compounds. This agrees with the reports from Kwon et al. [57] that natural α-glucosidase inhibitors from medicinal plants show strong inhibitory activity against α-glucosidase and therefore can be potentially used as an effective therapy for the management of postprandial hyperglycemia with minimal side effects. The kinetics of inhibition following Lineweaver-Burk plot established the non-competitive inhibition of α-glucosidase suggesting that the active components of the extract binds to a site other than the active site of the enzyme and combine either with free enzyme or enzyme substrate complex possibly interfering with the action of both [58], thus, indicating the slowing down of disaccharides to monosaccharides.

Antioxidants are endowed with the responsibility of fighting against free radicals protects the body's defence mechanism from various diseases [59]. Many synthetic drugs may control oxidative damage but these drugs are associated with adverse side effects [60]. Hence, the antioxidant activities of plants may come handy in preventing the excessive production of free radicals by neutralizing their effects and or scavenge them when produced in the body or chelate the transition metal composition [61]. Ascorbic acid (vitamin C) is a water-soluble micronutrient required for multiple biological functions [62]. Gallic acid is an antioxidant used as a remote astringent in cases of internal haemorrhage as well as in the treatment of albuminuria and diabetes [63]. Dai and Mumper [64] reported that natural antioxidants are more effective than the synthetic ones such as vitamin C, tocopherol etc., hence the need to evaluate the radical scavenging effects of potential antioxidative medicinal plants becomes germane.

In this present evaluation, the extracts scavenged superoxide uniformly at all concentrations, except at high concentration where the standard (gallic acid) had strong inhibition (Figure 1). The ability of the root extracts of *P. prunelloides* to inhibit this radical agrees with the findings of Giacco and Brownlee [65] and Khan et al. [66] suggesting the possibility of plant in reducing the production of superoxide while alleviating the potential damaging effects of the cellular components that contributes to tissue damage and other diseases such as diabetes mellitus. As reported by Kazeem and Ashafa [67], hydroxyl radicals are highly reactive in causing enormous biological damage to any living cell; however, it is evident that this untoward effect may be mitigated by *P. prunelloides* since the extracts even showed superior effectiveness than gallic acid (Table 1). DPPH assay is an important method at assessing the antioxidant potential of medicinal plants, the ability of the extract to scavenge this radical signifies the potential to reduce the stable free radical of DPPH to the yellow coloured diphenylpicrylhydrazine [68] and this indicates the capacity to inhibit oxidative stress, which consequently might ameliorate type II diabetes and its associated complications. Humans are unable to eliminate iron released from the breakdown of transfused red blood cells leading to excessive deposition in the liver, spleen and endocrine organs which ultimately causes tissue damage leading to various complications such as heart failure, endocrine abnormalities like diabetes, liver failure hypothyroidism and eventual death [69,70]. The ability of the extracts particularly aqueous-ethanol to inhibit metal ions (Table 1) and successfully interfere with the formation of ferrous-ferrozine complex is wonderful as noted in the report of Enein et al. [71] assist in removal of toxic ions formed, resulting in abnormalities that could pave way for onset of diabetes mellitus [70].

Secondary metabolites are one important factors contributing to the medicinal value of a plant [72]. The differences in the percentage composition of each phytochemicals in the screened extracts could be attributed to the extractant used, which might probably be due to degrees in polarity. The presence of phytochemicals such as saponins, tannins, terpenoids, flavonoids and phenolics in some or all the extracts may be responsible for the inhibitory potential of the plant on the studied enzymes and parameters. Numerous studies had established and confirmed the pharmacological attributes of these phytoconstituents as antioxidants, antidiabetic, antimalarial, antimicrobials etc. [51,52,53,73,74]. Saponins provide several health benefits such as reducing cholesterol levels in the intestinal tract and inhibiting the proliferation of cancer cells, therefore, help in mitigating against obesity and antimutagenicity [22]. Tannins have been reported to help in Alzheimer and diabetes conditions [75]. Flavonoids and phenolic acids, being the largest classes of plant phenolics and are known to possess good antioxidant activity both *in vitro* and *in vivo* experiments [76]. The detection of flavonoids only in ethanol extract coupled with the exhibited antihyperglycemic potency corroborates previous similar submissions [52,53,77] which reports flavonoids due to ease of scavenging superoxide anions [78] as effective antioxidants, antidiabetic and cytotoxic phytoconstituents.

5. Conclusion

Overall, the result from this investigation provides information indicating *P. prunelloides* as a possible anti-diabetic plant. It elicited its action through inhibition of hydrolyzing enzymes (α-amylase and α-glucosidase).

Additionally, the mitigation of free radicals by the various extract of the plant is suggestive of the use of the plant as a promising anti- oxidative agent. These findings also support the traditional use of *Pentanisia prunelloides* in the management of diabetes mellitus and further document the use of the plant in the eastern Free State Province of South Africa for this purpose and numerous other illnesses.

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Statement of Competing Interest

The authors declare no conflicting interest

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