HPTLC fingerprinting analysis and \textit{in vitro} pharmacological activities of \textit{Hermannia geniculata} roots phenols

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Abstract

\textit{Hermannia geniculata} is a herb that plays an important role in the treatment of an array of diseases including diabetes, ulcer, and colitis in the South African traditional medicine. The bioactive constituent and medicinal properties in phenols of \textit{Hermannia geniculata} (PoHG) roots were investigated using high pressure thin layer chromatography (HPTLC). The α-amylase inhibitory potentials of PoHG was determined by reacting different concentration of the plant extract with 1% starch solution containing α-amylase. The inhibitory effect of the extract on α-glucosidase was evaluated by pre-incubating α-glucosidase with varying extract concentrations followed by the addition of ρ-nitrophenylglucopyranoside. The reactive oxygen and free radical scavenging potentials of the extract were also analyzed. The result showed the presence of phenolic compounds in the extract with retention factor (Rf) values ranging from 0.14 to 0.95. The extract scavenged DPPH, ABTS, hydroxyl, and superoxide anion radicals. The extract was able to chelate metallic ions with a lower IC\textsubscript{50} value which differs significantly (p<0.05) from silymarin. Moreover, PoHG extract inhibited the key enzymes (α-glucosidase and α-amylase) involved in carbohydrate catabolism with IC\textsubscript{50} values of 1.76 ±0.14 and 7.52 ±0.23 mg/mL respectively while IC\textsubscript{50} value reported for acarbose were 7.62 ±0.12 and 4.38 ±0.25 mg/mL for glucosidase and α-amylase, respectively. The α-glucosidase exhibited non-competitive inhibition by PoHG extract while α-amylase showed uncompetitive inhibition. This study confirmed the presence of phenol in PoHG extract and also showed an appreciable antioxidant and antidiabetic activities \textit{in vitro}. Therefore, PoHG extract may be of nutraceutical importance.

Keywords: Phenols; antioxidants; antidiabetics; HPTLC; \textit{Hermannia geniculata}
1.0 Introduction

As early as the beginning of human existence, man has been familiar with the use of plants in a variety of ways. Knowledge of medicinal plants has improved worldwide as different diseases pose a great threat to human existence. Traditional medicine which relies mainly on the use of medicinal plants has been playing a crucial role in the health care delivery system worldwide (Mahomoodally, 2013). Dependence on the use of medicinal plants may be due to its affordability and also unavailability of health care facilities (Bairi et al., 2017; Jokar et al., 2017)). Plants secondary metabolites include flavonoids, phenols, saponins, anthraquinones, alkaloids and glycosides they are bioactive compounds with therapeutic efficacies on scavenging reactive oxygen species and several other endogenous enzymes (Elisha et al., 2014). Phenols are plant secondary metabolites that have been reported to possess several pharmacological activities which include antiinflammatory, anticancer, antioxidant and antidiabetic (Olaokun et al., 2016; Yao et al., 2013). It can also modulate lipid and carbohydrate metabolism (Moharram and Youssef, 2014).

Medicinal plants accounted for 9% of South African flora with over 30,000 medicinal plant species (Street and Prinsloo, 2013). Hermannia geniculata is among the plant species frequently in use in South Africa to treat different diseases such as blood glucose disorder, ulcer and colic (Essop et al., 2008; Moffett, 1993). It belongs to the genus of the flowering plant from the family Malvaceae. H. geniculata is readily identified by the hanging flowers, a typically green calyx encloses the base of free petals with five petals which are contorted with transversely expanded filament (Gwynne-Evans, 2015). The plant is endemic in all the provinces of South Africa. The plant can also be found in Madagascar, Kenya, Lesotho and Saudi Arabia (Essop et al., 2008; Gwynne-Evans, 2015).

H. geniculata is a useful plant in Sotho traditional medicine of South Africa, traditionally, a concoction of the dry root is made and taken thrice daily to treat diabetes (Moffett, 1993). Among the commonest disease worldwide affecting more than 463 million people is diabetes mellitus (DM). An increase in oxidative stress as a result of an increasing imbalance in the circulating free radicals which then overwhelm the endogenous antioxidant system has been implicated in the pathogenesis and progression of DM (Li et al., 2017). Studies on ROS have shown that it causes inflammation that may be responsible for β-cells destruction and or insulin resistance which is key to diabetic etiology and its associated complication (Egea et al., 2017). Therefore the use of agents from a natural sources that have antioxidant capabilities may be able to preserve β-cells function through suppression of β-cell apoptosis helping in managing DM. This research work seeks to investigate the in vitro pharmacological activities of the phenols present in H. geniculata root extract.

2.0 Experimental

Collection of Plants, Preparation and Extraction
The roots of H. geniculata were bought in the Puthaditjhaba market, Qwaqwa Northern Free State, South Africa. Authentication of the plant species was carried out by comparisons with an earlier voucher specimen (Sh/med/05/2013/QwHb) in the Herbarium unit of Department of Botany, University of Free State, Qwaqwa Campus, South Africa.

Chemicals and Reagents
The chemicals purchased from different suppliers were: sodium dodecyl sulphate, sodium nitrite and ferrous sulphate (Sigma and Aldrich, Munich, Germany). Porcine pancreatic α-amylase, rat intestinal α-glucosidase, 1,1-diphenyl-2-picrylhydrazyl, silymarin, acarbose, para nitrophenyl-glucopyranoside and starch soluble were products J.T Baker Inc, Phillipsburg, USA. Glass distilled water was obtained from Phytomedicine and Phytopharmacology Research Laboratory, Department of Plant Sciences, University of Free State, South Africa.

Extraction of Phenols
The roots were properly rinse with water, dried and chopped into pieces. It was air-dried at 25°C, pulverized into a fine powder with a blender (Labcon, South Africa) and kept at -4°C before extraction. Phenol extraction was carried out using a shake extraction procedure as previously described (Arceusz et al., 2013). In brief, a 5000 mg of the root fine powder of H. geniculata was added to 3:17 (vol/vol) mixture of 1 M HCl and 95% C₂H₂OH (ethanol). Thereafter, the mixture was centrifuged at 3000 rpm for 10 mins, and then sieved with Millex-HV syringe (0.45 μm) which was followed by evaporation of the filtrate under reduced pressure. The extract was stored at -4°C before analysis.

In vitro antioxidant activities assays
All experiments were carried out in triplicate (n=3), preparation of the control (blank) was done using a similar procedure but distilled water was used to replace the extract.

The percentage inhibition for all antioxidant assays was obtained as follows: Control absorbance (Ao) – PoHG/standard absorbance (A1) divided by Control Absorbance (Ao) X 100. ([(Ao – A1) / Ao] x 100). Calculation of 50% inhibitory concentration (IC50) was determined from linear regression equation (Y=mx + c). Y=50 (% activity); m=slope, c=intercept and x=IC50 value.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity
The free radical scavenging activity of the PoHG extract was evaluated based on its scavenging activities on the stable 1, 1-diphenyl-2-picyrlhydrazyl (DPPH) free radical. The method was described by (Braça et al., 2001). Briefly, 150 μL of the varying concentration of plant extract/standard (0.02 – 0.1 mg/mL) was added separately to 150 μL of 0.004% methanolic solution of DPPH in a 96-well microtiter plate. The absorbance at 517 nm was determined after 30 min using a 96-well microplate reader (BIORAD, model 680, Japan).

Metal Chelating Assay
The chelating of ferrous ions by PoHG extract were estimated as
described by (Dinis et al., 1994). Briefly, 40 μL of the different concentrations of the extract and standards (0.02–0.1 mg/mL) was dispensed into a 96-well microtitre plate, 200 μL of 2 mM FeCl₂
solution was afterward added to the mixture. The reaction was initiated by the addition of 80 μL 5 mmol/L ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then read at 562 nm using a BIO-RAD (model 680, Japan) microplate reader.

2.2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) Radical Scavenging Determination

The ability of the PoHG extract to scavenge ABTS cation chromophore obtained from the oxidation of ABTS solution and potassium persulphate was determined according to the already adopted method (Re et al., 1999). Briefly, 50 mL each of 7 mM ABTS stock solution was mixed with 2.45 mM potassium persulphate leaving the mixture for 4–16 h until the reaction was completed and absorbance was stable. The resultant mixture was diluted with ethanol to get an absorbance of 0.700±0.05. The absorbance reading was taken at 734 nm using microplate reader (BIO-RAD, model 680, Japan). 20 μL of different concentration (0.02-0.1 mg/mL) of PoHG and standard was then mixed with 200 μL ABTS solution in a 96- well microtitre plate and absorbance was read at 734 nm using a microplate reader (BIO-RAD, model 680, Japan) after 15 mins of incubation at 25°C.

Hydroxyl radical scavenging ability

The ability of the plant extracts to prevent Fe²⁺/H₂O₂ induced decomposition of deoxyribose was carried out using the modified method of (Mathew and Abraham, 2006). Briefly, 100 μL of different concentrations (0.02–0.1 mg/mL) of PoHG extract and standard, 120 μL of 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer, 40 μL of 20 mM hydrogen peroxide and 40 μL of 500 μM ferrous sulphate were taken and mixed in 2 mL Eppendorf tubes. Then 100 μL of distilled water was added and incubated for 30 min at 37 °C. Subsequently, 0.5 mL 2.8% of trichloroacetic acid (TCA) and 400 μL of 0.6% thiobarbituric acid (TBA) solutions were added to stop the reaction. From the mixture, 300 μL of the resultant mixture was dispensed into a 96-well microtitre plate and incubating it in boiling water for 20 min. The absorbance was taken at 532 nm using a microplate reader (BIO-RAD, model 680, Japan).

Superoxide Anion Scavenging Assay

The determination of superoxide anion radical scavenging potential of PoHG extract was achieved according to (Liu et al., 1997). Superoxide radicals were generated in 50 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 50 μL of nitroblue tetrazolium blue (NBT) (50 mM) solution, 50 mL of nicotinamide adenine dinucleotide dehydrogenase (NADH) (78 mM) solution and different concentrations (0.02–0.1 mg/mL) of PoHG extract and standard. The reaction was initiated by adding 1 mL of phenazine methosulphate (PMS) solution (10 mM) to the mixture. The reacting mixture was incubated at 25 °C for 5 min, then the absorbance was measured at 560 nm using a microplate reader (BIO-RAD, model 680, Japan).

In vitro Assays of Antidiabetic effect of PoHG α-glucosidase inhibitory activity assay

The inhibitory effect of PoHG extract on α-glucosidase was carried out as described previously (Apostolidis et al., 2007). Briefly, varying concentration of 0.125, 0.25, 0.50, 0.75 and 1.0 mg/mL of extract/standard were prepared in distilled water and 0.05 L of PoHG extract/standard mixture was added into the test tubes. Then 0.1 L of 0.1 M of 0.02 M Na₂PO₄ (pH 6.9) buffer containing 1.0 M of α-glucosidase mixture was added. The resultant mixture was dispensed into a labeled 96-well plate and incubated for 10 min at 25°C. Further addition of 50 mL of 5 nmoL of p-nitrophenyl- α-D-glucopyranoside (pNPg) solution prepared in 0.1 M Na₂PO₄ buffer (pH 6.9) was added into each well at different time intervals and the reaction mixture incubated for 5 min at 25°C. The absorbance of the mixture to determine the extract inhibitory effect was measured at 405 nm using a microplate reader (BIO-RAD, Model 680 Japan). The same methodology was applied to the control, where the extract was replaced with distilled water.

All experiments were carried out in triplicate, preparation of the control (blank) was done using a similar procedure while distilled water was used to replace the extract. The percentage inhibition was calculated as follows: Control absorbance (Ao) – PoHG/standard absorbance (A1) divided by Control Absorbance (Ao) X 100. [(Ao – A1) / Ao] x 100.

Calculation of 50% inhibitory concentration (IC₅₀) was determined from using linear regression equation (Y=mx + c). Y=50 (% activity); m=slope, c=intercept and x= IC₅₀ value.

α-amylase inhibitory activity assay

The α-amylase enzyme inhibition potential was assayed using the method of Apostolidis et al. (2007). Briefly, 250 mL of different concentrations of PoHG extract/standard 0.125, 0.25, 0.50, 0.75 and 1.0 mg/mL prepared in distilled water and put into a test tube and freshly prepared 0.25 L of 0.02 M Na₂PO₄ (pH 6.9) buffer containing the dissolved α-amylase mixture was added. The resultant mixture was incubated for 10 min at a temperature of 25°C this was followed by the addition of 50 mL solution of starch (1%) which was prepared in 0.02 M Na₂PO₄ (pH 6.9) buffer at a different timed interval. The whole mixture was incubated for 10 min at 25°C. Dinitrosalycylic acid (DNS) reagent (500 mL) was added and the whole mixture was boiled in a water bath for 5 min to terminate the reaction. Then absorbance was measured after diluting the mixture with 5 mL distilled water. Microplate reader (BIO-RAD, Model 680, Japan) was used to measure absorbance at 540 nm. A similar method was used to prepare the control but distilled water replaced the extract. All experiments were carried out with triplicate preparation, the control (blank) was done using similar procedure replacing extract with distilled water. The percentage inhibition was calculated as follows: Control absorbance (Ao) – PoHG/standard absorbance (A1) divided by Control Absorbance (Ao) X 100. [(Ao – A1) / Ao] x 100.

Calculation of 50% inhibitory concentration (IC₅₀) is determined by using linear regression equation (Y=mx + c). Y=50 (% activity); m=slope, c=intercept and x= IC₅₀ value.
Kinetic Studies

Determination of mode of α-glucosidase inhibition by PoHG extract

The kinetics of inhibition of α-glucosidase activity by PoHG extract was carried out using the method described by Nagata et al. (2015). Briefly, pre-incubation of 50 µL of 5 mg/mL PoHG extract with 100 µL of 0.1 M of α-glucosidase solution at 25 °C for 10 min in one set of test tubes. In another set of test tubes, a pre-incubated α-glucosidase in 50 µL of 0.1 M Na2PO4 buffer (pH 6.9). 50 µL of different concentrations ranging from 0.31–50.00 mg/mL of 0.05 M PnQ was added to both sets of the reaction mixture to start the reaction. After incubation at 25 °C for 10 mins, 500 µL of 0.1 M sodium carbonate was added to terminate the reaction. Measurement of the amount of reducing sugar was carried out colorimetrically using a p-nitrophenol standard curve. The rate of reaction velocities was determined and a double reciprocal plot of enzyme mode of inhibition was plotted using the Lineweaver-Burk plot (1/v vs 1/[S]). Km and Vmax values were determined (Lineweaver and Burk, 1934).

Determination of mode of α-amylase inhibition by PoHG extract

This assay was carried out using a described procedure (Nagata et al., 2015), in brief, 250 µL of (500 µg/mL) extract/standard of PoHG which had been initially incubated with 250 µL containing 50 µg/mL solution of α-amylase at 25 °C for 10 min in one set of test tubes, while in another test tube, α-amylase was pre-incubated with 0.25 L of 0.1 M Na2PO4 (pH 6.9) buffer. Initiation of the reaction in the two sets of test tubes occurs with the addition of 0.25 L solution of (5%) starch at concentration ranging from 0.31–50.00 mg/mL. After incubating the solution for 10 min at 25 °C, 0.01 mM of DNS (500 mL) was added. The resultant mixture was boiled for 5 min to stop the reaction. Measurement of the sugar (reducing) liberated was done spectrophotometrically using a microplate reader (BIO-RAD, Model 680 Japan) measured at 405 nm and the reaction velocities was obtained through its conversion from maltose standard curve. A double reciprocal plot (1/v vs 1/[S]); v = velocity of the reaction; [S] = concentration of the substrate was plotted to calculate the kinetics of inhibition (Lineweaver and Burk, 1934).

HPTLC fingerprint analysis of PoHG extract

This was carried out using the method of Reich et al. (2008). Briefly, 50 mg of PoHG extract was weighed accurately in an electronic balance (AFCoset), dissolved in 250 µL of ethanol and centrifuged at 3000 rpm for 5min. This solution was used as a test solution for HPTLC analysis. Then 2 µL of the test solution and 2 µL of standard solution were loaded as 5 mm band length in the 3 x 10 Silica gel 60 F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The sample loaded plate was kept in Thin Layer Plate (TLC) twin trough the developing chamber (after saturated with solvent vapor) with respective mobile phase (phenols) and the plate was developed in the respective mobile phase up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, ultraviolet (UV) 254 nm and UV 366 nm. The developed plate was sprayed with respective spray reagent (Flavonoid) and dried at 100°C in a hot air oven. The plate was photo-documented in visible light and UV 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in the scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm. The Peak table, Peak display were noted. The software used was winCATS 1.34 version. Toluene-Acetone-Formic acid (4.5: 4.5: 1) was used as the mobile phase while 1% ethanolic aluminum chloride was used as the spraying reagent.

Statistical analysis

This was carried out using Graph Pad Prism Version 5 Statistical Package (Graph pad software, USA). Data were presented as Mean ±SD (n=3). One-way analysis of variance (ANOVA) and Bonferroni (Post-hoc Test) was employed to compare and analysed the significance of the mean difference. p<0.05 was considered statistically significant.

3.0 Results

In vitro Antioxidant activities of PoHG roots extract

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical decolorization assay was used to evaluate the in vitro antioxidant activity of PoHG root extract. The IC50 values obtained in this experiment for DPPH radicals scavenging properties of PoHG was 320±0.00 µg/mL which is similar to the IC50 value 380±0.02 µg/mL recorded for the standard (Table 1, Figure 1A). IC50 values of ABTS cations and superoxide anion radicals scavenging capabilities of PoHG extracts are 130±0.01 and 200±0.00 respectively which is similar to the corresponding values for the standard (Table 1, Figures 1B and 1E). The result of the metal-chelating capabilities of PoHG as shown in Table 1 and Figure 1C shows that phenol has a better activity when compared to the standard with the IC50 value of (60±0.01) µg/mL which is lower and significantly different (p<0.05) from the standard with IC50 value of 180±0.01 µg/mL. Also, PoHG had a better hydroxyl radical scavenging capability with IC50 20±0.02 µg/mL compared to the standard with IC50 value of 120±0.01 µg/mL (Table 1, Figure 1D).

Table 1: Free radical scavenging activities of phenolic extract of Hermannia genculata root and silymarin (IC50 values in µg/mL)

| Oxidants       | Phenols (µg/mL) | Silymarin (µg/mL) |
|----------------|----------------|-------------------|
| DPPH           | 320±0.01        | 380±0.02           |
| Metal Chelating| 60±0.01         | 180±0.01           |
| ABTS           | 130±0.01        | 110±0.01           |
| Hydroxyl Radicals| 20±0.02         | 120±0.01           |
| Superoxide anion | 200±0.00        | 220±0.01           |

The values are expressed as mean ± SD of triplicate determination. *Means along the row not sharing a common superscript are significantly different (p<0.05) from each other. Silymarin is a standard antioxidant agent for the antioxidant assay.
Figure 1A: DPPH radical scavenging capability; Figure 1B. ABTS radical scavenging activities; Figure 1C. Metal chelating activities; Figure 1D. Hydroxyl radical scavenging capabilities and Figure 1E. Superoxide anion radical scavenging capabilities of the phenols of *Hermannia geniculata* root extracts. Values are means ± standard deviation (SD) of triplicate determinations.
Figure 2A: Inhibitory potential of *Hermannia geniculata* root phenols extract on specific α-glucosidase activity.

Figure 2 B: Inhibitory potential of *Hermannia geniculata* root phenols extract on specific α-amylase activity.

Values are Mean ± standard deviation (SD) for triplicate determinations. Values with different alphabet are significantly different (p<0.05).

Figure 3A: Lineweaver-Burk plot of total phenols extract eliciting uncompetitive inhibition on α-amylase activity.

Figure 3B: Lineweaver-Burk plot of total phenols extract eliciting non-competitive inhibition on α-glucosidase activity. Results represent mean± standard deviation (SD); (n=3); (p>0.05)
Figure 4A: Track A – Sample A peak densitogram display (Scanned at 254nm) of Phenols of *Hermannia geniculata* roots extract. Figure 4B: Track STD – Phenolic standard Peak densitogram display (Scanned at 254nm) of the standard quercetin. Figure 4C: 3D display of all Tracks of Phenols of *Hermannia geniculata* root extract and the standard quercetin.
which is similar to the standard IC₅₀ 380± 0.02 µg/mL. Commendable inhibition of ABTS radical and metal chelating effect of PoHG was also observed, with IC₅₀ values of 130±0.01 µg/mL for ABTS which is similar to standard with IC₅₀ values of 110±0.01 µg/mL. One of the possible mechanism through which phenolic compounds reduced oxidants is by transferring H⁺ from the polyhydroxy group of the extract phenols to the carbon carrying the reactive oxygen radicals (Rice-Evans et al, 1996). The metal chelating capabilities of PoHG indicated that the extract has better metal chelating properties compared to the standard with [IC₅₀ 180 ±0.01] µg/mL. Antioxidant properties of the extract helps the survival of the cells from the destructive effect of reactive oxygen species which distort cells membrane lipids, modify the activities of cell organelles and metabolites resulting in several cellular degenerative diseases like diabetes and cancer (Mikali et al, 2013) The result of this study suggested that PoHG may be a useful agent in ameliorating oxidative stress-induced diseases.

Diabetes mellitus is a disease characterized by chronic hyperglycaemia due to the unregulated hydrolysis of carbohydrate and defective insulin release. The key enzymes involved in carbohydrate catabolism are α-amylose and α-glucosidase. Presently, several antidiabetic drugs in use has its defect, therefore, critical search for a natural product with less side effect is highly needed. Natural product with minimal inhibition of α-amylase but maximum effect on α-glucosidase has been suggested to be effective in treating excessive carbohydrate catabolism which occurs in postprandial hyperglycaemia and also eliminate the unwanted side effect associated with the use of acarbose. PoHG extract exhibited higher inhibition of α-glucosidase than acarbose with their respective IC₅₀ 1.70±0.14 and 4.38± 0.25 mg/mL while the extract mildly inhibited α-amylose compared to acarbose with IC₅₀ 7.52± 0.25 and 5.62± 0.02 mg/mL, the observed activities of the phenols in PoHG was similar to the previous observation of (Ademiluyi and Oboh, 2013) which recorded bound and free phenols exerted stronger inhibition of α-glucosidase enzyme. The IC₅₀ values obtained from this study showed that PoHG extract may be a useful natural product that can be used in the management of diabetes mellitus especially type 2 through its capabilities to inhibit the key carbohydrate metabolizing agents capable of causing postprandial hyperglycaemia.

In addition, kinetics of α-amylase inhibition by PoHG extract showed uncompetitive as reflected by binding of the inhibitor to the enzyme-substrate (ES) complex which occurs when the ES is exposed. Binding of the inhibitor to the ES complex increases the affinity of the enzyme for the substrate thereby reducing the Km value. The decrease in the value of Vmax showed that there is reduced enzyme turnover. The Vmax and Km decrease at the same rate. The velocity of the enzyme decreased from 0.155 to 0.150 mM/min while the Km value decreased from 15.2 to 14.0 mM.

Intestinal α-glucosidase has four catalytic domains (Roskar et al., 2015) its mode of inhibition by PoHG extract was by non-competitive
inhibition. The inhibitor does not affect the availability of the binding site to the substrate (Km is constant). However, it has a significant inhibitory effect on the speed of the reaction by reducing the Vmax. The maximum velocity was reduced from 1.82 nM/min to 0.95 nM/min while the Km remain constant.

HPTLC fingerprint analysis provides an improved high-resolution detection and quantification of plants secondary metabolites. The result of chemical profiling of PoHG root extract showed phenolic compounds are present in the extract with retention factor (RF) values which range from 0.14-0.95. Several studies have established the presence of phenols in medicinal plants like Daucus carota, Frankenia laevis extracts (Brezáni et al., 2017; Qasim et al., 2017). The existence of different types of phenolic compounds in the extract was linked to plants genotypic variation and also environmental factors like climate, temperature, stress from pest, microbial and other plants' pathogens, choice of the part tested and time of sample collection (Samatha et al., 2012). The variation in the phenolic constituent in different plants could be responsible for its various pharmacological activities (Ahmed and Akhtar, 2016).

Conclusion
Phenols of H. geniculata roots extract was found to possess appreciable antioxidant activity against hydroxyl radicals and displayed a strong metal chelating activity. This suggests that PoHG may be useful in the management of diseases like diabetes mellitus whose pathogenesis is linked to oxidative stress induced by reactive oxygen and free radicals. The in vitro α-amylase and α-glucosidase inhibitory activity of PoHG indicated that it may be used in controlling postprandial hyperglycaemia. This work has demonstrated the antioxidant and antidiabetic properties potential of PoHG which may be explored for the development of nutraceuticals.

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Declaration of Conflict of Interests
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Authors’ Contributions
Conception: ALA, AAOT
Design: ALA, AAOT
Execution: ALA, AAOT
Interpretation: AAOT
Writing the paper: ALA

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