Kinetics of α -amylase and α -glucosidase Inhibitory Potential of *Hermannia geniculata* Eckl. & Zehlroot Extracts Used in Basotho Traditional Medicine.

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ABSTRACT

The scientific investigation of the folkloric use of *Hermannia geniculata* roots in the management of diabetes mellitus was conducted. Phytochemical analyses, in vitro antioxidant and hyperglycaemic studies were carried out on the crude extracts of *H. geniculata*. Qualitative phytochemical analysis revealed the presence of saponins, phenols, flavonoids, alkaloids, tannins, phytosterols, triterpenes and anthraquinones. The ethanol extract exhibited the highest free radical scavenging capability with the lowest IC_{50} values (0.52, 0.38, 0.59, 0.63, 0.39) mg/mL for 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-Azino-bis(3-ethylbenzothiazoline-6-Sulphonic acid (ABTS), hydroxyl radical, superoxide anion radical and metal chelating ability which is significantly different ($p < 0.05$) from the standard (silymarin). In antidiabetic studies, ethanol extract is a potent inhibitor of α -glucosidase (IC_{50} : 0.15 mg/mL) which is lower and significantly different ($p < 0.05$) from the standard (acarbose) IC_{50} value of (0.52 mg/mL). Ethanol extract exhibited a milder inhibition of α -amylase enzyme with IC_{50} (0.57mg/mL) which is higher and significantly different ($p < 0.05$) from acarbose with IC_{50} (0.47 mg/mL). Kinetic studies revealed *H. geniculata* ethanol extract exhibited competitive inhibition of α -amylase and uncompetitive inhibition of α -glucosidase enzymes. All these findings provided the scientific basis which support the use of the root extract of *H. geniculata* in the management of diabetes mellitus and oxidative stress induced ailments like colitis and ulcers by the Basotho traditional medicine of South Africa.

Keywords: *Hermannia geniculata*; antioxidant; diabetes mellitus; enzymes; α -amylase; α -glucosidase.

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INTRODUCTION

Medicinal plant is an important part of traditional health care system and a veritable health care source for the vast majority of the world population. It was estimated that 70-80% of people worldwide use herb for management of mild to moderate illnesses [1-5]

The phenomenal rise of the alternative medicine industry responds to some of the shortcomings in what modern medicine has to offer. In several North American and European countries, the production and sale of herbal medicines, dietary supplements, and other so-called "natural" products have become a huge and profitable industry, amounting to \$32 billion a year in the USA alone [6]. Therefore, South Africa with about 9% of the world vegetation [7] must tap into this emerging market through conscious and systematic investigation of medicinal plants in her domain. Thus, the need for scientific investigation of *Hermannia geniculata* which has been frequently used in the folkloric medicine for the cure of several diseases like diabetes, colitis, severe wound, gastrointestinal disorder and skin diseases [8-10].

It is a flowering plant from the family *Malnaceae* [11,12]. The plant is seen across South Africa and its vast majority being endemic in Eastern Cape, Free State, Guateng, KwaZulu-Natal, Limpopo and Mpumalanga. It is also found in Madagascar, East Africa, North-East Africa and Saudi Arabia and North America [12].

Studies on safety and toxicity of the aqueous roots extract of *H. geniculata* on rats showed that it is safe [9]. However, there is dearth of scientific information to authenticate its wide use as a medicine by the Basotho tribe.

This present study seeks to validate the folkloric use of *H. geniculata* in the management of diabetes mellitus and several oxidative stress induced diseases. This study will also demonstrate the kinetics of inhibition of α -amylase and α -glucosidase enzymes using in vitro models by *H. geniculata*.

MATERIALS AND METHODS

Plant collection, Preparation and Extraction.

Hermannia geniculata roots were purchased from a local market in Puthaditjhaba, Qwaqwa,

Northern Free State Province, South Africa. Confirmation of the species identity was carried out the Department of Plant Sciences, University of Free State, Qwaqwa Campus, South Africa. It was compared with the herbarium specimen with voucher specimen file number (5056.000-10700) at the University of Free State, Qwaqwa Campus, South Africa. It was also compared with our earlier Voucher specimen (Ash/med/05/2013/QwHB) [9] at the herbarium.

The roots were separated, washed under running tap to remove all debris and chop into smaller pieces before being dried in the oven for nine days at 45°C to a constant weight. The dried roots material was pulverised into fine powder using waring laboratory blender (Labon, Durban, South Africa). 30 g each of the dried powdered material was extracted in 300 mL of distilled water, warm water of 40°C (decoction), ethanol and hydro-ethanol (50-50), with constant shaking on a Labcon platform shaker (Laboratory Consumables, PTY, Durban, South Africa). The mixture was filtered using No. 1 Whatman filter paper. The ethanol extract was concentrated to dryness *in vacuo* at 40 °C using a rotary evaporator (Cole-Palmer, South Africa). All other extracts were air dried and stored at -4°C until use.

Chemicals and Reagents

Porcine pancreatic α -amylase, rat intestinal α -glucosidase, 1,1-diphenyl-2-picrylhydrazyl, gallic acid, acarbose, rutin, quercetin, and paranitrophenyl-glucopyranoside were products of Sigma-Adrich, South Africa. Starch, ferric chloride (FeCl_3), potassium ferricyanide, dinitrosalicylic acid (DNS) and maltose were products of J. T. Baker Inc., Phillipsburg, USA. Distilled water was obtained from Phytomedicine and Phytopharmacology Research Group Laboratory, Plant Sciences Department, University of Free State, QwaQwa campus, South Africa.

Measurement of Percentage Yield.

The percentage yield of the extract was calculated as $((c-b)/a) \times 100$. Where a = weight of sample; b = weight of beaker and c = weight of beaker + sample.

Qualitative Phytochemical Screening

Using described procedure [13] the root extracts of *Hermannia geniculata* was subjected to qualitative phytochemical screening. Alkaloids, anthraquinones, flavonoids, phenols, saponins, tannins, triterpenes, phytosterols were screened for.

Quantitative Phytochemical Analysis**Assessment of total phenolic content**

The quantification of phenolic content of *H. geniculata* extracts was carried out using the procedure reported [14].

Determination of total flavonoids

The total flavonoids of the extracts were determined using the method already adopted [15].

In vitro Antioxidant Assays

All experiments were conducted in triplicates and all the negative controls (blank) were prepared using the same procedure replacing the extract with distilled water. The percentage inhibitory activity of the extract/standard was calculated using $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The half maximal inhibitory concentration (IC_{50}) value were calculated from the linear regression equation using $y = m x + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept and x is the IC_{50} value.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activity of the *H. geniculata* root extracts were evaluated based on its scavenging activities on the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The method was described [16].

Hydroxyl Radical Scavenging Ability

The ability of the plant extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of [17].

Superoxide Anion Scavenging Assay

Determination of superoxide anion radical scavenging potential of *H. geniculata* root extracts were achieved according to [18].

Metal Chelating Assay

The chelating action of ferrous ions by root extracts of *H. geniculata* was estimated as described by [19].

Ferric Ions Reducing Power

Ferric ions reducing power (FRAP) of the extracts and standards were determined according to the method adopted by [20].

2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) Radical Scavenging Determination

The ability of *H. geniculata* root extracts to scavenge ABTS cation chromophore obtained from the oxidation of ABTS solution and potassium persulphate was determined according to already adopted method [21].

In vitro Antidiabetic Assays **α -Amylase Inhibitory Assay**

This assay was carried out using the procedure of [22]. 250 ml of varying concentration of the extract/ standard (0.125 – 1.0 mg/ml) was placed in a test tube and 250 ml of 0.02 M sodium phosphate buffer (pH 6.9) containing α -amylase solution was added. This solution was incubated at 25°C for 10 min, followed by addition of 250 ml of starch (1%) solution in 0.02 M sodium phosphate buffer (pH 6.9) at timed intervals, the resulting reaction mixture was then incubated at 25°C for 10 min. The reaction was terminated by adding 500 ml of dinitrosalicylic acid (DNS) reagent before incubating the tubes in boiling water for 5 min and cooled to 25°C. 5 ml distilled water was added to the reacting mixture after cooling and the absorbance was measured at 540 nm using a microplate reader (BIO-RAD, model 680, Japan). The control was prepared using the same procedure replacing the extract with distilled water. The α -amylase inhibitory activity was done in triplicate and was calculated as percentage inhibition, thus; % Inhibition = (Absorbance (control) – Absorbance (extract))/ Absorbance (control) $\times 100$. Concentrations of extract resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically using the linear regression equation $y = m x + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept and x is the IC_{50} value.

α -Glucosidase Inhibitory Assay

The effect of the extracts on α -glucosidase activity was determined according to the method described by [22] with slight modification. In brief, different concentrations (0.125 – 1.0 mg/ml) of the extract/ standard were prepared in distilled water. Then, 50 ml from the stock solution was mixed with 100 ml of 0.1 M phosphate buffer (pH6.9) containing 1.0 M of α -glucosidase solution. The mixtures were then incubated in 96-well plates at 25°C for 10 min. Following this, 50 ml of 5 mM p-nitrophenyl- α -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. The inhibitory effect of the extract/standard on the enzyme activities were determined by measuring the absorbance of the mixtures at 405 nm using a microplate reader (BIO-RAD, model 680, Japan). The control was prepared using the same procedure replacing the extract with distilled water. The experiments were conducted in triplicate and the α -glucosidase inhibitory activity was expressed as % inhibition using the expression:

% Inhibition = $[(A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}}] \times 100$, where A_{control} and A_{extract} are the absorbance's of the control and extract respectively. Concentrations of the extract/standard resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically using the linear regression equation $y = m x + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept and x is the IC_{50} value.

Kinetic Studies

Mode of α -amylase Inhibition

This assay was conducted using previously reported method [23]. Briefly, 250 ml of the (5 mg/mL) extracts/standard were pre-incubated with 250 ml of 0.5 mg/mL α -amylase solution for 10 min at 25°C in one set of tubes while α -amylase was pre-incubated with 250 ml of 0.1M phosphate buffer (pH 6.9) in another set of tubes. The reaction of the two sets of the mixtures was initiated by adding 250 ml of 5% starch solution at increasing concentrations (0.31 – 50.00 mg/ml). The mixture was then incubated for 10 min at 25°C, followed by

addition of 0.01mM DNS (500 ml). The reaction was terminated after boiling for 5 min. The amount of reducing sugars released was determined spectrophotometrically using maltose 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.

Mode of α -Glucosidase Inhibition

The α -glucosidase mode of inhibition by ethanol extract was determined using the method of [24]. Briefly, 50 μ L of 5 mg/ml extract was pre-incubated with 100 μ L of 0.1M α -glucosidase solution for 10 min at 25°C in one set of tubes. In another set of tubes, α -glucosidase was pre-incubated with 50 μ L of 0.1M phosphate buffer (pH6.9). 50 ml of 0.05 M pNpG at different concentrations (0.31 – 50.00 mg/ml) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25 °C, and 500 ml of 0.1M Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined colourimetrically using a p-nitrophenol standard curve. Reaction rates (v) were thereafter calculated and double reciprocal plots of enzyme kinetics. K_m and V_{max} values were also calculated from Lineweaver-Burkplot ($1/v$ versus $1/[S]$) [25].

Statistical Analysis

Statistical analysis was performed using a Graph Pad Prism 5 statistical package (Graph Pad Software, San Diego, MA, USA). Data were expressed as means of replicate determinations $\pm$ SD, for all assays and were subjected to one-way analysis of variance (and nonparametric) followed by Bonferroni: compare all pair of column. Statistical significance was considered at $p < 0.05$.

RESULTS

The percentage yield by the different solvents used in the extraction is presented in Table 1

Phytochemicals

The qualitative and quantitative analyses of the root extracts of *H. geniculata* are presented in Tables 2 and 3 respectively. Saponins, phenols, flavonoids, anthraquinones, alkaloids, tannins,

triterpenes and phytosterols were detected at varying degree in all the tested extracts while anthraquinones and phytosterols were found in trace amount in the ethanol and hydro-ethanol extracts.

Antioxidant Activity

The *in vitro* antioxidant potentials of the root extracts of *H. geniculata* are shown in Figures 1-6. The extracts scavenged/inhibited/chelated the generated radicals/ions/metals in all assays evaluated. Ethanol extracts showed better capability to scavenge DPPH and hydroxyl radicals in a concentration dependent manner (0.125, 0.25, 0.50, 0.75 and 1.00) mg/ml (Figures 1 and 2). Their corresponding IC₅₀ values are (0.52 and 0.59) mg/ml respectively which is lower and significantly different ($p < 0.05$) from the standard (silymarin) IC₅₀: (1.09 and 1.12) mg/ml (Table 4).

However, hydro-ethanol showed remarkable capability in scavenging superoxide anion radical, its IC₅₀ value is 0.49 mg/mL which is comparable to silymarin with IC₅₀:1.12 mg/mL. *H. geniculata* root extracts also showed significant metal chelating potential against ferrous ion (Figure 3 and Table 4). The reducing power and ABTS cation scavenging capability of the extracts competed well with silymarin in a dose dependent manner (0.125, 0.25, 0.50, 0.75 and 1) mg/ml with the highest concentration of 1mg/mL showing the best activity (Table 4, Figures 5 and 6).

In vitro antidiabetic assays

The inhibitory potentials of *H. geniculata* root extracts on both α - amylase and α - glucosidase enzymes is concentration dependent (0.125, 0.25, 0.50, 0.75 and 1 mg/ml; Figures 7 and 8). Ethanol extract has the lowest IC₅₀ (0.15) mg/mL which is significantly different ($p < 0.05$) from all other extracts and acarbose (Table 5). Ethanol and decoction extracts show milder inhibition of α -amylase with their respective IC₅₀ values of (0.57 and 0.62) mg/ml which is higher and significantly different ($p < 0.05$) from acarbose and hydro-ethanol (IC₅₀:0.47 and 0.42) mg/ml respectively. The mode of inhibition depicted by Lineweaver-Bulk Plot is competitive and uncompetitive inhibition of α - amylase and α - glucosidase enzymes respectively (Figures 9 and 10).

DISCUSSION

The use of plants in treating diseases is as old as civilization [26] and herbal medicine is still a major part of habitual treatment of different diseases [27]. The process in the preparation of herbs like pulverization and solvents deployed in the extraction of raw material for drugs affects the percentage yield of the biologically active compound present in the extracts. In this experiment, we use the local solvents deployed in herbs remedies such as ethanol, hydro-ethanol, decoction and distil water as solvent for extraction. The percentage yield indicated that hydro-ethanol has the highest yield of 29.71% from the 30g dry weight of the plant sample extracted while decoction extract yield 8.05% of the 30g dry weight of the plant sample. It is worthy of note that the traditional healer use decoction (boil the dry root of *Hermannia geniculata* as their method of extracting the biologically active component of the plant [9].

Result of the quantitative phytochemical assays indicated the concentration of different phytochemicals (PC) found in the root extracts of *H. geniculata*. Phytochemicals are known to possess varying antioxidant activities [28-32]. Antioxidant activity of a medicinal plant cannot be concluded based on a single antioxidant test model [32] as such, several *in vitro* antioxidant tests were conducted on the extracts using silymarin as positive control for all assays except metal chelating assay where citrate was used as the standard. We determine the free radical scavenging capability of *H. geniculata* on the molecules of DPPH radicals, ABTS cations radical, the reducing power, superoxide anion radicals. We also assay for hydroxyl radical which is one of the most potent reactive oxygen species in the biological system that react with polyunsaturated fatty acid moieties of cell membrane phospholipid causing cellular damage.

The result of the assay showed that ethanol extract of *Hermannia geniculata* has better performance in free radical scavenging activity compared to the standard and other extract tested for DPPH, hydroxyl radical and metal chelating activities while hydro ethanol showed superior activity compared to the

standard and other extracts tested in ABTS, superoxide anion and reducing power. All these predictions are based on the standard curve of percentage inhibition/scavenging effect and IC_{50} value of the tested extract which revealed a decrease in concentration of the reactive oxygen species (ROS) which may be due to the scavenging ability of *H. geniculata* extracts. Similar findings have documented the antioxidant and anti-inflammatory properties of some *Hermannia* species [10]. Phytochemical like polyphenols has the capability to scavenge superoxide and other ROS like hydroxyl and peroxy radicals [33-35], saponins, triterpenes and phytosterols have also been demonstrated to scavenge superoxide anion [36-38]. Flavonoid are currently receiving attention as a potential protector against variety of human disease, major flavonoid has been shown to have neutralizing effect on free radical and ROS like hydroxyl radical, superoxide radical, hydrogen peroxides [32, 38-40].

Marked postprandial hyperglycaemia is important in the pathogenesis of type 2 diabetes, it induces mitochondrial superoxide overproduction which potentially inhibit the glycolytic enzyme glyceraldehyde-3-phosphate (GAPDH) thus, diverting upstream metabolites from glycolytic pathway into pathway of glucose overutilization resulting in formation of diacylglycerol (DAG) from dihydroxyacetone phosphate (DHAP) a potent activator of protein kinase C (PKC) which ultimately causes β -cells destruction and insulin resistance [41-43]. The unregulated hydrolysis of starch by α -amylase and α -glucosidase which catalyze the rate limiting step in the conversion of oligosaccharides and disaccharides into monosaccharides is responsible for the elevated blood glucose seen in type 2 diabetes mellitus. Therefore, controlling hyperglycaemia via inhibition of carbohydrate hydrolysing enzymes is an important strategy in the management of type 2 diabetes mellitus [44-46]. *In vitro* evaluation of the inhibitory effect of the extracts on α -glucosidase and pancreatic α -amylase enzymes was carried out on all the extracts using acarbose as the standard to determine its percentage inhibition and their respective IC_{50} value. Mild inhibition of α -amylase and strong

inhibition of α -glucosidase enzymes is targeted as a way of reducing postprandial hyperglycaemia, and elimination of the unwanted effect like gastrointestinal discomfort flatulence, diarrhoea associated with the use of acarbose⁴⁷. In this study, ethanol and decoction extracts mildly inhibit α -amylase with their respective IC_{50} values of (0.57 and 0.62) which is higher and significantly different ($p < 0.05$) from acarbose with lower IC_{50} (0.47 mg/ml). The result of the inhibitory potentials of the extracts on α -glucosidase showed ethanol and decoction extracts have potent inhibition of the enzyme activity. Thus, may be employed in the management of postprandial hyperglycemia. This finding is consistent with findings of many authors [44, 46-48] who described moderate inhibition of α -amylase and strong inhibition of α -glucosidase as a better therapeutic approach to be deployed in the delay and regulation of carbohydrate hydrolysis in the intestine which is responsible for glucose toxicity observed in type 2 diabetes mellitus.

The ethanol extract which possess the highest IC_{50} for α -amylase enzyme and lowest IC_{50} for α -glucosidase compared to acarbose and other tested extracts of *H. geniculata* was used to determine the mode of inhibition of α -amylase and α -glucosidase enzymes in order to investigate its enzyme inhibition kinetics.

Result for the mode of inhibition of α -amylase enzyme showed that the ethanolic extract competitively inhibit the breakdown of disaccharides and oligosaccharides which are substrate for α -amylase. The V_{max} values obtained with inhibitor and without inhibitor in the reaction pathway is the same, the K_m values decreased from $4.85 \times 10^{-2} (\mu M)^{-1}$ for reaction pathway without inhibition to $1.44 \times 10^{-2} (\mu M)^{-1}$ with inhibitor. Decreased K_m value signify increase affinity. This result suggested competitive mode of inhibition.

However, the mode of inhibition of α -glucosidase by ethanolic extract of *H. geniculata* is by uncompetitive inhibition. The proposed model is the binding of the *H. geniculata* extract (inhibitor) to a site other than the active site and only when the substrate is binding to ES complex thereby inhibiting the

formation of product. The kinetic further shows that there is a decrease in K_m from $(7.10 \times 10^{-2} (\mu M)^{-1})$ to $4.69 \times 10^{-2} (\mu M)^{-1}$ without inhibitor and with inhibitor respectively) and also a decrease in V_{max} from $19.76 \mu M/min$ without inhibitor to $14.66 \mu M/min$ with inhibitor which suggests a 39.74% decrease in overall activity of α -glucosidase enzyme in the presence of ethanol extract of *Hermannia geniculata* roots.

Conclusion

Our findings scientifically justify the use of *H. geniculata* in Basotho traditional medicine. Moreover, the overall result revealed better performance of the extracts *in vitro* than all the standards (acarbose/silymarin/citrate) in both antidiabetic and antioxidant assays. Therefore, the root extract of *Hermannia geniculata* may play an important role in the development of nutraceuticals and also in the management of oxidative stress induced illnesses and diabetes.

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Figure 1. DPPH scavenging effect of the root extracts of *Hermannia geniculata*. Values are mean $\pm$ standard deviation (SD) of triplicate determinations. N=3; (p<0.05).

Figure 2. Scavenging effect of the root extracts of *Hermannia geniculata* on hydroxyl radical. Values are mean $\pm$ standard deviation (SD) of triplicate determinations. N=3; ($p < 0.05$).

Figure 3. Scavenging effect of the root extracts of *Hermannia geniculata* on superoxide anion radical. Values are mean $\pm$ standard deviation (SD) of triplicate determinations. N=3; ($p < 0.05$).

Figure 4. Metal chelating capability of the root extracts of *Hermannia geniculata*. Values are mean $\pm$ standard deviation (SD) of triplicate determinations. N=3; (p<0.05).

Figure 5. Reducing potential of the root extracts of *Hermannia geniculata*. Values are mean $\pm$ standard deviation (SD) of triplicate determinations. N=3; (p<0.05).

Figure 6. ABTS scavenging effect of the root extracts of *Hermannia geniculata*. Values are mean and standard deviation (SD) of triplicate determinations. N=3; (p<0.05).

Figure 7. The inhibitory potentials of *H. geniculata* root extracts on α -amylase activity. Value are mean and standard deviation (SD) of triplicate determination. N=3; (p<0.05).

8. The inhibitory potentials of *H. geniculata* root extracts on α -glucosidase activity. Value are mean and standard deviation (SD) of triplicate determination. N=3; ($p < 0.05$).

Figure 9. Lineweaver-Burk plot of ethanolic extract of *Hermannia geniculata* eliciting competitive inhibition on α -amylase activity. Result represent mean $\pm$ standard deviation: (n=3;

Figure 10. Lineweaver-Burk plot of ethanolic root extract of *Hermannia geniculata* eliciting uncompetitive inhibition on α -glucosidase activity. Result represent mean $\pm$ standard deviation: (n=3; (p<0.05)

Table 1: The percentage yield from different extracting solvents used in the root extract of *Hermannia geniculata*

	Ethanol	Hydro-ethanol	Decoction	Aqueous
Percentage yield (%)	11.12	29.71	8.05	18.55

Table 2. Phytochemical constituents of the root extracts of *Hermannia geniculata*.

Phytochemicals	Ethanol	Hydro-ethanol	Decoction	Aqueous
Alkaloids	+++	+++	++	++
Phenols	+++	+++	+++	++
Flavonoids	+	+	-	-
Anthraquinones	++	++	+++	+++
Tannins	++++	++++	++	++
Triterpenes	-	++	+++	++++
Phytosterols	-	++	+++	+++

Key: +: detected; +++: degree of intensity; -: not detected or in trace amount.

Table 3 . The result of the quantitative phytochemical screening of *Hermannia geniculata* root extracts.

Phytochemicals	Ethanol	Hydro-ethanol	Decoction	
Aqueous				
Total Flavonoid (mg quercetin in g-1)	0.36	1.10	0.61	0.30
Total phenol (mg gallic acid g-1)	8.35	10.29	10.79	10.41

Table 4 . The IC₅₀ values of the free radical scavenging/chelating capabilities of different extracts of *Hermannia geniculata*

Samples	IC ₅₀ (mg/mL)				
	DPPH	ABTS	HYDROXYL	SUPEROXIDE	METAL CHELATING
Silymarin	1.09 ± 0.02 ^a	0.39 ± 0.05 ^a	1.12 ± 0.02 ^a	1.12 ± 0.02 ^a	
Citrate					1.5± 0.01 ^a
Ethanol	0.52 ± 0.05 ^b	0.38 ± 0.02 ^a	0.59 ± 0.01 ^b	0.63 ± 0.10 ^b	0.39± .01 ^b
Hydro-ethanol	1.15 ± 0.03 ^a	0.30 ± 0.02 ^b	0.94 ± 0.01 ^a	0.49 ± 0.00 ^c	0.41± .05 ^b
Decoction	1.78 ± 0.01 ^c	0.49 ± 0.02 ^c	1.03 ± 0.01 ^a	0.57 ± 0.01 ^b	1.73± .02 ^c
Aqueous	1.05 ± 0.01 ^a	0.49 ± 0.05 ^c	1.76 ± 0.01 ^c	0.60 ± 0.01 ^b	0.67± .01 ^d

The values are expressed as mean ± standard deviation (SD) of triplicate determination. Means down vertical column not sharing a common superscript are significantly different (p<0.05) from each other. Silymarin is the standard antioxidant agent for all the antioxidant assays except metal chelating that has citrate as the standard.

Table 5 . The IC₅₀ values for the root extracts of *Hermannia geniculata* on specific activities of α-amylase and α-glucosidase enzymes

Samples	IC ₅₀ (µg/mL)	α- glucosidase	α-amylase
Acarbose		0.52 ± 0.04 ^a	0.47 ± 0.01 ^a
Ethanol		0.15 ± 0.00 ^b	0.57 ± 0.01 ^b
Hydro-ethanol		0.39 ± 0.00 ^a	0.42 ± 0.05 ^a
Decoction		0.46 ± 0.01 ^a	0.62 ± 0.03 ^d
Aqueous		0.45 ± 0.04 ^a	0.53 ± 0.08 ^a

The values are expressed as mean ± standard deviation (SD) of triplicate determination. Means down vertical column not sharing a common superscript are significantly different (p<0.05) from each other.