

## Antioxidative activity and inhibition of key enzymes linked to type-2 diabetes ( $\alpha$ -glucosidase and $\alpha$ -amylase) by *Khaya senegalensis*

MOHAMMED AUWAL IBRAHIM<sup>1,3</sup>  
NEIL ANTHONY KOORBANALLY<sup>2</sup>  
MD. SHAHIDUL ISLAM<sup>1\*</sup>

<sup>1</sup> School of Life Sciences and  
<sup>2</sup> School of Chemistry and Physics  
University of KwaZulu-Natal  
(Westville Campus)  
Durban, 4000, South Africa

<sup>3</sup> Department of Biochemistry  
Ahmadu Bello University, Zaria  
Nigeria

This study evaluated the *in vitro* antioxidative activity of *Khaya senegalensis* extracts and inhibitory effects of some solvent fractions on  $\alpha$ -glucosidase and  $\alpha$ -amylase activities. The stem bark, root and leaf samples of the plant were sequentially extracted with ethyl acetate, ethanol and water and then tested for antioxidative activity. Our findings revealed that the ethanolic extract of the root had the highest antioxidative activity. Solvent-solvent fractionation of the root ethanolic extract yielded a butanol fraction that showed higher antioxidative activity than other fractions. Furthermore, the butanol fraction had significantly higher ( $p < 0.05$ )  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities with  $IC_{50}$  values of  $2.89 \pm 0.46$  and  $97.51 \pm 5.72 \mu\text{g mL}^{-1}$ , respectively. Enzyme kinetic studies indicated that the butanol fraction is a non-competitive inhibitor for  $\alpha$ -glucosidase with an inhibition binding constant  $K_i$  of  $1.30 \mu\text{g mL}^{-1}$  and a competitive inhibitor of  $\alpha$ -amylase with a  $K_i$  of  $7.50 \mu\text{g mL}^{-1}$ . GC-MS analysis revealed that the butanol fraction contained two chromones, *p*-anilinophenol and 3-ethyl-5-(3-ethyl-(3*H*)-benzothiazol-2-ylidene)-2-(*p*-tolylvinylamino)-4-thiazolidinone. Data obtained in the study suggest that the butanol fraction derived from the ethanolic extract of *K. senegalensis* root possessed excellent antioxidative as well as  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities while chromones and/or *p*-anilinophenol could be the main bio-active compounds responsible for the observed activities.

**Keywords:** *Khaya senegalensis* (Meliaceae), type-2 diabetes,  $\alpha$ -glucosidase,  $\alpha$ -amylase, oxidative stress

Accepted May 9, 2014

Diabetes mellitus is among the major global public health problems and its prevalence is currently increasing at an alarming rate. According to the International Diabetes Federation, about 366 million people are living with diabetes and this figure is projected to increase to 552 million by the year 2030 (1). Between the two major types of diabetes, type-

\* Correspondence; e-mail: islamd@ukzn.ac.za; sislam1974@yahoo.com

2 diabetes (T2D) is most prevalent and accounts for > 95 % of the total diabetic population worldwide. Type-2 diabetes is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of pancreatic  $\beta$ -cells to compensate for insulin resistance ( $\beta$ -cell dysfunction) leading to hyperglycemia (2) while oxidative stress is a major contributor to the  $\beta$ -cell damage (3).

Oxidative stress refers to the existence of products called free radicals (molecules possessing an unpaired electron) and reactive oxygen species (ROS), which are formed in normal physiological processes but become deleterious when they are not quenched by a cascade of antioxidant systems. This can result either from an overproduction of ROS or from the inactivation of the antioxidants (AO), thereby shifting the ROS/AO balance in favor of stress (4). In T2D, hyperglycemia induces generation of free radicals, including ROS, hydroxyl and nitric oxide (NO) radicals (5), which are responsible for oxidative stress induced pancreatic  $\beta$ -cell destruction as well as the activation of all major pathways underlying the different components of chronic vascular diabetic complications such as glycation and sorbitol pathways among others (4). The concept of oxidative stress as an important trigger, and postprandial hyperglycemia at the onset and progression of diabetes, offer a unique therapeutic strategy for the treatment of T2D and for reducing chronic vascular complications (6). Current therapeutic strategy for the control of postprandial hyperglycemia is the inhibition of two members of exo-acting glycoside hydrolases ( $\alpha$ -glucosidase and  $\alpha$ -amylase), resulting in an aggressive delay of carbohydrate digestion to absorbable monosaccharides (7). Starch and other complex polysaccharides are hydrolyzed by  $\alpha$ -amylase to oligosaccharides, which are further hydrolyzed to liberate glucose by intestinal  $\alpha$ -glucosidase before being absorbed into the intestinal epithelium and entering blood circulation. Therefore,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors will ultimately reduce the flow of glucose from complex dietary carbohydrates into the bloodstream, diminishing the postprandial hyperglycemia.

However, the leading glucosidase inhibitors, acarbose and miglitol, are often reported to produce diarrhea and other intestinal disturbances, with the corresponding bloating, flatulence, cramping and abdominal pain (8). Randomized controlled trials with glucosidase inhibitors report these gastrointestinal side effects as the most common reasons for non-compliance and early subject withdrawal (9). Interestingly, plant based agents were reported to be more acceptable source of glucosidase inhibitors due to their low cost and relatively better safety levels, including lower incidence of serious gastrointestinal side effects (10). The foregoing therefore makes the search for alternative agents with potent antioxidative properties that could also decrease postprandial hyperglycemia, thereby providing a holistic avenue to control hyperglycemia and other diabetic complications resulting from oxidative stress, of the utmost importance.

*Khaya senegalensis* (Desr.) A. Juss. also known as African mahogany, is a member of the *Meliaceae* family and is highly reputed for its numerous medicinal effects. Ethnopharmacological surveys have revealed that the stem bark, root and leaves of the plant are used in the traditional treatment of diabetes, malaria, anemia, diarrhea, gastrointestinal diseases and fever amongst others (11, 12). Scientific investigations have revealed the anti-hyperglycemic activity of the aqueous stem bark extract of this plant in a type-1 diabetes model of rats (13) as well as an *in vitro*  $\alpha$ -amylase inhibitory potential (14). However, detailed investigation of the complete antioxidative and  $\alpha$ -glucosidase or  $\alpha$ -amylase inhibitory activity of the extracts from different parts of this plant has not been conducted to date.

Hence, in this study, we conducted a comprehensive and systematic investigation of the *in vitro* antioxidative as well as  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of various extracts and solvent fractions of *K. senegalensis* using complementary *in vitro* models, along with identification of active compounds in the most active fraction as well as establishing the mechanisms of  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitions by the most active fraction *via* the enzyme kinetics approach.

## EXPERIMENTAL

### *Chemicals and reagents*

Yeast  $\alpha$ -glucosidase, porcine pancreatic amylase, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG), *p*-nitrophenol, acarbose, gallic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2-deoxy-D-ribose, ascorbic acid and potassium ferricyanide were obtained from Sigma-Aldrich, Germany. Starch, dinitrosalicylic acid (DNS), maltose, absolute ethanol, ethyl acetate, trichloroacetic acid, hydrogen peroxide, ferric chloride, Griess reagent, sodium nitroprusside, dimethylsulfoxide (DMSO), thiobarbituric acid and Folin Ciocalteu reagent were obtained from Merck Chemical Company, Germany.

### *Plant material*

The stem bark, root and leaf samples of *K. senegalensis* (*Meliaceae*) were collected in January 2011 from the Botanical garden of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, and the species was identified at the herbarium unit of the same department; herbarium voucher specimen was deposited. The stem bark, root and leaves were immediately washed and shade-dried for four weeks to constant mass. The dried samples were pounded to fine powder using a kitchen blender and then stored individually in air-tight containers for subsequent analyses.

### *Preparation of crude extracts and solvent partitioning of the crude extract*

Forty grams of a fine powdered sample from each part of the plant were separately defatted with hexane. Defatted materials were sequentially extracted with ethyl acetate, ethanol and water by soaking for 48 h in 200 mL of the relevant solvent, followed by shaking for 2 hours at 200 rpm. After filtration through Whatmann filter paper No. 1, the respective solvent was evaporated in vacuum using a rotary evaporator (Büchi Rotavapor II, Büchi, Switzerland) at 40 °C under reduced pressure. Aqueous extracts were however dried on a water bath at 50 °C. Based on the results of the antioxidative assays (described below), the most active ethanolic extract of the root was further fractionated. Ten grams of the crude ethanolic extract of the root was dissolved in 200 mL of distilled water/methanol (9:1) and successively partitioned with dichloromethane (2 x 200 mL), ethyl acetate (2 x 200 mL) and *n*-butanol (2 x 200 mL). The resulting fractions were evaporated to dryness at 40 °C under reduced pressure while the remaining aqueous fraction was dried on a water bath. The crude extracts and fractions in each case were weighed, transferred to micro tubes and stored at 4 °C until further analysis.

### *Estimation of total phenolic content*

The total phenolic content of each extract or fraction was determined (as gallic acid equivalent) according to the method described by McDonald *et al.* (15) with slight modifications. Briefly, 200  $\mu\text{L}$  of the extract or fraction dissolved in 10 % DMSO (240  $\mu\text{g mL}^{-1}$ ) was incubated with 1 mL of Folin Ciocalteu reagent (diluted 10 times) and 800  $\mu\text{L}$  of 0.7 mol  $\text{L}^{-1}$   $\text{Na}_2\text{CO}_3$  for 30 min at room temperature. Then, the absorbance was measured at 765 nm on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu, Japan). All measurements were done in triplicate.

### *DPPH radical scavenging activity*

The total free radical scavenging activity of the tested extracts and fractions was determined and compared to that of ascorbic and gallic acids as well as Trolox using a slightly modified method described by Tuba and Gulcin (16). A 0.3 mmol  $\text{L}^{-1}$  solution of DPPH was prepared in methanol and 500  $\mu\text{L}$  of this solution was added to 1 mL of the extract or fraction (dissolved in 10 % DMSO) at different concentrations (15–240  $\mu\text{g mL}^{-1}$ ). These solutions were mixed and incubated in the dark for 30 min at room temperature. Absorbance was then measured at 517 nm against a blank samples lacking scavenger.

### *Hydroxyl radical scavenging (HRS) activity (deoxyribose assay)*

The HRS activity was measured by studying the competition between deoxyribose and the extracts or fractions for hydroxyl radical generated by the ascorbate/EDTA/ $\text{H}_2\text{O}_2$  system (Fenton reaction) as described by Hinnerburg *et al.* (17). The assay was performed by adding 200  $\mu\text{L}$  of premixed 100  $\mu\text{mol L}^{-1}$   $\text{FeCl}_3$  and 100  $\mu\text{mol L}^{-1}$  EDTA (1:1, V/V) solution, 100  $\mu\text{L}$  of 10 mmol  $\text{L}^{-1}$   $\text{H}_2\text{O}_2$ , 360  $\mu\text{L}$  of 10 mmol  $\text{L}^{-1}$  2-deoxy-D-ribose, 1 mL of different extracts or fractions dissolved in 10 % DMSO (concentration 15–240  $\mu\text{g mL}^{-1}$ ), 400  $\mu\text{L}$  of 50 mmol  $\text{L}^{-1}$  sodium phosphate buffer (pH 7.4) and 100  $\mu\text{L}$  of 1 mmol  $\text{L}^{-1}$  ascorbic acid as per the above-mentioned sequence. The mixture was incubated at 50  $^\circ\text{C}$  for 2 h. Thereafter, 1 mL of 2.8 % TCA and 1 mL of 1.0 % thiobarbituric acid (in 0.025 mmol  $\text{L}^{-1}$  NaOH) were added to each tube. The samples were further incubated in a water bath at 50  $^\circ\text{C}$  for 30 min to develop the pink chromogen. The extent of oxidation was estimated from the absorbance of the solution at 532 nm and the HRS activity of the extract is reported as percentage inhibition of deoxyribose degradation.

### *Nitric oxide (NO) radical scavenging assay*

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen, leading to reduced production of NO (18). The assay was carried out by incubating 500  $\mu\text{L}$  of 10 mmol  $\text{L}^{-1}$  sodium nitroprusside in phosphate buffer (pH 7.4) and 500  $\mu\text{L}$  of different extracts or fractions dissolved in 10 % DMSO (concentration 15–240  $\mu\text{g mL}^{-1}$ ) at 37  $^\circ\text{C}$  for 2 h. The reaction mixture was then mixed with 500  $\mu\text{L}$  of Griess reagent. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm. The percentage inhibition of

NO generated was measured in comparison with the absorbance value of a control (10 mmol L<sup>-1</sup> sodium nitroprusside in phosphate buffer).

#### *Determination of $\alpha$ -glucosidase inhibitory activity of fractions of the root ethanolic extract*

The  $\alpha$ -glucosidase inhibitory activity was determined according to the method described by Ademiluyi and Oboh (6), with slight modifications. Briefly, 250  $\mu$ L of each fraction or acarbose, at different concentrations (30–240  $\mu$ g mL<sup>-1</sup>), was incubated with 500  $\mu$ L of 1.0 U mL<sup>-1</sup>  $\alpha$ -glucosidase solution in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250  $\mu$ L of pNPG solution (5 mmol L<sup>-1</sup>) in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) was added and the mixture was further incubated at 37 °C for 20 min. The absorbance of the released *p*-nitrophenol was measured at 405 nm and the inhibitory activity was expressed as percentage of a control sample without inhibitors.

#### *Determination of $\alpha$ -amylase inhibitory activity of fractions of the root ethanolic extract*

The  $\alpha$ -amylase inhibitory activity was determined according to the method described by Shai *et al.* (19), with slight modifications. A volume of 250  $\mu$ L of each fraction dissolved in 10 % DMSO or acarbose at different concentrations (30–240  $\mu$ g mL<sup>-1</sup>) was incubated with 500  $\mu$ L of porcine pancreatic amylase (2 U mL<sup>-1</sup>) in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) at 37 °C for 20 min. Two hundred and fifty  $\mu$ L of 1 % starch dissolved in 100 mmol L<sup>-1</sup> phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 37 °C for 1 h. One mL of DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm and the inhibitory activity was expressed as percentage of a control sample without inhibitors. All assays were carried out in triplicate.

#### *Mechanism of $\alpha$ -glucosidase and $\alpha$ -amylase inhibitions*

The most active (butanol) fraction was subjected to kinetic experiments to determine the type of inhibition exerted on  $\alpha$ -glucosidase and  $\alpha$ -amylase. The experiment was conducted according to the protocols as described above at a constant concentration of sample fraction (30  $\mu$ g mL<sup>-1</sup>) with a variable concentration of substrate. For the  $\alpha$ -glucosidase inhibition assay, 0.625–5 mmol L<sup>-1</sup> of pNPG was used and 0.125–1 % of starch was used for the  $\alpha$ -amylase inhibition assay. The initial rates of reactions were determined from calibration curves constructed using varying concentrations of *p*-nitrophenol and maltose for the  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition assays, respectively. The initial velocity data obtained were used to construct Lineweaver-Burke's plot to determine the  $K_M$  (Michaelis constant) and  $v_{max}$  (maximum velocity) of the enzyme as well as the  $K_i$  (inhibition binding constant as a measure of affinity of the inhibitor to the enzyme) and the type of inhibition for both enzymes.

#### *Gas chromatography-mass spectrometric (GC-MS) analysis*

The most active (butanol) fraction was further subjected to GC-MS analysis. The analysis was conducted with an Agilent Technologies 6890 GC coupled with an Agilent 5973

mass selective detector and driven by Agilent Chemstation software (Agilent Technologies, USA). A DB-5SIL MS capillary column was used (30 m  $\times$  0.25 mm i.d.,  $\times$  0.25  $\mu$ m film thickness). The carrier gas was ultra-pure helium at a flow rate of 0.7 mL min<sup>-1</sup> and a linear velocity of 37 cm s<sup>-1</sup>. The injector temperature was set at 250 °C. The initial oven temperature was 60 °C, which was programmed to 280 °C at the rate of 10 °C min<sup>-1</sup> with a hold time of 3 min. Injections of 2  $\mu$ L were made in the splitless mode with a manual split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 4 min and scan range 50–700 amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology (NIST) library.

### Statistical analysis

Data were analyzed using a statistical software package (SPSS for Windows, version 18, IBM Corporation, USA) using Tukey's-HSD multiple range *post-hoc* test. Values were considered significantly different at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The present study revealed that the butanol fraction of ethanolic extract of the root of *K. senegalensis* contained powerful antioxidative agents as well as inhibitors of  $\alpha$ -glucosidase and  $\alpha$ -amylase.

Ethanolic extracts were found to contain significantly ( $p < 0.05$ ) higher amounts of total phenolics than other extracts, with the highest amount being present in the ethanolic extract of the stem bark  $107.31 \pm 3.05$  mg g<sup>-1</sup> GAE (Table I). The antioxidative activities (using DPPH, HRS and NO models) of the extracts from the different parts of *K. senegalensis* are summarized in Table II. All the extracts from the plant scavenged DPPH radicals; however, the ethanolic extracts of the stem bark and root as well as the aqueous extract of the leaves displayed a statistically similar but significantly ( $p < 0.05$ ) higher DPPH radical scavenging activity than all other extracts from the plant. Further, all the extracts could scavenge hydroxyl radicals generated by Fenton's reaction except the ethyl acetate extracts of the stem bark and leaves, which showed pro-oxidative tendencies in this model. The ethyl acetate extract of the root showed the best HRS activity with an  $IC_{50}$  value of  $1.68 \pm 0.27$   $\mu$ g mL<sup>-1</sup>, followed by the ethanolic extracts from all the parts of the plant, which had higher HRS activity than the standard antioxidant Trolox. The highest NO scavenging activity was observed with the ethanolic extract of the leaves ( $IC_{50}$  value of  $6.92 \pm 1.42$   $\mu$ g mL<sup>-1</sup>) followed by the stem bark aqueous extract and ethanolic extract of the root, which had a statistically similar NO scavenging activity to that of ascorbic acid. Three experimental models for *in vitro* antioxidative studies were used because a single model cannot provide a full evaluation of the antioxidative capabilities of the different extracts tested due to the involvement of multiple mechanisms. Interestingly, analysis of the results obtained from the three complementary models for antioxidative studies revealed that the ethanolic extract of the root contained active constituents which could reasonably scavenge all the various forms of the reactive species tested. This further suggests that the components of

Table I. Percentage yield and total polyphenol content of fractions of *K. senegalensis* parts

Sample	Yield (%)	Total polyphenols (mg g <sup>-1</sup> GAE)
Stem bark		
Ethyl acetate	1.4	50.62 ± 0.08 <sup>c</sup>
Ethanollic	4.8	107.31 ± 3.05 <sup>§</sup>
Aqueous	3.5	59.64 ± 1.03 <sup>d</sup>
Root		
Ethyl acetate	1.0	13.70 ± 0.37 <sup>b</sup>
Ethanollic	2.9	97.76 ± 0.66 <sup>f</sup>
Aqueous	2.6	58.32 ± 1.76 <sup>d</sup>
Leaves		
Ethyl acetate	1.4	12.08 ± 0.12 <sup>a</sup>
Ethanollic	3.0	77.38 ± 1.27 <sup>e</sup>
Aqueous	3.8	14.08 ± 0.74 <sup>b</sup>

GAE – gallic acid equivalents. Data are presented as mean ± SD values of triplicate determinations.

<sup>a-§</sup> Different letters stand for significantly different values from each other within a column (Tukey's-HSD multiple range *post hoc* test,  $p < 0.05$ ); the same letters stand for non-significant difference.

this plant with high redox potentials are ethanol extractable. Guided by these activities, solvent fractionation of the ethanolic extract of the root was carried out.

Using solvent-solvent fractionation, the ethanolic extract of the root was fractionated to obtain a dichloromethane, ethyl acetate, butanol and aqueous fraction. The butanol fraction had a significantly ( $p < 0.05$ ) higher DPPH radical scavenging activity ( $IC_{50}$  value of  $3.11 \pm 0.77 \mu\text{g mL}^{-1}$ ) than the other fractions (Table III). However, the ethyl acetate and dichloromethane fractions had significantly higher ( $p < 0.05$ ) hydroxyl and NO radical scavenging activities than the other fractions (Table III). However, the overall results suggested that the butanol fraction possessed better antioxidant and scavenging activities than the other fractions because its antioxidant activity cuts across the experimental models used. This is indicative of the presence of powerful compounds that might have the ability to inhibit free radical upsurge as well as oxidative stress. These compounds, either in pure form or in combination, could be useful therapeutic agents for treating oxidative stress based metabolic disorders.

Fig. 1 shows the inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase by fractions of the ethanolic extract of the root. All the fractions inhibited  $\alpha$ -glucosidase and  $\alpha$ -amylase activities *in vitro* in a dose dependent pattern. The more polar (aqueous and butanol) fractions consistently maintained higher inhibitory activities against the enzymes than the less polar (ethyl acetate and dichloromethane) fractions. Furthermore, as shown by the  $IC_{50}$  values, the butanol fraction had a significantly ( $p < 0.05$ ) higher inhibitory activity than other frac-

Table II.  $IC_{50}$  values of various extracts of *K. senegalensis* parts in different antioxidative models

Sample	$IC_{50}$ ( $\mu\text{g mL}^{-1}$ )		
	DPPH	HRS	NO
Stem bark			
Ethyl acetate	7.15 $\pm$ 2.12 <sup>c</sup>	P	3205.41 $\pm$ 760.44 <sup>h</sup>
Ethanol	1.99 $\pm$ 0.87 <sup>a</sup>	2.08 $\pm$ 0.28 <sup>a</sup>	63.75 $\pm$ 4.81 <sup>c</sup>
Aqueous	195.01 $\pm$ 45.60 <sup>f</sup>	15.13 $\pm$ 1.25 <sup>d</sup>	28.50 $\pm$ 7.71 <sup>b</sup>
Root			
Ethyl acetate	48.20 $\pm$ 3.47 <sup>b</sup>	1.68 $\pm$ 0.27 <sup>a</sup>	95.32 $\pm$ 6.08 <sup>d</sup>
Ethanol	1.59 $\pm$ 0.64 <sup>a</sup>	2.18 $\pm$ 0.45 <sup>a</sup>	29.23 $\pm$ 1.87 <sup>b</sup>
Aqueous	22.71 $\pm$ 3.47 <sup>d</sup>	4.85 $\pm$ 0.61 <sup>c</sup>	192.70 $\pm$ 33.91 <sup>e</sup>
Leaves			
Ethyl acetate	44.67 $\pm$ 4.62 <sup>e</sup>	P	47.75 $\pm$ 5.92 <sup>c</sup>
Ethanol	3.25 $\pm$ 0.57 <sup>b</sup>	2.44 $\pm$ 0.53 <sup>a</sup>	6.92 $\pm$ 1.42 <sup>a</sup>
Aqueous	1.40 $\pm$ 0.22 <sup>a</sup>	59.31 $\pm$ 12.43 <sup>e</sup>	183.45 $\pm$ 15.18 <sup>e</sup>
Ascorbic acid	2.56 $\pm$ 0.26 <sup>ab</sup>	ND	26.40 $\pm$ 6.46 <sup>b</sup>
Gallic acid	1.40 $\pm$ 0.43 <sup>a</sup>	ND	1053.48 $\pm$ 239.92 <sup>g</sup>
Trolox	8.47 $\pm$ 2.88 <sup>c</sup>	3.23 $\pm$ 0.49 <sup>ab</sup>	629.76 $\pm$ 63.98 <sup>f</sup>

DPPH – 1,1-diphenyl-2-picrylhydrazyl radical, HRS – hydroxyl radical scavenging, NO – nitric oxide radical  
P – extract showed pro-oxidative properties in the experimental model.

ND – not determined. Data are presented as mean  $\pm$  SD values of triplicate determinations.

<sup>a-g</sup> Different letters stand for significantly different values from each other within a column (Tukey's-HSD multiple range *post hoc* test,  $p < 0.05$ ); the same letters stand for non-significant difference.

tions with  $IC_{50}$  values of  $2.89 \pm 0.46$  and  $97.51 \pm 5.72 \mu\text{g mL}^{-1}$  for  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition, respectively (Table IV). Thus, the strong inhibition of  $\alpha$ -glucosidase by the butanol fraction would slow down the breakdown of disaccharides to liberate glucose, thereby reducing glucose absorption from the small intestine (20). Furthermore, in spite of abundant reports on the  $\alpha$ -glucosidase inhibitory activities of extracts and pure compounds from plants from different parts of the world, only a few authors (21) reported such a low  $IC_{50}$  value as found with the butanol fraction in our study. This observation might be connected to the high phenolic content recorded in this fraction, because polyphenolic fractions from plants have been shown to inhibit  $\alpha$ -glucosidase activity, allowing for tighter control of blood glucose (22). Furthermore, Mai *et al.* (23) reported a strong positive correlation between the polyphenolic content and  $\alpha$ -glucosidase inhibitory effects of 28 extracts from Vietnamese edible plants, but also suggested that the enzymatic inhibition might depend on the type of polyphenolic. In the same vein, the butanol fraction had the



Table III.  $IC_{50}$  values of various fractions of the ethanolic extract of *K. senegalensis* root in different antioxidative models

Fraction	Total phenolics (mg g <sup>-1</sup> GAE)	$IC_{50}$ ( $\mu$ g mL <sup>-1</sup> )		$IC_{50}$ (mg mL <sup>-1</sup> )
		DPPH	HRS	NO
Aqueous	128.23 $\pm$ 1.73 <sup>b</sup>	27.71 $\pm$ 0.87 <sup>b</sup>	62.45 $\pm$ 0.72 <sup>c</sup>	6.92 $\pm$ 0.4 <sup>c</sup>
Butanol	201.24 $\pm$ 4.64 <sup>d</sup>	3.11 $\pm$ 0.77 <sup>a</sup>	41.20 $\pm$ 2.27 <sup>b</sup>	35.89 $\pm$ 3.24 <sup>d</sup>
Ethyl acetate	182.15 $\pm$ 2.78 <sup>c</sup>	3.78 $\pm$ 1.12 <sup>a</sup>	31.53 $\pm$ 4.22 <sup>a</sup>	3.58 $\pm$ 0.5 <sup>b</sup>
Dichloromethane	7.71 $\pm$ 3.30 <sup>a</sup>	91.00 $\pm$ 1.09 <sup>c</sup>	58.31 $\pm$ 4.74 <sup>c</sup>	104.84 $\pm$ 0.04 <sup>a</sup>

DPPH – 1,1-diphenyl-2-picrylhydrazyl radical, HRS – hydroxyl radical scavenging, NO – nitric oxide radical. Data are presented as mean  $\pm$  SD values of triplicate determinations.

<sup>a-d</sup> Different letters stand for significantly different values from each other within a column (Tukey's-HSD multiple range *post hoc* test,  $p < 0.05$ ); the same letters stand for non-significant difference.

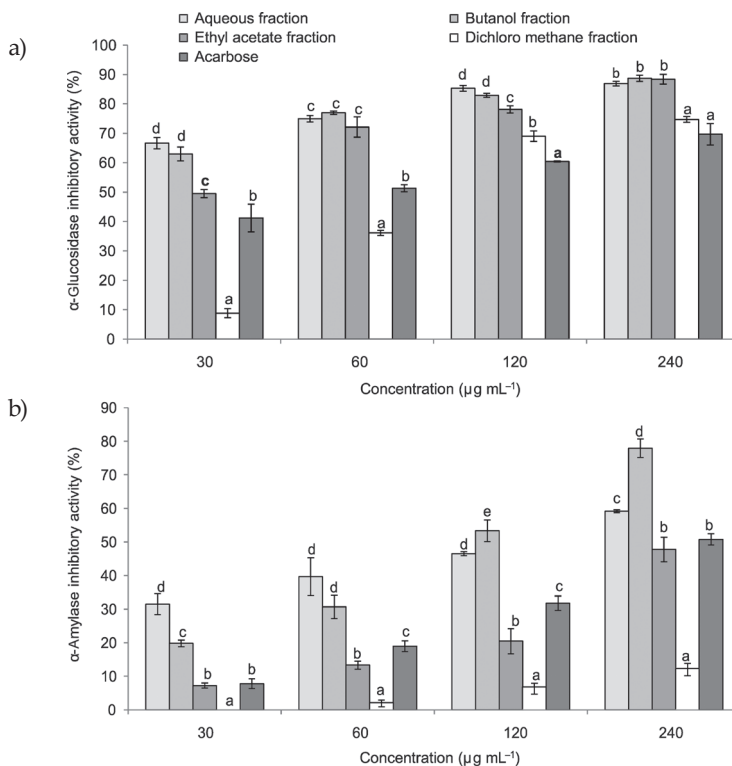


Fig. 1. a)  $\alpha$ -glucosidase and b)  $\alpha$ -amylase inhibitory activities of different solvent fractions of ethanolic extract of the *K. senegalensis* root. Data are presented as mean  $\pm$  SD of triplicate determinations. <sup>a-d</sup> Different letters over the bars for a given concentration for each extract indicate a significant difference from each other (Tukey's-HSD multiple range *post hoc* test,  $p < 0.05$ ).

Table IV.  $IC_{50}$  values for the inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase by various solvent fractions of the ethanolic extract of *K. senegalensis* root

Fractions/standard	$IC_{50}$ ( $\mu\text{g mL}^{-1}$ )	
	$\alpha$ -glucosidase	$\alpha$ -amylase
Aqueous	$5.15 \pm 1.10^b$	$130.23 \pm 12.90^b$
Butanol	$2.89 \pm 0.46^a$	$97.51 \pm 5.72^a$
Ethyl acetate	$23.89 \pm 0.70^c$	$394.97 \pm 13.46^d$
Dichloromethane	$91.23 \pm 2.17^e$	$154.64 \pm 6.67^{e*}$
Acarbose	$55.59 \pm 5.22^d$	$256.66 \pm 20.52^c$

Data are presented as mean  $\pm$  SD of triplicate determinations.

<sup>a-e</sup> Different letters stand for significantly different values from each other within a column (Tukey's-HSD multiple range *post hoc* test,  $p < 0.05$ ); the same letters stand for non-significant difference.

\*  $IC_{50}$  in  $\text{mg/mL}^{-1}$ .

Table V. Identified components of the butanol fraction of the *K. senegalensis* root ethanolic extract by GC-MS

Peak No.	Compound	$t_R$ (min)	$M_r$
1	7,8-dihydroxy-2,3-dihydrochromone	9.35	181.05
2	5,7-dihydroxychromone	12.53	179.03
3	<i>p</i> -anilinophenol hydrochloride	16.49	221.06
4	3-ethyl-5-(3-ethyl-(3 <i>H</i> )-benzothiazol-2-ylidene)-2-( <i>p</i> -tolylvinylamino)-4-thiazolidinone	22.76	423.14

highest  $\alpha$ -amylase inhibitory activity, which suggests the presence of more active compounds that could inhibit the breakdown of complex carbohydrates to oligosaccharides, thereby diminishing the effect of carbohydrate consumption on postprandial hyperglycemia. Moreover, the observed higher  $\alpha$ -glucosidase inhibitory activity of the butanol fraction than the corresponding  $\alpha$ -amylase activity is of great pharmaceutical interest. This is because some of the side effects associated with the currently available drugs for the management of T2D are linked with excessive inhibition of  $\alpha$ -amylase activity (6).

The enzyme kinetic studies revealed that the butanol fraction is a non-competitive inhibitor of  $\alpha$ -glucosidase with  $v_{\max}$  decreasing from 655.09 to 27.38  $\mu\text{mol min}^{-1}$ . The  $K_M$  remained unchanged at 2.00  $\text{mmol L}^{-1}$  and the computed  $K_i$  value for inhibiting  $\alpha$ -glucosidase was 1.30  $\mu\text{g mL}^{-1}$  (Fig. 2a). This observation could indicate that the fraction bound the  $\alpha$ -glucosidase at separate site(s) of the enzyme (rather than the active site) but caused conformational modification at the active site, thereby preventing effective binding of the pNPG and consequently reducing the  $\alpha$ -glucosidase activity. It further suggests that the fraction contains some components capable of interacting with the  $\alpha$ -glucosidase-pNPG complex (24). Conversely, the mechanism of inhibition of  $\alpha$ -amylase by the butanol fraction

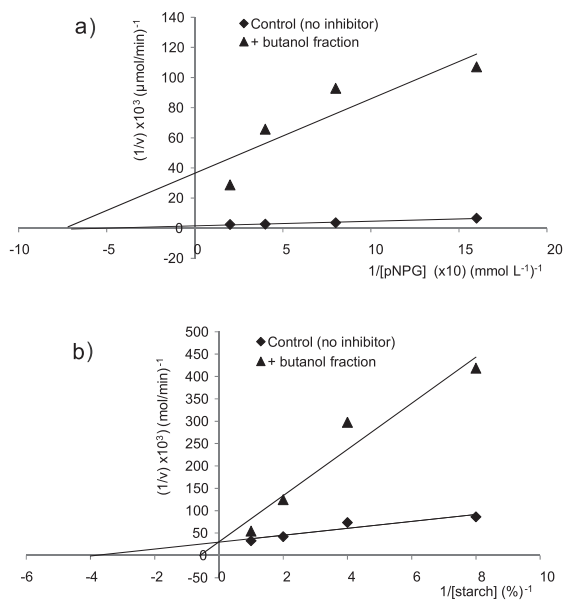


Fig. 2. Lineweaver-Burke's plot of a)  $\alpha$ -glucosidase and b)  $\alpha$ -amylase catalyzed reactions in the presence and in the absence of the butanol fraction derived from the *K. senegalensis* root ethanolic extract.

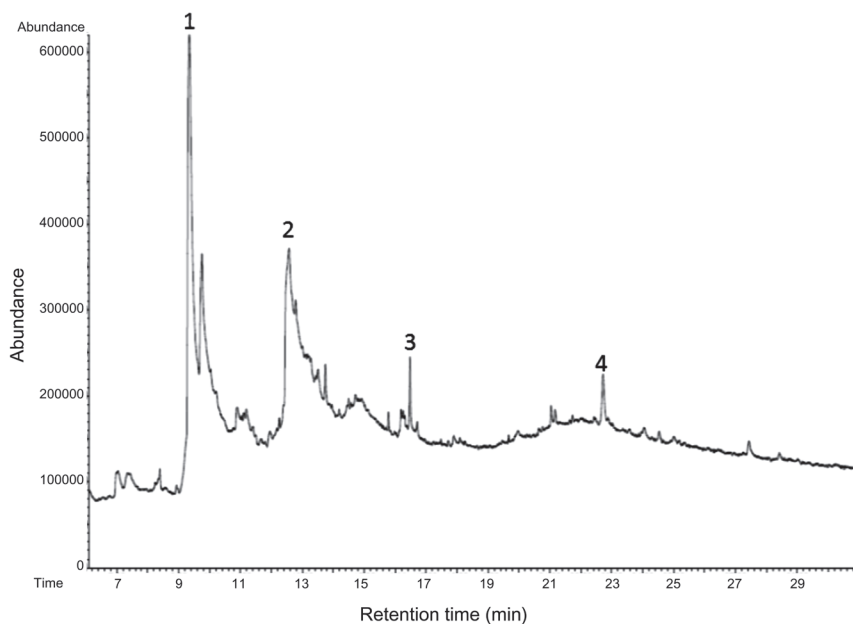


Fig. 3. GC-MS chromatogram of the butanol fraction of the *K. senegalensis* root ethanolic extract.

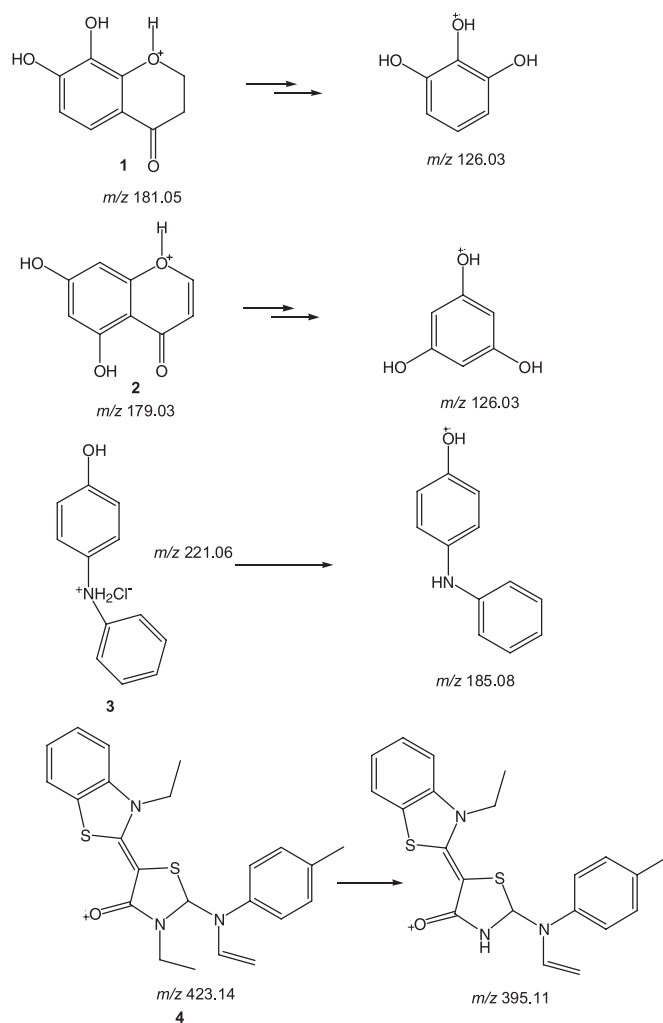


Fig. 4. Structures of components identified by GC-MS.

revealed a competitive inhibition pattern with a  $K_M$  value of 1.25 and 0.25 % in the presence and absence of the inhibitor, respectively. The  $v_{max}$  of the  $\alpha$ -amylase was not affected ( $33.70 \mu\text{mol min}^{-1}$ ) and  $K_i$  for inhibiting  $\alpha$ -amylase by the fraction was  $7.50 \mu\text{g mL}^{-1}$  (Fig. 2b). Thus, this pattern of  $\alpha$ -amylase inhibition by the fraction suggests that the active site of the enzyme is directly involved in the inhibitory action and the fraction most likely contains some compounds that could serve as substrate analogues, thereby competing for the active site of  $\alpha$ -amylase (25).

The GC-MS chromatogram of the butanol fraction is presented in Fig. 3. The major peaks detected in the chromatogram of the butanol fraction were those of the chromones

7,8-dihydroxy-2,3-dihydrochromone (peak 1), 5,7-dihydroxychromone (peak 2), *p*-anilinophenol hydrochloride (peak 3) and 3-ethyl-5-(3-ethyl-(3*H*)-benzothiazol-2-ylidene)-2-(*p*-tolylvinylamino)-4-thiazolidinone (peak 4). They were identified by their fragmentation pattern and in conjunction with the NIST library (Fig. 4). The retention time and molecular mass of the detected components are provided in Table V. Phenolics are very important constituents among the secondary metabolites of plants because of their multiple biological effects and direct contribution to antioxidative and  $\alpha$ -glucosidase inhibitory activities (23). While not discounting the possible contributions of the other detected phytochemicals, it is possible to suggest that the bioactive agents of the butanol fraction contain phenolic groups such as those present in the two chromones 7,8-dihydroxy-2,3-dihydrochromone and 5,7-dihydroxychromone identified by GC-MS.

## CONCLUSIONS

The results of this study suggests that the butanol fraction derived from the ethanolic extract of *K. senegalensis* root contains the most potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory agents and that the inhibitions were mediated *via* non-competitive and competitive patterns for  $\alpha$ -glucosidase and  $\alpha$ -amylase, respectively. Further, the chromones and/or *p*-anilinophenol could be bioactive agents and hence they can be investigated as novel anti-diabetic therapeutics.

*Acknowledgements.* – This study was supported by the Competitive Research Grant from the Research Office, University of KwaZulu-Natal (UKZN), Durban; an Incentive Grant for Rated Researchers and a Grant Support for Women and Young researchers from the National Research Foundation (NRF), Pretoria, South Africa. The first author was awarded a PhD study scholarship by the Ahmadu Bello University, Zaria, Nigeria, and also received a doctoral research grant from the Research Office, University of KwaZulu-Natal (Westville Campus), Durban, South Africa.

## REFERENCES

1. International Diabetes Federation: *Diabetes Atlas 2011*, 5<sup>th</sup> ed., International Diabetes Federation, Brussels 2011.
2. K. Srinivasan, B. Viswanad, A. Lydia, C. L. Kaul and P. Ramarao, Combination of high-fat diet-fed and low-dose streptozotocin treated rat: A model for type 2 diabetes and pharmacological screening, *Pharmacol. Res.* **52** (2005) 313–320; DOI: 10.1016/j.phrs.2005.05.004.
3. D. Quilliot, E. Walter, J. P. Bonte, J. C. Fruchart, P. Duriez and O. Ziegler, Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis, *Am. J. Clin. Nutr.* **81** (2005) 1111–1117.
4. N. F. Wiernsperger, Oxidative stress as a therapeutic target in diabetes: revisiting the controversy, *Diabetes Metab.* **29** (2003) 579–585; DOI: 10.1016/S1262-3636(07)70072-1.
5. A. Ceriello, Oxidative stress and diabetes-associated complications, *Endocr. Pract.* **1** (2006) 60–62.
6. A. O. Ademiluyi and G. Oboh, Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*, *Exp. Tox. Pathol.* **65** (2013) 305–309; DOI: 10.1016/j.etp.2011.09.005.
7. Y. Kawamura-Konishi, N. Watanabe, M. Saito, N. Nakajima, T. Sakaki, T. Katayama and T. Enomoto, Isolation of a new phlorotannin, a potent inhibitor of carbohydrate-hydrolyzing enzymes, from the brown algae *Sargassum patens*, *J. Agric. Food Chem.* **60** (2012) 5565–5570; DOI: 10.1021/jf300165j.

8. T. Fujisawa, H. Ikegami, K. Inoue, Y. Kawabata and T. Ogihara, Effect of two  $\alpha$ -glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms, *Metabolism* **54** (2005) 387–390; DOI: 10.1016/j.metabol.2004.10.004.
9. D. Neuser, A. Benson, A. Bruckner, R. B. Goldberg, B. J. Hoogwerf and D. Petzinna, Safety and tolerability of acarbose in the treatment of type 1 and type 2 diabetes mellitus, *Clin. Drug Invest.* **25** (2005) 579–587; DOI: 10.2165/00044011-200525090-00003.
10. W. Benalla, S. Bellahcen and M. Bnouham, Antidiabetic medicinal plants as a source of  $\alpha$ -glucosidase inhibitors, *Curr. Diabetes Rev.* **6** (2010) 247–254; DOI: 10.2174/157339910791658826.
11. E. U. Etuk, S. O. Bello, S. A. Isezuo and B. J. Mohammed, Ethnobotanical survey of medicinal plants used for the treatment of diabetes mellitus in the north western region of Nigeria, *Asian J. Exp. Biol. Sci.* **1** (2010) 55–59.
12. T. van Andel, B. Myren and S. van Onselen, Ghana's herbal market, *J. Ethnopharmacol.* **140** (2012) 368–378; DOI: 10.1016/j.jep.2012.01.028.
13. O. T. Kolawole, S. O. Kolawole, A. A. Ayankunle and O. I. Olaniran, Anti-hyperglycemic effect of *Khaya senegalensis* stem bark aqueous extract in Wistar rats, *Eur. J. Med. Plants* **2** (2012) 66–73.
14. I. Funke and M. F. Melzig, Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of  $\alpha$ -amylase activity, *Rev. Bras. Farmacogn.* **16** (2006) 1–5.
15. S. McDonald, P. D. Prezier, M. Autokiwich and K. Robards, Phenolics content and antioxidant activity of olive oil extracts, *Food Chem.* **73** (2001) 73–84; DOI: 10.1016/S0308-8146(00)00288-0.
16. A. K. Tuba and I. Gulcin, Antioxidant and radical scavenging properties of curcumin, *Chemico-Biol. Inter.* **174** (2008) 27–37; DOI: 10.1016/j.cbi.2008.05.003.
17. I. Hinneburg, H. J. Damien and R. Hiltunen, Antioxidant activities of extracts from selected culinary herbs and spices, *Food Chem.* **97** (2006) 122–129; DOI: 10.1016/j.foodchem.2005.03.028.
18. A. G. Kurian, S. Suryanarayanan, A. Raman and J. Pidakalla, Antioxidant effects of ethyl acetate extract of *Desmodium gangeticum* root on myocardial ischemia reperfusion injury in rat hearts, *Chin. Med.* **5** (2010) 1–7; DOI: 10.1186/1749-8546-3.
19. L. J. Shai, P. Masoko, M. P. Mokgotho, S. R. Magano, A. M. Mogale, N. Boaduo and J. N. Eloff, Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa, *South Afr. J. Bot.* **76** (2010) 465–470; DOI: 10.1016/j.sajb.2010.03.002.
20. Y. I. Kwon, E. Apostolidis, Y. C. Kim and K. Shetty, Health benefits of traditional corn, beans and pumpkin: *in vitro* studies for hyperglycemia and hypertension management, *J. Med. Food.* **10** (2007) 266–275; DOI: 10.1089/jmf.2006.234.
21. W. Kang, Y. Song and X. Gu,  $\alpha$ -glucosidase inhibitory *in vitro* and antidiabetic activity *in vivo* of *Osmanthus fragrans*, *J. Med. Plants Res.* **6** (2012) 2850–2856; DOI: 10.5897/JMPR11.1402.
22. O. Kamiyama, F. Sanae, K. Ikeda, Y. Higashi, Y. Minami, N. Asano, I. Adachi and A. Kato, *In vitro* inhibition of  $\alpha$ -glucosidases and glycogen phosphorylase by catechin gallates in green tea, *Food Chem.* **122** (2010) 1061–1066; DOI: 10.1016/j.foodchem.2010.03.075.
23. T. T. Mai, N. N. Thu, P. G. Tien and N. V. Chuyen, Alpha-glucosidase and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents, *J. Nutr. Sci. Vitaminol.* **53** (2007) 267–276; DOI: 10.3177/jnsv.53.267.
24. M. A. Ibrahim, A. B. Aliyu, A. Abusufiyanu, M. Bashir and A. B. Sallau, Inhibition of *Naja nigricolis* (Reinhardt) venom protease activity by *Luffa egypitiaca* (Mill) and *Nicotiana rustica* (Linn) extracts, *Indian J. Exp. Biol.* **49** (2011) 552–554.
25. A. B. Sallau, G. C. Njoku, A. R. Olabisi, A. U. Wurochekke, A. A. Abdulkadir, S. Isah, M. S. Abubakar and S. Ibrahim, Effect of *Guiera senegalensis* leaf extract on some *Echis carinatum* venom enzymes, *J. Med. Sci.* **5** (2005) 280–283.