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Khaya Senegalensis Inhibitory Activity Against α-Glucosidase

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α-glucosidase is an enzyme responsible for the hydrolysis of oligosaccharides to simple absorbable sugars. The inhibition of the enzyme is implicated in the management of postprandial hyperglycaemia. The current study evaluates the α-glucosidase inhibitory potentials of *Khaya senegalensis* stem bark. The result of an *in vitro* analysis using α-glucosidase from *Saccharomyces cerevisiae* and *p*-nitrophenyl α-D-glucopyranoside as substrate showed that the stem bark extract of *K. senegalensis* (IC_{50} = 7.7 ± 0.02 µg /ml), had a significant inhibitory effect on α-glucosidase in a concentration dependant manner. From the study of the enzyme kinetics, the stem bark extract of *K. senegalensis* exhibited a non-competitive inhibition with a *Ki* of 0.33µg/ml. In an *in vivo* study, using Wistar albino rats, the extract significantly attenuated postprandial hyperglycaemia of area under glucose curve ($AUC_{Glucose}$) following maltose administration. Two hitherto unknown bioactive compounds with α-glucosidase inhibitory activity were obtained from the methanol partition of the aqueous extract. The study concludes that the blood glucose-lowering effect of *K. senegalensis* is partly mediated by inhibiting the α-glucosidase activity in the intestine.

Keywords: *Khaya senegalensis,* Meliaceae; α-glucosidase inhibition; Postprandial hyperglycaemia.

1. Introduction

Type 2 diabetes is one of the major public health challenges of the 21st century and comprises 90% of people with diabetes around the world (Shaw *et al.,* 2010). The prevalence of Type 2 diabetes is on the increase and literally, it can be said that most developing countries are also facing a diabetic explosion. It is estimated that African countries will experience the highest prevalent rate of diabetes in the next few decades (Shaw *et al.,* 2010). This implies that an appropriate, effective and urgent action(s) is needed in the control of the disease spectrum particularly in the African countries. One of the therapeutic approaches in Type 2 diabetes is to lower the corresponding postprandial hyperglycemia (Scheen, 2003). Postprandial hyperglycemia plays an important role in the development of type 2 diabetes, and complications associated with these diseases have been proposed as an independent risk factor for cardiovascular disease (Bonora and Muggeo, 2001). The control of hyperglycemia is, therefore of great benefit to reduce the progression of the disease. This can be achieved by inhibiting *α*-glucosidase located in the small intestine's brush border, which is required to break down carbohydrates into absorbable monosaccharides (Varsha*et al.,* 2011). The inhibitors of this enzyme are among the available glucose-lowering medications in the market, for the treatment of postprandial hyperglycemia (Casirola and Ferraris, 2006). However, their safety and tolerability is questionable (Johnston *et al.,* 1998). Other oral hypoglycemic medications available for the treatment of Type 2 diabetes include sulphonylureas, biguanides, thiazolidinediones etc. Their usage has also exhibited several undesired side effects (Selvin *et al.*, 2008), thus, suggesting other effective alternatives.

Plants are natural reservoirs of untapped sources of lead compounds for drug discovery (Newman and Cragg (2016). The extracts of these plants have been used effectively by traditional practitioners to treat several diseases for several hundreds of years and are thought to be almost free from disconcerting side effects (Bhutani and Gohil 2010). So, speaking, plants used in folklore medicine have been a guide in the isolation of present-day drugs.

Khaya senegalensis (Desr) A. Juss., a dry zone mahogany belonging to the family; Meliaceae, has been highly reputed for its medicinal uses for the treatment of hyperglycaemia, malaria, diarrhoea, and fever (Gill, 1992; Olayinka *et al.*, 1992; Iwu, 1993). It has been reported that the stem bark contains scopoletin, scoparone, limonoid, bitter principle, tannins, saponins and sterols (Gbile,

1986). Limonoids with growth inhibitory properties (El-Aswad *et al*., 2004), anticancer activity (Huaping et al., 2007), anti-sickling activity (Fall *et al*., 1999) and anti-feedant (Abdelgaleil *et al*., 2001; Abdelgaleil, and Nakatani 2003) have been isolated from different part of the plant. The flavonoids found in *K. senegalensis* are responsible for immune-stimulating activity (Kayser and Abreu, 2001). Its extracts, phytochemical contents and pharmacological importance have been the subject of extensive research for the past fifty years. However, there is a dearth of information on the Pharmacological action of *K. senegalensis* stems bark, as an *α*-glucosidase inhibitor. The current study reports the *in vitro* effects of methanol stem bark extract of *K. senegalensis* on the activity of α-glucosidase. The work studied the in vitro α-glucosidase inhibitory effect of the extract by isolating bioactive compounds through fractionation and also in vivo effect of the extract on postprandial blood glucose levels in alloxan-induced diabetic rats following maltose administration.

2. General Experimental Procedures

The study was carried out in the Department of Biochemistry, Usmanu Danfodiyo
University, Sokoto (UDUS), Nigeria, between the months of September and December 2011.

2.1 *Chemicals and reagents*

All chemicals and reagent used for this study were of analytical grade. Methanol was purchased from BDH chemicals, Potassium Phosphate monobasic (P5379), Sodium carbonate (S2127), *Saccharomyces cerevisiae*α-glucosidase (G0660), 4-nitrophenyl α-D-glucopyranoside (N1377), arcabose (A8980), L-Glutathione (G4251) and Alloxanmonohydrate (A7413), was purchased from Sigma Aldrich Germany. ACUU CHECK Advantage was purchased from Roche Diagnostic Indianapolis, USA.

2.2 *Plant materials*

The stem bark of *K. senegalensis* (UDUS/VS/11/21) was collected in and around the Sokoto metropolis (130 21’ 16” N and 50 5’ 37” E) in June. The plants were authenticated by a taxonomist; Dr Mohammed Lawal of the botanical unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (UDUS), Nigeria.

2.3 *Preparation of plant extracts*

K. senegalensis stem bark was washed and air-dried at room temperature until a constant weight was obtained. The samples were ground using pestle and mortar and the powdered sample was then used for the extraction. 300 g of plant samples were separately placed in an Erlenmeyer flask and one litre of methanol was added into the flask and allowed to soak for a minimum of 24 hours. The mixture was filtered using a piece of clean, sterile muslin cloth to remove debris and then filtered on Whatman No.1 filter paper. The resultant filtrate was evaporated to dryness at room temperature (Harbone, 1973). The dry weight of the extract was reconstituted in distilled water to obtain the needed concentration (mg/l) for the study.

2.4 *Assay of K. Senegalensis for α-glucosidase inhibitory activity and determination of the IC_{50} value*

The spectrophotometric Stop Rate Determination was employed. Here, α-glucosidase inhibition was determined by the method of Adams *et al*. (2010) with modifications. The reaction mixture contained 5 ml, 67 mM potassium Phosphate Buffer, pH 6.8, 0.2 ml 3 mM Glutathione (GSH) and 0.2 ml α-glucosidase (0.15 U/ml) from *Saccharomyces cerevisiae.* The mixture was equilibrated to 37^0C for 5min. The reaction mixture was activated by the addition of 0.5 ml 10 mM *p*-nitrophenyl-α-glucoside in the absence or presence of an increasing concentration of methanol partition of *K. senegalensis* (01.25 – 50 µg/ml) for 20 minutes at 37^0C. In a test tube containing 8 ml, 100 mM Na_2CO_3 was added to 2 ml of the reaction mixture to terminate the reaction. The enzyme activity was monitored by taking the spectrophotometric absorbance of *p*-nitrophenol at 400 nm using optima sp-300 spectrophotometer. One unit of α-glucosidase was taken as the amount of enzyme liberating 1.0 µmol of *p*-nitrophenyl from *p*-nitrophenyl-α-glucoside per minute at pH 6.8 and 37^0C. The concentration of *K. senegalensis* that caused 50% inhibition (IC_{50} value) of α-glucosidase activity was determined through a nonlinear regression analysis of the dose response curve.

2.5 *Bioassay guided fractionation of K. senegalensis extract*

The method of Abdelgaleil *et al*., (2001) with modification was employed. A portion of the methanol extract was partitioned with a separating funnel apparatus using equal volumes of different organic solvents, successively starting with hexane, ethyl acetate, and then methanol. All partitions were evaporated to dryness and were assayed for α-glucosidase inhibitory activity. The methanol partition was subjected to column chromatography on silica gel (mesh size 60 – 120). The column was eluted in a gradient manner, using an increasing solvent polarity system, starting from hexane, hexane-ethyl acetate mixture, ethyl acetate, ethyl acetate-methanol mixture and finally methanol. The concentration of the combined solvents used was prepared in a stepwise manner with 5% difference between both solvents. Fractions that indicated the presence of a single spot-on TLC were tested for inhibitory activity, fractions that indicated no spots on TLC were discarded, while fractions with similar TLC profiles having more than one spot were combined and also tested for inhibitory activity. The

95:5 ethyl acetate-methanol from the first column was pooled, dried and subjected to fresh column chromatography on silica gel (mesh size 32 – 63) and then eluted with chloroform, methanol and water. The elution was conducted in an increasing polarity solvent system following the same procedure as described above in a stepwise manner by 50%. The fractions eluted resulted in 5 fractions (F3.1 - F3.5). The fraction F3.2 was further separated using Droplet Counter Current Chromatography (DCCC). In a descending mode, droplets of a denser mobile phase gotten from a previous mixture of ethyl acetate, methanol and water (5:1:5) with sample are allowed to fall through a column of the lighter stationary phase using only gravity. Two fractions were obtained, and the fraction with the highest activity was further subjected to DCCC adjusting the solvent mixture of ethyl acetate, methanol and water step-wisely to 5:2:4, and 5:3:3. A reddish crystals and a white amorphous compound, both having α-glucosidase inhibitory activity were obtained. The melting point, ultraviolent/visible as well as infra-red spectroscopy were further determined.

2.6 *Kinetic analysis of α-glucosidase inhibition and determination of inhibition constant (Ki)*

The mechanism of inhibition of α-glucosidase by the methanol extract of *K. senegalensis* was measured in the presence of different concentrations of extract (0 µg/ml, 2 µg/ml and 4 µg/ml) with increasing concentrations (2.5 mM, 5 mM and 10 mM) of 4-nitrophenyl-α-d-glucopyranoside (4-NPGP). The type of inhibition exhibited by the active extract as well as the inhibition constant (*Ki*) were analysed using Line weaver-Burk plots.

2.7 *Experimental animals*

Albino Wistar rats weighing 150-200 g were purchased from the farmhouse Usmanu Danfodiyo University, Sokoto, Nigeria, and housed in metal cages. The rats were allowed free access to a standard laboratory pellet diet (ECWA Feed Nig Ltd) and water *ad libitum* under a 12 hours light: dark cycle throughout the experimental period.

2.8 *Induction of experimental diabetes in rats*

Wistar albino rats were injected with alloxan monohydrate dissolved in normal saline at a dose of 80 mg/kg body weight per day intraperitoneally for 3 consecutive days (Saidu *et al*., 2007). The fasting blood glucose was checked from tail venule blood 7 days after administration of the last dose of alloxan, and the rats that had blood glucose levels of 9 -11 mmol/L were considered diabetic and employed for the experiment.

2.9 *Experimental protocol*

Effect of *K. senegalensis* on blood glucose levels following maltose loading in diabetic rats was examined. The rats were randomly divided into five equal groups (five rats in each group). Group 1 (control group) were treated with 3 mg/kg body weight of maltose without the extract, by oral intubation. Group 2 were treated with 3 mg/kg body weight of maltose with 3 mg/kg body weight of acarbose by oral intubation. Groups 3, 4 and 5 were subjected to the same treatment of 3 mg/kg body weight of maltose with corresponding 200,400 and 800 mg/kg body weight of the extract respectively by oral intubation. The extract used was suspended in normal saline and all groups were administered their respective doses by oral intubation 3-5 mins before the administration of the 3 mg/kg of maltose. Blood samples were collected for the measurement of glucose concentration using an electronic glucometer at 0 min before the oral administration of the extract, and then at 30, 60, 90 and 120 min after the tested materials have been administered. The area under the curve ($AUC_{Glucose}$) of extract-treated groups was compared with that of the vehicle-treated (control) group and the percentage of anti-hyperglycemic activity was determined as follows.

Anti-hyperglycemic activity (% Attenuation) =

$$\frac{\text{AUC of control group} - \text{AUC of extract treated group}}{\text{AUC of control group}} \times 100$$

2.10 *Data analysis*

Data are expressed as mean ± standard deviation for the given number of observations. The IC_{50} value was determined using a nonlinear regression analysis of the dose-response curve. The result was analysed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using Graph Pad Instat software. Differences were considered significant when $p < 0.05$.

3. Results

3.1 *Inhibitory activity of K. senegalensis on α-glucosidase and determination of the IC_{50} value*

Fig. 1 shows the result of the inhibitory activity of the methanol fraction of *K. senegalensis*. The extract exhibited a concentration dependent inhibitory effect against the activity of α-glucosidase from *Saccharomyces cerevisiae.* The optimal concentrations of *K. senegalensis* required for the 50% inhibition (IC_{50}) against the enzyme was 7.7 ± 0.02 µg/ml.

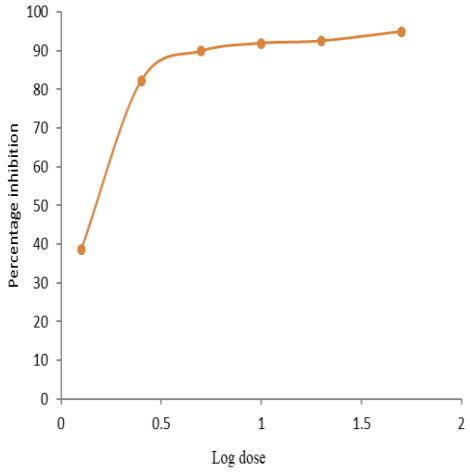

Figure. 1. Log dose-response and IC_{50} of stem bark extract of *K. senegalensis* on S. cerevisiaeα-glucosidase activity.

3.2 *Nature of the inhibition of the stem bark extract of K. senegalensis on α-glucosidase.*

The inhibitory mechanism and inhibition constant (*Ki*) of *K. senegalensis* against α-glucosidase are presented in Figs. 2A and 2B respectively. Analysis of the Lineweaver-Burk plot revealed that the extract of *K. senegalensis* possesses a non-competitive type of inhibition against α-glucosidase (Fig. 2A). This indicates that the inhibitors bind at a different site other than the catalytic site of the enzyme. The values of Vmax and Km were determined from the Linweaver-Burlk plot. The result (Fig. 2A) showed that all the concentrations (0, 0.2 and 0.4 µg/ml) of *K. senegalensis* had the same Km (1.1 mM) values, implying that, the inhibitor does not affect substrate binding; the inhibitor can bind to either the free enzyme or the enzyme-substrate complex at the same rate. A corresponding decrease in Vmax as the concentration of the inhibitor increases was also observed. The secondary plot of the Lineweaver-Burk plots in Fig. 2B evaluates the inhibition constant (K*i*) of the enzyme system to be 0.33 µg/ml, it shows that the K*i* (0.33 µg/ml) is the inhibitor concentration that halves the Vmax of the enzyme.

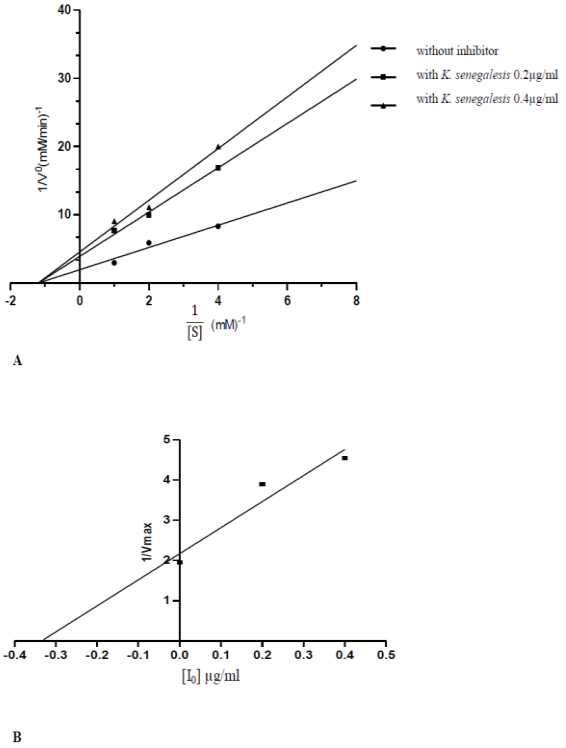

Figure 2. Primary (A) and Secondary (B) plot of Lineweaver-Burk plots of the activity of α-glucosidase in the presence of different concentrations (0, 0.2 and 0.4 µg/ml) of K. senegalensis.

3.3 *α-glucosidase inhibitory effect of stem bark sub-fractions.*

Percentage inhibitory effects of the stem bark sub-fractions of *K. senegalensis* on α-glucosidase activity are presented in Table 1. The aqueous extract of *K. senegalensis* was partitioned into three partitions (F1.1 - F1.3), then fractionated twice (F2.1 – F2.18 and F3.1 – F3.5) and then divided into six fractions using Droplet Counter Current Chromatography to yield two relatively pure compounds: a white amorphous compound (**F5.1**; 4mg) and a brownish crystal (**F6.2**; 18mg) both soluble in polar solvents and having α-glucosidase inhibitory activity.

UV/vis (methanol) spectra λ_{max} nm (abs) showed

Table 1.0: Extraction log of *K. senegalensis* and α-glucosidase inhibitory activity at 10 µg/ml of its corresponding fractions

FRACTIONS	SOLVENT	FRACTION	WEIGHT RECOVERED (mg)	α-GLUCOSIDASE INHIBITION (%)
Partition 1	Hexane	F1.1	1500	0
Partition 2	Ethylacetate	F1.2	5300	7
Partition 3	Methanol	F1.3*	16500	18
Rinses	Hexane	200ml	Discarded	XXX
15 -30	Hexane	F2.1	Not measured	XXX
36 – 45	Hexane	F2.2	24	0
49 – 57	Hexane : Ethylacetate (90 : 10)	F2.3	78	13
66 – 81	Hexane : Ethylacetate (85 : 15)	F2.4	36	Active
98 – 109	Hexane : Ethylacetate (60 : 40)	F2.5	101	0
121 – 126	Hexane : Ethylacetate (15 : 85)	F2.6	26	21
143 -148	Ethylacetate	F2.7	122	14
151 -169	Ethylacetate	F2.8	1200	0
173 – 179	Ethylacetate	F2.9	81	4
180 – 187	Ethylacetate	F2.10	97	Not tested
203 -219	Ethylacetate : Methanol (95 : 5)	F2.11*	1100	41
220 – 225	Ethylacetate : Methanol (80 : 20)	F2.12	53	9
230 – 245	Ethylacetate : Methanol (75 : 25)	F2.13	18	8
246 – 259	Ethylacetate : Methanol (75 : 25)	F2.14	Not measured	XXX
260 – 266	Ethylacetate : Methanol (50 : 50)	F2.15	58	23
269 – 281	Ethylacetate : Methanol (45 : 55)	F2.16	79	0
282 – 288	Ethylacetate : Methanol (10 : 90)	F2.17	44	28
294 – 220	Methanol	F2.18	180	Not tested
221 – 233	Chloroform	F3.1	318	0
239 – 241	Chloroform : Methanol (50 : 50)	F3.2*	242	64
243 – 249	Methanol	F3.3	19	13
252 – 261	Methanol : Water (50 : 50)	F3.4	74	31
262 – 287	Water	F3.5	36	Active
DCCC less dense 1.1	Ethylacetate : Methanol : Water (5 : 1 : 5)	F4.1	61	14
DCCC dense 1.2	Ethylacetate : Methanol : Water (5 : 1 : 5)	F4.2*	20	71
DCCC less dense 2.1	Ethylacetate : Methanol : Water (5 : 2 : 3)	F5.1#	4	63
DCCC dense 2.2	Ethylacetate : Methanol : Water (5 : 2 : 3)	F5.2*	8	74
DCCC less dense 3.1	Ethylacetate : Methanol : Water (5 : 3 : 3)	F6.1	9	6
DCCC dense 3.2	Ethylacetate : Methanol : Water (5 : 3 : 3)	F6.2*#	18	88

Fractions with * as superscript indicates that the fraction has the highest inhibitory activity in that set of subfraction. They were further fractionated if determined not to be pure, while fractions with # as superscript indicates that the fraction are relatively pure being determined with TLC. Fractions that were unable to dissolve in suitable solvent were not pursued hence reported as 'Not tested'. Some fractions were below measurable limit hence were 'Not measured'. Fractions that were sparingly soluble as such the exact concentrations were unable to be determined but had inhibitory activity were reported as 'Active'.

3.4 Description *of bioactive fractions.*

Fraction (**F5.1)** is a white amorphous compound with a melting point of 273 - 280^{0}C, that fraction (F5.1) had maximum intensity at 211nm (2.5) and 273 nm (0.6). IR (ethanol) V_{max} spectra showed maximum absorption at 3248, 2856, 2582, 2333, 1447 and 1018 cm^{-1}

Fraction (**F6.2)** is a brownish crystal with a melting point of 105 -107^{0}C, UV/vis (methanol) spectra λ_{max} nm (abs) showed that fraction (F6.2) had maximum intensity between the range of 200 – 260 nm (4.0). IR (ethanol) V_{max} spectra showed maximum absorption at 3254, 2956, 2341, 1443, 1190, and 623 cm^{-1}

3.5 *In vivo effects of K. senegalensis on postprandial blood glucose level*

The *in vivo* effects of the *K. Senegalensis* aqueous extract and Acarbose on the postprandial blood glucose level following the oral administration of maltose in alloxan-induced diabetic rats is shown in Fig. 3. The result indicates that following treatment with all the experimental doses (200, 400 and 800 mg/kg bw) of *K. senegalensis* aqueous extract as well as 3 mg/kg bw of acarbose; the postprandial blood glucose level of the experimental groups significantly ($P < 0.01$) prevented postprandial blood glucose level from increasing at 60, 90 and 120 min as compared to the levels observed at the corresponding times in the diabetic untreated group (control group). Meanwhile, there was no significant ($P > 0.05$) difference in postprandial blood glucose level in all the treated groups 30 min after oral administration of maltose, as compared to the normal control.

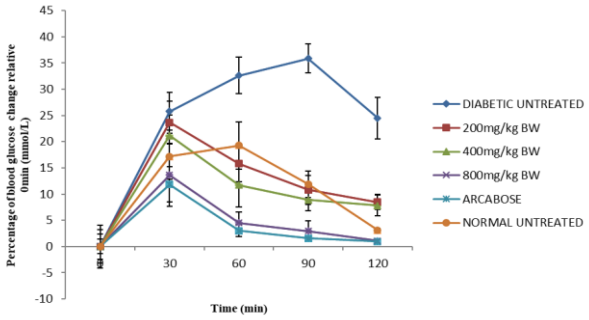

Figure 3. *In vivo* effect of *K. senegalensis* extract and Acarbose on the blood glucose levels
following the administration of maltose to alloxan-induced diabetic rats. The data are expressed as Mean ± SD, n=5. The level of significance was set at $p<0.05$, relative to diabetic untreated and normal untreated rats.

The area under the glucose curve ($AUC_{glucose}$) (Table 2.0) for each individual rat of the control and treated groups were deduced from Fig 3. The 200, 400, 800 mg/kg body weight extract and acarbose (3 mg/kg bw) attenuated the area under the glucose curve ($AUC_{glucose}$) significantly ($p < 0.01$) by 48.79, 57.09, 79.72 and 83.13% respectively, compared to the diabetic untreated group (control).

Table 2.0: The Effect Of K.Senegalensis Aqueous Extract On The Area Under Glucose Curve following oral administration of maltose

Treatent Group	($AUC_{Glucose}$)	% Attenuation
DIABETIC UNTREATED	3195 ± 86.33	0
200mg/kg BW	1636 ± 49.04	48.79[a]
400mg/kg BW	1371 ± 53.72	57.09[a]
800mg/kg BW	648 ± 13.11	79.72[a]
ARCABOSE	507 ± 18.00	84.13[a]
NORMAL UNTREATED	1498 ± 66.4	53.11[a]

Values are expressed as Mean ± SD; n= 5. The level of significance was set at aP< 0.05 when compared with the diabetic untreated dose group (control at 0mg/kg body weight) Donnette's multiple comparison test.

4. Discussion

Decoctions of *K. senegalensis* stem bark have been used in West African traditional medicine for centuries without overt toxicity (Xiao, 2006) and the plant has been widely exploited for its varied medicinal properties (Gill, 1992). Interestingly, *K. senegalensis* extract has been the subject of extensive phytochemical and pharmacological investigations for over 50 decades (Iwu, 1993; Olayinka *et al*., 1994; Kayser and Abreu 2001). Existing studies have shown that more than 45 liminoids have being extracted from *K. senegalensis* and many more novel compounds are being isolated from this plant (Zhang *et al.,* 2007a, b). Ironically, there is no extensive scientific report on the possible mode of action of this plant as an anti-diabetic agent; none of the isolated compounds have been screened as an inhibitor of α-glucosidase activity and no bioactivity guided fractionation to isolate compounds that inhibit α-glucosidase activity from *K. senegalensis* have been reported. These findings necessitated the need for this study.

The data (Fig. 1) demonstrates that *K. senegalensis* stem bark extract exhibited a concentration-dependent inhibition effect against the activity of α-glucosidase from *Saccharomyces cerevisiae.* The IC_{50} were in the range of microgram values and above that of
acarbose. This confirms the findings that acarbose shows milder interference of α-glucosidase
from both yeast and bacteria. Several plant glucosidase inhibitors have been reported (Kim *et al.,* 2005). It is worthy to mention, that Sequence comparison between enzyme orthologs of human and *S. cerevisiae* showed higher identity (49%) at the putative catalytic domain (Romero *et al.,*1997).

K. senegalensis was observed (Fig. 2A) to be a non-competitive type of inhibitor of α-glucosidase; as such its inhibitory effect may not be affected by increasing the concentration of the substrate. It binds the free enzyme and the enzyme-substrate complex as against the case of acarbose which is a competitive inhibitor, which bind only to the free enzyme (Clissold and Edwards, 1988). The ability of *K. senegalensis* to bind to sites other than the active site probably gives them a broader specificity of inhibition as was also observed in *Cymbopogon martini* (Ghadyale *et al*., 2012). The benefit of this mechanism of inhibition is that the glycaemic control goal can be achieved if the therapeutic dose is titrated accurately without the fear of hypoglycaemia as a risk factor, which is usually observed in other oral glycaemic drugs (Smith & Doe, 2020). The *Ki* value was in the microgram range. This was very much lower than the IC_{50} and below the toxicity dose carried out by previous toxicity studies by Adebayo *et al.* (2003) and Abubakar *et al*., (2010). It can be speculated that *K. senegalensis* at this dose may be a very potent inhibitor.

The aqueous plant extracts were obtained from raw plants of *K. senegalensis* which were further partitioned in hexane, ethyl acetate and methanol in a stepwise manner (Table 1). The reason is to isolate first the polar aprotic (positively charged) compounds, the non-polar compounds, and finally the polar protic (negatively charged) compounds respectively. Although, one fraction may exhibit the highest activity and others slight to no activity. The fractions with higher inhibitory activity in each sub-fraction were noted. This way, sensible results are likely to be obtained in a given time frame. In all, as the fraction with the highest activity was further fractionated stepwise the inhibitory activity increased in the corresponding step probably as a result of the purification procedures employed with a corresponding increased concentration and purity of the bioactive compounds obtained.

The two bioactive compounds isolated were analysed by UV and IR spectroscopic techniques. The IR spectrum showed characteristic absorption bands for phenol groups as a broad hump centred at 3470 - 3200 cm^{-1} (Abdelgaleil, *et al.,* 2001). IR peaks were indicative of 2950 - 2850 (C-H stretching), a characteristic broad feature in the range 3300 – 2500 cm^{-1}, that overlaps the C-H stretching region, and with a secondary absorption close to 2600 cm^{-1}, is observed for the hydrogen-bonded O-H of most carboxylic acids. The other IR peaks were indicative of 1440 - 1450 (Aromatic ring vibration), 1190 (C-N) stretch and 1010-1025 (C-O). The IR spectrum of both compounds (F5.1 and F6.2) had similar characteristics and peaks with differences in transmittance and broadening at corresponding peaks. This may be due to differences in the number of functional groups and their different placement around rings. The presence of phenolic compounds and other electron donor functional groups on both compounds may be the reason for their α-glucosidase inhibitory activity (Lee and Kim, 2019). Phenolic compounds have an electron donor capability and are readily oxidized to form phenolate ions or quinone, which is an electron acceptor. Thus, they may have the ability to bind at sites on the enzyme, thereby impairing the enzyme activity. The area under the glucose curve ($AUC_{glucose}$) (Table 2) for each rat was calculated from Fig. 3 to determine the increase in the blood glucose concentration during the 120 min observation period. These values showed that all several doses of the extract of *K. senegalensis* could attenuate the $AUC_{glucose}$ values significantly ($p < 0.01$). This implies that *K. senegalensis* controls postprandial glucose concentration and would be particularly beneficial to obese diabetes (Sodowski *et al*., 2007)

5. Conclusion

This research work provides scientific proof that *K. senegalensis*, a traditional medicinal plant used in the management of diabetes mellitus in Nigeria as a potential source of novel inhibitor(s) of α-glucosidase an intestinal brush border enzyme, which is important in regulating postprandial blood glucose level and thus diabetic mellitus.

Conflict of interest

The authors declare no conflict of interest.

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