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Inhibitory Effect of the Stem Bark Extracts of *Albizia chevalieri* Harms on the Activity of Alpha-glucosidase Obtained from *Aspergillus niger*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YS and OA designed the study. Author ADO performed the experiment, statistical analysis of the study. Author IB wrote the protocol and wrote the first draft of the manuscript. Authors ADO and IB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

The aim of the current study is to evaluate the inhibition of α-glucosidase activity by stem bark extract of *Albizia chevalieri*. The activity of alpha glucosidase was assayed *in vitro* using 50 mM acetate buffer pH 6.0 (prepared from acetic acid and sodium acetate) and various concentration of maltose (0.5 mM to 10 mM). Five test tubes, labeled T_A – T_E, each containing 1.5 ml of acetate buffer, 0.5 ml of alpha glucosidase and 0.5 ml of a known concentration of plant extract and control tubes (C_A – C_E) were assessed for Alpha glucosidase activity. The results showed that hexane, ethyl acetate and methanol extracts inhibited α-glucosidase activity. The results further indicated that the extracts act by competitive inhibition with inhibition constant of 232 mg/ml, 157 mg/ml and 67 mg/ml for hexane, ethyl acetate and methanol extracts, respectively. The value for the inhibition

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constants shows that there is a strong binding of the enzyme to the inhibitor as the polarity of solvent increases. The inhibitory activity of *Albizia chevalieri* may be due one or more of the phytochemicals present in the extracts.

Keywords: Diabetes mellitus; Albizia chevalieri; alpha-glucosidase; Aspergillus niger; pharmacokinetic.

1. INTRODUCTION

Glucose metabolism is catalyzed by the membrane bound intestinal alpha-glucosidase enzyme found in the brush border of the small intestine. Alpha-glucosidase enzyme hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other mono-saccharides in the small intestine [1,2]. Diabetes mellitus is an endocrine disorder due to insufficient synthesis of insulin or loss of sensitivity to the insulin produced [3-5] and is associated with major chronic complications such as accelerated macro-vascular diseases, retinopathy, renal disease and neuropathy [6,7]. Diabetes is characterized by hyperglycemia; an abnormal increase in the levels of glucose in the bloodstream [8,9]. Since alpha-glucosidase is the major enzyme responsible for the catabolism of starch (Carbohydrate) into glucose units and diabetes mellitus is a chronic metabolic disorder that results in hyperglycemia (rise in blood glucose level) and other complications, which account for the major death in the world [10-12], inhibition of this enzyme will go a long way in the management of diabetes mellitus.

Albizia (A.) chevalieri is a tree of acacia type belonging to the pea family (Fabaceae). It is native to tropical and subtropical regions, and grows up to 12m high or a as a shrub under harsher conditions of the dry savannah from Senegal, Niger and Nigeria. It has an open and rounded umbrella shaped canopy, pale-greyish bark, twigs pubescent with white lenticels, leaves with 8-12 pairs of pinnae and 20-40 pairs of leaflets each [13].

A decoction of the leaves of *A. chevalieri* is used in Northern Nigeria as remedy for dysentery, while the bark is used for diarrhea and as a laxative [14]. The bark was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal [15]. A cold water decoction of *A. chevalieri* bark or a grounded and sieved leaf mixed with pap, is used for the management of diabetes mellitus by traditional medical practitioners in some parts of Niger Republic and Nigeria [16,17]. The aqueous leaf extract of *A. chevalieri* have also shown hypoglycemic effect on alloxan-induced diabetic rats. Such plant and others may serve as an alternative medicine in the management of diabetes mellitus because of their abundance within the locality and due to the fact that they are relatively cheap. In this study, we evaluate the inhibitory activity of *A. chevalieri* extract on alpha-glucosidase as the possible mechanism through which it exerts its antidiabetic activity.

2. MATERIALS AND METHOD

2.1 Apparatus and Chemicals

Spectrophotometer, Weighing balance, Micro pipette (0-200 µL) Pipette 1 ml, Autoclaving machine, Test tubes, rack measuring cylinder and Glass rod. Dimethyl Sulfoxide, Ethyl Acetate, Hexane, Methanol, Lugols Reagent, Ammonium Nitrate, Magnesium Sulphate, Soluble Starch, Activated Charcoal, Flour, Sodium Acetate, Acetic Acid, Potassium dihydrogen phosphate were purchased from BDH Chemical ltd, Poole England. Fehling Solution A & B (HopKin & Williams) and Glucose Oxidase Kit (Biotest). All the reagents used are of analytical grade.

2.2 Preparation and Extraction of Plant Material

Fresh stem bark of *A. chevalieri* were collected from Sanyinna village in Sokoto State of Nigeria and taken to the Usmanu Danfodiyo University Sokoto Herbarium for authentication (Voucher No: 00412-2-2013). 70 g stem bark of *A. chevalieri* were air dried after which it was pulverized using a mortar and pestle. The pulverized plant was then sieved and weighed. The pulverized *A. chevalieri* was serially extracted using a soxhlet extractor for eight hours with 300 ml each of hexane, ethyl acetate and methanol consecutively. The extract was left to stand on air for complete evaporation of the solvent after which the percentage recovery was calculated.

2.3 Production and Isolation of Alpha-glucosidase from *Aspergillus niger*

The filamentous fungus *Aspergillus niger* is known to produce large amount of the starch-degrading enzymes, alpha-glucosidase [18], thus was used as a source to generate the enzyme for this study. The method of Rudick et al. [19] was adopted for this purpose. *Aspergillus niger* was cultivated aerobically in a standard medium with appropriate supplements. One gram (1 g) of ammonium nitrate (NH_4NO_3), 0.5 g of potassium dihydrogen phosphate ($KH_2PO_4.2H_2O$), 0.1 g of magnesium sulphate ($MgSO_4$), 0.5 g of soluble starch were dissolved in a conical flask containing 200 ml of distilled water. The medium was gently stirred and autoclaved at 117°C for 15 minute. The medium was then allowed to cool to room temperature and 5 ml was taken for Starch and Glucose test with Lugols reagent and Fehling solution respectively, then the medium was inoculated with *Aspergillus niger.* Subsequent test for glucose and starch were carried out on 3rd, 5th, 7th and 10th day to confirm if the *Aspergillus niger* is producing the enzyme.

The vegetative growth of *Aspergillus niger* was sieved through a 15 cm Whatman filter paper. The fungal growth was discarded while 5 g of activated charcoal added to the filtrate and gently stirred for 15 minute. The immobilized enzyme was separated by filtering with filter paper. Phosphate buffer solution of a pH 6.0 was gradually added to the filtrate to stabilize the enzyme in the solution. 5 ml of 10% sodium chloride (NaCl) was added to the enzyme extract and stirred for about 10 minute, this was then filtered with a filter paper and stored in the refrigerator [20,21].

2.4 Assay for Alpha-glucosidase

The method adopted for the assay were that of Calzyme laboratories Ltd, 2004 [22]. The activity of alpha glucosidase was assayed *in vitro* using 50 mM acetate buffer pH 6.0 (prepared from acetic acid and sodium acetate) and maltose as the substrate. To each three replicate set of five test tubes labeled T_A–T_E, 1.5 ml of acetate buffer, 0.5 ml of alpha glucosidase and 0.5 ml of a known concentration of plant extracts (5, 25 and 50 mg/ml) were added. Similar tubes for the control experiment were labeled C_A – C_E and each contains 0.5 ml of alpha glucosidase, 1.5 ml acetate buffer and 0.5 ml distilled water was added in place of the extract. The tubes were incubated at 25°C for 5 minutes and maltose of various concentrations (0.5 mM to 10 mM) were added and incubated for another 5 minutes at same temperature.

The above procedure was repeated for the reaction blank (in the absence of alpha glucosidase) for both the test and the control experiments. In the blank control tubes (labeled C_1–C_5), 0.5 ml alpha glucosidase was replaced with 0.5 ml of distilled water. Similarly, in the blank test tubes (labeled T_1 – T_5), 0.5 ml of distilled water was added to the tubes in place of 0.5 ml of alpha glucosidase. The enzyme activity was quantified by measuring the absorbance (Abs.) difference of the experimental tubes against the respective reaction blank tubes at 500 nm. One unit of alpha glucosidase is defined as the amount of enzyme which catalyzes the release of one micromole of glucose per minute at 25°C, pH 6.0. Percentage inhibition of alpha glucosidase by the extract was calculated.

Glucose concentration (mmol/L):

$$\frac{(\text{Abs. of Test} - \text{Abs. of blank}) \times \text{Conc. of control}}{\text{Abs. of control}}$$

2.5 Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Waller Duncan's multiple range test using the Statistical Analysis System (SAS) 2003 version. Difference at $p< 0.05$ was regarded as significant.

3. RESULTS AND DISCUSSION

Among the biological macromolecules that one can envisage as drug targets, enzymes hold a preeminent position because of the essentiality of their activity in many disease processes, and because the structural determinants of enzyme catalysis lend themselves well to inhibition by small molecular weight, drug-like molecules. Not surprisingly, enzyme inhibitors represent almost half the drugs in clinical use today. In diabetes mellitus, a major therapeutic option for its management is through inhibiting the activity of the starch hydrolyzing enzyme, alpha-glucose.

Knowledge of the dynamic properties of enzyme catalysis is a prerequisite for the design of inhibitors (drugs) directed against a certain

enzyme. The kinetic activity of an enzyme provides information on its reaction rate (V) and the effects of an antagonist presence or varying the conditions of the reaction can be investigated. This present study evaluate the putative inhibitory effect of various concentrated organic extracts of *A. chevalieri* stem bark on the kinetics activity and mechanism of action of alpha-glucosidase.

Michaelis Menten Kinetics Equation: $$v_o = \frac{(V_{max}[S])}{(K_M + [S])}$$ .....A

Lineweaver-Burk plot: $$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$ .....B

Equation 1. Michaelis Menten Kinetics equation (A) and Lineweaver-Burk double reciprocal plot equations (B) for enzyme kinetic study showing the relationship between the initial velocity (Vo), Maximum velocity (Vmax), Km and substrate concentration ([S])

In biochemistry, the Michaelis–Menten kinetics equation (
Equation **1**A) relates the reaction rate (V) of an enzyme to the concentration of the enzyme-targeted substrate [S], and is a model that is widely employed in interpreting data from kinetic study [23,24]. A double reciprocal plot of this equation, known (
Equation **1**B), can be used to give a graphical representation of the data from such studies [23-25]. Employing the aforementioned techniques, the effect of various concentrations of stem bark extracts of *A. chevalieri* on Alpha-glucosidase activity is depicted in Figs. 1, 3 and 5 for the various solvents used (hexane, ethyl acetate and methanol respectively). The intercept on Y-axis were extrapolated against the various concentration of stem bark extract used and the intercept on X-axis represent the K_i (K_i is the dissociation constant depicted in Figs. 2, 4 and 6).

Enzyme inhibition is categorize base on the mode of effect on the maximal rate of the enzyme reaction attainable (Vmax) and also on the corresponding affinity of the enzyme to the substrate (Km) [24]. Drugs and substances that inhibit an enzymatic reaction by competing with the substrate for the binding site of the enzyme, thus reducing the enzyme-substrate affinity are said to exhibit a competitive inhibition while these that bind to an allosteric site on the enzyme, different from the binding site, thereby attenuating the attainment of Vmax without affecting the Km are said to show non-competitive inhibition. A mix inhibition occur when both the Vmax and Km of the enzyme are altered upon binding of the antagonist [26].

The hexane extract showed a significant increase in the Km (lower enzyme-subtrate affinity) as the concentration of extract increases, hence, it showed a competitive inhibition. A slight increase in the Vmax was also observed at 50 mg/ml, which signifies a shift from competitive to

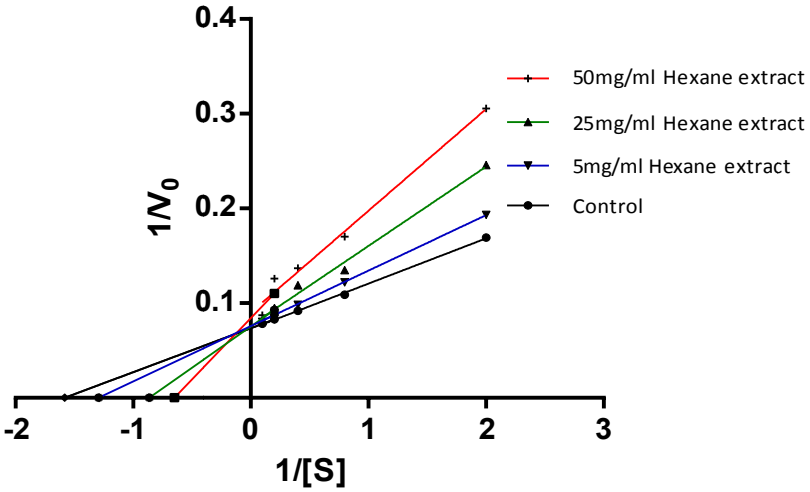

Fig. 1. Lineweaver- burk plot of various concentration of hexane extract of *A. chevalieri* stem bark. V_0 represents the initial rate and [S] represents the concentration of the substrate

Table 1. Kinetics parameter of various concentration of hexane extract of *A. chevalieri* stem bark

	Control	50 mg/ml Hexane extract	25 mg/ml Hexane extract	5 mg/ml Hexane extract
Vmax	13.62	12.11	13.28	13.21
Km	0.6321	1.542	1.158	0.7729

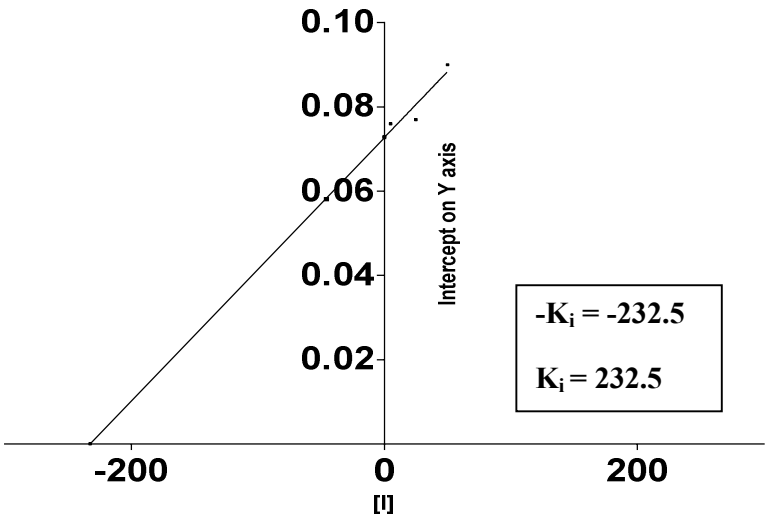

Fig. 2. The intercept on Y axis against Inhibitor concentration of hexane extract of *A. chevalieri* stem bark

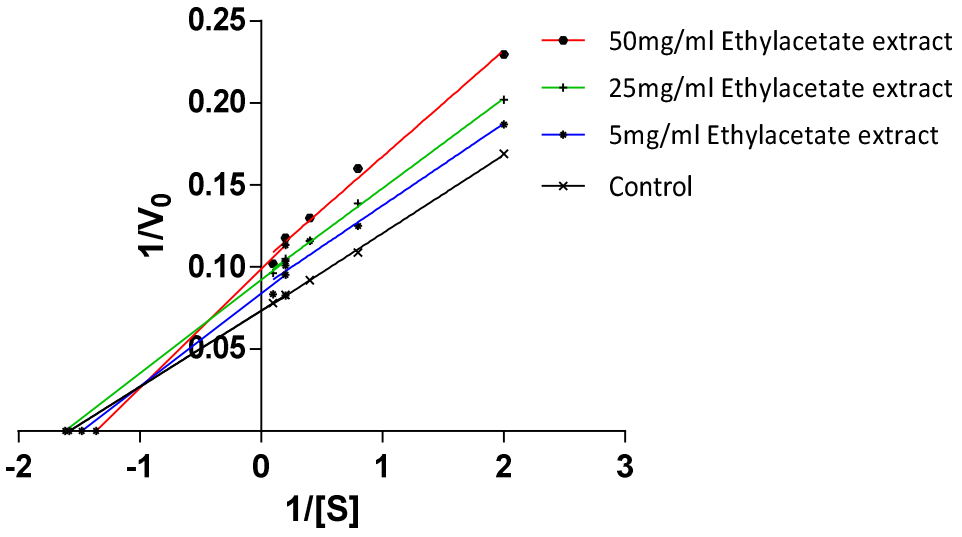

Fig. 3. Lineweaver burk plot of various concentration of ethyl acetate extract of *A. chevalieri* stem bark

mixed inhibition with increase in concentration (Fig 1). The ethyl acetate extract showed an increase in Vmax as the concentration increases (non-competitive inhibition). However, no significant increase in Km at a concentration of 25 mg/ml and 5 mg/ml, but at the highest concentration of 50 mg/ml, the affinity reduces (Fig 3).

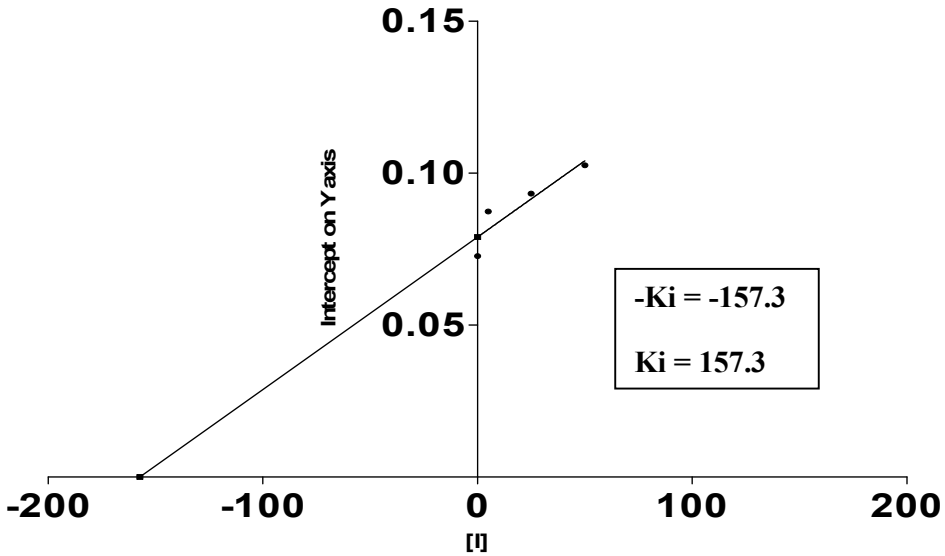

Fig. 4. The intercept against various concentration of ethyl acetate extract of *A. chevalieri* stem bark

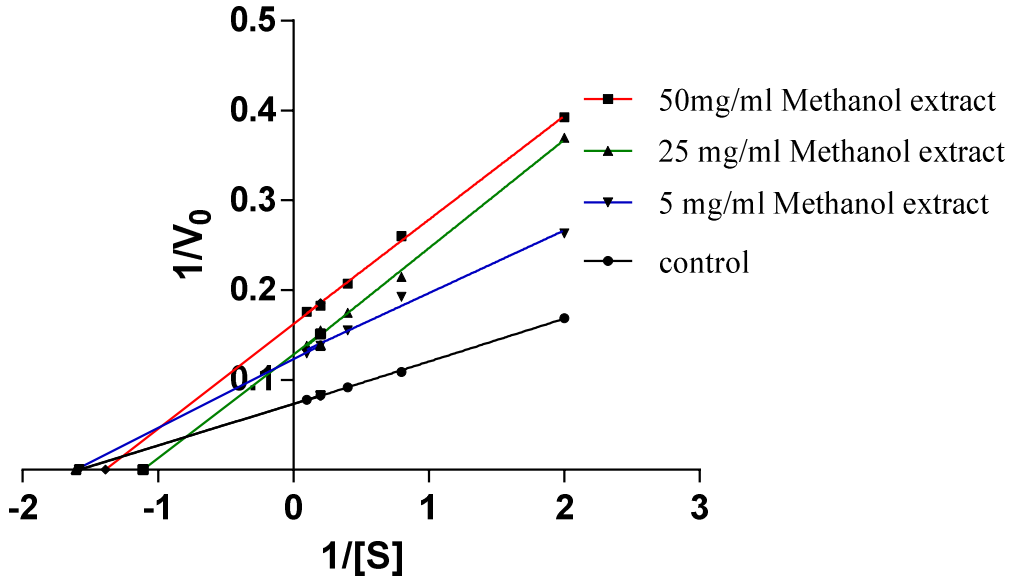

Fig. 5. Lineweaver burk plot with various concentration of methanol extract *A. chevalieri* stem bark

The methanol extract showed inhibition of α-glucosidase activity with the different concentration of methanol extract used. The highest concentration of extract (50 mg/ml) seems to affect both the affinity Km and maximum velocity Vmax (Mixed inhibition). At a concentration of 25 mg/ml, the Vmax increased compared to that of the first concentration but lower than the control (in the absence of inhibitor) but the km increased (the affinity reduced). The final concentration of extract used seems not to affect the Km but the Vmax was reduced compared with that of the control, thus it exhibited a non-competitive inhibition (Fig. 5).

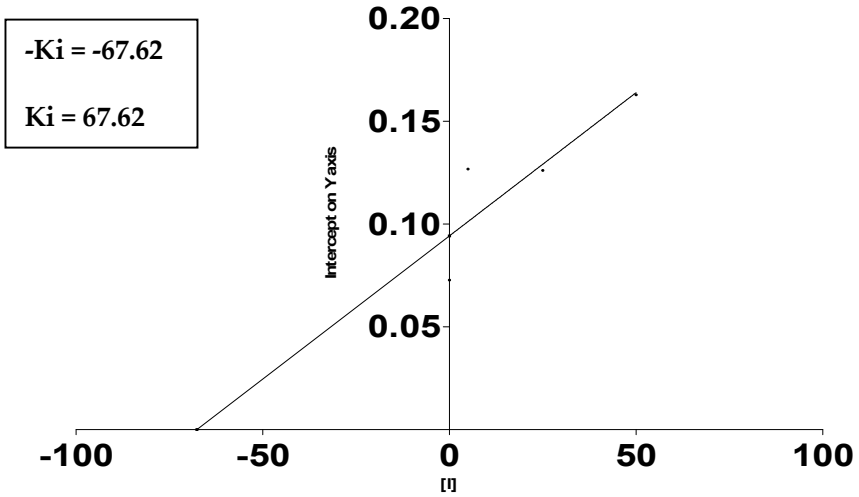

Fig. 6. The intercept against inhibitor concentration [I] of *A. chevalieri* stem bark

Table 2. Kinetics parameter of various concentration of ethyl acetate extract of *A. chevalieri* stem bark

	Control	50 mg/ml Ethyl acetate extract	25 mg/ml Ethyl acetate extract	5 mg/ml Ethyl acetate extract
Vmax	13.62	10.13	10.85	11.90
Km	0.6321	0.7505	0.6196	0.6766

Table 3. Kinetics parameter of various concentration of methanol extract of *A. chevalieri* stem bark

	Control	50 mg/ml Methanol extract	25 mg/ml Methanol extract	5 mg/ml Methanol extract
Vmax	13.62	6.167	7.792	8.109
Km	0.6321	0.7203	0.9021	0.6236

The concentration of inhibitor needed to inhibit an enzyme depends on how tightly the inhibitor binds to the enzyme. The inhibition constant (Ki) determines the nature of binding between the inhibitor and the enzyme, and it is used to determine how tightly the inhibitor binds to the enzyme. The value for the Ki for the different concentration of hexane extract shows that the binding between the inhibitor components present within the hexane extract is weak because the value for the Ki is very large (Fig. 2). The ethyl acetate extract showed a lesser value for the Ki indicating that the nature of binding between the enzyme and the inhibitor is stronger when compared to that of hexane extract (Fig. 4). The value of Ki for the methanol extract is the lowest (Fig. 6), showing that the interaction between the enzyme and the inhibitor is reasonably strong due to the small value of Ki.

Alpha glucosidase inhibitors such as acarbose, 1-deoxy norjirimycin and genistein have been isolated from natural source [27,28]. *Euphobia hirta* L herb have also shown inhibitory activity on alpha glucosidase [29]. A number of other medicinal plant have shown to possess antidiabetic effect which has been attributed to their alpha glucosidase inhibitory activity; such plants include *Arachis hypogea* [30], *Rosa damascene* [31], *Rosmarinus officinalis* [32] *Thymus serphyllu* [33] and *Piper trioicum* [34].

It is evident from the results obtained that *A. chevalieri* possess alpha glucosidase inhibitory activity. It can be deduced that a synergy from

multicomponents of the extract using several mechanism of inhibition, according to their concentration and solvent of extraction, are involve and this may augment the potency of their inhibitory activity. It had been reported that synthetic alpha-glucosidase inhibitors have undesirable side-effects such as abdominal cramping and diarrhea and some of these may increase the chance of hepatic syndrome and renal tumors [35,36]. While toxicity study conducted on *A. chevalieri* indicate a mild alteration in the hematological composition, no drastic and detrimental effect was found associated [37]; thus it's a good candidate as a medicinal plant that can be used to suppress postprandial hyperglycemia in diabetic patients. This study provides evidence that corroborate the use of hyperglycemia as an alternative and/or complementary medicine in the management of diabetic patients and for decreasing diabetes complication such as retinopathy.

4. CONCLUSION

A. chevalieri showed inhibitory activity against alpha glucosidase. This may be due to synergism of component of the bark extract and may serve as a modern alternative option for the treatment and control of postprandial hyperglycemia in diabetic patients with possibly less adverse effect compared to modern convectional drugs. Nonetheless, further cytotoxicity and genotoxicity study on the plant will shed more light on the safety profile the plant for use as herbal remedy or otherwise consumption.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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