

Original Research Article

In vitro Studies on the Inhibition of α -Amylase and α -Glucosidase by Leaf Extracts of *Picralima nitida* (Stapf)

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Abstract

Purpose: To investigate the anti-diabetic potential of *Picralima nitida* leaf extracts *in vitro*.

Methods: The current study evaluated the anti-diabetic potential of *Picralima nitida* leaf *in vitro* inhibition of α -amylase and α -glucosidase using the acetone, water and ethanol extracts. Preliminary phytochemical analysis was performed on the acetone, aqueous and ethanol extracts of *Picralima nitida* leaf. The α -amylase inhibitory potentials of the extracts were investigated by reacting different concentrations of the extracts with α -amylase and starch solution while α -glucosidase inhibition was determined by pre-incubating α -glucosidase with different concentrations of the extracts followed by the addition of *p*-nitrophenylglucopyranoside (pNPG). The mode(s) of inhibition of both enzymes was determined using Lineweaver-Burke plot.

Results: The acetone extract of *Picralima nitida* displayed the most effective inhibition of both α -amylase and α -glucosidase activities with half-maximal inhibitory concentration (IC_{50}) of 6.50 and 3.00 mg/ml, respectively. The results also showed that the extracts contain both non-competitive and competitive inhibitors of α -amylase and α -glucosidase respectively. The results of phytochemical analysis of the extract revealed the presence of saponins, flavonoids, tannins, terpenoids and reducing sugars.

Conclusion: The observed inhibitions of α -amylase and α -glucosidase suggest that the leaf extracts of *P. nitida* may be useful in the management of diabetes mellitus. This buttresses the traditional use of *P. nitida* for the management of sugar-related disorders in some parts of Nigeria.

Keywords: *Picralima nitida*, Diabetes, α -Amylase, α -Glucosidase, Hyperglycemia

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INTRODUCTION

Diabetes mellitus is a complex disease characterized by gross derangement in carbohydrate, fat and protein metabolism due to deficiency in insulin secretion and/or action [1]. Mammalian α -amylase is a prominent enzyme in the pancreatic juice which breaks down large and insoluble starch molecules into absorbable molecules ultimately maltose [2]. α -glucosidase, on the other hand, anchored in the mucosal

brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet [3]. Inhibitors of α -amylase and α -glucosidase delay the breakdown of carbohydrate in the small intestine and decrease the postprandial blood glucose excursion levels in diabetic patients [4]. The inhibition of these two prominent enzymes has been found as a useful and effective strategy to lower the levels of postprandial hyperglycemia [6]. The incidence of diabetes mellitus in the

world is increasing at an alarming rate, affecting close to 5 % of its population [5].

Picralima nitida (Staph) (Apocynaceae) otherwise known as *Akuama* or 'Abeere' among the Yoruba speaking people of South-Western Nigeria, is a medicinal plant with diverse uses [7]. It is used in traditional medicines in the treatment of inflammation, otitis, pulmonary bronchitis, venereal diseases, various fevers, hypertension, jaundice, gastrointestinal disorders and malaria [8, 9]. Previous studies have shown that the extracts from its seeds, fruit rind and stem bark possess antimicrobial, anti-inflammatory, antipyretic, anti-trypanosomal, anti-plasmodial [10], anti-leishmanial, larvicidal [11] and hypoglycaemic potentials [12].

Although several studies have been conducted on the biological and pharmacological potentials of *Picralima nitida*, there is no report on its effect on α -amylase and α -glucosidase activities *in vitro*. Yet the species is used in the traditional management of sugar-related disorders in Nigeria. The objective of this study, therefore, was to evaluate the α -amylase and α -glucosidase inhibitory potentials of *Picralima nitida* leaf extracts

EXPERIMENTAL

Plant material

The leaves of *Picralima nitida* were obtained from Ikoga-Zebbe village, Badagry Local Government Area of Lagos State, Nigeria in May 2012, and authenticated by Dr AB Kadiri of the Department of Botany, University of Lagos, Nigeria. A voucher specimen (LUH 4725) was deposited in the University herbarium of the Department of Botany, University of Lagos.

Chemicals and reagents

α -Amylase from *Aspergillus oryzae*, α -glucosidase from *Saccharomyces cerevisiae* and para-nitrophenyl-glucopyranoside were products of Sigma-Adrich Co, St Louis, USA while soluble starch (extra pure) was obtained from JT Baker Inc, Phillipsburg, USA. Other chemicals and reagents used were of analytical grade while the water used was glass-distilled.

Preparation of plant extracts

Fresh leaves of *Picralima nitida* were cut and washed under running tap to remove all foliar contaminants, dried at room temperature and pulverized to fine powder. The powdered leaves were divided into three portions of 50 g each and

extracted with 500 ml each of acetone, ethanol or water and kept on orbital Labcon Platform shaker (Laboratory Consumables, PTY, Durban, South Africa) in covered 1000 ml flasks for 24 h at room temperature. The resulting infusions in each case was decanted, filtered and evaporated to dryness in a rotary evaporator (Cole Parmer SB 1100, Shanghai, China) in respect of the organic solvent extracts. For the water extract, it was freeze-dried using Virtis Bench Top freeze dryer (SP Scientific Series, USA). The yield was 3.99, 6.62 and 2.41 g for acetone, ethanol and water extracts, respectively. The dried crude extract was dissolved in 10 % dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared.

Phytochemical screening

The phytochemical composition of each extract was determined using methods variously described by Trease and Evans [13] and Sofowara [14].

Test for tannins

In the test for tannins, 0.5 g of dried powdered sample was boiled in 20 ml of water in a test tube and filtered. A few drops of 0.1 % ferric chloride were added and observed for brownish green or a blue black colouration.

Test for saponin

Approximately 2 g of powdered material was boiled in 20 ml of distilled water in a water bath and filtered. Next, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion.

Test for flavonoids

A portion of the powdered material was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Development of yellow colouration is an indication of the presence of flavonoids.

Test for steroids

In this test, 2 ml of acetic anhydride was added to 0.5 g of extract with 2 ml concentrated H_2SO_4 . The colour change from violet to blue or green is indication of steroids.

Test for terpenoids

In brief, 5 ml of extract (0.5 mg/ml) was mixed with 2 ml chloroform and 3 ml concentrated H₂SO₄ was carefully added to form a layer. A reddish brown colouration of the interface was indication of terpenoids.

Test for anthraquinones

5 ml of chloroform was added to 0.5 g of the extracts of each specimen. The resulting mixture was shaken for 5 min after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of anthraquinones.

Test for reducing sugar

To about 1 g of each sample in the test tube was added 10 ml distilled water and the mixture boiled for 5 min. The mixture was filtered while hot and the cooled filtrate made alkaline to litmus paper with 20 % sodium hydroxide solution. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The formation of a brick red precipitate depicted the presence of reducing compound.

α-Amylase inhibitory assay

This assay was carried out using a modified procedure of McCue and Shetty [15]. A total of 250 μL of extract (1.25 – 10 mg/ml) was placed in a tube and 250 μL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/ml) was added. The content of the tubes were pre-incubated at 25 °C for 10 mins, after which 250 μL of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals. The reaction mixtures were incubated at 25 °C for 10 min. The reaction was terminated by adding 500 μL of dinitrosalicylic acid (DNS) reagent and further incubated in boiling water for 5 min and cooled to room temperature. The content of each test tube was diluted with 5 ml distilled water and the absorbance measured at 540 nm in a spectrophotometer (Spectrumlab S23A, Globe Medical, England). A control was prepared using the same procedure except that the extract was replaced with distilled water. The α-amylase inhibitory activity was calculated as in Eq 1.

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}100 \dots\dots\dots(1)$$

where Ac and Ae are the absorbance of the control and extract, respectively.

The concentration of extract resulting in 50 % inhibition of enzyme activity (IC₅₀) was determined graphically using Microsoft Excel 2007.

Mode of α-amylase inhibition

The mode of inhibition of the leaf extract was conducted using the extract with the lowest IC₅₀ according to the modified method described by Ali *et al.*, [16]. Briefly, 250 μL of the (5 mg/mL) extract was pre-incubated with 250 μL of α-amylase solution for 10 mins at 25 °C in one set of tubes. In another set of tubes, 250 μL of phosphate buffer (pH 6.9) was also pre-incubated with 250 μL of α-amylase solution. Starch solution (250 μL) of increasing concentrations (0.30 – 5.0 mg/mL) was added to both sets of reaction mixtures to initiate the reaction. The mixture was then incubated for 10 min at 25 °C, and then boiled for 5 min after addition of 500 μL of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double reciprocal plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

α-Glucosidase inhibitory assay

The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim *et al.*, [17], using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenyl glucopyranoside (pNPG) (3.0 mM) was prepared in 20 mM phosphate buffer, pH 6.9. 100 μL of α-glucosidase (1.0 U/ml) was pre-incubated with 50 μL of the different concentrations of the extracts (acetone, ethanol and water) for 10 mins. Then 50 μL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37 °C for 20 mins and stopped by adding 2 ml of 0.1 M Na₂CO₃. The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 405 nm. The results (% Inhibition) are expressed as percentage of the blank (control) as in Eq 2.

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}100 \dots\dots\dots(2)$$

where Ac and Ae are the absorbance of the control and extract, respectively.

The concentration of extract resulting in 50 % inhibition of enzyme activity (IC_{50}) was determined graphically using Microsoft Excel.

Mode of α -glucosidase inhibition

The mode of inhibition of the extracts was determined using the extract with the lowest IC_{50} according to the modified method described by Ali et al [16]. Briefly, 50 μ L of the (5 mg/mL) extract was pre-incubated with 100 μ L of α -glucosidase solution for 10 mins at 25 °C in one set of tubes. In another set of tubes α -glucosidase was pre-incubated with 50 μ L of phosphate buffer (pH 6.9). 50 μ L of PNPG at increasing concentrations (0.63 – 2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixtures were then incubated for 10 mins at 25 °C, and 500 μ L of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a paranitrophenol standard curve and converted to reaction velocities. A double reciprocal plot ($1/v$ versus $1/[S]$) where v is reaction velocity and $[S]$ is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α -glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). The data were analysed by one-way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as mean \pm SE of triplicate determinations. The level of significance was taken at 5 % confidence interval ($p < 0.05$)

RESULTS

The results obtained from the phytochemical screening conducted on the *P. nitida* leaf extracts are presented in Table 1. Anthraquinone was absent in all the extracts while reducing sugar was common to all. The acetone extract contains only saponin and terpenoids, whereas the ethanol extract contains flavonoids, steroids and terpenoids.

The inhibitory potentials of different extracts of *P. nitida* on α -amylase activity are presented on Figure 1. At lower concentrations (1.25 – 2.5 mg/ml), there were no significant differences ($p > 0.05$) among the values generated for all the extracts. However, there was significant difference ($p < 0.05$) between the percentage

inhibition of acetone and ethanolic extract at concentration of 5.00 mg/ml. When the concentration of the extract was increased to 10 mg/ml, acetone extract exhibited very high inhibitory activity (88.39%) against α -amylase which is significantly different ($p < 0.05$) when compared with ethanol and aqueous extract. The α -amylase inhibition is dose-dependent but the acetone extract displayed most effective inhibition against α -amylase with an IC_{50} value of 6.50 mg/ml (Table 2). The mode of inhibition of α -amylase by acetone extract of *P. nitida* leaf was determined by Lineweaver-Burke plot which showed that the mode of inhibition of this enzyme by the extract is non-competitive (Fig. 2).

Table 1: Phytochemical constituents of acetone, ethanol and aqueous extracts of *Picralima nitida*

Phytochemical type	Inference		
	Acetone	Ethanol	Water
Anthraquinones	-	-	-
Flavonoids	-	+	+
Reducing sugar	+	+	+
Saponins	+	-	+
Steroids	-	+	-
Tannins	-	-	+
Terpenoids	+	+	-

(+) = present and (-) = not detected

Table 2: IC_{50} values of α -amylase and α -glucosidase inhibition by leaf extracts of *Picralima nitida*

Extract	IC_{50} (mg/ml)	
	α -Amylase	α -Glucosidase
Acetone	6.50 \pm 0.52	3.00 \pm 0.36
Ethanol	12.35 \pm 2.05	6.15 \pm 0.83
Water	15.05 \pm 1.85	7.05 \pm 1.05

Similar to α -amylase inhibition, there was no significant difference in α -glucosidase inhibition at lower concentrations of the extracts and the results. The inhibitions of α -glucosidase by all the plant extracts at lower concentrations (0.63 - 1.25 mg/mL) were not significantly different ($p > 0.05$) from one another. At 2.50 mg/mL, the percentage inhibition of acetone and aqueous extract were significantly different ($p < 0.05$) when compared to ethanolic extract. The acetone extract displayed the highest inhibition (67.3 %) at the highest concentration (5.0 mg/mL) while it was between 40.0 and 45.0 % for aqueous and ethanol extracts, respectively. Further investigation into the mode of α -glucosidase inhibition by the plant extracts provided a characteristic inhibition profile in which acetone extract was the most potent α -glucosidase inhibitor with an IC_{50} value of 3.00 mg/ml (Table 2). The mode of inhibition of α -glucosidase by this acetone extract of *P. nitida*

leaf was determined using Lineweaver-Burke plot and the plot displayed near competitive inhibition of the enzyme activity.

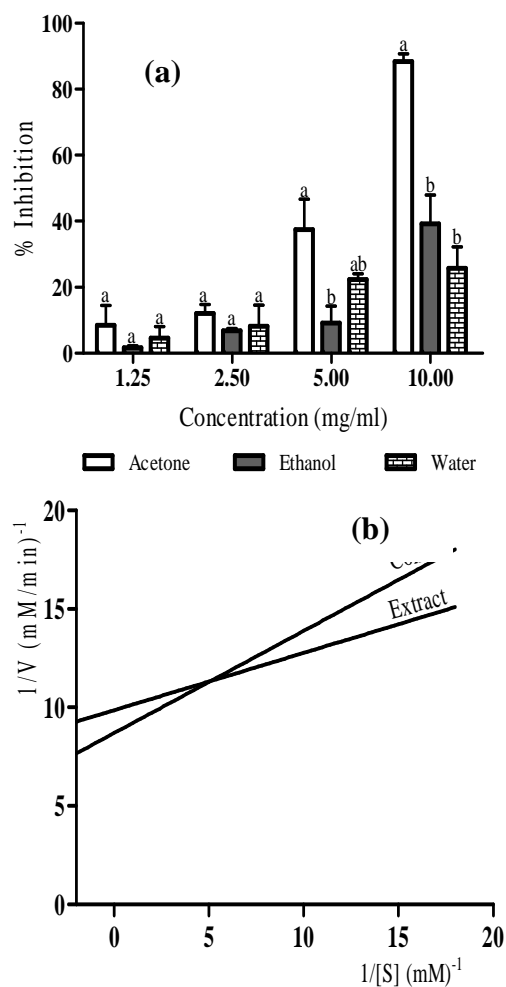


Fig 1: α -Amylase inhibitory activities of *Picralima nitida* leaf extracts (a), and mode of inhibition of α -amylase by acetone extract of *Picralima nitida* leaf (b).

DISCUSSION

Diabetes mellitus is a common metabolic disorder which may eventually lead to multiple organ damage and syndromes [18]. Elevated postprandial glucose level increases the risk of cardiovascular disease which is the most common cause of death among the people with diabetes. Postprandial spikes can also result into micro-vascular damage through oxidation of low density lipoprotein (LDL) and pro-atherogenic mechanism [19]. Management of the blood glucose level is a critical strategy in the control of diabetes and its complications.

Diet rich in carbohydrate causes sharp rise in the blood glucose level as the complex carbohydrate in food is rapidly absorbed in the intestine aided

by the α -amylase and α -glucosidase enzyme which break carbohydrate to simple absorbable sugars (monosaccharides) [19]. Inhibitors of saccharide hydrolyzing enzymes (α -amylase and α -glucosidase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with Type II diabetes mellitus [20]. These inhibitors delay carbohydrate digestion and prolong overall carbohydrate digestion time causing reduction in the rate of glucose absorption and consequently reducing the post prandial plasma glucose rise [21].

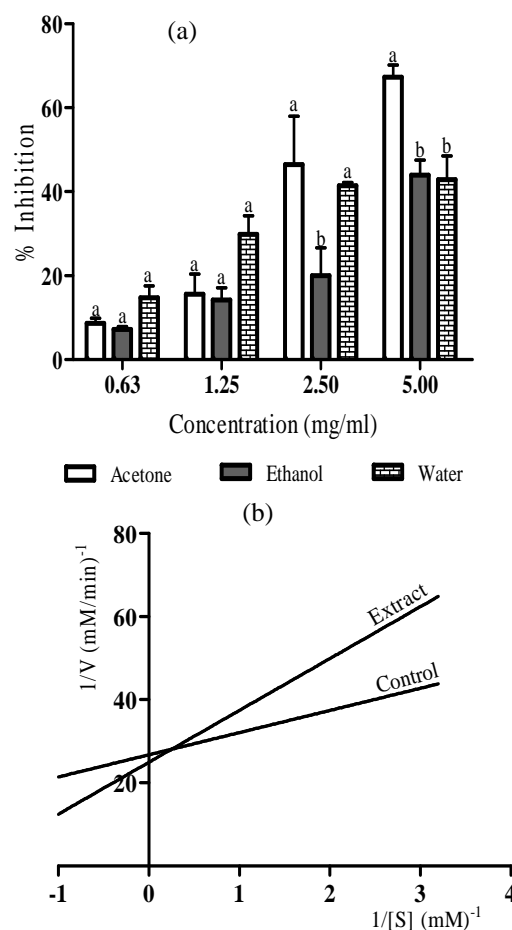


Fig 2: α -Glucosidase inhibitory activity of *Picralima nitida* leaf extracts (a), and mode of inhibition of α -glucosidase by acetone extract of *Picralima nitida* leaf (b)

Synthetic hypoglycemic drugs like acarbose, miglitol and voglibose are used in conjunction with other antidiabetic drugs, but these inhibitors have been found to possess gastrointestinal side effects like abdominal discomfort, flatulence and diarrhoea. As a result of this, there is growing interest in discovering new and effective α -

amylase and α -glucosidase inhibitors from plants with minimal or no side effects [22].

In the present study, the acetone extract showed the most potent inhibition of α -amylase activity at all concentrations tested. However, at high concentration (10.00 mg/ml), the inhibition was strong which is undesirable of α -amylase inhibitor. This was also attested to by the high IC_{50} for the α -amylase inhibition. In order for hypoglycemic agents to elicit their pharmacological effects and prevent the side effects experienced with the use of synthetic drugs, medicinal plants should be mild inhibitors of α -amylase [19]. To determine the mode of inhibition of this enzyme, the double reciprocal plot indicated a non-competitive inhibition of α -amylase by *P. nitida* extracts. This suggests that the active component of the extract binds to a site other than the active site of the enzyme and combine with either free enzyme or enzyme-substrate complex possibly interfering with the action of both [23].

The *in vitro* α -glucosidase inhibitory results demonstrated that all the extracts of *P. nitida* leaf possess α -glucosidase inhibitory activities. However, acetone extract exhibited the strongest inhibition of the enzyme as attested to by the lowest IC_{50} generated. This is in agreement with previous reports that phytochemicals from plants are strong inhibitors of α -glucosidase [19]. The curve generated from the Lineweaver-Burke plot of this inhibition showed a near competitive mode of inhibition of the α -glucosidase. This implies the active components of the extract compete with the substrate for binding to the active site of the enzyme, thereby preventing or slowing down the breakdown of oligosaccharides to disaccharides [22]. The phytochemical screening revealed the presence of saponins and terpenoids. The inhibitory effect of the extracts of *P. nitida* may not be unconnected with the presence of saponins and terpenoids in it.

CONCLUSION

Although the acetone extract exhibited the most potent α -amylase and α -glucosidase inhibitory activity which is an indication that the solvent is capable of extracting the active constituents in *P. nitida*, the use of infusion and decoction is to be encouraged for a mild to moderate activity since a higher dosage of the alcohol extract can lead to undesirable α -amylase and α -glucosidase inhibitory effects in humans. The inhibitory effect of these extracts on both enzymes may be linked to the presence of saponins and terpenoids which may be acting individually or in synergy. This study further validates the folkloric use of *P.*

nitida in the management of sugar related disorders in Nigeria.

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CONFLICT OF INTEREST

We declare there are no conflicts of interest

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