

Original Article***Helianthus annuus* Leaf Ameliorates Postprandial Hyperglycaemia by Inhibiting Carbohydrate Hydrolyzing Enzymes Associated with Type-2 Diabetes**Oluwafemi Adeleke Ojo ^{*1}, Adebola Busola Ojo ², Basiru Ajiboye ¹, Israel Olayide ¹, Adewale Fadaka ¹

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ABSTRACT

Background: Diabetes mellitus is a chronic disease. Decreasing postprandial hyperglycemia by retarding glucose absorption through inhibiting carbohydrates digesting enzymes (α -amylase and α -glucosidase) is one of many approaches used for the management of this disease. This study was aimed at evaluating the normoglycaemic potential of *Helianthus annuus* leaf.

Methods: The effect of the *in vitro* inhibitory of different extracts (acetone, ethyl acetate and hexane) of the plant was assessed on the activities of diabetes-related enzymes (α -amylase and α -glucosidase).

Results: The hexane extract of *H. annuus* leaf displayed the best inhibitory activity against α -amylase and α -glucosidase as indicated by the IC_{50} values (3.92 ± 0.02 mg mL⁻¹) and (3.29 ± 0.12 mg mL⁻¹), respectively. Lineweaver-Burk plot of inhibition of α -amylase and α -glucosidase by this extract showed that it was competitive and non-competitive mode, respectively.

Conclusion: *H. annuus* leaf possesses hypoglycaemic potential which may be due to the inhibition of pancreatic α -amylase and intestinal α -glucosidase.

Keywords: α -Amylase, α -Glucosidase, *Helianthus Annuus*, Postprandial Hyperglycaemia.

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INTRODUCTION

The prevalence of type-2 diabetes mellitus is increasing at an alarming rate such that is currently being estimated to be responsible for 90–95% of all diabetes cases worldwide [1]. This occurs as a result of lifestyle and socio-economic changes mainly characterized by lower physical activity and higher intake of fat and saturated carbohydrate containing diets among many other factors [2]. Hyperglycemia is a hallmark of type-2 diabetes mellitus and plays a vital role in most of the pathogenic features of the disease. This condition prevails when there is decreased insulin sensitivity or decreased insulin secretion from pancreatic β -cells, which can inhibit insulin secretion from the pancreas and reduce insulin mediated glucose uptake in peripheral tissues [3, 4]. All diabetic complications (nephropathy, neuropathy, microangiopathy, macroangiopathy, retinopathy, and cataract) are strongly linked to hyperglycemia. Therefore, the need for the improved treatment of hyperglycemia, type 2 diabetes mellitus related risk factors and the long term degenerative disorders so as to drastically lower the risk of both micro and macro vascular

complications. An important strategy to control hyperglycemia is through the inhibition of key carbohydrates digesting enzymes such as α -amylase and α -glucosidase, which also play a vital role in preventing diabetic complications. The inhibitors of these enzymes delay the digestion of carbohydrates reduce the rate of glucose absorption from the small intestinal tract, therefore, reducing postprandial blood glucose level. Thus, inhibition of α -amylase and α -glucosidase is a key in the management and treatment of hyperglycemia and type 2 diabetes mellitus [3].

Helianthus annuus L. is a folk remedy for bronchiectasis, bronchitis, carbuncles, catarrh, cold, colic, cough, diarrhea, dysentery, dysuria, epistaxis, eyes, fever, flu, fractures, inflammations, laryngitis, lungs, malaria, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, urogenital ailments, whitlow, and wounds [5]. Anti-hyperglycaemic effects of these plants are due to their capability to improve the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or the facilitation

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of metabolites in insulin dependent processes [6]. *H. annuus* leaves are extensively used for whooping cough, asthma, anti- malaria, insect bites, snake bites, fevers and lung problems. Elsewhere, the roots are used for treatment of diabetics [7]. Despite the usage of *H. annuus* leaf in the management of diabetes mellitus, there is no scientific report on the antidiabetic potential of this plant.

Therefore, the present study aimed to assess the hypoglycaemic potential of *H. annuus* leaf, through the evaluation of inhibitory potential of its extracts on key enzymes linked to diabetes mellitus (α -amylase and α -glucosidase).

MATERIALS AND METHODS

Sample Collection

Fresh Leaves of *H. annuus* were collected at a Ijadu farm in the suburbs of Ado Ekiti, Nigeria. The plant was identified and authenticated by a plant scientist in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria and a voucher specimen number was deposited accordingly at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Chemicals and Reagents

α -glucosidase from *Saccharomyces cerevisiae*, porcine pancreatic amylase, *p*-nitrophenyl- α -D-glucopyranoside (pNPG), *p*-nitrophenol, gallic acid, and potassium ferricyanide were obtained from Sigma-Aldrich, Germany. Starch, dinitrosalicylic acid (DNS), maltose, ethanol, ethyl acetate, trichloroacetic acid reagents were obtained from Merck Chemical Company, Germany.

Preparation of Plant Extracts

The powdered leaves were divided into three portions of 50 g each and these were extracted with acetone, ethyl acetate and hexane. They were all left to steep in covered conical flasks for 24 h, the flasks were shaken and kept still to allow the plant material settle at the bottom of the flask. The resulting infusions were decanted, filtered and evaporated in a rotary evaporator. Dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared.

$$\% \text{ yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of powdered leaves}} \times 100\%$$

Preliminary Phytochemical Screening

Helianthus annuus L., was subjected to qualitative chemical screening to identify the various major classes of active chemical constituents, namely tannins, steroid, terpenoids, saponins, cardiac glycosides, flavonoids, and alkaloid [8, 9].

Determination of α -Amylase Inhibitory Activity

The α -amylase inhibitory activity was determined [10], with slight modifications. A volume of 250 μ L of ethyl acetate fraction at different concentrations (1–5 mg mL⁻¹) was incubated with 500 μ L of porcine pancreatic amylase (2 U mL⁻¹) in 100 mmol L⁻¹ phosphate buffer (pH 6.8) at 37 °C for 20 min. Two hundred and fifty μ L of 1 % starch dissolved in 100 mmol L⁻¹ 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.

$$\alpha - \text{ amylase inhibition (\%)} = \frac{A540_{\text{control}} - A540_{\text{sample}}}{A540_{\text{control}}} \times 100$$

Mode of α -Amylase Inhibition

The mode of inhibition of α -amylase by the leaf extract was conducted using the most potent extract according to a modified method [11]. Briefly, 250 μ L of the (2.5 mg mL⁻¹) extract was pre-incubated with 250 μ L of α -amylase solution for 10 min at 25 °C in one set of tubes. In another set of tubes, α -amylase was pre-incubated with 250 μ L of phosphate buffer (pH 6.9). Two hundred and fifty microliter of starch solution at increasing concentrations (0.3-5.0 mg mL⁻¹) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25 °C and then boiled for 5 min after addition of 500 μ L 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 (Lineweaver-Burk)

plot ($1/v$ versus $1/[S]$) where, v is reaction velocity and $[S]$ is substrate concentration was plotted to determine the mode of inhibition.

Determination of α -Glucosidase Inhibitory Activity

The α -glucosidase inhibitory activity was determined [12], with slight modifications. Briefly, 250 μL of ethyl acetate fraction, at different concentrations ($1\text{--}5\text{ mg mL}^{-1}$), was incubated with 500 μL of 1.0 U mL^{-1} α -glucosidase solution in 100 mmol L^{-1} phosphate buffer (pH 6.8) at $37\text{ }^{\circ}\text{C}$ for 15 min. Thereafter, 250 μL of pNPG solution (5 mmol L^{-1}) in 100 mmol L^{-1} phosphate buffer (pH 6.8) was added and the mixture was further incubated at $37\text{ }^{\circ}\text{C}$ for 20 min. The absorbance of the released p -nitro phenol was measured at 405 nm and the inhibitory activity was expressed as percentage of a control sample without inhibitors.

$$\alpha\text{-glucosidase inhibition (\%)} = \frac{A405_{\text{control}} - A405_{\text{sample}}}{A405_{\text{control}}} \times 100$$

Mode of α -Glucosidase Inhibition

The mode of inhibition of α -glucosidase by the extracts was determined using the extract with the lowest IC_{50} according to a modified method [11]. Briefly, 50 μL of the (2.5 mg mL^{-1}) extract was pre-incubated with 100 μL of α -glucosidase solution for 10 min at $25\text{ }^{\circ}\text{C}$ in one set of tubes. In another set of tubes, α -glucosidase was pre-incubated with 50 μL of phosphate buffer (pH 6.9). 50 μL of pNPG at increasing concentrations ($0.63\text{--}2.0\text{ mg mL}^{-1}$) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at $25\text{ }^{\circ}\text{C}$ and 500 μL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a para-nitrophenol standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot ($1/v$ versus $1/[S]$) where v is reaction velocity and $[S]$ is substrate concentration was plotted to determine the mode of inhibition.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). All the results were expressed as Mean \pm SEM for triplicate determinations.

RESULTS

Phytochemical screening of the extracts of *H. annuus* L. showed the presence of various chemical constituents, mainly alkaloids, saponins, polysaccharides, flavonoids, polyphenols. The results obtained were comparable and satisfied the standard literature.

Figure 1 shows the result of the α -amylase inhibitory potential of different extracts of *H. annuus* leaf. At the lowest concentration tested (1 mg mL^{-1}), there were no significant differences among all the extracts. At higher concentrations, there were no significant differences between the acetone and ethyl acetate extracts. However, the percentage inhibition of the enzyme by hexane extract was significantly higher ($P < 0.05$) compared to other extracts. This is corroborated by the lowest IC_{50} value ($3.92 \pm 0.02\text{ mg mL}^{-1}$) exhibited by the hexane extract compared to other extracts (Table 1). The Lineweaver-Burk plot showed that hexane extract of *H. annuus* leaf displayed competitive inhibition of the enzyme, α -amylase (Figure. 2).

Figure 3 shows the inhibitory effect of different extracts of *H. annuus* leaf on α -glucosidase. Across all the concentrations, hexane extract displayed significantly higher ($P < 0.05$) percentage inhibition of the enzyme than acetone and ethyl acetate extracts while there was no significant difference between the inhibitions by acetone and ethyl acetate. The higher percentage inhibition exhibited by the hexane extract resulted in its lowest IC_{50} ($3.29 \pm 0.12\text{ mg mL}^{-1}$). The hexane extract of *Helianthus annuus* leaf also inhibited α -glucosidase in a mixed non-competitive manner as shown in (Figure. 4).

Table 1. IC_{50} values for the inhibition of α -amylase and α -glucosidase by *Helianthus annuus* leaf.

Extracts	IC_{50} (mg/ml)	
	α -amylase	α -glucosidase
Acetone	5.42 ± 0.22^a	4.43 ± 0.52^a
Ethyl acetate	5.20 ± 0.18^a	4.05 ± 0.68^a
Hexane	3.92 ± 0.02^b	3.29 ± 0.12^b

Data are presented as mean \pm SD of duplicate determinations. Different letters (a,b) over the bars for a given concentration for each extract indicate a significant difference from each other (Duncan multiple range post hoc test ($P < 0.05$)).

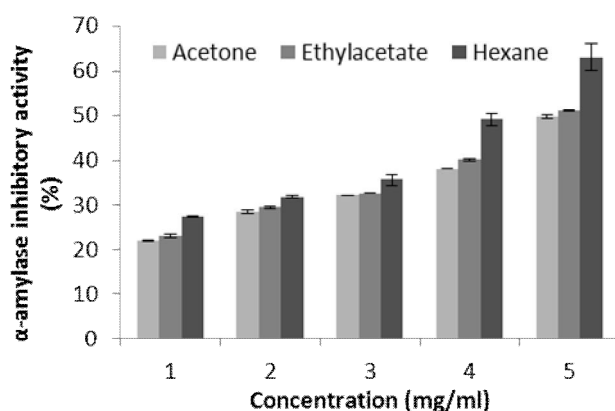


Figure 1. α -amylase inhibitory activities of extract of *Helianthus annuus* leaf. Data are presented as mean \pm SD of triplicate determinations.

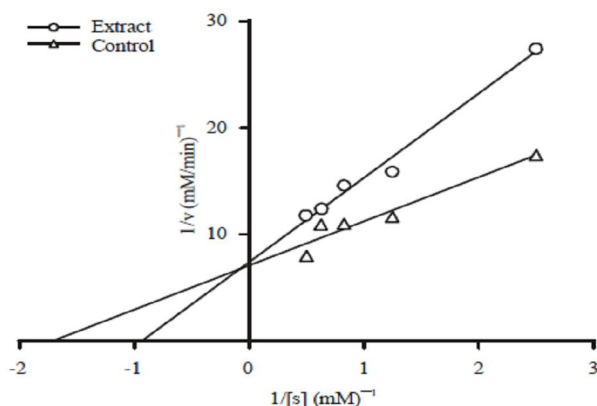


Figure 2. Mode of inhibition of α -amylase by hexane extract of leaf of *Helianthus annuus*.

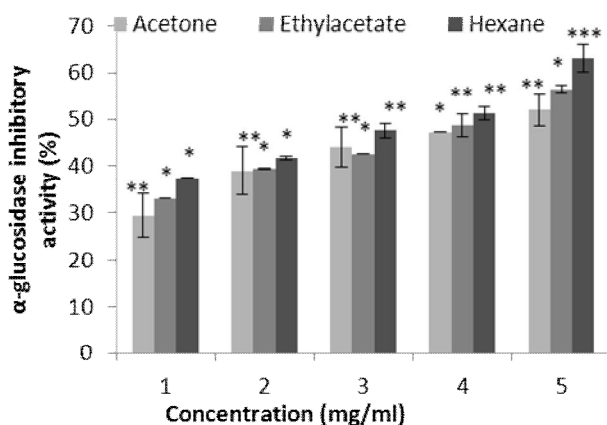


Figure 3. α -glucosidase inhibitory activities of ethanol extract of *Helianthus annuus* leaf. Data are presented as mean \pm SD of triplicate determinations. (*-***) Different symbol over the bars for a given concentration for each extract indicate a significant difference from each other (Duncan multiple range *post hoc* test, $P < 0.05$).

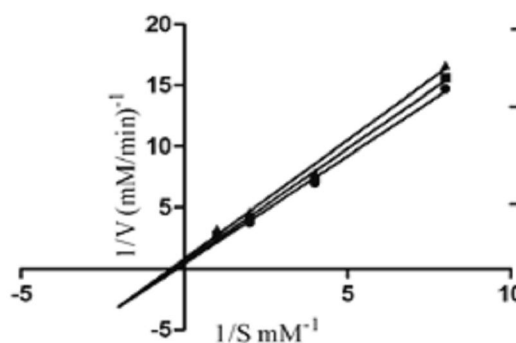


Figure 4. Mode of inhibition of α -glucosidase by hexane extract of leaf of *Helianthus annuus*.

DISCUSSION

The anti-diabetic effect of *H. annuus* leaf of different extracts (acetone, ethyl acetate and hexane) was investigated *in vitro*.

Although, pharmacological activities of medicinal plants are as a result of their chemical components, this is what informed the phytochemical screening of the three extracts of *H. annuus* leaf used in this study. The presence of only alkaloids, cardiac glycosides, tannins and saponins was detected in the hexane extract. Alkaloids have been shown to display wide spectrum antidiabetic potentials by inhibiting diabetes-related enzymes like α -glucosidase and preventing protein glycation [13, 14]. Tannins on the other hand, exhibits insulin-mimetic effect by improving the sensitivity of insulin receptors [15, 16] while cardiac glycosides prevent diabetes-related complications by inhibiting Na^+/K^+ pump, thereby improving cardiac output and preventing cardiovascular diseases [17]. Saponins on the other hand have been reported to having insulin sensitization and anti-hyperlipidemic effects in diabetic state [18, 19].

α -Glucosidase and α -amylase inhibitors have been useful as oral hypoglycemic drugs for the control of hyperglycemia in patients with type 2 diabetes. Inhibitions of these enzymes delay carbohydrate digestion and overall prolong the digestion time causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose [20]. However, all the extracts of *H. annuus* leaf inhibited both α -amylase and α -glucosidase. The highest percentage inhibition of the α -amylase by the hexane extract resulted in its low IC_{50} value ($3.92 \pm 0.02 \text{ mg mL}^{-1}$) which implies that it is the most potent inhibitor of the enzyme out of the three extracts. However, it is undesirable of an

antidiabetic agent to be a strong inhibitor of α -amylase so as to prevent some of the drawbacks of synthetic drugs which might be due to excessive inhibition of the enzyme [21]. The competitive inhibition of α -amylase by the hexane extract of *H. annuus* leaf suggests that the active inhibitory component(s) of the extract are structurally similar to the normal substrate of the enzyme. However, it binds reversibly to the active site of the enzyme and occupies it in a mutually exclusive manner with the substrate [10].

However, for α -glucosidase inhibition, the strong inhibition of the enzyme displayed by the extracts as shown by the IC_{50} (Table 1) values (3.29 ± 0.12 mg mL⁻¹) suggests that hexane extract is a potent α -glucosidase inhibitor. This is in conformity with previous reports that antidiabetic agents from plants are strong inhibitors of α -glucosidase [21, 22]. This implies that hexane extract of *H. annuus* leaf offer better pharmacological effect than the common synthetic drugs. Further study to ascertain the mode of inhibition α -glucosidase (Figure 4) by the hexane extract of *H. annuus* leaf showed the extracts inhibited the enzyme in a mixed non-competitive inhibition. The mixed non-competitive mode of inhibition obtained from the Lineweaver-Burk plot shows that the active components in the hexane extract do not compete with the substrate for binding to the active site rather the inhibitors bind to a separate site on the enzyme to retard the conversion of disaccharides to monosaccharides [23].

CONCLUSION

H. annuus leaf possesses anti-diabetic potential in Wistar rats and the possible mechanism of action in the inhibition of pancreatic α -amylase and α -glucosidase, thereby slowing down the absorption of carbohydrates and preventing hyperglycaemia. The hypoglycaemic activity of this plant may also be due to the presence of alkaloids, tannins and saponins.

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