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Evaluation of *Ficus exasperata* Vahl. Leaf Extracts in the Management of Diabetes Mellitus *in vitro*

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The sharp increase in the incidence and prevalence of diabetes mellitus has led to antidiabetic therapeutic investigations. Therefore, the aim of this study was to evaluate different extracts of *Ficus exasperata* leaves for their inhibitory potential against α -amylase and α -glucosidase activities. Phytochemical screening of the various extracts of *Ficus exasperata* was performed and their inhibitory potential on the activities of α -amylase and α -glucosidase was determined *in vitro*. The results revealed that aqueous extract of *Ficus exasperata* has the lowest IC₅₀ against α -amylase (3.70 mg mL⁻¹) and α -glucosidase (1.70 mg mL⁻¹) which makes it most potent inhibitor compared to the other extracts. Kinetic studies performed on the aqueous extract of *Ficus exasperata* in order to determine its modes of inhibition of the enzymes showed that it is a non-competitive and competitive inhibitor of α -amylase and α -glucosidase respectively. It is proposed that the inhibitory potential of aqueous extract of the plant might be due to the synergistic effect of its phytochemical constituents. Therefore it can be concluded that part of the mechanisms by which *Ficus exasperata* displayed its antidiabetic potential is through the inhibition of α -amylase and α -glucosidase.

Key words: *Ficus exasperata*, diabetes mellitus, α -amylase, α -glucosidase, tannins

INTRODUCTION

Diabetes mellitus is responsible for about 5% of global deaths (WHO, 2005). The underlying symptom of this condition is chronic hyperglycemia which eventually culminates into abnormal fat and protein metabolism due to defects in insulin secretion or action. Type 1 diabetes mellitus result from failure of pancreatic β -cells to produce insulin while the type 2 diabetes mellitus (which is more common) is caused by a decreased sensitivity of target cells to insulin (Burcelin *et al.*, 2010).

The available conventional therapies for diabetes include stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues, administration of oral hypoglycemic agents such as biguanides, thiazolidinediones, sulfonyleureas and α -glucosidase inhibitors (Zimmet, 2009). One of the methods employed to treat diabetes mellitus is the inhibition of carbohydrate-digesting enzymes such as α -amylase and α -glucosidase in the gastro-intestinal tract, thereby slowing down intestinal glucose absorption and decreasing postprandial blood glucose levels (Rhabasa-Lhoret and Chiasson, 2004).

Since, time immemorial, medicinal plants have been employed in the management of many diseases such as malaria, inflammations and diabetes mellitus (Babu *et al.*, 2006). These plants include *Abrus precatorius*, *Ageratum conyzoides*, *Allium sativum*, *Alstonia boonei*, *Bridelia micrantha* and *Ficus exasperata* (Gbolade, 2009). *Ficus exasperata* is a plant found in the tropics and sub-tropics region and it has been found to improve glucose in tolerance as assessed by glucose index (Taiwo *et al.*, 2010). It possesses anti-oxidant, anti-convulsant, anti-arthritic, anti-bacterial, anti-inflammatory, anti-pyretic, antinociceptic, anti-candidal, anti-diabetic and hypotensive properties (Woode *et al.*, 2009). Its leaves has also been reported to have anti-diabetic, lipid-lowering and anti-fungal potentials (Sonibare *et al.*, 2006).

Though, several studies have reported the antidiabetic potential of *Ficus exasperata* (Harati *et al.*, 2003; Taiwo *et al.*, 2010; Adewole *et al.*, 2011) but none has been able to clarify the mechanism by which this plant elicits its hypoglycemic potential. Therefore, the aim of this study was to investigate the α -amylase and α -glucosidase inhibitory potentials of *Ficus exasperata*, as a possible mechanism behind the hypoglycemic action of the plant and its usage in the management of diabetes mellitus.

MATERIALS AND METHODS

Plant material: The leaf of *Ficus exasperata* was obtained from Badagry Area of Lagos in Nigeria in May

2012. It was identified and authenticated by Dr. A. B. Kadiri of the Department of Botany, University of Lagos, Nigeria and voucher specimen (LUH 4720) was deposited in the University herbarium.

Chemicals and reagents: Alpha-amylase from *Aspergillus oryzae*, α -glucosidase from *Saccharomyces cerevisiae* and paranitrophenyl-glucopyranoside were products of Sigma-Adrich Co., St Louis, USA while starch soluble (extra pure) was obtained from J.T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and water used was glass-distilled.

Preparation of plant extracts: Fresh leaves of *Ficus exasperata* were cut and washed with water to remove all contaminants; they were dried under room temperature and grounded to powder. The powdered leaves were divided into three portions and each portion was extracted with acetone, ethanol or water. They were all left to steep in covered containers for 24 h; the resulting infusions were decanted, filtered and evaporated in a rotary evaporator (Cole Parmer SB 1100, Shanghai, China). The extracts were freeze-dried using Virtis Bench Top (SP Scientific Series, USA) freeze dryer. Dried extracts were weighed and dissolved in 10% dimethylsulphoxide to yield a stock solution from which lower concentrations were prepared.

Phytochemical screening: Phytochemical compositions of the leaves were determined using the methods variously described by Trease and Evans (1996) and Sofowara (2006).

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. Few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration as indication of tannins.

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 as indication of saponin.

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 H₂SO₄. The colour change from violet to blue or green is indication of steroids.

Test for terpenoids: In brief, 5 mL of extract was mixed with 2 mL chloroform and 3 mL H₂SO₄ was carefully added to form a layer. A reddish brown colouration of the interface was indication of terpenoids.

Test for anthraquinones: Briefly, 5 mL of chloroform was added to 0.5 g of the powdered plant materials 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.

Alpha-Amylase inhibitory assay: This assay was carried using a modified procedure of McCue and Shetty (2004). A total of 250 µL of extract was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25°C for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition:

$$\text{Inhibition (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}})]}{\text{Abs}_{\text{control}}} \times 100$$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

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.* (2006) Briefly, 250 µL of the (5 mg mL⁻¹) 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 α-amylase was pre-incubated with 250 µL of phosphate buffer (pH 6.9). Also, 250 µL of starch solution at increasing concentrations (0.30-5.0 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 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.

Alpha-Glucosidase inhibitory assay: The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim *et al.* (2005), using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. 100 µL of α-glucosidase (E.C. 3.2.1.20) 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 then added to start the reaction. The reaction mixture was incubated at 37°C for 20 min 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 were expressed as percentage of the blank control.

Percentage inhibition calculated as:

$$\text{Inhibition (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}})]}{\text{Abs}_{\text{control}}} \times 100$$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

Mode of α-glucosidase inhibition: The mode of inhibition of the extracts was determined using the extract with the lowest IC₅₀ according to the modified method described by Ali *et al.* (2006). Briefly, 50 µL of the (5 mg mL⁻¹) extract was pre-incubated with 100 µL of α-glucosidase solution for 10 min at 25 °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 (0.63-2.0 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 500 µL of Na₂CO₃ 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 ± SE for triplicate determinations.

RESULTS

From Table 1 which shows the yield of various leaf extracts, ethanolic extract of *Ficus exasperata* has the highest yield of 9.47% compared to the other extracts. The phytochemical constituent of each extract of *Ficus exasperata* was tested for the presence of anthraquinones, flavonoids, reducing sugar, saponins, steroids, tannins, and terpenoids (Table 2). However, it is only reducing sugar was present in all of the three extracts whereas anthraquinone was not detected in any these extracts. Ethanolic extract of *Ficus exasperata* has more phytochemical constituent when compared with acetone and aqueous extracts of *Ficus exasperata*. However, saponins were only detected in the aqueous extract of the plant.

Figure 1 shows the percentage inhibition of α-amylase activity by acetone, ethanol and aqueous extracts of *Ficus exasperata*. At concentrations of 0.63 and 2.50 mg mL⁻¹, the values obtained from the three extracts are not significantly different from one another (p > 0.05). At all the concentrations tested except at 5 mg mL⁻¹, acetone extract exhibited the highest percentage inhibition of the enzyme while aqueous displayed the highest value at 5 mg mL⁻¹. The IC₅₀ values generated from the percentage inhibition reveals that out of all the extracts, aqueous extract of *Ficus exasperata* has the lowest IC₅₀ value (3.70 mg mL⁻¹) (Table 3). Kinetic analysis of the α-amylase inhibition by aqueous extract of *Ficus exasperata* using Lineweaver-burke plot shows that it displayed a non-competitive mode of inhibition (Fig. 2).

Figure 3 shows the percentage inhibition of α-amylase activity by acetone, ethanol and aqueous extracts

of *Ficus exasperata*. At all concentrations tested (1.25-10.00 mg mL⁻¹), aqueous extract of *Ficus exasperata* displayed significantly higher inhibition (p < 0.05) from acetone and ethanolic extracts. This was corroborated by the lowest IC₅₀ value (1.70 mg mL⁻¹) generated by the aqueous extract compared to the other extracts (Table 3). This is because the lower the IC₅₀, the more potent the extract will be for treatment of the disease. The kinetics of inhibition by the aqueous extract of *Ficus exasperata*

Table 1: The percentage yield of different extracts of *Ficus exasperata* leaf

| Extracts | Initial weight (g) | Final weight (g) | Yield (%) |
|----------|--------------------|------------------|-----------|
| Acetone | 44.83 | 0.82 | 1.83 |
| Ethanol | 47.62 | 4.51 | 9.47 |
| Water | 37.93 | 1.15 | 3.03 |

Table 2: The phytochemical constituent of different extracts of *Ficus exasperata* leaf

| Phytochemicals | Extracts inference | | |
|----------------|--------------------|---------|-------|
| | Acetone | Ethanol | Water |
| Anthraquinones | - | - | - |
| Flavonoids | - | + | + |
| Reducing sugar | + | + | + |
| Saponins | - | - | + |
| Steroids | + | + | - |
| Tannins | - | + | + |
| Terpenoids | + | + | - |

+: Present, -: Not detected

Table 3: IC₅₀ values of various extracts of *F. exasperata* against α-amylase and α-glucosidase

| Extracts | IC ₅₀ (mg mL ⁻¹) | |
|----------|---|---------------|
| | α-amylase | α-glucosidase |
| Acetone | 5.45±0.24 | 21.50±1.87 |
| Ethanol | 8.55±0.50 | 18.25±2.25 |
| Water | 3.70±0.12 | 1.70±0.03 |

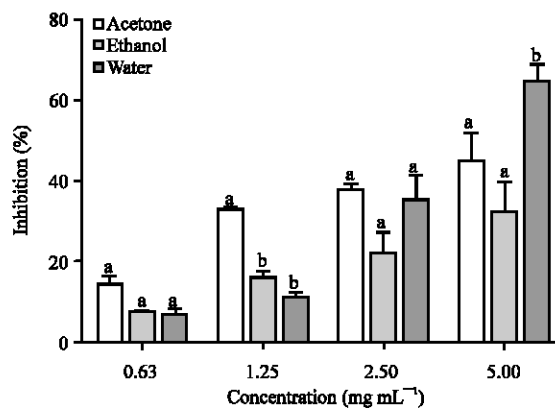


Fig. 1: Percentage inhibition of α-amylase by different extracts of *Ficus exasperata*. Bars carrying different letters at the same concentration are significantly different

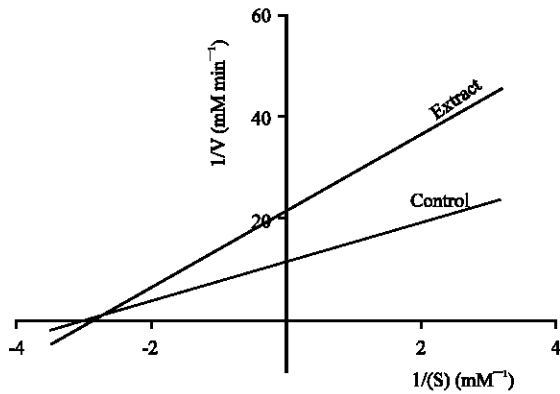


Fig. 2: Mode of inhibition of α -amylase by aqueous extract of *Ficus exasperata*

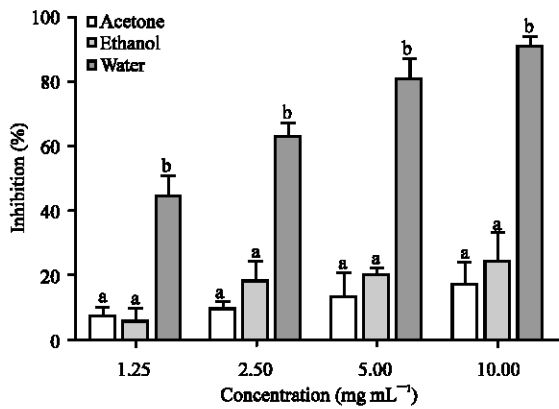


Fig. 3: Percentage inhibition of α -glucosidase by different extracts of *Ficus exasperata* Bars carrying different letters at the same concentration are significantly different

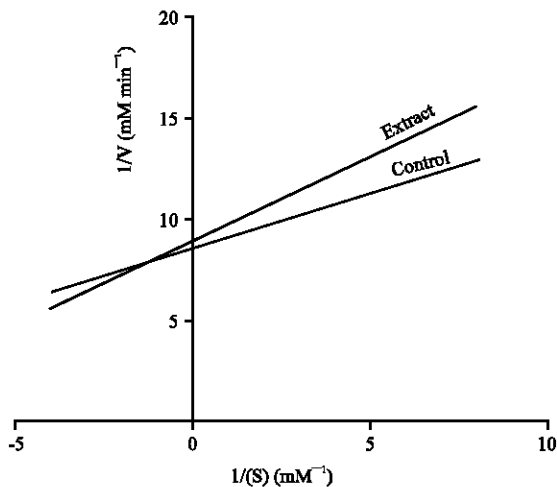


Fig. 4: Mode of inhibition of α -glucosidase by acetone extract of *Ficus exasperata*

against α -glucosidase using Lineweaver-Burke plot reveals that the mode of inhibition of the enzyme is near competitive (Fig. 4).

DISCUSSION

Hyperglycemia is a state characterized by an abnormal postprandial increase of blood glucose level and it has been linked to the onset of type 2 and associated vascular complications (Dicarli *et al.*, 2003). Recent studies have indicated that hyperglycemia induced vascular complications are likely from oxidative dysfunction from reactive oxygen species (ROS) produced by the mitochondrial electron transport chain (Kwon *et al.*, 2007).

In order to manage diabetes, various inhibitors of disaccharide hydrolysing enzymes (α -amylase and α -glucosidase) had been used as oral hypoglycemic agents especially in patients with type 2 diabetes mellitus (Oboh *et al.*, 2012). Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion which causes reduction in the rate of glucose absorption and consequently reducing the postprandial blood glucose rise (Kwon *et al.*, 2007).

From this study, the results of the enzymes (α -amylase and α -glucosidase) inhibitory assay showed that the aqueous extract of *Ficus exasperata* is a mild inhibitor of α -amylase and strong inhibitor of α -glucosidase. This however, is in agreement with earlier reports that plant phytochemicals are mild inhibitors of α -amylase and strong inhibitors of α -glucosidase activity with minimal side effect (Kwon *et al.*, 2007). This has an advantage over synthetic drugs such as acarbose; used by diabetics in the management of postprandial blood glucose which strongly inhibit α -amylase (Oboh *et al.*, 2012). Strong inhibition of both α -amylase and α -glucosidase by acarbose causes indigested starch-linked complications such as abdominal distention, flatulence, meteorism and possibly diarrhoea (Pinto *et al.*, 2009) which results to abnormal bacteria fermentation of undigested carbohydrate in the colon (Kwon *et al.*, 2007).

The characteristic non-competitive inhibition displayed by the aqueous extract of this plant towards α -amylase indicates that aqueous extract of *Ficus exasperata* binds to a site other than the active site of the enzyme and combines with either free enzyme or the enzyme-substrate complex, possibly interfering with the action of both (Mayur *et al.*, 2010). Lineweaver-Burke plot also showed that aqueous extract of this plant inhibited α -glucosidase competitively. This suggests that the active components in the extract compete with the substrate for binding to the active site of the enzyme

thereby preventing the breaking down of oligosaccharides to disaccharides (Matsuda *et al.*, 2002; Shai *et al.*, 2010).

The presence of phytochemicals such as flavonoids, saponins and tannins may be responsible for the inhibitory potential of this plant (aqueous) extract on the activities of the enzymes studied. These phenolic compounds can protect the human from free radicals, chelate metal catalysts, activate antioxidant enzymes and inhibit the action of oxidases (Oboh *et al.*, 2012). Flavonoids is one of the most diverse and widespread group of natural compounds, having hydroxyl group present in it which confers scavenging ability and also plays an important role in preventing lipid peroxidation (Mayur *et al.*, 2010). They may preserve beta cell function by reducing oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes (Song *et al.*, 2005). On the other hand, tannin, which is composed of a central glucose molecule derivatized at its hydroxyl groups with one or more galloyl residue and in the presence of copper ions, act as an antioxidant suppressing hydroxyl radical formation (Andrade *et al.*, 2005).

CONCLUSION

It can be concluded from this study that only aqueous extract of *Ficus exasperata* exhibited the most effective inhibition of α -amylase and α -glucosidase and that these enzymes were inhibited non-competitively and competitively respectively. The study also suggests that the inhibitory potential of the aqueous extract of *Ficus exasperata* on the two enzymes may not be unconnected to the presence of phytochemicals like flavonoids, tannins and saponins. Therefore, this study suggests that one of the mechanisms of antidiabetic potential of *Ficus exasperata* is through the inhibition of α -amylase and α -glucosidase.

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