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EVALUATION OF THE EFFECTS OF *FICUS CAPENSIS* LEAF EXTRACT ON α-AMYLASE AND α-GLUCOSIDASE ENZYME ACTIVITY

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ABSTRACT

Medicinal plants have been generally employed as sources of natural drugs for decades. The α-amylase and α-glucosidase enzyme activities were assayed using standard methods. *F. capensis* leaves were harvested, washed, dried to constant weight, and ground to a coarse powder. The leaf extracts were prepared by soaking 100 grams of the dried powder in 1000 ml of ethanol or methanol for 72 hours with gentle stirring. Each was filtered and filtrates evaporated to dryness. The residues were dissolved in 0.02M Na phosphate buffer (pH 6.9) and used as stock solutions (10mg/ml) of ethanol and methanol extracts, respectively. The results showed both ethanol and methanol extracts exhibited potent inhibition of α-amylase (IC_{50} of 1.71 ± 0.04 mg/ml and 1.66 ± 0.007 mg/ml respectively) and α-glucosidase (IC_{50} of 0.91± 0.01 mg/ml and 1.01± 0.02 mg/ml respectively). Statistical analysis at ($p<0.05$), showed no significant difference between the percentage inhibition by ethanol and methanol extracts on either α-amylase or α-glucosidase activity. There was however a significant increase in the percentage inhibition by both ethanol and methanol extracts on α-amylase and α-glucosidase at ($p<0.05$), when compared with the inhibition of these enzymes by acarbose standard. This demonstrates that *F. capensis* can be used in management of postprandial hyperglycaemia in type II diabetes.

INTRODUCTION

Medicinal plants are used right from ancient times as they serve as very good source of drugs due to their high efficacy, ready availability at reduced cost and adverse effects (Gauresh *et al*., 2012).The use of herbs in prevention and management of various diseases is increasingly gaining acceptance not only in rural communities but also in urban cities (Oreagba *et al.,* 2011). This popularity is not necessarily based on any proven scientific evidence but often times, on acclaimed medicinal benefits of such herbs (Picking, 2011). Nevertheless, many of these herbs contain phytochemical constituents like saponins, phenols and flavonoids with useful bioactivities. Such phytochemicals in plants like *Euphorbia hirta* and *Cassia glauca* account for their potential antioxidant and other bioactivities which can be employed in the management of oxidative stress and chronic human diseases like cancer, hypertension and diabetes mellitus that are closely associated with it (Mitra *et al*., 2007).

Among the group of plants endowed with enormous potential for management of various diseases are the *Ficus* species. *Ficus religiosa* has been employed locally in the treatment of asthma, haematuria, cough and diarrhoea among other diseases (Damanpreet *et al.*, 2009). Research has shown that *F. religiosa* extract has anticholinesterase activity and methanol extract of the fruits exhibit anticonvulsant activity (Damanpreet *et al.,* 2009). *F. capensis* has been used both as antispasmodic and antisickling drug (Ayinde et *al.,* 2009). In Nigeria, *F. capensis* has been used by the Igede people as *treatment* for dysentery and in wound dressing (Igoli *et al.*, 2005). It is also used in circumcision, leprosy and epilepsy (Joshua, 2006).The need to affirm that *F. capensis* may well be useful in management and prevention of diabetes mellitus and other chronic diseases related to oxidative stress is the major motivating factor for this research.

F. capensis commonly known as the fig tree is a medicinal plant found in terrestrial zones mostly along rivers (Joshua, 2006). It belongs to the family Moroaceae. *F. capensis* is a spreading deciduous or evergreen tree with a thick bole and spreading roots (Juurlink, 2001). It produces fruits throughout the year and the leaves are broad and green. In comparison to other plants, the flowers are relatively inconspicuous. It produces both male and female flowers in a special hollow, pear-shaped inflorescence called syconia. Many people refer to this inflorescence as a fig (Adebayo-Tayo and Odeniyi, 2012). The fig has an ostiole or small opening at its tip, which is the site of entrance by a tiny stringless wasp called the fig wasp that serves as its sole pollinator (Njoku-oji et *al.,* 2015).

MATERIALS AND METHODS

Collection of plant materials; Sample preparation

Large quantity of *F. capensis* leaves were collected and thoroughly washed with running water. The leaves were air-dried to constant weight and then ground to very fine powder. The powder was then stored in a cool dry container for subsequent use.

Reagents and chemicals

The following reagents and chemicals were purchased and used for this research; alpha amylase enzyme from

Aspergillus oryzae, alpha glucosidase enzyme from *Saccharomyces cerevisiae*, methanol, starch, acarbose, DNSA (3,5-dinitrosalicylic acid), sodium phosphate buffer (sodium dihydrogen phosphate and disodium hydrogen phosphate), PNPG (p-nitrophenol-α-d-glucopyranoside), sodium carbonate, sodium hydroxide and distilled water.

The chemicals and reagents were purchased from Sigma-Aldrich Co. St Louis USA.

Extraction procedures; Ethanol and Methanol extraction

A crude ethanol and methanol extract was prepared by soaking 100 grams of the dried leaf powder of *F. capensis* in 1litre of ethanol or methanol for 72 hours with gentle stirring. They were then filtered using a Whatman No. 4 filter paper. The filtrates were allowed to stand in open air in the laboratory for a few days until they completely evaporated each leaving a small oily residue at the bottom. These residues were then dissolved in 100 ml of sodium phosphate buffer and used subsequently as stock solution for either the ethanol or methanol extract respectively.

Alpha amylase activity inhibition assay

The assay of α-amylase activity was carried out using the modified starch-iodine change method described by Mahomoodally *et al.* (2012) and Kotowaroo *et al*. (2006). Into a test tube containing 100 µl α-amylase (0.5U/ml) in 0.02 M sodium phosphate buffer (pH 6.9) was added 3ml soluble starch solution (1g soluble starch was suspended into 10 ml distilled water and boiled for 2 mins. The volume was made up to 100 ml with distilled water. The starch solution was stored in the refrigerator and used within 2-3 days) and then 2ml 0.02M sodium phosphate buffer (pH 6.9). The reaction mixture was incubated at 37^{o}C for 1 hour. Aliquots of 0.1 ml were taken at time interval of t = 0, 30 minutes and 1hour and discharged into10 ml of iodine solution, mixed thoroughly and the absorbance measured at 565 nm. One unit of enzyme inhibitor is that which reduced α-amylase activity by one unit as defined by $\{(A_0 - A_t)\}/A_0$ x 100; A_0 and at being absorbances of starch-iodine solution at t = 0 and t = 60 min, respectively (Mahomoodally *et al*., 2012). This served as the control test.

The inhibitory effect of graded concentrations of various extracts of *F. capensis* was carried out as follows. In a set of test tubes, 100 µL of ethanol and methanol extract were pre-incubated with 100 µl α-amylase solution (0.5U/ml) at 37^{o}C for 15 minutes. The final concentrations of the extract in the reaction mixtures were 2mg/ml, 4mg/ml, 6mg/ml, 8mg/lml and 10mg/ml. The reaction was started in each tube at intervals of 2 minutes by addition of 3 ml of soluble starch solution followed by 2 ml of 0.02 M sodium phosphate buffer (pH 6.9). The reaction mixture was thoroughly mixed. Then 0.1 ml of the mixture was drawn and discharged into 10 ml iodine solution mixed thoroughly and the absorbance measured at 565 nm. This procedure was repeated at timed interval of 30 minutes and 1hour for each concentration of the extract used. The test was repeated three times for each concentration of the extract and the average absorbance taken. The α-amylase inhibitory activity was calculated using the following equation;

$$\%\text{Inhibition} = \frac{Abs\ control - Abs\ extract}{Abs\ control} \times \frac{100}{1}$$

The concentration of the extract leading to 50% inhibition of enzyme activity ($1C_{50}$) was determined graphically.

Alpha glucosidase activity inhibition assay

The inhibitory activity of various leaves extract of *F. capensis* on the activity of α-glucosidase was determined according to the method described by Kim *et al*. (2005) using α-glucosidase from *Saccharomyces cerevisiae*. The reaction involved preincubating 50µl α-glucosidase (1.0U/ml) with 50 µL of the different concentrations of the extracts with final concentration ranges of 2mg/ml to 10mg/ml for 10 minutes. Then 50 µl of 3.0 mM (pNPG) as substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was further incubated at 37ºC for 20 minutes and stopped by adding 2 ml of 0.1M Na_2C0_3. The α-glucosidase activity was determined by measuring the yellow-coloured paranitrophenol released from pNPG by the enzyme at 405 nm. The results were expressed as percentage of the blank control.

Percentage inhibition is calculated as follows;

$$\%\ \text{Inhibition} = [\{Abs_{\text{control}} - Abs_{\text{extract}}\} / Abs_{\text{control}}] \times 100$$

The concentration of the extract leading to 50% inhibition of enzyme activity (IC_{50}) was determined graphically. Acarbose was used as reference and $1C_{50}$ was determined graphically.

Statistical analysis of results

The basic statistical analysis of data was carried out using Microsoft excel package. Charts were used to represent the data of the percentage inhibition of the extracts on alpha amylase and alpha glucosidase. Comparative analysis was also carried out on the level of inhibition of alpha amylase and alpha glucosidase activities by the methanol and ethanol extracts of *F. capensis* in comparison with the control (acarbose). This was done using the independent t - test (assuming unequal variances at $\alpha = 0.05$).

RESULTS

Result of alpha amylase inhibition assay

The methanol extract of *Ficus capensis* leaf exhibited stronger inhibitory effects against α-amylase than the ethanol extract (Table 1). The IC_{50} for methanol and ethanol extracts are 1.66± 0.007 mg/ml and1.75± 0.04 mg/ml, respectively. This indicates that the concentration of ethanol extract required to achieve 50% inhibition of α-amylase activity was slightly higher than that of the methanol extract needed to achieve the same purpose. The IC_{50} of acarbose for α-amylase is 4.366±0.02 mg/ml (Table 1) The inhibitory effectiveness of Ficus *capensis* extracts were greater than that of the standard inhibitor acarbose.

Table 1: Percentage inhibition of α-amylase by acarbose, methanol and ethanol leaf extracts of *Ficus capensis* and their IC_{50} values (tests were performed in triplicates and average taken)

Conc of acarbose ethanol or methanol extract (mg/ml)	**Inhibition Ethanol extract (%)**	**IC_{50} of Ethanol Extract (mg/ml)**	**Inhibition Methanol extract (%)**	**IC_{50} of methanol extract (mg/ml)**	**Inhibition Acarbose standard (%)**	**IC_{50} of Acarbose standard (mg/ml)**
0.00	0.00		0.00		0.00	
2.00	82.59		84.00		56.00	
4.00	83.73	1.75±0.04	85.30	1.60±0.70	60.20	4.36±0.02
6.00	86.10		86.20		65.00	
8.00	87.53		90.20		67.00	
10.00	87.10		90.40		74.00	

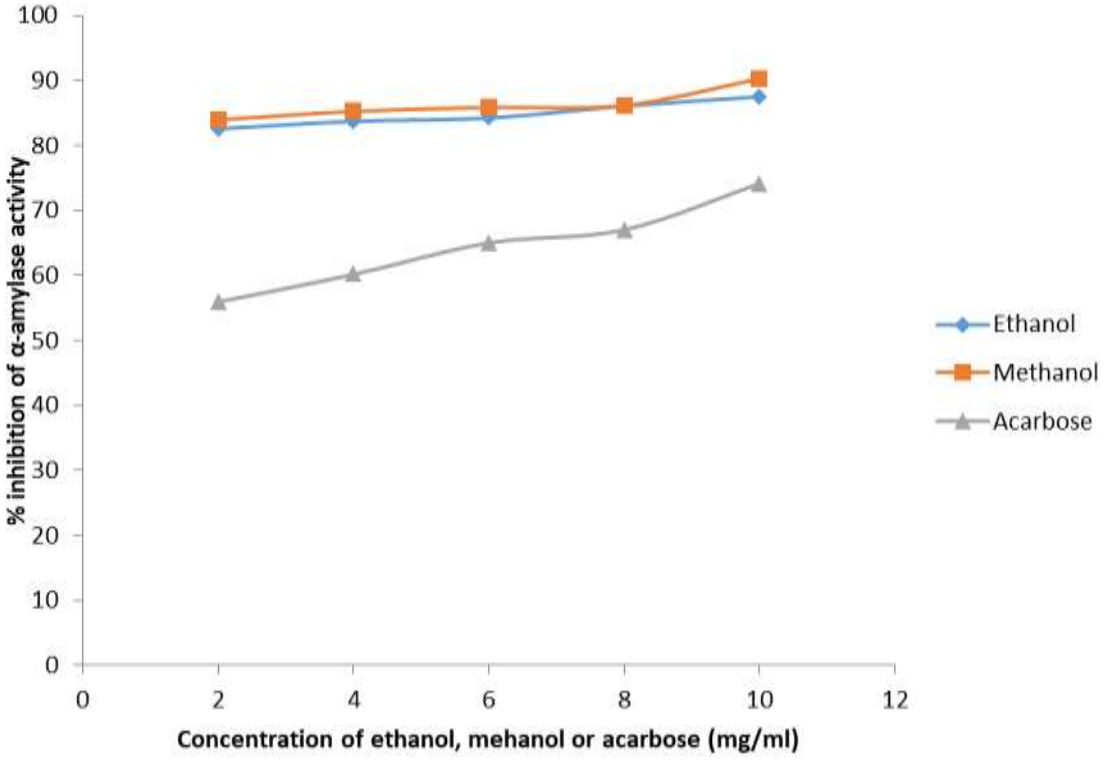


Figure 1: Inhibition of α-amylase activity by various concentrations of acarbose, ethanol and methanol leaf extracts of *Ficus capensis*.

Alpha glucosidase inhibition assay result

The ethanol and methanol leaf extracts of *Ficus capensis* exhibited stronger inhibitory activities against α-glucosidase activity compared to the standard inhibitor acarbose (Figure 2). The IC_{50} of ethanol and methanol leaf extract of *Ficus capensis* were 0.91 ± 0.01 mg/ml and 1.01 ± 0.02 mg/ml, respectively while that of acarbose is 11.13±0.01 (Table 2). This implies that a much lower concentration of the extracts was needed to achieve 50% of inhibition of enzyme activity when compared to acarbose. This invariably means that the leaf extracts of *Ficus capensis* are more potent inhibitors of α-glucosidase compared to acarbose.

Table 2: Table showing percentage inhibition of α-glucosidase enzyme activity by acarbose (std), ethanol and methanol extracts of *Ficus capensis* leaf. (tests were done in triplicates and the average taken)

Conc of acarbose, ethanol or methanol extract (mg/ml)	**Inhibition by Ethanol extract (%)**	**IC_{50} of Ethanol extract (mg/ml)**	**Inhibition by Methanol extract (%)**	**IC_{50} of Methanol extract (mg/ml)**	**Inhibition by Acarbose standard (%)**	**IC_{50} of Acarbose standard (mg/ml)**
0.00	0.00		0.00		0.00	
2.00	90.46		90.29		5.00	
4.00	92.13	0.91±0.01	90.65	1.01±0.02	32.00	11.13±0.01
6.00	93.50		90.74		34.00	
8.00	90.40		90.92		39.99	
10.00	87.26		87.62		37.00	

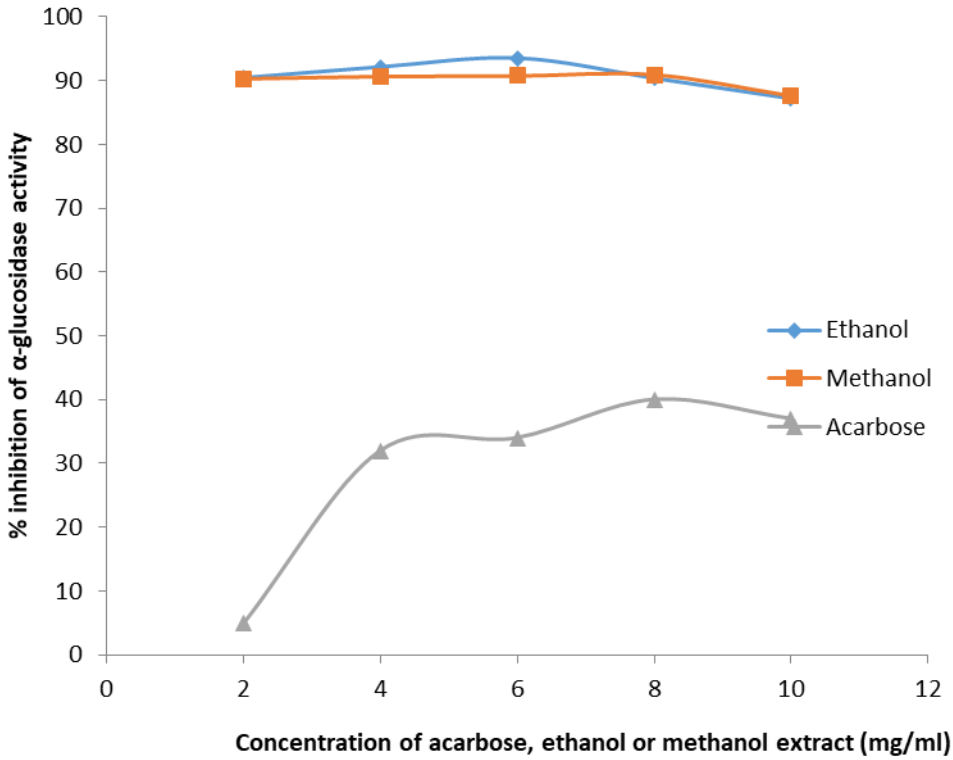


Figure 2: Inhibition of alpha-glucosidase activity by various concentrations of acarbose, ethanol and methanol leaf extract of *Ficus capensis*.

DISCUSSION

Management of blood glucose level was a critical strategy in the control of diabetes mellitus and its complications. The α-amylase and α-glucosidase inhibitors have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type II diabetes mellitus. Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time. This leads to a reduction in the rate of glucose release from complex carbohydrates, its absorption and consequently brings about a reduction in postprandial glucose rise.

The result of this study showed that *Ficus capensis* leaf extract inhibited both α-amylase and α-glucosidase enzyme activities very strongly. These are enzymes involved in carbohydrate hydrolysis. Many inhibitors of these enzymes like acarbose, miglitol and voglibiose are known to be useful in the control of postprandial glucose concentration which is the major problem in type II diabetes as they delay the release of glucose from disaccharides and oligosaccharides and so retard glucose absorption from the gut. By virtue of its antioxidant and enzyme (α-amylase and α-glucosidase) inhibitory capabilities, *F. capensis* doubles as a preventive measure for many diseases and as management therapy for diabetes mellitus.

The high percentage inhibition of α-amylase by the extracts of *F. capensis* leaf showed that they may contain very potent α-amylase inhibitor substances. This means that the plant *Ficus capensis* may essentially contain herbal bio-active compounds that cause inhibition of the enzyme activity.

When subjected to statistical analysis, there was no significant difference between the percentage inhibition by the methanol and ethanol extracts in alpha amylase activity ($p>0.05$). There was, however, a significant difference in the increase between the percentage inhibition of α-amylase activity by methanol extract of *F. capensis* leaves compared with that of the control (acarbose). Similarly, there was a significant difference in the increase between the percentage inhibition of ethanol extract and acarbose (standard) in the activities of α-amylase. Hence, the inhibitor substances in both extracts are more potent inhibitors of α-amylase activity than the standard inhibitor, acarbose. Similarly, the inhibitory effectiveness is far greater compared with that of acarbose.

The high percentage inhibitions of α-glucosidase by the extracts (methanol and ethanol), suggest that these extracts also contain potent α-glucosidase inhibitor component. These extracts therefore have the potential to function as good antidiabetic drugs for the control of postprandial hyperglycaemia with minimal side effects.

When subjected to statistical analysis at ($p>0.05$), there was no significant difference between percentage inhibition of the methanol and ethanol extracts of *Ficus capensis* against α-glucosidase enzyme activity. There was however a significant increase in the percentage inhibition of the enzyme, α-glucosidase ($p> 0.05$) by the ethanol extract when compared with the control (acarbose). Also, there was a significant increase in the percentage inhibition of α-glucosidase by the methanol extract compared with acarbose. Again, this showed that the α-glucosidase enzyme inhibitor component of the extract is far more potent than the standard inhibitor, acarbose. There was also significant difference in the inhibitory effectiveness (IC_{50}).

It was also observed statistically that at $p>0.05$, on the average, there was no significant difference between the percentage inhibition of alpha amylase and alpha glucosidase by the methanol leaf extract of *Ficus capensis*. But, there was a significant difference in the increase between the percentage inhibition of alpha glucosidase and alpha amylase by the

ethanol extract of *Ficus capensis*. Inherent from the foregoing was that the inhibitory effectiveness of the methanol extract of *Ficus capensis* was about equal for both α-amylase (IC_{50}, 1.66 ±0.007 mg/ml) and α-glucosidase (IC_{50}, 1.01±0.02 mg/ml). On the contrary, the inhibitory effectiveness of the ethanol extract was far greater for α-glucosidase (IC_{50}, 0.91±0.01 mg/ml) than for α-amylase ($IC_{50,}$1.75. ± 0.04 mg/ml). The likely implication of this is that the ethanol extract of *Ficus capensis* leaf may well be a better tolerated antidiabetic drug than the methanol extract with relatively reduced side effects (Kwon *et al.,* 2007; Mongaleet *et al*., 2011). This is because, much smaller concentration of the ethanol extract is required to achieve substantial inhibition of the α-glucosidase as distinct from α-amylase. At such low concentration, the inhibition of α-amylase activity will be relatively small. Hence the adverse gastrointestinal symptoms like bloating of the abdomen, flatulence and belching usually attributable to strong inhibition of α-amylase activity will be reduced (Oboh *et al.,* 2010). Much of the complex carbohydrates will be broken down to dextrin, maltose and maltotriose by α-amylase whose activity remains reasonably high on account of its minimal inhibition by low concentration of the ethanol extract. Relatively very few complex carbohydrates will be left for the intestinal flora to act upon, and troublesome gastrointestinal symptoms are averted or becomes minimal (Oboh *et al.,* 2010). Patients are likely to tolerate such drugs more and compliance to treatment will likely improve.

CONCLUSION

The methanol and ethanol extracts inhibit α-glucosidase strongly and both can function as potential antidiabetic drugs for the control of postprandial hyperglycaemia especially in type II diabetes. Nevertheless, the ethanol extract promises to be the drug of preference given its high potency and the possibility of less severe adverse effects which may guarantee greater patient compliance .This inhibitory property of the extract may be attributed to the presence of phytochemicals such as Flavonoids and Saponins.

It has been shown that the leaf of *Ficus capensis* is rich in nutrients and have the ability to scavenge free radicals. The free radical scavenging property of *Ficus capensis* leaf may prove to be useful in the prevention and management of oxidative stress and other chronic diseases associated with it. This coupled with the ability to inhibit alpha amylase and alpha glucosidase demonstrates that *Ficus capensis* may find use in the management and prevention of diabetes.

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