

## Inhibitory Effects of *Oncoba Spinosa* on Key Enzymes Related to Diabetes Mellitus ( $\alpha$ -Amylase and $\alpha$ -Glucosidase) and Obesity (Pancreatic Lipase) *in Vitro*

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### Abstract

**Aim and Objective:** *Oncoba spinosa* Forssk. (Flacourtiaceae) (OS) is traditionally used for the management of diabetes mellitus. In previous studies, we studied the effect of OS on  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition, however the effect of OS on pancreatic lipase inhibition and kinetics of  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase inhibition were not studied. This study determined the *in vitro* inhibitory effects of OS against pancreatic lipase and characterized the kinetics of  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase inhibition.

**Materials and Methods:** Dried powdered roots were successively extracted with various solvents such as petroleum ether, chloroform, ethyl acetate, and 70% ethanol. The successive extracts obtained (62.5-1000  $\mu$ g/ml) were subjected to *in vitro* pancreatic lipase inhibitory assay and the most active extract was selected for kinetic studies.

**Results:** Of all the successive extracts studied, the ethanolic extract showed highest pancreatic lipase inhibitory activity. The ethanolic extract showed mixed type of inhibition against  $\alpha$ -amylase and pancreatic lipase whereas non-competitive inhibition against  $\alpha$ -glucosidase.

**Conclusion:** The data of this study suggests that OS inhibits amylase, glucosidase and lipase activities, which leads to a reduction in the intestinal absorption of carbohydrates and lipids.

**Keywords:** *Oncoba spinosa*; Diabetes mellitus;  $\alpha$ -glucosidase;  $\alpha$ -amylase; Pancreatic lipase; Kinetics

### Introduction

Obesity and diabetes reduce quality of life as well as shorten lifespan. As a result, huge sums of money have been spent by individuals and governments to cure these diseases [1]. Acarbose and miglitol are the two currently marketed glucosidase inhibitors for the treatment of type 2 diabetes mellitus. Two major side effects-diarrhoea and abdominal pain- have limited their use in the clinical arena [2]. Orlistat, a pancreatic lipase inhibitor, is the only drug approved by the U.S. Food and Drug Administration for long-term treatment of obesity. However, orlistat may produce undesirable adverse events such as steatorrhea, fecal urgency or incontinence, and bowel movement urgency [3]. For these reasons several research groups addressed their activity on the discovery of natural products with inhibitory potential on key enzymes related to type 2 diabetes and obesity.

*Oncoba spinosa* Forssk (OS) belonging to the family Flacourtiaceae is a small tree of about 13 m high which grows under conditions of higher rainfall, of deciduous, secondary and fringing forest from Senegal to West Cameroon, and widely distributed in tropical Africa and Arabia [4]. Ethnopharmacological data has revealed that OS has

been extensively used for the treatment of diabetes and cancer in Nigeria region [5]; leaves and roots are used in African countries for the treatment of urethral discharges, infertility, epilepsy, dysentery and bladder conditions [6-8]; fruits are used for the treatment of syphilis, wounds, parasitic worms, and sexual impotence [9].  $\alpha$ -glucosidase inhibitory, radical scavenging and cytotoxicity activities of OS leaves have been reported. Earlier studies carried out by us have shown that ethanolic extract of OS roots demonstrated highest  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition when compared to other extracts studied [10]. Previous studies did not incorporate the inhibitory activity of the extracts on pancreatic lipase and the mode of inhibition on  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase. We report here the pancreatic lipase inhibitory activity of the extract and kinetics of all three enzymes  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase action of the plant extract.

### Materials and Methods

#### Chemicals

$\alpha$ -amylase, porcine pancreatic lipase, PNPG (4-nitrophenyl  $\alpha$ -d-glucopyranoside) and  $\alpha$ -glucosidase were purchased from Sigma Aldrich, USA. Other chemicals and reagents were purchased from Merck.

## Collection and identification of the plant materials

The roots of *Oncoba spinosa* were collected from Chittoor district, Andhra Pradesh and positively identified by Dr. Madhava chetty, Botanist, S.V. University, Tirupathi. A voucher specimen of the collected sample was deposited in the herbarium of the institution for future reference.

## Preparation of the extract

The roots of the plant were shade-dried, and powdered. The powdered material was sequentially extracted with petroleum ether, chloroform, ethyl acetate, and 70% ethanol for 72 h using maceration to produce the respective extracts. All the extracts were vacuum dried to obtain petroleum ether extract (PEOS), chloroform extract (CEOS), ethyl acetate extract (EAOS) and ethanol extract (EEOS) respectively.

## Pancreatic lipase inhibition

The pancreatic lipase inhibitory activity was determined as described by Bustanji et al 2010 with modifications [11]. A volume of 50  $\mu$ l pancreatic lipase solution (1 mg/ml in 2.5 mM tris-hydrochloride buffer pH 7.4 with 0.125 mM sodium chloride) was pre-mixed with 100  $\mu$ l extract (initial concentrations used ranged from 62.5-1000  $\mu$ g/ml for successive extracts) and incubated at 37°C for 15 min. Following pre-incubation, 100  $\mu$ l p-nitrophenyl butyrate (PNPB) (1.25 mM) was added to the enzyme-extract mixture and the volume was made up to 300  $\mu$ l using tris-hydrochloride buffer. The reaction mixture was incubated at 37°C for 60 min and the amount of p-nitrophenol released was measured at 405 nm. The concentration of sample required to inhibit pancreatic lipase activity by 50% (IC<sub>50</sub>) under assay conditions was calculated from the percentage inhibition values.

## $\alpha$ -Amylase kinetic studies

The mode of inhibition on the root extract was determined as described by Mohammed et al 2017 with slight modifications [12]. The extract with the lowest IC<sub>50</sub> value in our previous studies was selected for the study. The inhibition mode of the extract on  $\alpha$ -amylase was studied with increasing concentrations (0.063-1%) of starch substrate, in the presence and absence of the extract. 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 ethanolic extract on  $\alpha$ -amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

## $\alpha$ -Glucosidase kinetic studies

The mode of inhibition of the root extract was determined as described by Mohammed et al. with slight modifications [12]. The extract with the lowest IC<sub>50</sub> value in our previous studies was selected for the study. The inhibition mode of the extract on  $\alpha$ -glucosidase was studied with increasing concentrations (0.313-5 mM) of PNPB substrate, in the presence and absence of the extract. 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 ethanolic extract on  $\alpha$ -glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

## Pancreatic lipase kinetic studies

The extract with the lowest IC<sub>50</sub> value was selected for the study. The inhibition mode of the extract on pancreatic lipase was assayed with increasing concentrations (0.125-1.25 mM) of synthetic substrate, p-nitrophenyl butyrate (PNPB), in the presence and absence of the extract. 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 ethanolic extract on pancreatic lipase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

## Statistical analysis

The data were expressed as the mean  $\pm$  SEM of three replicates. Analysis was performed using Graphpad Software and Excel 2010. Values were considered significantly different at  $p < 0.05$ .

## Results

### Pancreatic lipase inhibition

Test Substance	% Inhibition Concentration ( $\mu$ g/ml)					IC <sub>50</sub> ( $\mu$ g/ml)
	62.5 $\mu$ g/ml	125 $\mu$ g/ml	250 $\mu$ g/ml	500 $\mu$ g/ml	1000 $\mu$ g/ml	
PEOS	-	-	-	-	-	-
CEOS	5.03 $\pm$ 1.21	8.33 $\pm$ 0.63	12.23 $\pm$ 1.00	20.26 $\pm$ 1.33	27.73 $\pm$ 1.70	>1000
EAOS	8.18 $\pm$ 1.01	11.13 $\pm$ 1.15	26.60 $\pm$ 0.72	35.93 $\pm$ 0.85	53.13 $\pm$ 0.39	909.46 $\pm$ 5.88
EEOS	12.43 $\pm$ 1.06	22.53 $\pm$ 1.27	39.13 $\pm$ 1.14	52.83 $\pm$ 0.31	70.40 $\pm$ 0.66	447.57 $\pm$ 6.97
Orlistat (Standard)	28.76 $\pm$ 0.86	37.60 $\pm$ 0.52	46.13 $\pm$ 0.44	59.36 $\pm$ 0.56	72.70 $\pm$ 1.15	322.98 $\pm$ 6.80

Values are expressed as mean  $\pm$  SEM of triplicate measurement.

**Table 1:** Pancreatic lipase inhibitory activity of *O. spinosa* root extracts.

The petroleum ether extract failed to inhibit pancreatic lipase activity. The pancreatic lipase inhibitory effect of CEOS was found to be ranging from 5.03  $\pm$  1.21% to 27.73  $\pm$  1.70%. The pancreatic lipase

inhibitory effect of EAOS was found to be ranging from 8.18  $\pm$  1.01% to 53.13  $\pm$  0.39% when studied at concentrations 62.5-1000  $\mu$ g/ml. At same concentration, the inhibitory effect of EEOS was found to be

ranging from  $12.43 \pm 1.06\%$  to  $70.40 \pm 0.66\%$  whereas the effect of the standard drug Orlistat, ranged from  $28.76 \pm 0.86\%$  to  $72.70 \pm 1.15\%$ . The  $IC_{50}$  value of ethyl acetate extract, EAOS was found to be  $909.46 \mu\text{g/ml}$  whereas the ethanolic extract, EEOS showed at  $447.57 \mu\text{g/ml}$ . The  $IC_{50}$  of Orlistat was found to be  $322.98 \mu\text{g/ml}$  (Table 1).

### $\alpha$ -Amylase kinetic studies

Since the highest inhibitory activity was observed with ethanolic extract in our previous studies, kinetic studies were performed on this extract. The double reciprocal plot displayed mixed type of inhibition of  $\alpha$ -amylase action by the ethanolic extract of OS (Figure 1). Both the  $K_m$  value and  $V_{max}$  value were altered.

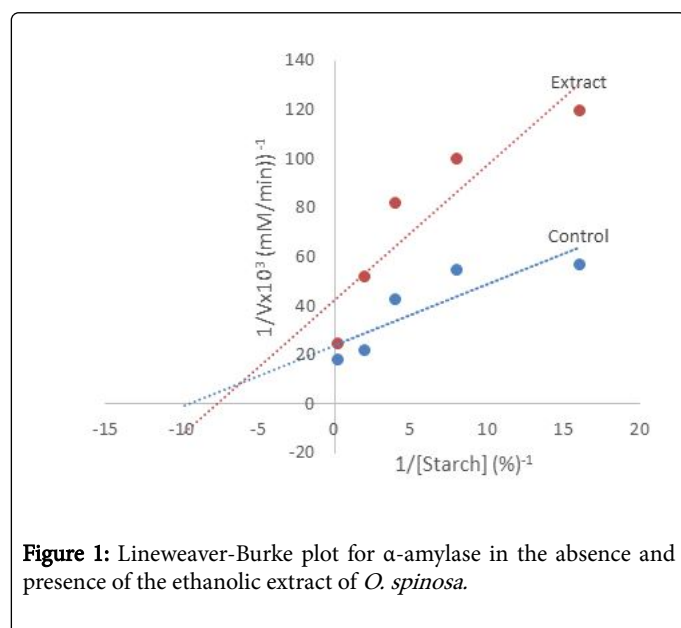


Figure 1: Lineweaver-Burke plot for  $\alpha$ -amylase in the absence and presence of the ethanolic extract of *O. spinosa*.

### $\alpha$ -Glucosidase kinetic studies

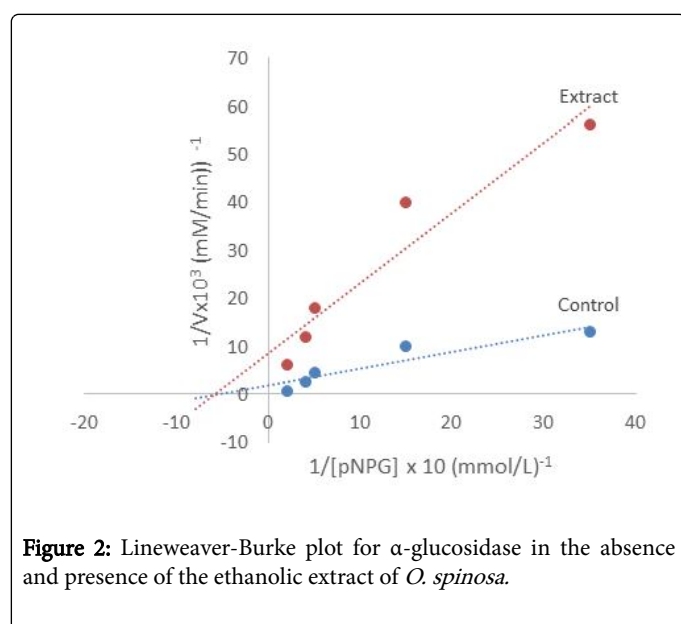


Figure 2: Lineweaver-Burke plot for  $\alpha$ -glucosidase in the absence and presence of the ethanolic extract of *O. spinosa*.

Since the highest inhibitory activity was observed with ethanolic extract in our previous studies, kinetic studies were performed on this extract. The double reciprocal plot displayed non-competitive inhibition of  $\alpha$ -glucosidase action by the ethanolic extract of OS (Figure 2). The  $K_m$  value was unaltered and  $V_{max}$  value was reduced.

### Pancreatic lipase kinetic studies

The highest inhibitory activity was observed with ethanolic extract, kinetic studies were performed on this extract. The double reciprocal plot displayed mixed type of inhibition of pancreatic lipase action by the ethanolic extract of OS (Figure 3). Both the  $K_m$  value and  $V_{max}$  value were altered.

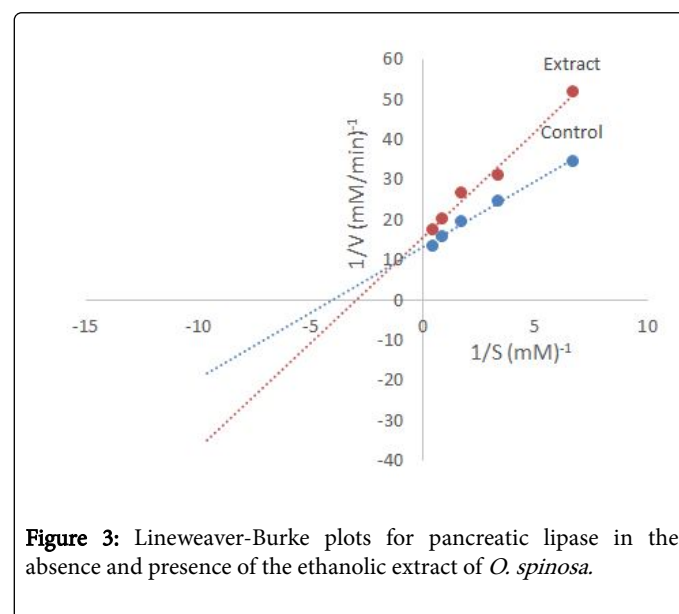


Figure 3: Lineweaver-Burke plots for pancreatic lipase in the absence and presence of the ethanolic extract of *O. spinosa*.

### Discussion

Medicinal plants were among the first plants to be utilized by humankind. Many species have been known from time immemorial to have medicinal properties. Over thousands of years, human observation has served as the basis for the preparation of medicines that led to the emergence of the production of modern industrial pharmaceuticals [13]. OS is a medicinal plant which has been extensively used in south west of Nigeria to manage various diseases including diabetes and cancer. However, there is no scientific data available on the inhibitory activities of OS on key enzymes linked to diabetes mellitus and obesity. Obesity is caused by excess caloric intake, and this can be improved by inhibiting pancreatic lipase activity and by inhibiting or delaying lipid absorption. Inhibition of carbohydrate hydrolysing enzymes and inhibition of carbohydrate absorption also play an important role in the prevention and treatment of diabetes. In this study, we performed *in vitro* experiments to study the effect of OS on pancreatic lipase activity and the kinetics of  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase inhibition.

Findings from our previous study showed that OS crude ethanolic extract displayed highest  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition when compared to other extracts studied. Interestingly, the crude ethanolic extract showed low inhibitory potential on  $\alpha$ -amylase and stronger action on  $\alpha$ -glucosidase [10]. These results are in agreement with previous reports which indicated that excessive inhibition of

pancreatic  $\alpha$ -amylase could result in the abnormal bacterial fermentation of undigested carbohydrates in the colon and eventually flatulence, abdominal distension and diarrhoea. Thus, stronger inhibition of  $\alpha$ -glucosidase activity and mild inhibition of  $\alpha$ -amylase activity could address the major drawback of currently used hypoglycaemic drugs [14,15]. The results of the present study showed that OS crude ethanolic extract showed highest pancreatic lipase inhibitory activity in a dose dependent manner when compared to other extracts studied. Interestingly, data from the present study demonstrated that OS was a potent inhibitor of enzymes directly linked to diabetes mellitus and obesity. Kinetic studies on the most active crude extract showed that the crude ethanolic extract exerted two modes of inhibitions, namely the noncompetitive and mixed type mechanisms. Lineweaver-Burk plot showed that crude ethanolic extract of OS displayed a mixed type of inhibition on  $\alpha$ -amylase and pancreatic lipase. On the other hand,  $\alpha$ -glucosidase was noncompetitively inhibited by the crude ethanolic extract. In mixed type of inhibition, the inhibitor binds to either free enzyme or enzyme substrate complex, with effect on  $K_m$  and  $V_{max}$  of the reaction. In non-competitive type of inhibition, the inhibitor binds to either free enzyme or enzyme substrate complex, with no effect on  $K_m$  but results in a decrease in  $V_{max}$  of the reaction [16]. It is therefore suggested that active constituents in the extract bind to other site(s), apart from the active site of the enzyme and induced a conformational change in the three-dimensional structure of the enzymes and ultimately slowed down their activities [12].

The inhibitory effect of OS on  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase could be as a result of the phytochemicals present such as tannins, flavonoids and phenolic compounds. The  $\alpha$ -glucosidase inhibition capacity of phenolic compounds is mainly due to their protein-binding capability [17]. Phenolic compounds have been shown to stimulate thermogenesis and body fat oxidation, thereby beneficial in the reduction of body weight [18,19]. Tannins induce insulin receptor phosphorylation as well as activation of translocation of glucose transporter 4 (GLUT-4), the main insulin-responsive glucose transporter [20]. The presence of hydroxyl group in the structure of flavonoids is also responsible for their antioxidant and antidiabetic effects [21]. Flavonoids, the powerful chain-breaking antioxidants (powerful electron donors) are also responsible for inhibition of lipid peroxidation. Flavonoids have been reported to prevent oxidative stress induced  $\beta$ -cell dysfunction, therefore alter the progression of insulin resistance to type 2 diabetes [22].

## Conclusion

It can be concluded from this study that out of the four extracts of *Oncoba spinosa* tested, the ethanolic extract displayed the most effective inhibition of pancreatic lipase *in vitro* and the mode of inhibition of both pancreatic lipase and  $\alpha$ -amylase is the mixed type one whereas the mode of inhibition of  $\alpha$ -glucosidase is the non-competitive one. The potent inhibitory activities of this plant may be due to the synergistic effect of phytochemicals present in it. Further *in vivo* antidiabetic and antiobesity studies of *O. Spinosa* are to be carried out in experimental models.

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