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Research Article

# *In-vitro* Pancreatic Lipase, Alpha-amylase and Alpha-glucosidase Inhibitory Activities of the Phytochemical Barbaloin

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ABSTRACT

The phytochemical barbaloin was studied for *in-vitro* pancreatic lipase, alpha (α)-amylase and alpha (α)-glucosidase inhibitory activities in the present study. The aim of this work is to evaluate the inhibitory activities of the phytochemical barbaloin at different concentrations. Pancreatic lipase is an enzyme that hydrolyzes the lipids obtained from the diet which acts as an important target to treat obesity. The natural medicines that can inhibit pancreatic lipase enzyme and thus decrease absorption of dietary fat in the body gained much attention for treating and preventing obesity. Diabetes mellitus is a metabolic disorder marked by an elevated level of glucose that circulates in the blood plasma. Alpha amylase and alpha glucosidase inhibitors are used to attain control over hyperglycemia in type 2 diabetes mellitus. The present study was designed to screen the novel pancreatic lipase, alpha-amylase and alpha-glucosidase inhibitors using a phytochemical, barbaloin, to minimize the toxicity and side effects of the inhibitors used at present to treat the disorders like obesity and hyperglycemia. The phytochemical, barbaloin exhibited significant pancreatic lipase, α-amylase and α-glucosidase inhibitory activities with an $IC_{50}$ value 5.52, 8.22 and 5.81 µg/mL, respectively and well compared with standard orlistat for pancreatic lipase and acarbose for alpha (α)-amylase and alpha (α)-glucosidase inhibitory activities, respectively.

## Introduction

Obesity and diabetes are two main disorders prevalent in the world and are interlinked to each other. Obesity causes diabetes to worsen faster. Obesity is also a major risk factor for many chronic diseases such as diabetes mellitus, coronary heart diseases, cancer (endometrial, breast, and colon) and respiratory disorders.[1] Barbaloin is reported to produce pharmacological activities such as histamine release inhibitory activity, anti-inflammatory, antiviral, antimicrobial, anticancer, cathartic, and antioxidant activities and used widely in cosmetic applications.[2] Obesity has nearly tripled worldwide since 1975, with about 13% of adults being obese and about 39% of adults being overweight. Common treatments for overweight and obesity include weight loss through healthy eating, weight-loss medicines, devices, bariatric surgery, etc.[3] Orlistat is lipase inhibitor used in treating obesity by decreasing the absorption of fats by the body. Due to its side effects like flatulence with fecal discharge, fecal incontinence, back pain, difficulty in moving, diarrhea etc., use of orlistat and other similar synthetic drugs are restricted.[4] Thus there is a need to explore new and newer natural drugs with less or no side effects for treating obesity. Diabetes mellitus is a metabolic disorder marked by an elevated level of glucose that circulates in the blood plasma. Over time leads to serious damage to the body's vital organs of the body *viz.,* heart, kidneys, eyes, blood vessels, and nerves. [5] The most common is type 2 diabetes, which occurs in adults when the body becomes resistant to insulin or doesn't make insulin sufficient to the body's needs.[6] The number of people with diabetes rose from 108 million in 1980 to 422 million in 2014 and is estimated to reach 643 million by 2030.[7] Among 7.7 billion of total population in 2019, around 463 million adult people have diabetes with

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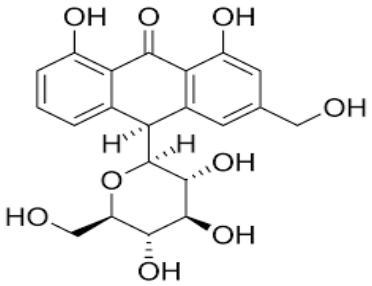


**Fig. 1:** Structure of barbaloin.

a global prevalence of 9.3% and is expected to rise 10.9% by 2045.[8] The conventional treatments for T2D include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target sites and the hindering the degradation of oligo- and disaccharides.[9] Inhibitors of the enzymes α-glucosidase and α-amylase are recognized as novel anti-diabetic drugs. The enzyme α-glucosidase is responsible for breaking oligo- and/or disaccharides into monosaccharides. The inhibitory action of this enzyme leads to a decrease of blood glucose level, because the monosaccharides are easily absorbed through the mucosal border in the small intestine.[10] an α-amylase enzyme is the major digestive enzyme in saliva. It helps in the hydrolysis of glycosidic linkages in starch to yield simpler compounds such as maltose and glucose, which results in increased glucose levels. The α-amylase inhibitors slow down the glucose absorption rate, decreasing the serum blood glucose levels in hyperglycemic individuals.[11] Some inhibitors currently in clinical use are acarbose, miglitol and voglibose etc. However, many of these synthetic hypoglycemic agents have their own limitations, which are non-specific, and produce serious side effects *viz*., bloating, abdominal discomfort, diarrhea and flatulence leading to diabetic complications.[12] Recently, herbal medicines are gaining more importance in the treatment of obesity and diabetes as they are free from side effects, easily available and less expensive when compared to synthetic drugs.

Barbaloin is the important phytoactive constituent of the *Aloe barbadensis*, belonging to the Liliaceae family and the structure of barbaloin is shown in Fig. 1. It is a shrubby perennial, xerophytic, pea-green color plant that grows mainly in the dry regions of Africa, Asia, Europe and America. In India, the plant is found in th states of Rajasthan, Andhra Pradesh, Gujarat, Maharashtra, Tamil Nadu, etc.[13] The present study was carried out to investigate the *in-vitro* pancreatic lipase, α-glucosidase and α-amylase inhibitory activities of the phytochemical barbaloin.

## Materials And Methods

### Chemicals and Reagents

Porcine pancreatic lipase (PPL), porcine pancreatic α-amylase (EC 3.2.1.1) (PPA) and α-glucosidase, 3,5-dinitrosalicylic acid (DNSA color reagent), soluble starch, p-nitrophenyl-α-D-glucopyranoside (p-NPG), were obtained from SRL Laboratories (Hyderabad, India). Acarbose from Sigma Aldrich (Mumbai, India), sodium potassium tartrate, dimethyl sulfoxide, and other chemicals are of analytical grade.

### Determination of *In-vitro* Pancreatic Lipase Enzyme Inhibitory Activity of Barbaloin

*In-vitro* pancreatic lipase enzyme inhibitory assay was based on the hydrolysis kinetics of an oleate ester of 4-methylumbelliferone used to determine pancreatic lipase activity.[14] The assay was performed as follows: A volume of 25 µL of the sample solution (plant extract, final concentration in the reaction mixture: 2.5 mg/mL) was dissolved in dimethylsulfoxide (DMSO), and 25 µL of pancreatic lipase solution were mixed in the well of a 96-well mL plate. The enzyme and extract solutions were prepared immediately before use. Porcine pancreas powder was suspended in tris–HCl buffer (13 mM Tris–HCl, 150 mM NaCl, 1.3 mM $CaCl_2$, pH 8.0) to givea concentration of 0.5 mg/mL. After a pre-incubation of PL with each particular extract for 10 minutes, 50 µL 4-methylumbelliferyl oleate (0.5 mM) was added to each well to initiate the enzyme reaction. The amount of 4-methylumbelliferone was measured at 37°C over 30 minutes using a fluorescence reader (Tecan Group Ltd., Maennedorf, Switzerland) at an excitation and emission wavelength of 360 and 465 nm. A 100% activity control, where the extract was replaced with the same volume of DMSO, was examined for all measurements. Orlistat was used as positive control.

### Determination of *In-vitro* Alpha-amylase Enzyme Inhibitory Activity of Barbaloin

The inhibition of α-amylase activity was determined according to the method[15] described in the literature with minor modifications. Stock solution of barbaloin was prepared by dissolving up to 100 mg of each extract in 10 mL of dimethyl sulfoxide. A total of 250 µL of extracts of varying concentrations (1, 2, 4, 6, 8, 10 µg/mL) was placed in a tube and 250 µL of 0.02M sodium phosphate buffer (pH- 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was pre-incubated at 25°C for 10 minutes. Then, 250 µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added and is incubated at 25°C for 10 minutes. The reaction was stopped by adding 500 µL dinitro salicylic acid (DNS) reagent. The tubes were incubated in boiling water (5 mL) and then cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Lab India). A control was prepared using the same procedure replacing the phytochemical with distilled water. The percentage inhibition was calculated by the formula:

**Table 1:** *In-vitro* pancreatic lipase enzyme inhibitory activity of barbaloin

| *S. No.* | *Name of sample* | *Concentration (mg/mL)* | *% Inhibition* | *$IC_{50}$ value (µg/mL)* |
|---|---|---|---|---|
| 1. | Barbaloin | 1 | 17.32 ± 0.02 | 5.52 ± 0.03 |
| | | 2 | 26.21 ± 0.04 | |
| | | 4 | 41.32 ± 0.06 | |
| | | 6 | 53.69 ± 0.18 | |
| | | 8 | 67.38 ± 0.05 | |
| | | 10 | 79.35 ± 0.15 | |
| 2. | Orlistat | 1 | 28.35 ± 0.04 | 3.87 ± 0.01 |
| | | 2 | 35.69 ± 0.06 | |
| | | 4 | 50.32 ± 0.04 | |
| | | 6 | 67.11 ± 0.05 | |
| | | 8 | 82.47 ± 0.03 | |
| | | 10 | 93.46 ± 0.13 | |

The values are expressed as means ± SD of triplicate determinations. orlistat is the standard pancreatic lipase enzyme inhibitor

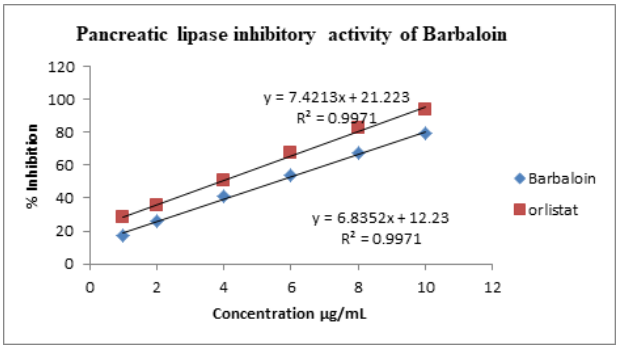


**Fig. 2:** *In-vitro* pancreatic lipase enzyme inhibitory activity of orlistat and barbaloin.

**Table 2:** *In-vitro* alpha- amylase enzyme inhibitory activity of barbaloin

| *S. No.* | *Name of sample* | *Concentration (µg/mL)* | *% Inhibition* | *$IC_{50}$ value (µg/mL)* |
|---|---|---|---|---|
| 1. | Barbaloin | 1 | 9.36 ± 0.02 | 8.22 ± 0.07 |
| | | 2 | 21.34 ± 0.15 | |
| | | 4 | 34.67 ± 0.24 | |
| | | 6 | 49.37 ± 0.35 | |
| | | 8 | 61.42 ± 0.28 | |
| | | 10 | 77.29 ± 0.36 | |
| 2. | Acarbose | 1 | 12.84 ± 0.27 | 3.68 ± 0.04 |
| | | 2 | 23.54 ± 0.25 | |
| | | 4 | 41.26 ± 0.22 | |
| | | 6 | 59.42 ± 0.35 | |
| | | 8 | 74.2 ± 0.14 | |
| | | 10 | 86.52 ± 0.22 | |

The values are expressed as means ± SD of triplicate determinations. Acarbose is the standard α-amylase inhibitor

% Inhibition = Absorbance (control) – Absorbance (Test)/Absorbance (control) × 100

### Determination of Alpha-glucosidase Enzyme Inhibitory Activity of Barbaloin

The inhibition of α-glucosidase activity was determined according to the method[15] described in the literature with minor modifications. Total of 1-mg of the enzyme, α-glucosidase was solubilized in 100 mL of phosphate buffer (pH 6.8). To 100 µL of phytochemical, barbaloin of varying concentrations (1, 2, 4, 8, 10, µg/mL), 200 µL α-glucosidase were added and the mixture was incubated at 37°C for 20 minutes. To the reaction mixture 100 µL of 3mM p-nitrophenyl-α-D-glucopyranoside (*p*-NPG) was added and incubated at 37°C for 10 minutes. The reaction was terminated by adding 2 mL sodium carbonate solution (0.1M) and the α-glucosidase activity was determined spectrophotometrically at 405 nm using a UV-vis spectrophotometer (Lab India) by determining the amount of p-nitrophenol released from *p*-NPG. Acarbose was used as positive control of α -amylase and α-glucosidase inhibitor. The concentration of the barbaloin was required to inhibit 50% of α-amylase and α-glucosidase activity under the assay conditions was defined as the $IC_{50}$ value.

% Inhibition = Absorbance (control) – Absorbance (test)/Absorbance (control) × 100

## Results And Discussion

### *In-vitro* Pancreatic Lipase Enzyme Inhibitory Activity of Barbaloin

The results of the study are shown in Table 1 and Fig. 2. The pancreas secretes the enzyme Lipase to digest the fat present in the food by hydrolyzing the lipids which acts as an important target to treat obesity. The primary role of lipase inhibitors is to decrease the gastrointestinal absorption of fats so that the fats are excreted without absorption.[16] Lipase enzyme is responsible for the digestion of about 50–70% of total fats in diet. Therefore, inhibition of the same has become an effective method for the treatment of obesity and is one of the widely studied mechanisms for the identification of natural products as anti-obesity agents.[17] Orlistat, a potent irreversible inhibitor of pancreatic lipase, was used as standard for *in-vitro* lipase inhibitory activity. Barbaloin has exhibited significant dose dependent anti lipase activity and the results were comparable to that of the standard drug, Orlistat. The $IC_{50}$ value of the phytochemical, Baraloin and standard (Orlistat) was found to be 5.52 and 3.87 µg/mL, respectively.

### *In-vitro* Alpha-amylase Enzyme Inhibitory Activity of Barbaloin

The study's results are presented in Table 2 and Fig. 3. α-amylase and α-glucosidase inhibitors play an important

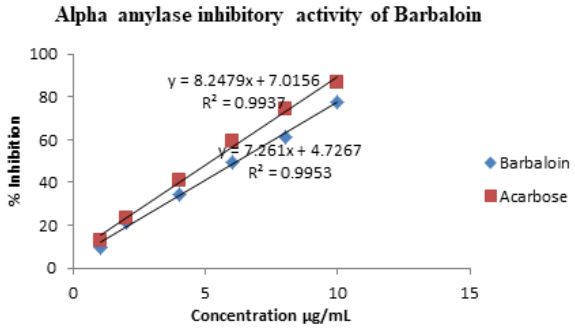


**Fig. 3:** *In-vitro* α-amylase inhibitory activity of acarbose and barbaloin.

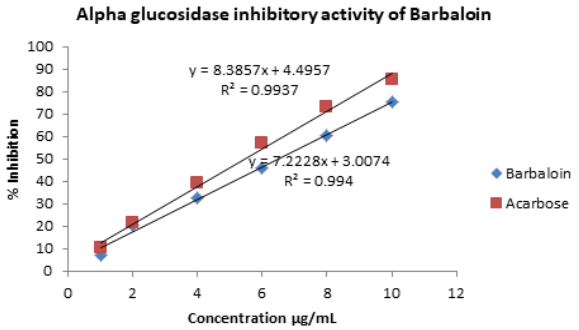


**Fig. 4:** *In-vitro* α-glucosidase enzyme inhibitory activity of acarbose and barbaloin.

**Table 3***: In-vitro* alpha-glucosidase enzyme inhibitory activity of barbaloin

| *S. No.* | *Name of sample* | *Concentration (mg/mL)* | *% Inhibition* | *$IC_{50}$ value (µg/mL)* |
|---|---|---|---|---|
| 1. | Barbaloin | 1 | 7.34 ± 0.02 | 5.81 ± 0.04 |
| | | 2 | 20.58 ± 0.14 | |
| | | 4 | 32.57 ± 0.06 | |
| | | 6 | 45.81 ± 0.18 | |
| | | 8 | 60.34 ± 0.11 | |
| | | 10 | 75.31 ± 0.11 | |
| 2. | Acarbose | 1 | 10.32 ± 0.12 | 3.64 ± 0.01 |
| | | 2 | 21.56 ± 0.41 | |
| | | 4 | 39.25 ± 0.13 | |
| | | 6 | 57.33 ± 0.22 | |
| | | 8 | 73.26 ± 0.18 | |
| | | 10 | 85.21 ± 0.16 | |

The values are expressed as means ± SD of triplicate determinations. Acarbose is the standard α-amylase inhibitor

role role in controlling high blood glucose levels. Alpha-amylase is an important enzyme found in the pancreatic juice and saliva that breaks down large insoluble starch molecules into small soluble, absorbable molecules. α-amylase promotes the process of digestion of carbohydrates by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides.[18]

In the present study, barbaloin inhibited the catalysis of α amylase at all concentrations (1, 2, 4, 8, 10 mg/mL) and the exhibited percentage inhibition was. Among all the test doses, barbaloin has shown remarkable α amylase enzyme inhibition i.e 59.4% at 10 mg/mL concentration and it was comparable with the standard drug acarbose (91.2% percentage inhibition at 10 mg/mL concentration). The $IC_{50}$ value of the phytochemical, barbaloin and standard (Acarbose) was found to be 8.22 and 3.683 mg/mL, respectively.

### In-*vitro* Alpha-glucosidase Enzyme Inhibitory Activity of Barbaloin

The results of the study are presented in Table 3 and Fig. 4. Alpha-glucosidase enzyme present in the mucosal brush border of the small intestine catalyzes the last step of digestion of starch and disaccharides, which are in surplus in the human diet. α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia.[19] The phytochemical barbaloin was assessed for alpha-glucosidase enzyme inhibitory activity at different concentrations ranging from (1–10 mg/mL) and it has shown potent α-glucosidase inhibitory activity in a dose-dependent manner comparable with that of the standard drug, acarbose. The $IC_{50}$ value of the phytochemical, baraloin and standard (Acarbose) was found to be 5.819 and 3.640 mg/mL, respectively.

## Conclusion

In the present study, the phytochemical barbaloin showed inhibition of pancreatic lipase, which may be used for the treatment of obesity after prior in-*vivo* studies. The drug also inhibited the action of the enzymes alpha-amylase and alpha-glucosidase which in turn decreases blood glucose levels, making the drug effective in the management of diabetic complications. Hence, the phytochemical barbaloin can be used as an adjuvant for the management of obesity and complications associated with diabetes mellitus.

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