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# Inhibitory Effect of *Capparis spinosa* Extract on Pancreatic Alpha-Amylase Activity

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## Abstract

**Background:** Diabetes mellitus is a metabolic disorder characterized by high blood glucose level caused due to deficiency of insulin secretion or insulin function. The inhibition of carbohydrate hydrolyzing enzymes such as $\alpha$-amylase can be an important strategy for decrease postprandial blood glucose level in patients with type II diabetes. Plants contains different chemical constituents with potential for inhibition of $\alpha$-amylase and hence maybe used as therapeutic.
**Objectives:** The aim of the present study is to investigate the effect of the ethanolic extract of *Capparis spinosa* on pancreatic $\alpha$-amylase activities to find out the relevance of the plant in controlling blood sugar.
**Materials and Methods:** In this experimental study, root and leaves of *C. spinosa* were tested for $\alpha$-amylase inhibition. Different concentrations (1.56, 3.12, 6.25, 12.5 and 25 mg/mL) of extracts were incubated with enzyme substrate solution and the spectrometric method used for measure enzyme activity. Also acarbose was used as the standard inhibitor.
**Results:** Both root and leaves extracts showed inhibition of $\alpha$-amylase (root = 97.31% and leaves = 98.92%). The root and leaves extracts of *C. spinosa* exhibited appreciable $\alpha$-amylase inhibitory activity with an $IC_{50}$ values 5.93 mg/mL and 3.89 mg/mL respectively, when compared with acarbose ($IC_{50}$ value 0.038 mg/mL).
**Conclusions:** This study supports that root and leaves extracts of *C. spinosa* exhibit considerable $\alpha$-amylase inhibitory activities. These results could be useful for developing functional foods by combination of plant-based foods for treatment of diabetes mellitus.

*Keywords:* Alpha Amylase, Inhibitory Effects, Diabetes Mellitus, *Capparis spinosa*

## 1. Background

Diabetes mellitus (DM) is an extended metabolic disease of several etiologies characterized by chronic hyperglycemia with disorder of carbohydrate, fat and also protein metabolism resulting from defects in insulin secretion, insulin action or both of them [1]. The control of hyperglycemia is very important in the treatment of all forms of diabetes by reason that in the long term, acute and chronic complications can happen when the blood glucose concentration is not kept in the normal range [2, 3]. The drugs widely used in clinic to manage or handle diabetes are insulin, sulfonylureas, biguanide, glycosidase inhibitors, aldosereductase inhibitor, thiazolidinediones, carbamoylmethyl benzoic acid [4]. Available therapies for treating type II diabetes consist of stimulation of endogenous insulin secretion, increase of activity of insulin at target tissues as well as inhibition of $\alpha$-amylase enzyme to reduce the degradation of starch to decreasing glucose [5, 6].

One unique approach for decreasing postprandial hyperglycemia is to reduce or slow down dietary carbohydrate digestion. Inhibiting the enzymes involved, such as the $\alpha$-amylase and $\alpha$-glucosidase enzymes, is a strong the therapeutic goal of controlling the postprandial glycemic reaction [7]. Also, $\alpha$-amylase inhibitors are one of the antidiabetic drug families, of which acarbose is the most well-known. These drugs have a very strong advantage and are suitable for healing noninsulin-dependent diabetes mellitus (type-2 diabetes) [8], but also induce gastrointestinal side effects that reduce their use in a preventive approach [9]. Accordingly, several researchers are investigating and developing nutritional strategies to perfectly control postprandial glycemia, without inducing negative effect in the digestive system, medicinal herbs due to easy accessibility and also lower negative effects have a special place in medicine to treat various diseases [10-12].

Caper (*Capparis spinosa*) is a long-lasting shrubby plant that belongs to the family Capparidaceae and growing extensively in the warm and dry weathers such as West and Middle Asia, the Mediterranean area and also numerous regions of Iran [13, 14]. It has been used since ancient times

for fragrant properties in food preparation, and besides it's utilizes as flavoring, *C. spinosa* has also been used as an ordinary natural medicine for its antihypertensive, poultice, tonic and diuretic characteristics [14, 15]. Methanol extract of *C. Spinosa* buds, containing more flavonoids, including many quercetin and kaempferol glycosides, have strong antioxidant/free radical scavenging effects in several in vitro tests [16]. Eddouks et al. showed that the *C. spinosa* extract has an effective action on the decrease of blood cholesterol, triglycerides and glucose in regular and serious hyperglycemia [17, 18]. Lemhadri et al. fed diabetic rats with the sodden fruit for three weeks and after measuring the glucose tolerance test, they discovered in sodden receiving rats, glucose tolerance was improved compared to control group. *C. spinosa* has been extensively studied in the control of blood glucose [19]. Several clinical studies have evaluated the chronic effects of *C. spinosa* and *C. spinosa* extracts in subjects suffering from type 2 diabete [20, 21].

## 2. Objectives

The aim of the present study was to examine the in vitro $\alpha$-amylase inhibitory activity of *C. spinosa*.

## 3. Materials and Methods

### 3.1. Chemical Materials

For this experimental study, all chemical substances were obtained from Sigma Aldrich (USA) and also Merck (Germany) companies. The chemicals were of analytical grade.

### 3.2. Plant Materials

*C. spinosa* young whole plants were collected from Ahvaz Province, Iran in May 2007. The plant was botanically identified and authenticated by local Plant Biotechnologist, department of natural resources, Khuzestan, Iran. The plants were dried at ambient temperature (30 - 40°C) for 25 - 30 days, then root and leaves of *C. spinosa* were into fine powder.

### 3.3. Extraction and Fractionation Procedure

The dried and powdered plants (100 g) were extracted with ethanol 90% v/v through soxhlet apparatus. The crude extracts were filtered and concentrated under reduced pressure at approximately 40°C.

### 3.4. Assessment of $\alpha$-amylase Inhibition

The starch solution (0.5% w/v) was obtained by stirring and boiling 0.25 g of soluble potato starch in 50 mL of deionized water for 15 minutes. The enzyme solution (0.5 unit/mL) was prepared by mixing 0.001 g of $\alpha$-amylase (EC 3.2.1.1) in 100 mL of 20 mM sodium phosphate buffer (pH = 6.9) containing 6.7 mM sodium chloride. The extracts and/or fractions were dissolved in DMSO to give suitable concentrations for the assay. The color reagent was a solution containing 96 mM 3, 5-dinitrosalicylic acid (20 mL), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 mL) and deionized water (12 mL). One milliliter of each the extracts and/or fractions and 1 mL of the enzyme solution were mixed in a test tube and incubated at 25°C for 30 minutes. To 1 mL of this mixture was added 1 mL of the starch solution and the tube was further incubated at 25°C for 3 minutes. Then, 1 mL of the color reagent was added and the stoppered tube was placed into an 85°C water bath. After 15 minutes, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 mL distilled water and the absorbance value determined at 540 nm using a Shimadzu Multispect-1501 spectrophotometer (Japan).

Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added before the addition of starch solution and then, the tube was placed into the water bath. Then, the method was followed as described above. Controls were conducted in an identical manner, replacing extracts and/or fractions with 1 mL DMSO. Acarbose solution (at the concentrations of 0.0094, 0.0184, 0.036, 0.07, 0.11, 0.21 $\mu$g/mL) was used as positive control. The inhibition percentage of $\alpha$-amylase was assessed by the following formula:

$$I\alpha - \text{amylase}\% = 100 \times \frac{\Delta\text{AControl} - \Delta\text{ASample}}{\Delta\text{AControl}} \quad (1)$$

$\Delta$AControl = ATest-ABlank; $\Delta$ASample = ATest-Ablank.

The I$\alpha$-amylase% was plotted against sample concentration and a logarithmic regression.

Curve was obtained in order to calculate the $IC_{50}$ value which is concentration of sample (mg/mL) necessary to decrease the absorbance of $\alpha$-amylase solution by 50%.

### 3.5. Statistical Analysis

All values were expressed mean $\pm$ SD. Statistical difference and linear regression analysis was performed using SPSS-16 statistical software and differences with a $P < 0.05$ were considered significant.

## 4. Results

The highest inhibitory activity for ethanol root extracts of *C. spinosa* was found to be 97.31% with an $IC_{50}$ values 5.93 mg/mL (Table 1). The highest inhibitory activities for ethanol leaves extracts of *C. spinosa* exhibited 98.92%) with an $IC_{50}$ values 3.89 mg/mL (Table 1). The $IC_{50}$ value of the positive control, acarbose, was measured as 0.038 mg/mL.

**Table 1.** $\alpha$-Amylase Inhibitory Percentage and $IC_{50}$ Values of Different Concentrations

| Plant Extracts Concentration, mg/mL | Inhibition, % | $IC_{50}$, mg/mL |
|---|---|---|
| **Root** | | 5.93 |
| 1.56 | 28.49 ± 0.58 | |
| 3.12 | 41.17 ± 1.1 | |
| 6.25 | 56.89 ± 1.08 | |
| 12.5 | 85.57 ± 0.58 | |
| 25 | 97.31 ± 1.25 | |
| **Leaves** | | 3.89 |
| 1.56 | 34.14 ± 0.38 | |
| 3.12 | 45.52 ± 0.74 | |
| 6.25 | 65.92 ± 1.99 | |
| 12.5 | 88.30 ± 0.89 | |
| 25 | 98.68 ± 1.24 | |

## 5. Discussion

In this study, the root extract showed 97.31% of $\alpha$-amylase inhibitory activity with $IC_{50}$ value 5.93 mg/mL and the leaves extract showed 98.92% of $\alpha$-amylase inhibitory activity with $IC_{50}$ value 3.89 mg/mL. At the same time, both root and leaves extracts showed appreciable $\alpha$-amylase inhibitory effects when compared with acarbose.

Diabetes mellitus is a chronic metabolic disorder identified by hyperglycemia due to insulin insufficiency and/or insulin resistance contributing to excess blood glucose. It affected approximately 171 million people all around the world in the year 2000 and the number is projected to increase to around 366 million by 2030 [22]. Management of the blood glucose level is an essential approach in the control of diabetes complications. Inhibitors of carbohydrates hydrolysing enzymes ($\alpha$-amylase and $\alpha$-glucosidase) have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patients with type-2 diabetes mellitus [5, 6, 8]. Inhibition of these enzymes holds of carbohydrate digestion and extends the total carbohydrate digestion time, leading to a decrease in the rate of glucose absorption and therefore reducing the postprandial plasma glucose rise [23], commonly used synthetic inhibitory drugs such as acarbose and miglitol [17] possessed negative effects. Traditionally, various parts of herbs were used directly as a medication. Clinically effective substances are now being obtained from plants, even those that have not been categorized before as medicinal herbs. Recently, traditional medicine (Phytotherapy) is often used to treat several diseases, besides modern medicine. A lot of natural extracts have been reported to have antidiabetic activities and are utilized for the treatment of diabetes. Herbal extracts have been used perfectly or ultimately for the processing of numerous modern medicines [10-12].

*C. spinosa* is the most widespread plant in the warm and dry weathers that its fruit, roots and barks are used for medical purposes. In traditional medicine, this plant is used as a diuretic, treatment of gout, rheumatism, hyperlipidemia, hyperglycemia, hypertension, spleen and liver disease [13, 15, 16, 19].

The present study was done to examine an in vitro inhibitory effect of different extracts of *C. spinosa* on porcine pancreatic amylase activities was evaluated. Antidiabetic activities of *C. spinosa* have been reported with its leaves and seeds. The fruit extract of *C. spinosa* were successively tested in diabetes in vivo in doses ranging from 200 and 800 mg/kg/day for 28 days in diabetic rats. These had lowered the blood glucose and triglycerides level successively [11]. Rahmani et al. and Huseini at el. showed fruit extract of *C. spinosa* (400 mg three times/day for two months) can significant decreased fasting blood glucose, glycosylated hemoglobin and triglyceride in diabetic patients [20, 21]. This study investigated for the first time the inhibitory effect of *C. spinosa* ethanol extract on $\alpha$-amylase activity. It may be due to the presence of more chemical constituents such as phenolic compounds, terpenes, tripenes, flavonoids and alkaloids in the root and leaves extracts. The plant-based $\alpha$-amylase inhibitor offers a prospective therapeutic approach for the management of diabetes [24, 25]. In this study, root and leaves extracts of *C. spinosa* showed considerable $\alpha$-amylase inhibitory effects when compared with acarbose.

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## Footnotes

**Authors' Contribution:** All authors had equal role in design, work, statistical analysis, and manuscript writing.

**Conflict of Interest:** The authors declare no conflict of interest.

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