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Research Article

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Investigating the Antioxidant Capacities, Total Phenolic Contents, and α-Amylase Inhibitory Activities of *Capparis spinosa* L. Methanolic Extracts

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ABSTRACT

Free radicals play an important role in developing or treatment of degenerative diseases. Using plants to treat various diseases has been done since thousand years ago, in which many of the human made drugs were obtained from plants. Accordingly, herbal medicines have been focused as new sources of antioxidants with limited complications. The present study was aimed to evaluate the total phenolic contents, antioxidant and α-amylase inhibitory activities of hydroalcoholic extract of Capparis (C.) spinosa L. The antioxidant power were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP) assay and the total phenolic contents were determined by Folin-Ciocalteu method. In the DPPH assay, the IC_{50} for leaves and root extracts were 13.3 and 15.8 mg/ml respectively. The IC_{50} for ascorbic acid was 1.02 mg/ml. The antioxidant capacities in FRAP method were 5.80 and 1.43 mg/ml for leave and root extracts, respectively. The total phenolic contents of roots and leaves were 268.6 mg and 259.7 mg galic acid per 100 grams powder, respectively. The IC_{50} of α-amylase inhibitory activities for leaves and root extracts were 5.93 and 3.89 mg/ml respectively. This study found that the antioxidant capacity of leaves extract is more than the root extract, while the phenolic contents of root is more than the leaves extract. On the other hand, the α-amylase inhibitory activities of leaves were more than the root. The findings of this study showed no relationship between phenol contents, antioxidant activities, and enzyme inhibitory power of extracts. In addition, notwithstanding a lower phenolic content in the leaves extract than the root extract, leaves extract has higher antioxidant capacity and inhibitory power.

Keywords: *Capparis spinosa*, phenolic contents, antioxidants capacity, α-amylase inhibitory, Folin-Ciocalteu

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INTRODUCTION

Using medicinal plants for the treatment of different diseases is as old as the history of mankind. Many of human made drugs were obtained from plants since a few past decades. In line with the development of science, synthetic drugs have entered the cycle of treatment and consequently the use of herbal medicines has decreased. Reactive oxygen species that have made many concerns nowadays, have been formed along with the emergence of oxygen in the atmosphere, prior to the formation of the Earth and much earlier than higher species start living life on earth. Oxidation is the transmission of an electron and is a major part of living and metabolism of the aerobic organisms. Oxygen is an electron acceptor in the electron transport system, in which produces energy from ATP in the body. Oxygen under certain conditions may become a single electron and produces free radical. Oxidative damage is the term generally indicates the attack of free radicals containing oxygen, such as superoxide anion, hydroxyl radical, hydrogen peroxide, and nitric acid and to the biological molecules. The imbalance in terms of oxidant-antioxidant systems occurs in the body during the severe oxidative conditions, that usually produces more oxidants. The produced radicals in the body destroy by its natural defenses, normally. These natural defenses include glutathione, desmotase superoxide, catalase and peroxidase [1, 2]. In some cases, that levels of free radical increased according to the different causes, defense system cannot act effectively, thus an antioxidant regimen is needed to eliminate the effect of excess free radicals. Antioxidants are compounds that considerably decrease the oxidation rate in low concentrations. These compounds can prevent and delay the oxidative damage, that their origin are free radicals which results in numerous chronic diseases and disorders such as atherosclerosis, stroke, myocardial infarction, Alzheimer's, diabetes, arthritis, Parkinson's disease, chronic inflammations, cancer and other degenerative diseases[3, 4]. Oxidative stress can be reduced with the provision of additional antioxidant[5]. Phenolic compounds are ubiquitous bioactive compounds and a diverse group of secondary metabolites present in higher plants[6]. Polyphenols have become an intense focus of research interest because of their beneficial effects on health. Recently, it has been shown that phenolics play a role in mediating amylase inhibition and therefore have potential to contribute to the management of type 2 diabetes[7].

Diabetes, a metabolic disorder with multiple causes that create pathological hyperglycemia and chronic disorganization metabolism of carbohydrates, proteins and fats, develop by defects in insulin secretion or its function or both. Diabetes mellitus is one of the important problems today that threatens global health, particularly in developed countries. Currently, about 177 million people are affected worldwide and are expected prevalence in 2010 reached over 240 million people and by 2030 to more than 366 million people will have diabetes mellitus as well [8, 9].

Medicinal plants are one category of such techniques with promising therapeutic effects. The α-amylase and α-Glucosidase are key enzymes in the metabolism of carbohydrates. One therapeutic approach for treating is to decrease the post-prandial hyperglycemia which could be done by retarding the absorption of glucose by inhibition of carbohydrate hydrolysis enzymes, α-amylase and α-glucosidase in the digestive tract, consequently blunting the post-prandial plasma glucose[7, 9]. These enzymes Inhibition with the delay in the process of hydrolysis and absorption of carbohydrates can be effective in controlling diabetes. Using plants for therapeutic and preventive purposes in traditional medicine and ethnically different countries has been occurred since centuries. *Capparis*(*C.) spinosa* is growing in different regions of Iran, especially in Sothern parts. The plant closed buds and other organs are used with food, just after three months storage in vinegar in southern region of Iran[10]. The *C. spinosa* is one of the plants in the treatment of non-insulin dependent diabetes that is used in traditional medicine of India and Pakistan[11]. The plant roots traditionally were used in the treatment of capillary flow weakness[12]. Many studies have been conducted in relation to *C. spinosa* effect on blood sugar[13, 14]. This study was conducted to investigate the antioxidant activities and measuring total phenolic contents, and investigation of inhibitory activities of methanolic extracts of *C. spinosa* L. on α-amylase enzyme.

MATERIALS AND METHODS

2.1. Chemicals:

All the chemicals used were purchased from Sigma-Aldrich Chemie Gmbh (Germany) and Merck (Germany) companies. The chemicals were of analytical grade.

2.2. Plant materials:

Capparis spinosa 's leaves and roots were collected in the range of Ahvaz University of Medical Sciences campus on May 2008 and authenticated in Medicinal Plant and Natural Product Research Center . The plant parts were dried in a drying room, with active ventilation at ambient temperature. A voucher specimen for plant was deposited at the Herbarium of the Department of Pharmacognosy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

2.3. Extraction

The dried and fine plant parts (100g) of leaves and roots were separately extracted with methanol using Soxhlet apparatus for 6 hours. The extracts were filtered and concentrated under reduced pressure at approximately 40°C. Each extract was dissolved in methanol to give stock solution (100 mg/ml) and by serial dilutions to give concentrations from 3.125 to 75 mg/ml (3.125, 6.25, 12.5, 25, 50, 75 mg/ml) .

2.4. Determination of antioxidant activity:

2.4.1. Free radical scavenging activity:

The DPPH (1, 1 - Diphonyl-2-Picrylhydrazyl) free-radical scavenging assay was carried out in triplicate, based on the Tepe et al. method[4]. The hydrogen atoms or electron-donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of a purple-coloured methanol solution of DPPH. The absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (Inh%) was calculated in following way:

$$\text{Inh. \%} = (A_{\text{Blank}} - A_{\text{Sample}} / A_{\text{Blank}}) \times 100$$

where A_{Blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound. The concentration of extract that provides 50% inhibition of free radical DPPH (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. The ascorbic acid was used as positive control.

2.4.2. The ferric reduction antioxidant potential assay:

The ability to reduce ferric ions was measured using a modified version of the method described by Benzie and Strain (1996)[15]. The FRAP method is done based on the Liu et al. according to the restoration of Fe^{+3} to Fe^{+2} in complex with Tripyridyltriazine (TPTZ) solution and in the presence of a reducing agent and acidic pH. The blue-colored ferrous form (Fe^{+2}-TPTZ complex) was measured at 593 nm. The measurement was compared to a standard curve of $FeSO_4.7H_2O$ solutions and expressed as an EC_1 value, which means the concentration of antioxidant in the reactive system having a ferric-TPTZ reducing ability equivalent to 1mM $FeSO_4.7H_2O$[3, 16].

2.4.3. Determination of total phenolic compounds:

In Folin-Ciocalteu method 0.1 ml of sample or standard solution of gallic acid is added to 2 ml of sodium bicarbonate 2 % and the tubes are incubated in the dark for 2 minutes. At the end of two minutes, 0.1 ml of Folin-Ciocalteu reagent is added to it. Then the prepared solution was incubated for 30 minutes and absorption intensity is measured at 750 nm. This experiment was repeated three times for every sample[17]. Results are expressed as mg galic acid equivalents per 100g dry sample (mg GAE/100g DS) using a standard curve of galic acid.

2.4.4. The inhibitory effect of α-amylase:

The inhibitory effect of α-amylase was measured using Nickavar *et al*. method [18]. One ml of each plant extract and 1 ml enzyme solution was mixed in a tube and incubated at 25°C for 30 min. One ml of this mixture was added to 1 ml of starch solution and the tube incubated at 25°C for 3 min. Then, 1 ml of the color reagent was added and the closed tube placed into an 85°C water bath. After 15 min, 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 in a Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan). Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing plant extracts with 1 ml DMSO. Acarbose solution, at the concentrations of 0.0094, 0.0184, 0.036, 0.07, 0.11, 0.21 μg/ml, was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:

$I_{\alpha\text{-amylase}}\,\% = 100 \times (\Delta A_{Control} - \Delta A_{Sample}) / \Delta A_{Control}$
$\Delta A_{Control} = A_{Test} - A_{Blank}$
$\Delta A_{Sample} = A_{Test} - A_{Blank}$

The $I_{\alpha\text{-amylase}}$ % was plotted against the sample concentration and a logarithmic regression curve established in order to calculated the IC_{50} value (inhibitory concentration). This would represent the concentration of sample necessary to decrease the absorbance of α-amylase by 50%.

2.5. Statistical analyses:
Experimental results were mean of three independent experiments, each with duplicate measurements. *P*-values <0.05 were considered as significant and *P*-values <0.01 very significant.

RESULTS AND DISCUSSION

3.1. Antioxidant activity by DPPH radical scavenging assay
Methanolic extracts were individually assessed for their possible antioxidative capacities using two complementary tests: DPPH free radical-scavenging and FRAP assays. Free radical-scavenging capacities of the corresponding extracts were measured by DPPH assay and the results are shown in Figure 1. The lower the IC_{50} values is, the greater the free radical-scavenging activities.

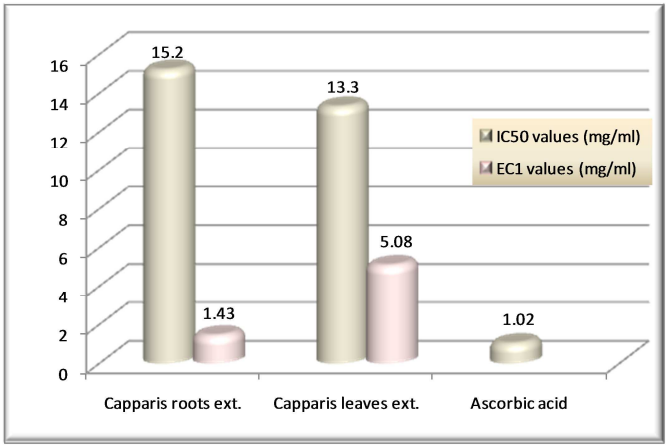

Figure 1. Antioxidant activities of the extracts measured in DPPH and FRAP assay (results are means of three different experiments)

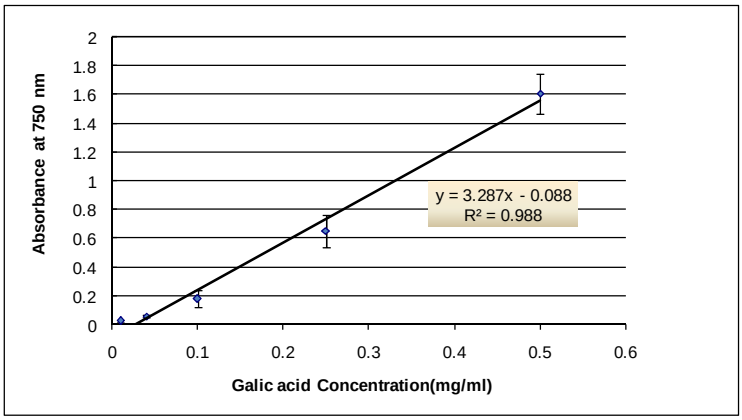

Figure 2: The standard curve of galic acid in Folin-Ciocaltue assay (results are presented as the means of three different experiments ± SEM)

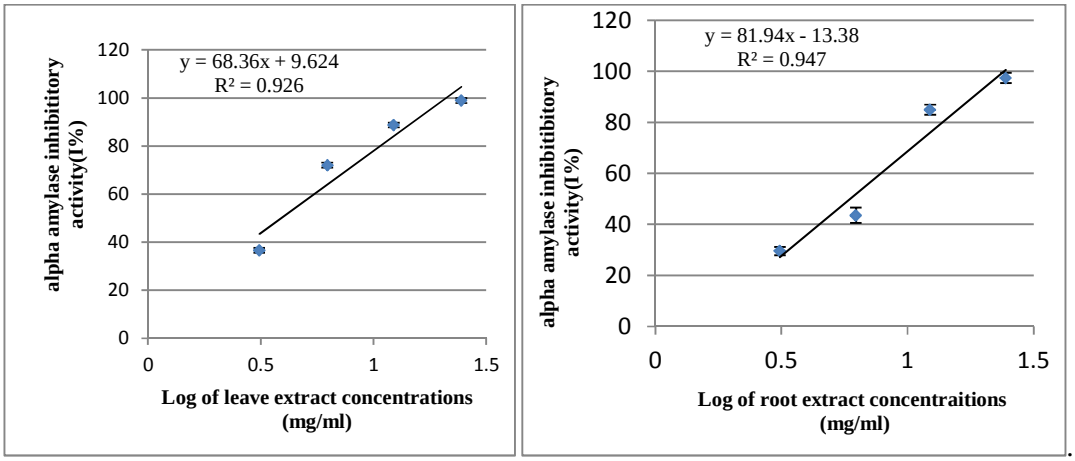

Figure 3. α-Amylase inhibitory activities of leaves and roots extract of *C. spinosa* (results are presented as the means of three different experiments ± SEM)

3.2. The ferric reduction antioxidant potential assay

In FRAP, the antioxidant activity was determined based on the ability of the antioxidant components in the samples to reduce the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Results showed that the EC_1 values for methanolic extract of leaves were more the methanolic extract of roots (Figure 1).

3.3. Total phenolic assay

The standard curve of galic acid absorbtion in Folin-Ciocaltue assay was shown in Figure 2.

The phenolic contents of plant leaves and roots were 259.7 and 268.6 mg GAE/100g dry samples, respectively.

3.4. α-Amylase inhibitory activity

All the concentrations of leaves and roots extract showed inhibitory effects on α-amylase enzyme. The α-amylase inhibitory activity ($I_{\alpha\text{-amylase}}$) of leaves and roots are shown in Figure 3.

The IC_{50} values of plant extracts and acarbose according to α-amylase enzyme inhibition were determined (Figure 4).

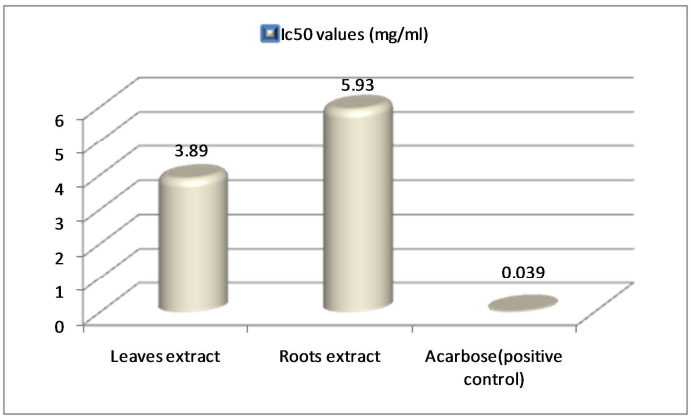

Figure 4. α-Amylase inhibitory activities of extract and positive control (each point represents the mean of three experiments)

CONCLUSION

Oxidative damage is the term generally indicates that the attack of free radicals containing oxygen to the biological molecules. Oxidative reactions play an important role in the presence of some diseases, and an incomplete level of natural antioxidants can observe in a number of these diseases[19]. Diabetes mellitus is a state of increased oxidative stress. Auto oxidation process created in diabetes mellitus, caused by free radicals to be either directly caused the cells destroy or indirectly created by the destruction of many toxic compounds [20, 21].

The α-amylase is one of the most important human enzymes that break down starch to simple sugars. Although α-amylase enzyme has no direct role in the etiology of diabetes, inhibitors of these enzymes can break down and digest the carbohydrates as well as delay the production rate and thus reduce the absorption of glucose, and eventually, decrease the increasing rate of blood sugar after food intake. This inhibitor also involves in improving the glucose tolerance in diabetic patients. Control of post-prandial hyperglycemia via modulation of pancreatic α-amylase or intestinal α-glucosidase to delay carbohydrate absorption by dietary anti-diabetic agents is an attractive strategy to control or prevent the onset of long-term complications of hyperglycemia and diabetes mellitus[22]. This study investigated the effects of methanolic extract of leaves and roots of *C. spinosa* L. on α-amylase enzyme. Our findings suggest that *C. spinosa* L. may be potentially useful to control postprandial hyperglycemia in patients with type 2 diabetes through inhibition α-amylase enzyme.

The values of the IC_{50}, obtained in the DPPH method, showed that leaves extract has higher antioxidant capacities than the roots. In addition, the values of the EC_1, determined in the FRAP method revealed the higher the antioxidant capacity of leaves than roots. The result of Folin-Ciocalteu method showed higher value in roots than leaves extract. The α-amylase enzyme percent inhibition showed the higher IC_{50} and consequently higher inhibitory strength of roots extract than leaves.

In conclusion, the findings of this study showed that the antioxidant capacity of leaves extract is more than the roots extract, while the phenol contents of roots are more than the leaves extract. On the other hand α-amylase inhibitory power in the leaves extract is more than the roots extract.

This study identified no relationship between phenol contents, antioxidant activity extracts and enzyme inhibitory power of extracts, because leaves extract has higher antioxidant capacity and inhibitory power, while a lower phenol contents than the roots extract.

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