α-Amylase Inhibitory Activities of Six Salvia Species

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Abstract

α-Amylase inhibitory activities of ethanol extracts from six selected Iranian Salvia species (S. hydrangea DC., S. hypoleuca Benth., S. officinalis L., S. reuterana Boiss., S. verticillata L. and S. virgata Jacq.) were examined, using in vitro model. In addition, antioxidant capacities (DPPH• radical scavenging) and total flavonoid contents of the extracts were also determined. The results showed that the extracts of S. verticillata and S. virgata, significantly and concentration dependently, inhibited α-amylase activity. All the extracts exhibited antioxidant activities, among which S. verticillata, S. virgata, S. officinalis, S. hypoleuca and S. hydrangea were found to be the most active in terms of assay. S. hypoleuca and S. reuterana extracts had the highest total flavonoid contents. However, a favorable correlation was not found between α-amylase inhibitory effects or antioxidant activities and the total flavonoid contents of the extracts.

Keywords: α-Amylase inhibitory activity; Antioxidant activity; Flavonoid content; Salvia species.

Introduction

Diabetes mellitus is a metabolic disorder characterized by a congenital (type I insulin-dependent diabetes mellitus/IDDM) or acquired (type II noninsulin-dependent diabetes mellitus/NIDDM) inability to transport glucose from the bloodstream into cells (1). The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (2). At the present time it is estimated that 150 million people, worldwide, have diabetes and that this will increase to 220 million by 2010 and 300 million by 2050 (3). Globally, type II diabetes afflicts approximately 90% of all diabetes (1).

Various pharmacological approaches are used to improve diabetes via different modes of action such as stimulation of insulin release, increase, the number of glucose transporters, inhibition of gluconeogenesis and reduction of absorption of glucose from the intestine (4). One of the most beneficial therapies for type II diabetes is said to be the control of postprandial hyperglycemia after a meal (5). Stabilization of blood glucose is important for diabetic patients, because it prevents hyperglycemia and the complications associated with diabetes (6). The best therapeutic approach to decrease postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes in the digestive organs (5). The enzymes are responsible for the breakdown of oligo- and disaccharides to monosaccharides. α-Amylase is one the enzymes that catalyses the breakdown of starch to maltose and finally to glucose, which is the only sugar that can be utilized by the body (7). The inhibition of these enzymes leads to a decrease in blood
glucose level, since monosaccharides are a form of carbohydrates which are absorbed through the small intestine (2). There are several reports of established or screened and developed enzyme inhibitors and its effects on blood glucose levels after food uptake (5).

In recent years, plants and their constitutions have received much attention in the treatment of diabetes for various reasons and many researchers have focused on hypoglycemic agents from medicinal plants (4). Based on the existing studies, it is found that polyphenols and flavonoids are among the natural active antidiabetic agents (8). These compounds have been reported to exert various biological effects, including carbohydrate hydrolyzing enzyme inhibition and antioxidant activity. Polyphenolic compounds are able to inhibit the activities of digestive enzymes due to their ability to bind with proteins. The inhibitory activities of plant phytochemicals, including polyphenols, against carbohydrate hydrolyzing enzymes contribute to the lowering of postprandial hyperglycemia in the management of diabetes, as observed in vivo (9). On the other hand, phenolic compounds and flavonoids constitute one of the major groups of herbal compounds acting as free radical scavengers and antioxidants. Free radicals and reactive oxygen species (ROS) can react with biological molecules, leading to cell and tissue injuries and pathological events. The role of free radicals and ROS in the etiology of many chronic diseases has been well-known. Therefore, free radical scavengers and antioxidants, especially phenolic compounds, are important for human health and have been proposed as health-promoting natural products (9, 10).

Several medicinal plants including Salvia species have been advocated in Traditional Iranian Medicine for their hypoglycemic effects (11). The genus Salvia commonly called sage is one the largest members of the family Lamiaceae that contains nearly 900 species spread throughout the world (12). In the Flora of Iran, the genus is represented by about 58 species of which 17 species are endemic (13). They are well known among people and widely used as flavoring agents or fragrances and for various medicinal purposes (10, 14). Salvia species are rich sources of polyphenolic flavonoids and phenolic acids (10, 12). The plants and their isolated constituents possess significant antioxidant activity in the enzyme dependent and enzyme independent systems (10, 15).

The aim of the present study was to evaluate the anti-α-amylase potential of six Iranian Salvia species (S. hydrangea DC., S. hypoleuca Benth., S. officinalis L., S. reuterana Boiss., S. verticillata L. and S. virgata Jacq.). To the best of our knowledge, there is no scientific published data on the inhibitory effects of the selected plants on carbohydrate hydrolyzing enzymes, especially α-amylase. Recently, the free radical scavenging activities of five Salvia species have been reported by us (10). This investigation was also designed to determine the antioxidant capacities of the aforementioned Salvia species by the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging method and estimate the total flavonoid contents of the selected species by the AlCl$_3$ assay and study the correlation between flavonoid contents and α-amylase inhibitory effects and/or antioxidant activities.

**Experimental**

**Chemicals**

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

**Plant materials**

The plants (S. hydrangea, S. hypoleuca, S. officinalis, S. reuterana, S. verticillata, S. virgata) were collected in Tehran province during the flowering period in summer 2004 and 2005 and authenticated by M. Kamalinejad. A voucher specimen for each plant was deposited at the Herbarium of the Department of Pharmacognosy, Shaheed Beheshti University (M. C.), Tehran, Iran. The plants were dried in a drying room, with active ventilation at ambient temperature. Only the aerial parts of the plants were used for investigation.

**Extraction**

The dried and fine plant parts (100 g) were extracted with a 90% ethanolic solution (500 ml)
at ambient temperature for 48 h. The extracts were filtered and concentrated under reduced pressure at approximately 40°C.

α-Amylase inhibition

The α-amylase inhibition assay was adapted and modified from Giancarlo et al. (16). The Starch solution (0.5% w/v) was obtained by boiling and stirring 0.25 g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α-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 were dissolved in DMSO to give concentrations from 14.7 to 36 mg/ml (14.7, 18.4, 23, 28.8, 36 mg/ml). 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).

1 ml of each plant extract and 1 ml enzyme solution were mixed in a tube and incubated at 25 °C for 30 min. To 1 ml of this mixture was added 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 Multispec-1501 spectrophotometer (Kyoto, Japan).

The inhibition percentage of α-amylase was assessed by the following formula:

\[ I_{\text{α-amylase}}\% = 100 \times \frac{\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}}{\Delta A_{\text{Control}}} \]

\[ \Delta A_{\text{Control}} = A_{\text{test}} - A_{\text{Blank}} \]

\[ \Delta A_{\text{Sample}} = A_{\text{test}} - A_{\text{Blank}} \]

The \( I_{\text{α-amylase}}\% \) 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 (μg/ml) necessary to decrease the absorbance of α-amylase by 50%.

Antioxidant and DPPH radical scavenging activity

The antioxidant activities of the plant extracts were assessed by their ability to bleach the stable DPPH radical (10). To 2.5 ml of each sample solution at different concentrations (5, 10, 25, 50, 100, 250, 500, 1000 μg/ml, in ethanol 90%), 1 ml of 0.3 mM DPPH• solution in ethanol was added. Test tubes were vortexed and incubated for 30 min at room temperature in the dark. The absorbance values were then determined at 518 nm. The inhibition percentage of DPPH radical was assessed, using the following formula:

\[ I_{\text{DPPH•}}\% = 100 \times \left[ \frac{A_{\text{Control}} - (A_{\text{Sample}} - A_{\text{Blank}})}{A_{\text{Control}}} \right] \]

Ethanol (1 ml) plus each sample solution (2.5 ml) was used as the blank. DPPH• solution (1 ml) plus ethanol (2.5 ml) was used as the negative control. Also, a rutin solution (at the concentrations of 2.5, 5, 10, 25, 50, 100 μg/ml) was used as the positive control.

The \( I_{\text{DPPH•}}\% \) was plotted against the sample concentration and a logarithmic regression curve established in order to calculated the IC\(_{50}\) value (inhibitory concentration), which is the concentration of sample (μg/ml) necessary to decrease the absorbance of DPPH• by 50%.

Determination of total flavonoid content

The amount of the total flavonoid content for each extract was determined based on the colorimetric assay, using rutin as the reference compound (10). 2.5 ml of a rutin solution (at the concentrations of 75, 50, 37.5, 25 μg/ml, in ethanol 90%) was mixed with 2.5 ml of the AlCl\(_3\) reagent in ethanol 90% (20 mg/ml). After 40 min, the absorbance was measured at 415 nm. A combination of ethanol 90% (2.5 ml) and the rutin solution (2.5 ml) was used as the blank. Then, a linear calibration curve (absorbance versus concentration) was constructed. The same procedure was carried out on 2.5 ml of
each sample in ethanol 90%, instead of the rutin solution. The total flavonoid content for each extract [as µg rutin equivalents (RE)/mg of extract] was determined on the basis of the standard curve.

**Statistical analysis**

The IC$_{50}$ values were estimated by non-linear curve-fitting and presented as their respective 95% confidence limits. Total flavonoid contents have been shown as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey’s post test was used to assess the presence of significant differences ($p<0.05$) between the extracts. All the statistical analyses were accomplished, using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, USA).

**Results and Discussion**

α-Amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars thus, the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Consequently, postprandial rise in blood glucose is decreased. Hence, they have long been though to improve glucose tolerance in diabetic patients (17, 18). On the other hand, Natural polyphenols have been reported to inhibit the activity of carbohydrate hydrolyzing enzymes (9). Besides, phenolic compounds are currently regarded natural antioxidants and considered to be important ingredients for human health (10). Therefore, in this study, α-amylase inhibitory activities, antioxidant capacities (based on the DPPH radical scavenging method) and total flavonoid contents (based on the AlCl$_3$ assay) of the ethanol extracts obtained from six Iranian Salvia species (S. hydrangea, S. hypoleuca, S. officinalis, S. reuterana, S. verticillata, S. virgata) were determined and compared to each other.

The quantification of total flavonoid content was developed, using the AlCl$_3$ reagent and rutin as standards. Table 1 presents the total flavonoid content, in rutin equivalent (RE), calculated for each extract. It varied from 53.16 to 8.54 µg RE/mg of the extract. When the flavonoid content of each extract was compared with the others, the S. hypoleuca (53.16 ±1.95 µg/mg) and S. reuterana (46.97±4.43 µg/mg) extracts were found to have significantly higher contents, while S. virgata (8.54 ± 0.99), S. verticillata (10.81±1.98) and S. officinalis (17.24 ±1.31) contained remarkably lower amounts of these compounds. The order obtained in terms of the total flavonoid content for the tested extracts was S. hypoleuca ≥ S. reuterana > S. hydrangea > S. officinalis ≥ S. verticillata ≥ S. virgata.

The antioxidant activity of each extract was monitored, using the DPPH radical assay. All the tested plants exhibited favorable concentration dependent radical scavenging and antioxidant activities and the IC$_{50}$ values ranged from 23.53 to 125.10 µg/ml (Table 1 and Figure 1). S. verticillata [23.53 (20.56–26.93) µg/ml], S. virgata [27.01 (24.08–30.29) µg/ml], S. officinalis [30.67 (27.88–33.73) µg/ml], S. hypoleuca [36.81 (33.63–40.29) µg/ml] and S. hydrangea [44.57 (40.63–48.89) µg/ml] showed the highest antioxidant capacities, with no significant difference among them. The

<table>
<thead>
<tr>
<th>Plant species</th>
<th>IC$_{50}$ (µg/ml)</th>
<th>Total Flavonoid content (µg/mg)</th>
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<tbody>
<tr>
<td>S. hydrangea</td>
<td>44.37 (41.67–48.45)</td>
<td>33.30 ± 1.25</td>
</tr>
<tr>
<td>S. hypoleuca</td>
<td>36.81 (31.63–42.05)</td>
<td>31.16 ± 1.94</td>
</tr>
<tr>
<td>S. officinalis</td>
<td>30.67 (27.16–33.73)</td>
<td>17.24 ± 1.33</td>
</tr>
<tr>
<td>S. reuterana</td>
<td>12.10 (8.99–16.72)</td>
<td>4.97 ± 4.19</td>
</tr>
<tr>
<td>S. verticillata</td>
<td>23.53 (20.56–26.93)</td>
<td>10.81 ± 1.98</td>
</tr>
<tr>
<td>S. virgata</td>
<td>27.01 (24.08–30.29)</td>
<td>8.34 ± 0.99</td>
</tr>
</tbody>
</table>

*Note: The IC$_{50}$ values of the plants were estimated as their respective 95% confidence limits (t = 1). The IC$_{50}$ values have been presented along with their respective 95% confidence limits (t = 1). The total flavonoid content values are mean ± SEM (t = 1). S. hydrangea, S. hypoleuca, S. officinalis, S. reuterana, S. verticillata, S. virgata*
The α-amylase inhibitory activities varied widely among the tested plants. As can be observed in Table 2 and Figure 2, *S. verticillata* [IC$_{50}$=18.34 (18.07–18.61) mg/ml] and *S. virgata* [IC$_{50}$=19.73 (19.30–20.17) mg/ml] exhibited noticeable concentration dependent effects and did not show any significant differences in their IC$_{50}$ values (p > 0.05). An increase in graded concentrations of the *S. reuterana* extract resulted in a decrease in α-amylase activity from 1.19 ± 0.45% to 25.01±1.68% (Table 2). However, the inhibitory activity of *S. reuterana* on α-amylase was weak and it did not reach the
The findings of the present study not only clarify the α-amylase inhibitory effect, the antioxidant activity and the total flavonoid contents of six Iranian *Salvia* species, but also introduce novel sources for the prevention of non-communicable diseases in Iran and in other countries. However, further in vitro and in vivo studies are needed to confirm the present observations.

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