EVALUATION OF IN VITRO ANTI-HYPERGLYCEMIC, ANTI-LIPASE AND ANTIOXIDANT ACTIVITIES OF SALVIA HISPANICA AND CHENOPODIUM QUINOA SEEDS

TANISHA AND MAJUMDAR M*

Department of Biotechnology, School of Sciences, Jain University, Bangalore, India- 560011

*Corresponding author: E Mail: mala.majumdar@jainuniversity.ac.in; Mob.: 9880184285

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. Defects in insulin action and increased glucose levels in diabetes may lead to high lipoproteins in blood. Subsequent increase in lipids might lead to progression of diabetic complications. Persistent hyperglycemia leads to production of excess free radicals by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation, resulting in impaired antioxidant defense and increased insulin resistance. Current antidiabetic therapy includes synthetic drugs that have undesirable side effects. Therefore, interest to find natural compound having anti-hyperglycemic, anti-lipase and antioxidant activities has increased which help in controlling diabetes and oxidative damages. Edible seeds can be a good option as they are rich in phytochemicals, having multiple health benefits including radical scavenging activities. In present study an effort was carried out to investigate in vitro anti-hyperglycemic, anti-lipase and antioxidant activities of methanolic extract of 2 edible seeds Salvia hispanica and Chenopodium quinoa. Preliminary phytochemical screening revealed the presence of alkaloid, flavonoid, phenol and saponins. S.hispanica exhibited highest phenolic (66.6±0.5 mg GAE/gm extract) and flavonoid (72.3± 0.8 mg QE/gm extract) contents. S. hispanica showed maximum DPPH and ABTS●+ scavenging activities (IC₅₀=1.02 μg/ml; 1.09 μg/ml respectively). S.hispanica also exhibited maximum α-amylase (IC₅₀=1.13 mg/ml) inhibitory activity whereas C.quinoa showed maximum α-glucosidase (IC₅₀= 0.86 mg/ml) and moderate lipase (IC₅₀=0.97 mg/ml) inhibitory activity compared to S.hispanica (IC₅₀=1.37 mg/ml). Present results suggest that S.hispanica and C.quinoa seed extracts may
have a great potential for further development as diabetes prevention agents or food supplements as a complementary pharmacological drug.

Keywords: *Salvia hispanica*, *Chenopodium quinoa*, Antioxidant, Antihyperglycemic, Lipase inhibition

INTRODUCTION

Type 2 diabetes mellitus is a metabolic disorder characterized by hyperglycemia which results either due to defect in insulin secretion and/or action or by both due to an alteration in carbohydrate, lipid and protein metabolism [1]. Persistent hyperglycemia is also related to higher risk of hyperlipidemia (a group of metabolic disorder characterized by elevated lipid and triglyceride levels). Diabetic hyperlipidemia describes the effects of insulin resistance on abnormal lipid levels. Defects in insulin action and increased glucose may result in higher amounts of lipoproteins in the blood. Subsequent increase in lipid level, secondary to glucose intolerance, increases the risk of progression of diabetic secondary complications such as cardiovascular diseases and atherosclerosis [2]. Additionally, consumption of diet rich in carbohydrate and fat results in a significant increase in postprandial glucose levels and oxidative stress by the formation of reactive oxygen species by several biochemical pathways such as non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation, resulting in impaired anti-oxidant defense and increased insulin resistance [3, 4]. One of the therapeutic approaches to control hyperglycemia and hyperlipidemia is the inhibition of carbohydrate digestive enzymes (α-amylase and α-glucosidase) and fat digestive enzymes (pancreatic lipase). Currently, acarbose and orlistat are potent inhibitors of α-amylase and α-glucosidase and pancreatic lipase respectively, but these have undesirable side effects such as abdominal pain, bloating, diarrhea, nausea, vomiting etc. [5, 6]. This has led to the search of biologically active antioxidants, anti-hyperglycemic and anti-hyperlipidemic agents from natural resources with less side effects [7, 8]. Edible seeds can be a good option for this purpose as edible seeds are rich in many bioactive compounds, specifically phenolics and flavonoids which have a positive correlation as antidiabetic agents [9, 10, 11]. Polyphenolic compounds may reduce the activity of α-amylase and α-glucosidase by the interacting or inhibiting the specific positions in enzymes [12]. *Salvia hispanica* (Chia) seeds are used as a traditional food in central and southern America and it possess various health
benefits specifically in maintaining healthy serum lipid level [13]. *Chenopodium quinoa* (Quinoa) is one of the most important Andean grain crops with high nutritional quality. Recent studies have identified quinoa seeds as a good source of polyphenol which might prevent oxidative stress [14]. Therefore, the present study was aimed to screen the anti-hyperglycemic, anti-lipase and antioxidant activity of *S. hispanica* and *C. quinoa* seeds.

**MATERIALS AND METHODS**

**Chemicals**

All chemicals used were of analytical grade 1,1-Diphenyl-2-picryl hydrazyl (DPPH) and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•⁺), potassium persulphate, Lipase, p-nitrophenyl butyrate (pNPB), DNSA reagent was obtained from Sigma Chemicals, Bangalore, India. Alpha amylase, alpha glucosidase, 4-Nitrophenyl-Β-D-Glucopyranoside (pNPG), quercetin, gallic acid were purchased from SISCO Research Laboratories Pvt, Ltd. Mumbai, India. Methanol, aluminium chloride (AlCl₃), Starch, Potassium acetate was obtained from Fischer scientific, Bangalore, India. Acarbose was obtained from Bayer pharmaceuticals. Orlistat was obtained from Roche. Sodium carbonate (Na₂CO₃), N,N, dimethylformamide was obtained from SDFCL. FC reagent was obtained from Merck. Ascorbic acid was obtained from HiMedia, Bangalore, India.

**Collection of Seeds**

Seeds were collected from in and around Bangalore and were identified and authenticated by Dr. Rama Rao from Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore.

**Preparation of seed extract:** The seeds were dried and ground to a fine powder. The powdered seeds were extracted with methanol using soxhlet apparatus. The filtrates were concentrated using a rotary evaporator and stored at -20°C for further studies.

**Phytochemical Analysis**

All the “seeds extract” were qualitatively screened for the presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids, quinine and phlobatannins using standard protocol [15].

**Determination of total phenolic contents (TPC)**

The total phenolic content of seed extracts was determined using Folin-Ciocalteu reagent [16]. The reaction mixture containing 0.3 ml of methanolic extracts (1 mg/ml) and 1.5 ml of 10% Folin-Ciocalteu reagent was incubated for 15 minutes in dark. Then, 7.5% Na₂CO₃ was added to terminate the reaction. The absorbance was determined at 765 nm. Gallic acid was used as standard. The concentration of phenols
was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

**Determination of total flavonoid contents (TFC)**
The total flavonoid content of seed extracts were determined using spectrophotometric method [17]. 0.5 ml of seed extracts (1 mg/ml) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was incubated at room temperature for 30 minutes. Quercetin was used as a standard. The absorbance was measured at 420 nm. The concentration of flavonoids was expressed in terms of Quercetin equivalent (mg of QE/g of extract).

**Determination of Antioxidant activity**

**DPPH Assay**
DPPH radical scavenging assay was performed according to the standard protocol [18]. 1 ml of 0.1 mM DPPH in methanol was added to 3 ml of methanolic seed extracts (12.5, 25, 50, 100 and 200 μg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Ascorbic acid was used as standard and absorbance was measured at 517 nm using spectrophotometer. The antioxidant capacity was expressed as percentage inhibition, using the following formula:

\[
\text{Scavenging activity (\%)} = \left( \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) \times 100
\]

Where \( A_{\text{control}} \) is the absorbance of the control reaction and \( A_{\text{sample}} \) is the absorbance of the sample.

**ABTS**\(^+\) Assay
ABTS\(^+\) scavenging activity was performed according to the standard protocol [19]. Briefly, ABTS\(^+\) was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium per sulphate and the mixture was allowed to react for 12 h at 37°C in the dark. The resulting solution was then diluted with 5 mM phosphate-buffered saline (pH 7.4). One ml of seed extracts (12.5, 25, 50, 100 and 200 μg/ml) were added to 0.5 ml of the diluted ABTS\(^+\) solution, incubated for 20 min and the absorbance was measured at 745 nm. The percentage (%) of radical scavenging activity was calculated by the following formula:

\[
\text{Scavenging activity (\%)} = \left( \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) \times 100
\]

Where \( A_{\text{control}} \) is the absorbance of the control reaction and \( A_{\text{sample}} \) is the absorbance of the sample.

**Antihyperglycemic activity**

**α-amylase inhibitory activity**
α-amylase inhibition assay was carried out using the standard method with minor modifications [20]. 100 μl of seed extract (0.25, 0.50, 1.0, 1.5 and 2 mg/ml) was added to 100 μl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (1 Unit/ml) and was
incubated for 10 min. After that, 100 μl of 1% (v/v) starch solution was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with addition of 200 μl DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature, diluted with 2 ml of distilled water and the absorbance was measured at 540 nm. Acarbose was used a positive control.

Percentage of inhibition was calculated as:

\[
\% \text{ Inhibition} = \left[ \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right] \times 100
\]

Where \( A_{\text{control}} \) is the absorbance of the control reaction and \( A_{\text{sample}} \) is the absorbance of the sample.

**α-glucosidase inhibitory activity**

α-glucosidase inhibition assay was performed according to standard protocol with minor modifications [21]. 50 μl of α-glucosidase (1 unit/ml) was pre-incubated with 100 μl of seed extracts (0.25, 0.50, 1.0, 1.5 and 2 mg/ml) for 20 minutes at room temperature. After pre-incubation, 50 μl pNPG (4-nitrophenyl β-D-glucopyranoside) was added to start the reaction and this reaction mixture was incubated for 20 minutes at room temperature. After incubation, 500 μl of 0.1 M sodium carbonate (Na₂CO₃) was added to stop the reaction. The absorbance was measured at 405 nm. Acarbose was used as standard.

Percentage of inhibition was calculated as:

\[
\% \text{ Inhibition} = \left[ \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right] \times 100
\]

Where \( A_{\text{control}} \) is the absorbance of the control reaction and \( A_{\text{sample}} \) is the absorbance of the sample.

**Statistical analysis**

All experiments were performed in triplicate. Data were presented as mean ± standard error of mean (SEM). Differences were evaluated by two ways analysis of variance (ANOVA) test followed by Tukey’s multiple comparison tests using Graph Pad Prism 6. Differences were considered significant at \( p \leq 0.05 \).

**RESULTS AND DISCUSSION**

Natural α-amylase and α-glucosidase inhibitors from food-grade herbal sources offer an attractive therapeutic approach to
the treatment of postprandial hyperglycemia, which may be potentially useful in the treatment of diabetes mellitus and obesity [23]. As an alternative therapy to treat diabetes and prevent weight gain as well as oxidative stress, in vitro potential of S.hispanica and C.quinoa to reduce oxidative stress as well as carbohydrate and lipid digestion via the inhibition of the enzymes α-amylase, α-glucosidase and lipase were investigated. The effective inhibitory activity of S.hispanica and C.quinoa may be due to phytochemicals like phenols, tannins, alkaloids, terpenoids and flavonoids which are also involved in radical scavenging activities [24].

In present study, the phytochemical screening of the tested seeds revealed that alkaloid, flavonoid, phenol and saponins were present (Table 1). S. hispanica exhibited highest TPC (66.6±0.5 mg GAE/gm extract) and TFC (72.3±0.8 mg QE/gm extract), when compared to C.quinoa (Table 2).

In diabetes, excess production of free radicals causes decline of antioxidant defense mechanisms which may lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance. These consequences of oxidative stress can promote the development of microvascular and macrovascular complications of diabetes mellitus. Therefore, use of antioxidants is an important aspect in the therapeutic management of diabetes mellitus [25]. DPPH method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant capacity. ABTS**+ assay is used to evaluate the scavenging activity of both polar and non-polar sample [26].

The antioxidant activity of the tested seeds were evaluated by DPPH scavenging and ABTS˙+ assay (Table 2). S.hispanica showed maximum DPPH and ABTS˙+ scavenging activities (IC₅₀=1.02 μg/ml; 1.09 μg/ml) respectively compared to C.quinoa (IC₅₀=1.09 μg/ml; 1.35 μg/ml) respectively. Ascorbic acid was used as standard for antioxidant assay and it showed significant DPPH (IC₅₀= 0.78 μg/ml) and ABTS˙+ (IC₅₀= 0.80 μg/ml) scavenging activity. Antioxidant and TPC of amaranth and C.quinoa grains were studied where the latter seeds exhibited moderate DPPH scavenging activity (IC₅₀=313.25 μg/ml. The antioxidant activity of S.hispanica seeds was evaluated in which the seed showed appreciable DPPH scavenging activity (IC₅₀=162±1.25 μg/ml) which is in accordance with the earlier findings [27, 28].

Among the seeds, S. hispanica exhibited maximum α-amylase (IC₅₀=1.13 mg/ml)
and *C*. *quinoa* showed maximum α-glucosidase (IC$_{50}$ = 0.86 mg/ml) inhibitory activity (Fig. 1). Acarbose was used as a positive control and it showed significant effect on α-amylase (IC$_{50}$ = 0.41 mg/ml) and α-glucosidase (IC$_{50}$ = 0.89 mg/ml) inhibition. In the current study, *C*. *quinoa* (IC$_{50}$ = 0.97 mg/ml) exhibited higher lipase inhibitory activity compared to *S*. *hispanica* (IC$_{50}$ = 1.37 mg/ml) (Fig. 2). Orlistat was used as positive control which exhibited higher inhibitory activity against the enzyme (IC$_{50}$ = 0.38 mg/ml) compare to tested seeds.

Free phenolic fractions from *S*. *hispanica* possessed significant α-glucosidase inhibitory activity (IC$_{50}$ = 56.54±9.4 mg/ml) and pancreatic lipase inhibitory activities (IC$_{50}$ = 10 mg/ml) [29]. Three different varieties of *C*. *quinoa* (white quinoa, red quinoa and black quinoa) were studied for their α-glucosidase inhibitory activity where IC$_{50}$ values for α-glucosidase ranged from 37.58 - 55.58 mg/ml. The seeds were also evaluated for their lipase inhibitory activity where IC$_{50}$ values for lipase ranged from 9 - 11 mg/ml [30]. Pancreatic lipase plays a key role in triglycerides metabolism [31]. Thus, inhibitions of pancreatic lipase along with inhibition of carbohydrases (α-amylase and α-glucosidase) are considered as a valuable method for the treatment of diet induced hyperglycemia [32]. Orlistat, a lipase inhibitor, exerts the therapeutic activity by forming a covalent bond with active serine residue site of gastric and pancreatic lipases making the enzymes inactive and unavailable to hydrolyze dietary fat (triglycerides) into absorbable free fatty acids and monoglycerides. This phenomenon is considered to have a positive effect on weight control.

### Table 1: Qualitative phytochemical screening of *S*. *hispanica* and *C*. *quinoa* seed extracts

<table>
<thead>
<tr>
<th>Name of Seeds</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Phenol</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Glycoside</th>
<th>Terpenoid</th>
<th>Quinone</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S</em>. <em>hispanica</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>C</em>. <em>quinoa</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+= present; - = absent

### Table 2: Total phenolic and flavonoid contents of seed extracts and IC$_{50}$ values of seed extracts by DPPH and ABTS$^{•+}$ method

<table>
<thead>
<tr>
<th>Seed Extract</th>
<th>mg GAE/g</th>
<th>mg QE/g</th>
<th>DPPH (µg/ml)</th>
<th>ABTS$^{•+}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S</em>. <em>hispanica</em></td>
<td>66.6±0.55</td>
<td>72.36±0.8</td>
<td>1.02</td>
<td>1.29</td>
</tr>
<tr>
<td><em>C</em>. <em>quinoa</em></td>
<td>58.75±1.06</td>
<td>56.99±0.9</td>
<td>1.09</td>
<td>1.35</td>
</tr>
</tbody>
</table>

GAE= Gallic acid equivalent; QE= Quercetin equivalent
CONCLUSION
The results of the present study might be useful for the search of specific enzyme inhibitors from edible seed extracts for effective management of Type-2 diabetes mellitus and related complications. Further, in vivo studies will be conducted to confirm the anti-diabetic, anti-lipase and antioxidant activities of these two seed extracts.

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