Antioxidant, antiglycation and alpha Amylase inhibitory activities of *Cassia absus* seeds

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**A B S T R A C T**

Being the natural reservoirs of metabolites, plants are the mainstay of health care system since antiquity and are potent in neutralizing free radicals and thus ameliorate certain degenerative diseases like diabetes mellitus. Present research was planned to assess antioxidant, antiglycation and enzyme inhibitory activities of *Cassia Absus* seeds (CAS). *Cassia Absus* seeds extract and solvent fractions were subjected to DPPH free radical scavenging assay, and phytochemical screening. In addition, antiglycation and alpha amylase inhibition potentials were evaluated by prescribed protocols. Antioxidant activity ranged from 49.8 to 74.2% and CAS n-hexane fraction had significantly highest scavenging activity. DPPH scavenging activity in descending order was n-hexane > n-butanol > ethylacetate > chloroform > aqueous methanol. Significant (P < 0.05) antiglycation activities were time-dependent. After first week incubations, 16.2-44.5% decline in glycation was observed and methanol aqueous extract was the strongest inhibitor. Three weeks glycation study further blocked BSA glycation by 24.2-60.21% and n-butanol fraction had most dominant antiglycation potency. CAS extract/fractions showed concentration-dependent amylase inhibitory effect. With 0.1 mL concentrations, 12.5-27.43% decline in enzyme activity was observed that progressed to 58.65-70.23% by 0.5 mL test sample. Most effectual in enzyme inhibition was n-butanol fraction. Other samples (0.5 mL) reduced amylase activity in descending order as n-hexane > chloroform > aqueous > ethylacetate. Screening showed that alkaloids, flavonoids, terpenoids, glycosides and tannins were present. The data offers some valuable bioactivities of *Cassia absus* seeds. CAS may be considered as a potential source of biological agents for developing different pharmaceutical applications.

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**Capsule Summary:** Different solvent fractions of *Cassia* absus seeds were studied for antioxidant, antiglycation and enzyme inhibitory activities. Variable degrees of bioactivities were observed for each fraction indicating plant as potential source to develop phytomedicine.

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**INTRODUCTION**

Being the natural reservoirs of phytochemicals and secondary metabolites, plants are the mainstay of health care system since antiquity. Current research has led to the credence that plant derived polyphenols are potent in neutralizing free radicals and thus ameliorates certain degenerative diseases like diabetes mellitus (Rechner et al., 2002; EL-Kamali, 2009; Leena and Jill, 2010; Murugan et al., 2013).

Diabetes is a serious disease which has reached epidemic proportions in many parts of the world. Oxidative stress, hyperglycemia and advanced glycation endproducts (AGEs) have been implicated in the progression of diabetes. The accumulation of AGEs is associated with more social acceptance than allopathic drugs due to fewer side effects. One of the successful methods to prevent of the onset of diabetes is to control postprandial
hyperglycemia by the inhibition of pancreatic alpha amylase activities, resulting in the aggressive delay of the carbohydrate digestion of absorbable monosaccharides (Ledwani and Oberoi, 2010; Chen et al., 2011; Meng et al., 2011).

*Cassia absus* L. (Leguminosae) seed (CAS) extracts are used to treat mucous diseases and blood glucose levels. Flavonoids, anthraquinone and polysaccharides are detected in all *Cassia* species and thus it is functional against numerous aliments (Ayyanar and Ignacimuthu, 2008). CAS has a bitter taste and having diuretic, cathartic and astringent properties, its efficacy to treat hepatic and renal diseases is already proven. Ocular infectious manifestation is also cured by *Cassia absus* seeds. *Cassia absus* L. extracts not only decreased systemic arterial blood pressure but it was also revealed that *Cassia absus* exerted centrally acting/ganglion blocking, anti-nicotinic, non-specific muscle relaxant and curare like activities (Aftab et al., 1996; Souri et al., 2008; Pandya et al., 2010).

Recently, there has been a resurgent interest in the herbal treatments of diabetes. Pharmaceutical industry and research worldwide are more interested in medicinal plants with multiple curative potentials. A plant having anti-oxidation, antiglycation and enzyme inhibitory potentials may serve as a better therapeutic agent for diabetes. While most research reports indicated that CAS manifests antioxidant and hypoglycemic activities, some of biochemical aspects such as antiglycation and alpha amylase inhibitory efficacies remain to be investigated. As there are no previous reports on antidiabetic activities of CAS, present study was planned to assess the phytochemical components of CAS along with their antioxidant and antidiabetic activities in various organic solvents.

**MATERIALS AND METHODS**

**Plant material and preliminary processing**

*Cassia absus* seeds collected from the local retail market were authenticated at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The seeds were ground to course powder and extracted with methanol. Solvent was evaporated by rotary evaporator, greyish material was dried on water bath and stored at -4°C. This processing sequel was performed thrice with 4 days interims. Extracts were reconstituted in distilled water, partitioned with different polarity based solvents using separating funnel and attained ethylacetate (45 g), *n*-hexane (120 g), *n*-butanol (45 g), chloroform (55 g) fractions.

**Antioxidant assay**

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was performed (Souri et al., 2008). From 10 to 500 µM/mL samples concentration were mixed with 90 µM DPPH (1mL) solution and final volume of 4 mL was made up with 95% methanol. Synthetic antioxidant, BHT (butylated hydroxy toluene) was used as standard control. After incubation at room temperature for 1 hour, the absorbencies were recorded at 517 nm.

**Antidiabetic profile**

**Inhibition of porcine pancreatic alpha amylase**

Porcine pancreatic alpha amylase (EC 3.2.1.1) was used to study enzyme inhibition phenomenon (Apostolidis et al., 2006). Extracts and fractions (100 µL) and 100 µL of 0.02M sodium phosphate buffer (pH 6.9) containing enzyme solution (0.5 mg/mL) were allowed to incubate for 10 minutes at 25°C. Then 100 µL of 1% starch solution was added. After incubation at 25°C for 10 minutes, dinitrosalicylic acid (DNS) reagent was added and heated in boiling water bath for 5 minutes. After dilution with distilled water absorbance was measured at 540 nm. Percent inhibition in enzyme activity was calculated as: 

\[
\text{Percent inhibition} = \left(1 - \frac{A_{	ext{Ac}}}{A_{	ext{As}}}ight) \times 100.
\]

Where, *Ac* represents the absorbance of control and *As* represents the absorbance of test samples. Sodium phosphate buffer (0.02 M, pH 6.9) and metformin drug served as negative and positive controls respectively. Same procedure was repeated with 300 and 500 µL CAS extract/fractions.

**Antiglycation assay**

Antiglycation assay was performed as reported previously (Ayatollahi et al., 2010) and the results were compared to those of the synthetic inhibitor aminoguanidine. 100 µL sample was prepared in dimethylsulfoxide (DMSO). Equal volumes (100 µL) of extracts/fractions, bovine serum albumin (10 mg/mL) in 67 mM sodium phosphate buffer pH 7) and glucose (50 mg/mL) solutions were mixed. In glycated (positive) control, sodium phosphate buffer was used, while negative control contained bovine serum albumin and sodium phosphate buffer only. After incubation at 37°C for 7 days, 100 µL of 100% trichloroacetic acid was added and centrifuged (15000 rpm) at 4°C for 4 minutes. Phosphate buffer saline (pH 10) was used to dissolve the pellets containing advanced glycation end-product. At 440 nm the absorbence was noted and percentage inhibition was calculated by the following formula:

\[
\text{Inhibition} = 100 - \left(\frac{\text{OD test}}{\text{OD blank}}\right) \times 100.
\]

Antiglycation activity was also monitored after 2 and 3 weeks incubation periods.

**Phytochemical screening**

Table 1: Phytochemical screening of *Cassia absus* seed extracts in different solvents

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Glycosides</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>n</em>-hexane</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>n</em>-butanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous methanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+): Presence
CAS extract and fractions were screened for the detection of active components like tannins, alkaloids, terpenoids and flavonoids by the method of Krishnaiah et al. (2009). Data was analyzed using SPSS (SPSS Inc. Chicago, IL, USA) software (version 15.0) with level of significance set at $p < 0.05$. All the results (mean values ± standard deviation) were average of three samples of each extract/fraction, analyzed individually in triplicate ($n = 1 \times 3 \times 3$).

**RESULTS**

The antioxidant profile is presented in Figure 1. Free radical scavenging activity ranged from 49.8 to 74.2% and CAS $n$-
hexane fraction had significantly highest scavenging activity. DPPH scavenging activity in descending order was n-hexane > n-butanol > ethylacetate > chloroform > aqueous methanol extract.

Starch hydrolyzing enzymes inhibitors may be valuable in the treatment of diabetes mellitus. Alpha amylase inhibition by CAS was investigated and presented in Figure 2. Cassia absus extract/fractions reflected concentration-dependent amylase inhibitory effect. With 0.1 mL concentrations, 12.5-27.43% decline in enzyme activity was observed that progressed to 58.65-70.23% by 0.5 mL test sample. Most effective in enzyme inhibition was n-butanol fraction. Other samples (0.5 mL) reduced amylase activity in descending order as n-hexane > chloroform > aqueous > ethylacetate.

The results of antiglycation assay are summarized in Figure 3. All the samples demonstrated different though significant (P < 0.05) antiglycation trends. Glycation impediment by CAS reflected time-dependent tendency. After first week incubations, 16.2-44.5% decline in glycation was observed and methanol aqueous extract was most strong inhibitor. Further reduction (18.5-55.17%) was detected after two weeks incubations and n-butanol fraction was most powerful in hindering glycation. Three weeks glycation study further blocked BSA glycation by 24.2-60.21% and again n-butanol fraction had most dominant obstruction potential.

Table I represents the phytochemicals prevalent in Cassia absus seeds. Fundamental chemicals detected included alkaloids, flavonoids, saponins, terpenoids and glycosides.

**DISCUSSION**

Interest in the role of antioxidants in human health has prompted research in the fields of medical science to assess plants antioxidants. Our results are similar to Jayaraman et al. (2014), as they indicated almost analogous antioxidant activities by CAS. It has already been revealed by the qualitative screening that the Cassia absus seeds like other plants, have a quite number of useful chemical constituent that may be beneficial in their antioxidant role. Our data also highlighted the varying extraction ability of different solvents as indicated by variable antioxidant potentials. The scavenging effect of the CAS revealed a range of its defense mechanism against the stable free radical DPPH and showed a significant level of activity of antioxidants which was in accordance with the earlier reports (Cho et al., 2003; Li et al., 2008; Gokani et al., 2011). Pancreatic α-amylase inhibitors offer an effective strategy to lower the levels of post-prandial hyperglycemia via control of starch breakdown. Regarding the enzyme inhibition phenomenon, some constituents like tannins, alkaloids, terpenoids and flavonoids could be responsible for inhibiting the effective alpha amylase (Hakkim et al., 2007; Myung-Hee et al., 2010). The remedial nature of Cassia absus exposed in current assay may be accredited to the presence of potent chemicals such as saponin, tannin, alkaloid, phenol, steroid and flavonoid (Ayyanar and Ignacimuthu, 2008). Alpha amylase inhibitory activities have been reported for many herbal plant extracts but no such report was found for Cassia absus.

Protein glycation leads to the excessive production of reactive oxygen species via lipoxidation and glycoxidation. Non-enzymatic reaction between carbonyl groups of glucose and epsilon amino group of amino acids results in Schiff base formation and ultimately advanced glycation endproducts (AGEs). AGEs are involved in the pathogenesis of diabetic and aging-related complications (Kim and Kim, 2003; Fujiwara et al., 2011). The results revealed the potential antihyperglycemic effect of the CAS due to the presence of fundamental phytochemicals. The current results are in accordance with the
earlier observations in different species of *Cassia* as well in other medicinal plants (Kim and Kim, 2003; Jayaraman et al., 2014). Phytoconstituents which have multiple biological effects are the most important group of secondary metabolites and are commonly present in most of the plants (Kim and Kim, 2003; Li et al., 2008). Phytochemical analysis is the most important topic of interest for the researchers because of its biological activities. Present inferences are in accordance with the findings of Jayaraman et al. (2014) and partly in agreement with data reported by Hakkim et al. (2007).

**CONCLUSIONS**

The present study revealed the evidence for antidiabetic and antioxidative activities of *Cassia absus* seeds in various organic fractions. These natural antioxidant properties of the plant extract support the therapeutic usage of the seeds for treating many diseases and will facilitate the researchers to develop new medicines.

**REFERENCES**


