Evaluation of the antioxidant activity of various extracts of aerial parts of Cassia absus: An in-vitro techniques

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ABSTRACT
Cassia absus (Linn) (family Fabaceae) is generally known as “chaksu” in an ayurvedic traditional system. The current study, aerial parts of different concentrates (Pet.ether, ethyl acetate and methanol) of Cassia absus, was evaluated for its in-vitro antioxidant potential by Diphenylpicrylhydrazyl radical, nitric oxide activity and total antioxidant activity taking ascorbate as the standard for all the three methods. The IC50 value was originated that methanolic concentrates of Cassia absus more efficient in Diphenylpicrylhydrazyl radical, nitric oxide activity, total antioxidant activity compared EA&PE concentrates. The methanolic concentrates of Cassia absus & ascorbic acid exhibited antioxidant potential possessing IC50 230 μg/ml & 130 μg/ml (Nitric oxide), 205 μg/ml & 57 μg/ml (total antioxidant), 195 μg/ml & 66 μg/ml (Diphenylpicrylhydrazyl radical) respectively. The difference in the scavenging potential of the extracts can be due to variation in the percentage of bioactive compounds present in different solvents. In vitro antioxidant studies obviously show the methanolic concentrates of Cassia absus have better antioxidant activity. This result indicates that aerial parts of methanolic concentrates Cassia absus could serve as a natural antioxidant, which may be useful in prevent free radical-induced diseases.

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INTRODUCTION
Oxidative stress ensuing from the poisonous effects of free radicals on the tissue plays a significant role in the pathogenesis of a variety of pathological conditions such as ageing process, anemia, arthritis, asthma, atherosclerosis, cancer, neuro degeneration, Parkinson’s disease, and perhaps dementia. Antioxidants are radical scavengers, which protect the human body against freeradicals (Mahaknakorn et al., 2004; Polterait, 1997). Ethnomedical literature contains a huge amount of herbs that may be used for the various diseases, in which ROS and free radical participate vital responsibility. Huge numerical herbs are used for strong antioxidant activity (Badami et al., 2003). Current reports revealed that there is a converse connection between the intake of antioxidant-rich foods and the occurrence of human diseases (Halliwell and Gutteridge, 1999).

Cassia absus (Linn) (family Fabaceae ) is generally known as “chaksu” in an ayurvedic traditional sys-
tem. (Kirtikar and Basu, 1918). Chaksine and iso
chaksine bothalkaloids were isolated from the seed
of Cassia absus (Siddiqui and Ahmed, 1935). Cassia
absus was used for different diseases like antibac-
terial, antimalarial and lowering the blood pres-
sure (Aftab et al., 1996). Cassia absus was used
antihistaminic activity of an eye drops (Abdul et al.,
2010). Still, no literature are available on the antiox-
idant activity of aerial parts Cassia absus. Thus,
the present study to assess antioxidant activities of
aerial parts Cassia absus.

METHODOLOGY

Gathering & Identification of Plant
The aerial parts Cassia absus(family Fabaceae) were
gathered form senkottai, Tirunelveli District of
Tamilnadu, India. Plant identification was
made from the Botanical investigation of India,
Palayamkottai. The Cassia absus were desic-
cated under shadowy, segregate, crushed through a
grinder (SatheeshKumar et al., 2011).
Preparation of Concentrates
The pulverized materials were packed in a muslin
cloth and extracted with pet.ether, ethyl acetate
and methanol as solvents respectively according to
the (Shajiselvin et al., 2010) increasing order of
polarity through hot constant percolation method
in Soxhlet equipment (Harborne, 1984) for twenty-
four hours. The concentrates were concentrated
through the rotational evaporator and subjected to
solidify drying in a lyophilizer till dry powder was
acquired (Alagumanivasagam et al., 2010; Sivakrish-
nan et al., 2014).
Assessment of Antioxidant potential through in
vito methods
The variety of concentrates of Cassia absus were
used assessment of antioxidant activity by Men-
sor et al. (2001) method was adopted for Diphenyl
picrylhydrazyl radical assay. Garrat et al. (1964)
method was adopted for NO radical assay & Prieto
et al. (1999) method described for total antioxidant
activity.

RESULTS AND DISCUSSION

DPPH scavenging activity
Diphenylpicrylhydrazyl is a stable N2-centered free
radical generally utilized for testing the antioxidant
potential of herbal concentrates. When the sta-
ble Diphenylpicrylhydrazyl radical accepts an elec-
tron from the antioxidant compound, the violet
colour of the Diphenylpicrylhydrazyl as reduced
to yellow colored diphenylpicrylhydrazine radical
which was measured colorimetrically. Substances
which are able to perform this reaction can be con-
sidered as antioxidants & therefore, radical scav-
engers (Mohammad et al., 2009). The DPPH activity
of PE concentrates of Cassia absus appeared in
Table 1. The PE concentrates of Cassia absus exhibit
a more DPPH activity of 49.16% at 800 µg/ml &
ascorbate was recorded 72.82% at 800 µg/ml. The
IC50 of the PE concentrates of Cassia absus & ascor-
bic acid were recorded 825µg/ml & 66µg/ml corre-
spondingly.
DPPH activity of EA concentrates of Cassia absus
summarized in Table 2. The EA concentrates of Cas-
ia absus exhibit more DPPH scavenging potential
of 55.76% at 800 µg/ml & ascorbate was recorded
72.82% at 800 µg/ml. The IC50 of the EA con-
centrates of Cassia absus & ascorbic acid were
recorded590µg/ml&66µg/m corre-
spondingly.
DPPH potential of methanolic concentrates of Cas-
ia absus appeared in Table 3. The methanolic
concentrates of Cassia absus having more DPPH scav-
enging potential of 62.56% at 800 µg/ml & ascor-
bate was recorded 72.82% at 800 µg/ml. The IC50 of
the methanolic concentrates of Cassia absus & ascor-
bic acid were recorded195µg/ml & 66µg/m corre-
spondingly.
The methanolic concentrates of Cassia absus was
recorded to more activity than PE&EA concentrates.
The IC50 of the methanolic concentrates of Cassia
absus & ascorbic acid were found to be 195 µg/ml &
66 µg/ml correspondingly. Among the three differ-
ent plant concentrates tested, interestingly, in the
DPPH radical activity of the methanolic of Cassia
absus having more Diphenylpicrylhydrazyl radical
potential comparable with that of ascorbic acid.

Nitric oxide scavenging activity
NO produced from sodium nitroprusside reacts with
oxygen to form nitrite. The nitrite ions diazotize
with sulphanilamide acid and couple with naphthyl
ethlenediamine, producing pink coloured, which
absorbs at 546 nm (Panda et al., 2009). Nitric
oxide scavenging activity of PE concentrates of Cas-
ia absus appeared in Table 4. The PE concentrates
of Cassia absus exhibit a more Nitric oxide scaveng-
ing activity of 52.67% at 750 µg/ml & ascorbate was
recorded 67.56% at 750 µg/ml. The IC50 of the PE
concentrates of Cassia absus & ascorbic acid were
recorded 822µg/ml &130µg/ml correspondingly.
Nitric oxide scavenging activity of EA concentrates
of Cassia absus appeared in Table 5. The EA con-
centrates of Cassia absus exhibit a more Nitric oxide
scavenging activity of 59.84% at 750 µg/ml & ascor-
bic acid was recorded 67.56% at 750 µg/ml. The
Table 1: DPPH radical activity of *Cassia absus* PE extract

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract (µg/ml)</th>
<th>PE concentrates</th>
<th>Ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>12.28±0.045</td>
<td>54.19±0.024</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>25.32±0.022</td>
<td>59.24±0.032</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>38.73±0.054</td>
<td>65.32±0.054</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>49.16±0.028</td>
<td>72.82±0.062</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 825 µg/ml</td>
<td>IC50 = 66 µg/ml</td>
</tr>
</tbody>
</table>

*Every value was articulated as mean± SEM for 3 experimentation

Table 2: DPPH radical activity of *Cassia absus* EA extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extract (µg/ml)</th>
<th>(EA concentrates)</th>
<th>(Ascorbate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>24.46±0.052</td>
<td>54.19±0.024</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>37.48±0.074</td>
<td>59.24±0.032</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>45.08±0.045</td>
<td>65.32±0.054</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>55.76±0.022</td>
<td>72.82±0.062</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 590 µg/ml</td>
<td>IC50 = 66 µg/ml</td>
</tr>
</tbody>
</table>

*Every value was articulated as mean± SEM for 3 experimentation

Table 3: DPPH radical activity of *Cassia absus* methanolic extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extract (µg/ml)</th>
<th>(Methanolic concentrates)</th>
<th>Ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>39.12±0.042</td>
<td>54.19±0.024</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>50.78±0.026</td>
<td>59.24±0.032</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>56.34±0.072</td>
<td>65.32±0.054</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>62.56±0.038</td>
<td>72.82±0.062</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 195 µg/ml</td>
<td>IC50 = 66 µg/ml</td>
</tr>
</tbody>
</table>

*Every value was articulated as mean± SEM for 3 experimentation

Table 4: Nitric oxide scavenging activity of *Cassia absus* PE Extract

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract (µg/ml)</th>
<th>PE concentrates</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>32.32±0.012</td>
<td>48.24±0.028</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>39.56±0.018</td>
<td>56.12±0.042</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>45.23±0.024</td>
<td>63.12±0.053</td>
</tr>
<tr>
<td>4</td>
<td>750</td>
<td>52.67±0.048</td>
<td>67.56±0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 822 µg/ml</td>
<td>IC50 = 130 µg/ml</td>
</tr>
</tbody>
</table>

*Every value was articulated as mean± SEM for 3 experimentation
Table 5: Nitricoxide scavenging activity of *Cassia absus* EA Extract

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract (µg/ml)</th>
<th>% of activity (±SEM)*</th>
<th>EA concentrates</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>29.24 ± 0.012</td>
<td>48.24 ± 0.028</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>38.42 ± 0.036</td>
<td>56.12 ± 0.042</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>51.65 ± 0.052</td>
<td>63.12 ± 0.053</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>750</td>
<td>59.84 ± 0.062</td>
<td>67.56 ± 0.022</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 530 µg/ml</td>
<td>IC50 = 130 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>

*Every value was articulated as mean ± SEM for 3 experimentation

Table 6: Nitricoxide scavenging activity of *Cassia absus* methanol Extract

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract (µg/ml)</th>
<th>% of activity (±SEM)*</th>
<th>(Methanolic concentrates)</th>
<th>(Ascorbate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>40.18 ± 0.024</td>
<td>48.24 ± 0.028</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>51.68 ± 0.030</td>
<td>56.12 ± 0.042</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>58.82 ± 0.045</td>
<td>63.12 ± 0.053</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>750</td>
<td>64.42 ± 0.052</td>
<td>67.56 ± 0.022</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 230mg/ml</td>
<td>IC50 = 130mg/ml</td>
<td></td>
</tr>
</tbody>
</table>

*Every value was articulated as mean ± SEM for 3 experimentation

Table 7: Total antioxidant activity of *Cassia absus* PE Extract

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract (µg/ml)</th>
<th>% inhibition (±SEM)*</th>
<th>PE concentrates</th>
<th>Ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>18.12 ± 0.022</td>
<td>50.76 ± 0.024</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>25.38 ± 0.034</td>
<td>61.68 ± 0.035</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>32.18 ± 0.020</td>
<td>74.64 ± 0.048</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>39.78 ± 0.015</td>
<td>98.12 ± 0.021</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 740 µg/ml</td>
<td>IC50 = 57 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>

*Every value was articulated as mean ± SEM for 3 experimentation

Table 8: Total antioxidant activity of *Cassia absus* EA Extract

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract (µg/ml)</th>
<th>% inhibition (±SEM)*</th>
<th>(EA concentrates)</th>
<th>Ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>14.28 ± 0.024</td>
<td>50.76 ± 0.024</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>22.18 ± 0.010</td>
<td>61.68 ± 0.035</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>29.34 ± 0.056</td>
<td>74.64 ± 0.048</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>42.43 ± 0.062</td>
<td>98.12 ± 0.021</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IC50 = 445 µg/ml</td>
<td>IC50 = 57 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>

*Every value was articulated as mean ± SEM for 3 experimentation
Table 9: Total antioxidant activity of Cassia absus methanol Extract

<table>
<thead>
<tr>
<th>S.no</th>
<th>Extract (µg/ml)</th>
<th>Methanol concentrates</th>
<th>% inhibition(±SEM)*</th>
<th>Ascorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>35.45 ± 0.043</td>
<td></td>
<td>50.76 ± 0.024</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>40.34 ± 0.024</td>
<td></td>
<td>61.68 ± 0.035</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>50.76 ± 0.037</td>
<td></td>
<td>74.64 ± 0.048</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>63.18 ± 0.028</td>
<td></td>
<td>98.12 ± 0.021</td>
</tr>
<tr>
<td></td>
<td>IC50 = 205µg/ml</td>
<td></td>
<td></td>
<td>IC50 = 57 µg/ml</td>
</tr>
</tbody>
</table>

*Every value was articulated as mean ± SEM for 3 experimentation

IC50 values & total antioxidant potential revealed that methanol concentrates of Cassia absus is a better activity in scavenging total antioxidant potential when compared ethyl acetate & PE extracts.

CONCLUSIONS

The current trends, antioxidative activity of the herbs having more interest due to their possible use as natural additives to substitute synthetic ones. Among the three various extracts, the methanolic extract of Cassia absus exhibited higher potency of antioxidant activity. These results indicate that methanol concentrates of Cassia absus might serve as a natural antioxidant, which may be useful in prevent free radical-induced diseases.

REFERENCES


