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

Mythri M¹, Sanal Dev K T¹, Kottai Muthu A*²

¹Alshifa College of Pharmacy, Perinthalmanna, Malapuram District, Kerala, India
²Department of Pharmacy, Annamalai University, Annamalainagar-608 002, Tamilnadu, India

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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 IC₅₀230μg/ml &130μg/ml (Nitric oxide). 205μg/ml &57μg/ml (total antioxidant),195μg/ml &66μg/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 obviouslyshow the methanolic concentrates of Cassia absushave better antioxidant activity. This result indicates that aerial parts of methanolic concentrates Cassia absuscould serve as a natural antioxidant, which may be useful in prevent free radical-induced diseases.

*Corresponding Author
Name: Kottai Muthu A
Phone: +919443171712
Email: akottaimuthu@gmail.com

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 (Mahakunakorn 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 antibacterial, antimalarial and lowering the blood pressure (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 antioxidant 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 desiccated 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; Sivakrishnan et al., 2014).

**Assessment of Antioxidant potential through in vitro methods**

The variety of concentrates of Cassia absus were used assessment of antioxidant activity by Mensor et al. (2001) method was adopted for Diphenylpicrylhydrazyl 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 a stable N₂-centered free radical generally utilized for testing the antioxidant potential of herbal concentrates. When the stable Diphenylpicrylhydrazyl radical accepts an electron 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 considered as antioxidants & therefore, radical scavengers (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 & ascorbic acid were recorded 825µg/ml & 66µg/ml correspondingly.

DPPH activity of EA concentrates of Cassia absus summarized in Table 2. The EA concentrates of Cassia 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 concentrates of Cassia absus & ascorbic acid were recorded 590µg/ml & 66µg/m correspondingly.

DPPH potential of methanolic concentrates of Cassia absus appeared in Table 3. The methanolic concentrates of Cassia absus having more DPPH scavenging potential of 62.56% at 800 µg/ml & ascorbate was recorded 72.82% at 800 µg/ml. The IC₅₀ of the methanolic concentrates of Cassia absus & ascorbic acid were recorded 195µg/ml & 66µg/m correspondingly.

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 different 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 ethylenediamine, producing pink coloured, which absorbs at 546 nm (Panda et al., 2009). Nitric oxide scavenging activity of PE concentrates of Cassia absus appeared in Table 4. The PE concentrates of Cassia absus exhibit a more Nitric oxide scavenging 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/m correspondingly.

Nitric oxide scavenging activity of EA concentrates of Cassia absus appeared in Table 5. The EA concentrates of Cassia absus exhibit a more Nitric oxide scavenging activity of 59.84% at 750 µg/ml & ascorbic acid was recorded 67.56% at 750 µg/ml. The
Table 1: DPPH radical activity of Cassia absus PE extract

| S.no | Extract (µg/ml) | PE concentrates | % of activity (±SEM)* |
|------|----------------|----------------|----------------------|
| 1    | 100            | 12.28±0.045    | 54.19±0.024          |
| 2    | 200            | 25.32±0.022    | 59.24±0.032          |
| 3    | 400            | 38.73±0.054    | 65.32±0.054          |
| 4    | 800            | 49.16±0.028    | 72.82±0.062          |

IC50 = 825 µg/ml

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

Table 2: DPPH radical activity of Cassia absus EA extract

| S.No | Extract (µg/ml) | (EA concentrates) | % of activity (±SEM)* |
|------|----------------|------------------|----------------------|
| 1    | 100            | 24.46±0.052      | 54.19±0.024          |
| 2    | 200            | 37.48±0.074      | 59.24±0.032          |
| 3    | 400            | 45.08±0.045      | 65.32±0.054          |
| 4    | 800            | 55.76±0.022      | 72.82±0.062          |

IC50 = 590 µg/ml

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

Table 3: DPPH radical activity of Cassia absus methanolic extract

| S.No | Extract (µg/ml) | (Methanolic concentrates) | % of activity (±SEM)* |
|------|----------------|--------------------------|----------------------|
| 1    | 100            | 39.12±0.042              | 54.19±0.024          |
| 2    | 200            | 50.78±0.026              | 59.24±0.032          |
| 3    | 400            | 56.34±0.072              | 65.32±0.054          |
| 4    | 800            | 62.56±0.038              | 72.82±0.062          |

IC50 = 195 µg/ml

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

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

| S.no | Extract (µg/ml) | PE concentrates | % of activity (±SEM)* |
|------|----------------|----------------|----------------------|
| 1    | 125            | 32.32±0.012    | 48.24±0.028          |
| 2    | 250            | 39.56±0.018    | 56.12±0.042          |
| 3    | 500            | 45.23±0.024    | 63.12±0.053          |
| 4    | 750            | 52.67±0.048    | 67.56±0.022          |

IC50 = 822 µg/ml

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

| S.no | Extract (µg/ml) | % of activity (±SEM)* | Ascorbic acid (µg/ml) |
|------|----------------|-----------------------|-----------------------|
| 1    | 125            | 29.24 ± 0.012         | 48.24 ± 0.028         |
| 2    | 250            | 38.42 ± 0.036         | 56.12 ± 0.042         |
| 3    | 500            | 51.65 ± 0.052         | 63.12 ± 0.053         |
| 4    | 750            | 59.84 ± 0.062         | 67.56 ± 0.022         |
|      |                | IC50 = 530 µg/ml      | IC50 = 130 µg/ml      |

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

### Table 6: Nitric oxide scavenging activity of *Cassia absus* methanol Extract

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

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

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

| S.no | Extract (µg/ml) | % inhibition (±SEM)* | Ascorbate (µg/ml) |
|------|----------------|----------------------|-------------------|
| 1    | 50             | 18.12 ± 0.022        | 50.76 ± 0.024     |
| 2    | 100            | 25.38 ± 0.034        | 61.68 ± 0.035     |
| 3    | 200            | 32.18 ± 0.020        | 74.64 ± 0.048     |
| 4    | 300            | 39.72 ± 0.015        | 98.12 ± 0.021     |
|      |                | IC50 = 740 µg/ml     | IC50 = 57 µg/ml   |

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

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

| S.no | Extract (µg/ml) | % inhibition (±SEM)* | Ascorbate (µg/ml) |
|------|----------------|----------------------|-------------------|
| 1    | 50             | 14.28 ± 0.024        | 50.76 ± 0.024     |
| 2    | 100            | 22.18 ± 0.010        | 61.68 ± 0.035     |
| 3    | 200            | 29.34 ± 0.056        | 74.64 ± 0.048     |
| 4    | 300            | 42.43 ± 0.062        | 98.12 ± 0.021     |
|      |                | IC50 = 445 µg/ml     | IC50 = 57 µg/ml   |

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

| S.no | Extract (µg/ml) | Methanol concentrates | % inhibition (±SEM)* |
|------|----------------|-----------------------|---------------------|
| 1    | 50             | 35.45 ± 0.043         | 50.76 ± 0.024       |
| 2    | 100            | 40.34 ± 0.024         | 61.68 ± 0.035       |
| 3    | 200            | 50.76 ± 0.037         | 74.64 ± 0.048       |
| 4    | 300            | 63.18 ± 0.028         | 98.12 ± 0.021       |
|      | IC50 = 205 µg/ml |                      | IC50 = 57 µg/ml     |

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

IC50 of the EA concentrates of Cassia absus & ascorbic acid were recorded 530 µg/ml & 130 µg/ml correspondingly.

Nitric oxide scavenging activity of methanol concentrates of Cassia absus appeared in Table 6. The methanol concentrates of Cassia absus exhibit a more Nitric oxide scavenging activity of 64.42% at 750 µg/ml & ascorbic acid was recorded 67.56% at 750 µg/ml. The IC50 of methanol concentrates of Cassia absus & ascorbic acid were recorded 230 µg/ml & 130 µg/ml correspondingly.

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

**Phosphomolybdic acid method**

The total antioxidant activity of PE concentrates of Cassia absus appeared in Table 7. The PE concentrates of Cassia absus exhibit a more total antioxidant activity of 39.78% at 300 µg/ml & ascorbic acid was recorded 98.12% at 300 µg/ml. The IC50 of the PE concentrates of Cassia absus & ascorbic acid were recorded 740 µg/ml & 57 µg/ml correspondingly.

The total antioxidant activity of the EA concentrates of Cassia absus appeared in Table 8. The EA concentrates of Cassia absus exhibit a more total antioxidant activity of 42.43% at 300 µg/ml & ascorbic acid was recorded 98.12% at 300 µg/ml. The IC50 of the EA concentrates of Cassia absus & ascorbic acid were recorded 445 µg/ml & 57 µg/ml correspondingly.

The total antioxidant activity of methanol concentrates of Cassia absus appeared in Table 9. The methanol concentrates of Cassia absus exhibit a more total antioxidant activity of 63.18% at 300 µg/ml & ascorbic acid was recorded 98.12% at 300 µg/ml. The IC50 of the methanol concentrates of Cassia absus & ascorbic acid were recorded 205 µg/ml & 57 µg/ml correspondingly.

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.

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