ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

# Estimation of total phenolic content and evaluation of *in vitro* antioxidant activity of different extracts of *Cassia absus* (Linn) by Superoxide radical, Iron chelating activity

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*Article History:*

Received on: 05 Dec 2019
Revised on: 11 Jan 2020
Accepted on: 20 Jan 2020

*Keywords:*

Cassia absus,
Superoxide radical,
Iron chelating activity,
total phenol

ABSTRACT

*Cassia absus* (Linn) (family Fabaceae) is generally known as "chaksu" in ayurvedic traditional system. The current study, aerial parts of different concentrates (concentrates (Pet.ether, ethyl acetate and methanol) of *Cassia absus* were evaluated for its *in-vitro* antioxidant potential by Superoxide radical, Iron chelating activity taking activity taking quercetin , Ethylenediamine tetraacetate as the standard respectively and total phenol content was estimated as equivalent to gallic acid. An $IC_{50}$ value was originate that methanolic concentrates of *Cassia absus* more efficient in Superoxide radical, Iron chelating activity compared EA & PE concentrates. The methanolic concentrates of *Cassia absus* and standard exhibited antioxidant potential possessing $IC_{50}$ 217$\mu$g/ml & 60$\mu$g/ml (Superoxide radical). 184$\mu$g/ml & 65$\mu$g/ml (Iron chelating) respectively. The methanolic and EA concentrates of *Cassia absus* showed the total phenolic content (10.22 $\pm$ 0.40, 4.03 $\pm$ 0.47) respectively. The difference in scavenging potential of the extracts can be due to variation in the percentage of bioactive compound present in different solvents. *Invitro* antioxidant studies obviously show methanolic concentrates of *Cassia absus* have better antioxidant activity due to the presence of total phenolic content. This result indicate that aerial parts of methanolic concentrates *Cassia absus* could serve as natural antioxidant, which may be useful in prevent free radical induced diseases.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i2.2037

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## INTRODUCTION

Free radicals are fundamental in modulating biochemical reactions and have been continuously generated in the body by normal usage of oxygen such as in aerobic respiration and some cell-mediated immune functions (Tiwari, 2001). ROS such as superoxide, Singlet oxygen, $H_2O_2$ and hydroxyl radicals, Nitric Oxides radicals ($NO^{\cdot}$) as well as non-free-radicals species such as Hydrogen Peroxide ($H_2O_2$) and Nitrous oxide generated in cell were responsible for damaging oxidative reactions. Due to the excess of ROS they pony up maximum diseases ege.g. Diabetes, cancer, cardiac damage and immunological disease (López-Alarcón and Denicola, 2013). Plants are enriched with bioactive ingredients which have broad spectrum of pharmacological, antioxidant activity and therapeutic function. The secondary metabolites from plant sources like carotenoids, polyphenols, ascorbate and natural and natural phenolic compounds exert a strong chemical defense

defence mechanism against ROS (Benzie, 2003).

*Cassia absus* (Linn) (family Fabaceae ) is generally known as "chaksu " in ayurvedic traditional system (Kirtikar and Basu, 1918). Chaksine and iso chaksine both alkaloidswere alkaloids were isolated from seed of *Cassia absus* (Siddiqui and Ahmed, 1935). *Cassia absus* was used for different diseases like antibacterial, 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, There are no literature 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 Tirunelveli District of Tamilnadu, India. Plant identification was made from Botanical investigation of India, Palayamkottai The *Cassia absus* were desiccated under shadowy, segregate, crushed through grinder (Sivakrishnan and KottaiMuthu, 2014).

### Preparation of Concentrates

The pulverized materials were packed in muslin cloth and extracted with pet.ether, ethyl acetate and methanol as solvents respectively according to the increasing order of polarity (Shajiselvin *et al.*, 2010) through hot constant percolation method in Soxhlet equipment (Harborne, 1984)for twenty four hours. The concentrates were concentrated through rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired. (SatheeshKumar *et al.*, 2010; Alagumanivasagam *et al.*, 2012).

### Assessment of Antioxidant potential through invitro methods

The variety of extract of *Cassia absus* were used assessment of antioxidant activity by Winterbourn *et al.* (1975) method described for Superoxide radical ($O_2^-$) assay and Benzie and Strain (1996) method was adopted to determine the Iron chelating activity and determination of total phenolic compound were estimated by the methods of Mallick and Singh (1980).

## RESULTS AND DISCUSSION

### Superoxide activity

Superoxide radical activity were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. Superoxide radical potential of PE extract of *Cassia absus* shown in Table 1. The more Superoxide radical potential of PE extract and standard at 800 $\mu$g/ml was recorded 49.67% and 91.23%. %. $IC_{50}$ of PE extract and standard was recorded as 776$\mu$g/millilitre and 60$\mu$g/ millilitre correspondingly.

Superoxide radical potential of EA extract of *Cassia absus* appeared in Table 2. The more SO scavenging potential of EA extract and standard 800 $\mu$g/ml was recorded 59.45% and 91.23% correspondingly. EA extract and Quercetin $IC_{50}$ was recorded as 380$\mu$g/ml and 60$\mu$g/ml correspondingly.

Superoxide radical scavenging potential of methanolic extract of *Cassia absus* appeared in Table 3. Superoxide radical scavenging potential was more in methanolic extract and Quercetin (standard) at 800$\mu$g/ml was recorded 67.17 recorded 67.17% and 91.23% . %. Methanolic extract and standard $IC_{50}$ standard $IC_{50}$ was recorded as 217$\mu$g/ml and 60$\mu$g/ml correspondingly.

$IC_{50}$ values and Superoxide radical potential revealed that methanol extract of *Cassia absus* is better activity in scavenging superoxide radical when compared EA and PE extracts. Superoxides could be produced in huge amounts by various biological processes. It is known to be more injurious to cellular components as an originator of the most ROS, contributing to tissue damage and many disorders (Halliwell, 1999). The methanolic extract of *Cassia absus* exhibited higher ability in scavenging superoxide anion radical when compared to the standard quercetin.

### Iron chelating potential

The iron chelating potential of the all the extract was measured by Fe-ferrozine complex formation. Ferrozine-Fe complex is producing red coloured which absorbs at 562nm (Yamaguchi *et al.*, 2000). Iron complex potential of PE extract *Cassia absus and* Ethylenediamine tetraacetate were appeared as shown in Table 4. Iron chelating potential were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. The more iron binding potential of PE extract and Ethylenediamine tetraacetate 800 $\mu$g/ml were recorded 48.97% and 84.48%. The $IC_{50}$ of PE extract of *Cassia absus* and Ethylenediamine tetraacetate were found as 745$\mu$g/ml and 65$\mu$g/ml correspondingly.

Iron complex potential of EA extract of *Cassia absus* and Ethylenediamine tetraacetate were is presented

 

**Table 1: Activity of PE extract of *Cassia absus* on Superoxide radical method**

| S.no | Extract ($\mu$g/ml) | % inhibition (±SEM)* | |
|---|---|---|---|
| | | (PE extract) | (Quercetin) |
| 1 | 100 | 24.54±0.012 | 64.32±0.018 |
| 2 | 200 | 33.22±0.028 | 71.12±0.024 |
| 3 | 400 | 44.36±0.036 | 83.44±0.046 |
| 4 | 800 | 49.67±0.012 | 91.23±0.016 |
| | | $IC_{50}$ = 776 $\mu$g/ml | $IC_{50}$ = 60 $\mu$g/ml |

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

**Table 2: Activity of EA extract of *Cassia absus* on Superoxide radical method**

| S.no | Extract ($\mu$g/ml) | % of inhibition (±SEM)* | |
|---|---|---|---|
| | | (Ethyl acetate extract) | (Quercetin) |
| 1 | 100 | 29.22±0.022 | 64.32 ±0.018 |
| 2 | 200 | 42.38±0.044 | 71.12 ±0.024 |
| 3 | 400 | 50.18±0.038 | 83.44 ±0.046 |
| 4 | 800 | 59.45±0.010 | 91.23 ±0.016 |
| | | $IC_{50}$ = 380 $\mu$g/ml | $IC_{50}$ = 60 $\mu$g/ml |

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

**Table 3: Activity of Methanolic extract *Cassia absus* on Superoxide radical method radical method**

| S.no | Extract ($\mu$g/ml) | % inhibition (±SEM)* | |
|---|---|---|---|
| | | Methanolic extract | Quercetin |
| 1 | 100 | 35.34±0.028 | 64.32 ±0.018 |
| 2 | 200 | 50.98±0.022 | 71.12 ±0.024 |
| 3 | 400 | 62.33±0.034 | 83.44 ±0.046 |
| 4 | 800 | 67.17±0.062 | 91.23 ±0.016 |
| | | $IC_{50}$ = 217 $\mu$g/ml | $IC_{50}$ = 60 $\mu$g/ml |

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

**Table 4: Iron-binding potential of *Cassia absus PE* absus PE extract**

| S.no | Extract ($\mu$g/ml) | % inhibition (±SEM)* | |
|---|---|---|---|
| | | PE extract | Ethylenediamine tetraacetate |
| 1 | 100 | 25.24±0.019 | 57.12±0.018 |
| 2 | 200 | 33.65±0.024 | 63.34±0.022 |
| 3 | 400 | 40.29±0.036 | 71.46±0.016 |
| 4 | 800 | 48.97±0.056 | 84.48±0.034 |
| | | $IC_{50}$ = 745 $\mu$g/ml | $IC_{50}$ = 65 $\mu$g/ml |

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

**Table 5: Iron-binding potential of Cassia absus of absus of *EA* extract**

| S.no | Extract ($\mu$g/ml) | % inhibition (±SEM)* | |
|---|---|---|---|
| | | EA extract | Ethylenediamine tetraacetate |
| 1 | 100 | 28.46±0.012 | 57.12±0.018 |
| 2 | 200 | 39.29±0.036 | 63.34±0.022 |
| 3 | 400 | 50.02±0.056 | 71.46±0.016 |
| 4 | 800 | 64.38±0.017 | 84.48±0.034 |
| | | $IC_{50}$ = 405$\mu$g/ml | $IC_{50}$ = 65$\mu$g/ml |

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

**Table 6: Iron-binding potential of *Cassia absus* Methanolic extract**

| S.no | Extract ($\mu$g/ml) | % inhibition(±SEM)* | |
|---|---|---|---|
| | | Methanol extract | EthyleneDiamine tetra acetate |
| 1 | 100 | 35.46±0.013 | 57.12±0.018 |
| 2 | 200 | 51.21±0.042 | 63.34±0.022 |
| 3 | 400 | 60.25±0.012 | 71.46±0.016 |
| 4 | 800 | 67.56±0.033 | 84.48±0.034 |
| | | $IC_{50}$ = 184$\mu$g/ml | $IC_{50}$ = 65$\mu$g/ml |

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

**Table 7: The total Phenolic content of various extracts of aerial parts of *Cassia absus***

| S.No | Extracts | Total phenol content (mg/g of Gallic acid) (±SEM)* |
|---|---|---|
| 1 | Petroleum ether extract of Cassia absus | 0.74 ± 0.38 |
| 2 | Ethyl acetate extract of Cassia absus | 4.03 ± 0.47 |
| 3 | Methanolic extract of Cassia absus | 10.22 ± 0.40 |

*All values are expressed as mean ± SEM for three determinations

in Table 5. The more iron binding capacity of EA extract and Ethylenediamine tetraacetate 800 $\mu$g/ml was recorded 64.38% and 84.48%.%. The $IC_{50}$ value of ethyl acetate extract of *Cassia absus* and Ethylenediamine tetraacetate were tetraacetate found were found 405$\mu$g/ml and 65$\mu$g/ml correspondingly.

Iron complex potential of methanolic extract of *Cassia absus* and Ethylenediamine tetraacetate were is presented in Table 6. The more iron binding potential of methanolic extract and Ethylenediamine tetraacetate 800 $\mu$g/ml were recorded 67.56% and 84.48%. %. The $IC_{50}$ value of methanol extract of *Cassia absus* and Ethylenediamine tetraacetate waste traacetate was recorded as 184$\mu$g/ml and 65$\mu$g/ml correspondingly.

$IC_{50}$ values and iron binding potential revealed that methanol extract of *Cassia absus* is huge activity in iron chelating potential when compared ethyl acetate and acetate and petroleum ether extract. But when compare to the all the three extract, the methanol extract of the *Cassia absus* showed the better result better results.

**Total phenol**

Phenolic compounds are famous as powerful chain breaking antioxidants. The phenolic compounds might contribute directly to antioxidative action. The total amount of phenolic content of various extract of aerial plant of *Cassia absus* is was present in presented in Table 7 .

Based on the result the methanolic extract of *Cassia absus* was found higher content of phenolic components than that of petroleum ether and ethyl acetate extract of *Cassia absus.* Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [groups] (Hatano

 

*et al.*, 1989).

## CONCLUSIONS

Among the three various extracts, methanolic extract of *Cassia absus* exhibited higher potency of antioxidant activity due to presence of total phenolic compounds. Phenolic compounds are known to act as antioxidant. This results indicateresults indicate that aerial parts of methanolic extract *Cassia absus* could serve as natural antioxidant, which may be useful in prevent free radical induced diseases.

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