

## Estimation of Flavonoids and Screening of In Vitro Antioxidant Activities of Various Extracts of Aerial Parts of *Cassia absus* (Linn)

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### ABSTRACT

#### BACKGROUND

Free radicals are generated by both enzymatic and non-enzymatic reactions. The main source of free radicals in enzymatic reactions include those involved in phagocytosis, respiratory chain, in prostaglandin synthesis, and in cytochrome p<sup>450</sup> system. Current investigation was to examine invitro antioxidant potential of *Cassia absus* (Linn).

#### METHODS

The aerial parts of *Cassia absus* (Linn) were powdered and the dry powder was subjected to extraction with various solvents (PE, EA and methanol) through Soxhlet extractor. The aerial parts of different concentrates (pet. ether, ethyl acetate and methanol) of *Cassia absus* was evaluated for its in-vitro antioxidant potential by hydroxyl radical, FRAP activity taking ascorbate used as standard for the both methods and total flavonoids content was estimated as equivalent to rutin.

#### RESULTS

The methanolic concentrates of *Cassia absus* & standard exhibited antioxidant potential possessing IC<sub>50</sub> 196 µg/mL & 65 µg/mL (hydroxyl radical) 216 µg/mL & 50 µg/mL (FRAP activity) respectively. Methanolic concentrates of *Cassia absus* were more efficient in hydroxyl radical, FRAP activity compared EA & PE concentrates. The methanolic and EA concentrates of *Cassia absus* showed the total flavonoids content (10.22 ± 0.40, 4.03 ± 0.47 respectively). The difference in scavenging potential of the extracts can be due to variation in the percentage of bioactive compound flavonoids present in methanolic extracts. Invitro antioxidant studies show that methanolic concentrates of *Cassia absus* have better antioxidant activity due to the presence of total flavonoids content.

#### CONCLUSIONS

This result indicates that methanolic concentrates of aerial parts of *Cassia absus* could serve as natural antioxidant, which may be useful in preventing free radical induced diseases.

#### KEY WORDS

*Cassia absus*, Hydroxyl Radical, FRAP, Flavonoids

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## BACKGROUND

Free radicals are generated by both enzymatic and non-enzymatic reactions. The main source of free radicals in enzymatic reactions include those involved in phagocytosis, respiratory chain, in prostaglandin synthesis, and in cytochrome p<sup>450</sup> system. Non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations result in free radical production.<sup>1</sup> Elimination and neutralization of ROS is handled by both enzymatic and non-enzymatic antioxidant mechanisms. The human body is in constant battle to keep from free radicals. The first line of defence is the preventive antioxidants which quench the free radicals generated in the body. An early stage Augmentation of antioxidant status should either prevent or greatly curtail tissue injury.<sup>2</sup> Flavonoids & Phenolic acids are known to act as antioxidant, not only for their good efficacy to donate hydrogen or electrons, but also their contribution as stable radical intermediates.<sup>3</sup> The current trend of research is the investigation of medicines of plant origin which is affordable and access able with minimal side effects. As crude drugs of herbs and other plant materials rich in secondary metabolites like flavonoids, phenolic compounds, alkaloids etc are increasing interest in the pharmaceutical industry because they prevent the degradation of lipids and enhance their free radical scavenging potential thereby improving its medicinal values.<sup>4</sup>

*Cassia absus* (Linn) (family c) is generally known as "chaksu" in ayurvedic traditional system.<sup>5</sup> Chaksine and iso chaksine both alkaloids were isolated from seed of *Cassia absus*.<sup>6</sup> *Cassia absus* was used for different diseases like antibacterial, antimalarial and lowering the blood pressure.<sup>7</sup> *Cassia absus* was used antihistaminic activity of an eye drops.<sup>8</sup> 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*.

## METHODS

### Gathering & Identification of Plant

The aerial parts *Cassia absus* (family *Fabaceae*) were gathered from Senkottai, 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.<sup>9</sup>

### 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<sup>10</sup> through hot constant percolation method in Soxhlet equipment<sup>11</sup> for twenty-four hours. The concentrates were concentrated through rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired.<sup>12</sup>

## Assessment of Antioxidant Potential through In Vitro Methods

The variety of concentrates of aerial parts *Cassia absus* were used assessment of antioxidant activity by Elizabeth and Rao (1990)<sup>13</sup> method was adopted for Hydroxyl radical assay & Benzie and Strain (1996)<sup>14</sup> was adopted for the FRAP assay and Cameron GR et al 1943 method described for total flavonoids content.<sup>15</sup>

## RESULTS

### Hydroxyl Radical Scavenging Activity

Hydroxyl radical activity was expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. Hydroxyl radical potential of PE, EA and methanol extract of *Cassia absus* appeared Table 1. The more Hydroxyl radical potential of PE extract and standard at 800 µg/mL was recorded 49.64% and 84.37%. IC<sub>50</sub> of PE extract and standard was recorded as 820 µg/millilitre and 65 µg/millilitre correspondingly. The more Hydroxyl radical scavenging potential of EA extract and standard 800 µg/mL was recorded 59.04% and 84.37% correspondingly. EA extract and Quercetin IC<sub>50</sub> was recorded as 445µg/mL and 65µg/mL correspondingly.

Sl. No.	Concentration of Plant Extract/Ascorbate (µg/mL)	% of Effect ( ± SEM)*			
		PE Extract	EA Extract	Methanolic Extract	Ascorbate
1	100	26.58 ± 0.48	33.76 ± 0.013	36.28 ± 0.028	57.34 ± 0.024
2	200	32.41 ± 0.26	40.23 ± 0.038	49.92 ± 0.034	63.12 ± 0.028
3	400	39.56 ± 0.44	51.18 ± 0.076	56.43 ± 0.054	71.40 ± 0.033
4	800	49.64 ± 0.32	59.04 ± 0.052	66.12 ± 0.022	84.37 ± 0.018
		IC <sub>50</sub> = 820 µg/mL	IC <sub>50</sub> = 445 µg/mL	IC <sub>50</sub> = 445 µg/mL	IC <sub>50</sub> = 65 µg/mL

**Table 1. Activity of PE, EA and Methanol Extract of *Cassia absus* on Hydroxyl Radical Method**

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

Hydroxyl radical scavenging potential was more in methanolic extract and Quercetin (standard) at 800 µg/mL was recorded 66.12% and 84.37%. Methanolic extract and standard IC<sub>50</sub> was recorded as 196 µg/mL and 65 µg/mL correspondingly. IC<sub>50</sub> values and Hydroxyl radical potential revealed that methanol extract of *Cassia absus* is better activity in scavenging superoxide radical when compared EA and PE extracts. The methanolic extract of *Cassia absus* exhibited higher ability in scavenging Hydroxyl radical when compared to the standard quercetin.

### FRAP Assay

Ferric reducing ability of PE, EA and methanol extract *Cassia absus* and ascorbate were appeared in table 2. Reducing ability were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. The more Reducing ability of PE extract and ascorbate 800 µg/mL were recorded 48.14% and 81.43%. The IC<sub>50</sub> of PE extract of *Cassia absus* and ascorbate were found as 865 µg/mL and 50 µg/mL correspondingly.

Sl. No.	Concentration of Plant Extract/ Ascorbate ( $\mu\text{g/mL}$ )		% of Effect ( $\pm$ SEM)*			
	PE Extract	EA Extract	Methanolic Extract	Ascorbate		
1	100	31.54 $\pm$ 0.032	21.56 $\pm$ 0.024	37.34 $\pm$ 0.022	59.45 $\pm$ 0.056	
2	200	36.57 $\pm$ 0.024	32.50 $\pm$ 0.032	49.42 $\pm$ 0.034	64.56 $\pm$ 0.034	
3	400	43.64 $\pm$ 0.016	47.08 $\pm$ 0.036	62.44 $\pm$ 0.012	73.34 $\pm$ 0.022	
4	800	48.14 $\pm$ 0.065	59.12 $\pm$ 0.042	69.44 $\pm$ 0.028	81.43 $\pm$ 0.062	
		IC <sub>50</sub> = 865 $\mu\text{g/mL}$	IC <sub>50</sub> = 498 $\mu\text{g/mL}$	IC <sub>50</sub> = 216 $\mu\text{g/mL}$	IC <sub>50</sub> = 50 $\mu\text{g/mL}$	

**Table 2. Reducing Ability of *Cassia absus* PE, EA and Methanol Extract by FRAP Method**

\*Every value was articulated as Mean  $\pm$  SEM for 3 experimentation

The more Ferric reducing ability of EA extract and ascorbate 800  $\mu\text{g/mL}$  was recorded 59.12% and 81.43%. The IC<sub>50</sub> value of ethyl acetate extract of *Cassia absus* and ascorbate were found 498  $\mu\text{g/mL}$  and 50  $\mu\text{g/mL}$  correspondingly. The more Ferric reducing ability of methanolic extract and ascorbate 800  $\mu\text{g/mL}$  were recorded 69.44% and 81.43%. The IC<sub>50</sub> value of methanol extract of *Cassia absus* and ascorbate was recorded as 216  $\mu\text{g/mL}$  and 50  $\mu\text{g/mL}$  correspondingly.

IC<sub>50</sub> values and Ferric reducing ability revealed that methanol extract of *Cassia absus* is huge activity in Ferric reducing ability when compared ethyl 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.

### Total Flavonoids

The total amount of flavonoid content of various extract of aerial plant of *Cassia absus* was present in Table 3. The EA and methanolic extract of *Cassia absus* were found 3.438  $\pm$  0.32 and 9.618  $\pm$  0.64 correspondingly.

Sl. No.	Extracts	Total flavonoids content (mg Rutin/g) ( $\pm$ SEM)*
1	Petroleum ether extract of <i>Cassia absus</i>	0.176 $\pm$ 0.45
2	Ethyl acetate extract of <i>Cassia absus</i>	3.438 $\pm$ 0.32
3	Methanolic extract of <i>Cassia absus</i>	9.618 $\pm$ 0.64

\*All values are expressed as mean  $\pm$  SEM for three determinations

**Table 3. Total Flavonoids Content of Various Extracts of Aerial Parts of *Cassia absus***

Based on the result the methanolic extract of *Cassia absus* was found higher content of flavonoid than that of PE and EA extract of *Cassia absus*.

### DISCUSSION

Hydroxyl radical is the most ROS and causes severe injury to adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radicals were produced by the oxidation reaction with the DMSO to give in HCHO, which provides a suitable method to identify hydroxyl radicals by treatment with Nash reagent.<sup>16</sup> Among the three different plant concentrates tested, interestingly, in the OH radical activity of the methanol extract of *Cassia absus* exhibited more OH radical potential comparable with that of ascorbic acid. The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyl triazine ( $\text{Fe}_3^+$ -TPTZ) complex and produce a coloured ferrous tripyridyl triazine ( $\text{Fe}_2^+$ -TPTZ).<sup>12</sup> Generally, the reducing

properties are linked with the presence of compounds which exert their action by breaking free radical chain by donating a hydrogen atom.<sup>17</sup> Total antioxidant potential of methanol extract of *Cassia absus* exhibited higher ability in scavenging compared to ascorbic acid. The antioxidant activity of phenolics and flavonoids is largely due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators.<sup>18</sup> Among the three various extracts, methanolic extract of *Cassia absus* exhibited higher potency of antioxidant activity due to presence of total flavonoid compounds.

### CONCLUSIONS

Among the three extracts, methanolic extract of *Cassia absus* exhibited higher potency of antioxidant activity due to presence of total flavonoid compounds. These results indicate that methanolic extract of aerial parts of *Cassia absus* could serve as a natural antioxidant, which may be useful in preventing free radical induced diseases.

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