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## *In vitro* anti-oxidant activity of various solvent fractions of *Cassia fistula* L. pods

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### Abstract

*Cassia fistula* L. is a common medicinal plant has been used for the treatment of various ailments such as convulsion, inflammation, pain, infectious diseases etc. The Present study was aimed to estimate the total phenolic and flavonoid content and to examine the antioxidant activity of such as n-hexane (NHF), ethyl acetate (EAF) and aqueous fractions (AQF) of *Cassia fistula* L. pods. The antioxidant activity was assessed by superoxide scavenging and reducing power abilities. The antioxidant potency was found to be decreasing order of EAF, NHF and AQF and had good co-relation with their polyphenolic and flavonoid content. This study shows that *Cassia fistula* L pods can serve as a good source of antioxidants and thus used for preventing oxidative stress related disorders.

**Keywords:** *Cassia fistula*, Polyphenol estimation, Superoxide scavenging activity, Reducing power assay, Flavonoid content estimation

### 1. Introduction

Natural antioxidants present in the plant parts such as whole grains, fruits and vegetables are important ingredients of food as they scavenge free radicals [1]. Free radicals are frequently generated in our body during normal cellular metabolism as well as under certain environmental conditions, these radicals are more reactive as they lack of an electron and try to become neutral by accepting an electron from or donate an electron to adjacent molecules and create new free radicals. This in turn initiates a chain of reaction that can damage several molecules through accepting or donating an electron leads to degenerative diseases. The phytochemicals such as phenolic acids, polyphenols and flavonoids are generally known as anti-oxidants and their beneficial effects in several chronic ailments have been reported [2]. In addition, the synthetic anti-oxidants available at present are known to produce negative health effects. Thus, the search for antioxidants from plant source has been increased recently. Plants, owing to their healing potential have been greatly by the practitioners of Indian traditional medicinal system [3].

*Cassia fistula* L. commonly known as Indian laburnum belonging to the family Caesalpiniaceae has been widely used in traditional medicine for worm infestation, wound healing, convulsion, antipyretic, haematemesis, pruritus, intestinal disorders, leucoderma, diabetes, analgesic, antipyretic and as laxative [4]. The various medicinal implications have been mainly attributed due to the presence of alkaloids, triterpene derivatives, anthraquinone derivatives, polyphenolics, comprising flavonoids, catechins and proanthocyanidins [5-7]. The phytoconstituents, particularly polyphenols and flavonoids have been shown to possess anti-oxidant activity [8]. Hence, the present study was aimed to estimate the amount of polyphenols and flavonoids present and to study the antioxidant activity of various fractions of *Cassia fistula* pods.

### 2. Materials and Methods

#### 2.1 Collection of plant material and authentication

With the support of professional collectors, pods of *Cassia fistula* were collected from the surroundings of Coimbatore and shade dried. The plant material was taxonomically identified by a Scientist F (Dr. G.V.S. Murthy), Botanical survey of India, Coimbatore, Tamil Nadu, India and the voucher specimen BSI/SRC/5/23/2011-12/Tech 782 was retained in our laboratory for future reference.

## 2.2 Preparation of hydroalcoholic extract

Fresh samples were air-dried and ground, yielding 1500 g of powder. From this, 500 g of powder was defatted with petroleum ether (40–60 °C) for 6 hours [9] and filtered. The resultant marc dried and extracted with a mixture of ethanol (700 ml): water (300 ml) (70:30) as solvent for 24 h at room temperature and placed in a rotary shaker to get hydroalcoholic extract by maceration method for 2 days. The extract was filtered by using Whatman No.1 filter paper and the filtrate was taken. The extraction was repeated two times and the filtered hydro-ethanolic extracts were mixed and evaporated under reduced pressure (56 g) and denoted as HAE.

## 2.3 Fractionation of hydroalcoholic extract of *Cassia fistula* L.

50.0 g of HAE was dissolved in 200 mL of methanol/water (7:3). N-Hexane, ethyl acetate and aqueous fractions were made from the solution obtained through liquid/liquid solvent partition of increasing polarity. From each fraction, the solvent was evaporated in rotary evaporator and dried. Fractions were designated as follows – hexane fraction (NHF), ethyl acetate fraction (EAF) – aqueous fraction (AQF), dried to constant weight and stored at –10 °C until used for experiments.

## 2.4 Total phenolic content estimation.

The total phenolic content of all fractions was estimated by using Folin catechu reagent and measured at 765 nm using gallic acid as standard [10]. Aliquots of fractions or gallic acid were mixed with 0.5 ml of Folin catechu reagent (diluted 1:1 with water) and 2.5 ml of 20% aqueous sodium carbonate and allowed to stand for 45 min in dark room and absorbance was measured by spectrophotometer. The total phenolic content in each fraction was calculated as gallic acid equivalent from the calibration curve.

## 2.5 Determination of total flavonoid content

Flavonoid content in all the three fractions was determined by aluminium chloride colorimetric method [11]. Aliquot quantity of fractions was diluted with 1.50 ml of distilled water and 0.50 ml of 10% (w/v) aluminium chloride was added along with 0.10 ml of 1 M potassium acetate and 2.80 ml of distilled water. This mixture was incubated at room temperature for 30 min. The absorbance of the resulting reaction mixture was measured at 415 nm UV spectrophotometer. Quantification of flavonoids was done on the basis of standard curve of quercetin prepared in 80% methanol and results were expressed in milligram quercetin equivalent (QE) per gm of dry fruits.

## 2.6 Superoxide radical scavenging activity

Measurement of superoxide anion scavenging activity of fractions was based on the method described by [12]. Phenazine methosulphate (PMS)–nicotinamide adenine dinucleotide (NADH) system generates superoxide radicals by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 µM) solution, 1 ml NADH (78 µM) solution and 0, 50, 100, 150, 200, 250 µg/ml of fractions were mixed. The reaction was started by adding 1 ml of PMS

solution (10 µM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm in a UV-spectrophotometer was measured against blank samples. Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula.

$$\text{Percentage inhibition} = [A_0 - A_1/A_0] \times 100$$

Where,  $A_0$  was the absorbance of control (blank), and  $A_1$  was the absorbance of EAF/ NHF/ AQF or standard.

## 2.7 Reductive ability

Reducing power ability was measured by mixing 50, 100, 150, 200 and 250 µg/ml of EAF, NHF and AGF prepared with distilled water and mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 500 °C for 30 minutes. Trichloroacetic acid (2.5 ml, 10%) (TCA) were added to the mixture and centrifuged for 10 min at 3000 RPM and 2.5 ml from the upper portions were diluted with 2.5 ml water and shaken with 0.5 ml fresh 0.1%, ferric chloride. The absorbance was measured at 700 nm using UV-spectrophotometer. The blank was prepared as above, but contained water instead of the samples. Increase in the absorbance by reaction mixture indicates increased reducing power. All experiments were done in triplicate using butylated hydroxy toluene as standard [13].

## 3. Results and discussion

### 3.1 Polyphenolic content estimation

EAF and NHF were found to contain more polyphenols than AQF. The amount of phenolic constituent present in EAF was found to 61.5 µg/mg in terms of gallic acid equivalent and in NHF and AQF it was found to 53.5 µg/mg and 13.5 µg/mg gallic acid equivalents respectively. Phenolic compounds are one of the most significant phytoconstituent because their hydroxyl groups confer scavenging ability [14] and they are known as powerful chain breaking antioxidant [15]. The calibration curve of standard polyphenol, gallic acid is shown in figure 1

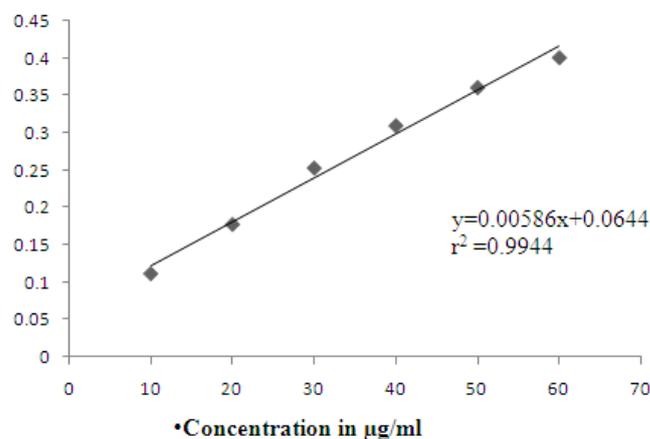


Fig 1: Standard curve of gallic acid

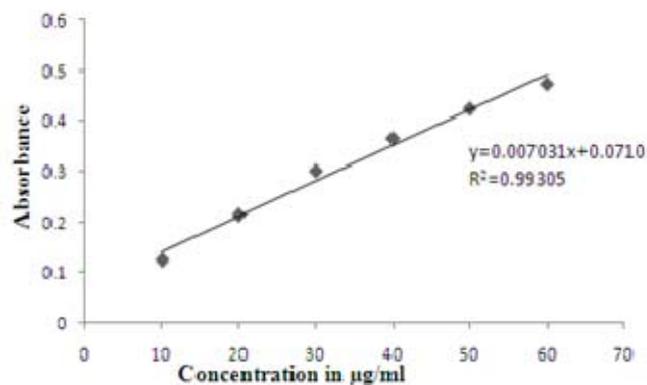


Fig 2: Standard curve of quercetin

### 3.2 Flavonoid content estimation

Flavonoids are considered to be the most important group of polyphenolic compounds and have been shown to exhibit

several pharmacological activities such as neuroprotective, cardioprotective, anti-diabetic, anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anticancer activities [16]. Their phenolic hydroxyl groups effectively scavenge the  $O_2$  free radicals and considered to be most potent antioxidants [17]. The total flavonoids content of CAF, NHF and AQF was estimated to be 30.5, 25.5 and 5.5 mg/mg respectively in terms of quercetin equivalent.

### 3.3 Reducing power ability

The ability with which a natural anti-oxidant to donate electron is designated as reducing power activity [18-19]. Several research reports stated the direct correlation between the anti-oxidant activity and reducing power ability of plant extracts [1, 18]. The order reducing power ability was found as follows BHT>EAF>NHF>AQF. The reducing power ability of fractions was well correlated with their polyphenolic and flavonoid content. The results were shown in table 1.

Table 1: Reductive ability of various fractions of *Cassia fistula* L. pods

Sl. NO	Conc µg/mg	Absorbance (Mean±SEM)*			
		EAF	NHF	AQF	BHT
1	20	0.183±0.0004	0.178±0.0102	0.083±0.0007	0.193±0.0050
2	40	0.221±0.0030	0.189±0.0180	0.100±0.0069	0.247±0.0071
3	60	0.254±0.0013	0.239±0.0012	0.128±0.0074	0.260±0.00038
4	80	0.278±0.0014	0.260±0.0029	0.150±0.0032	0.386±0.0006
5	100	0.348±0.0017	0.329±0.0017	0.193±0.0074	0.569±0.00189

‡ Data expressed as mean±SEM of three observations EAF= Ethyl acetate fraction, NHF= n-hexane fraction, AQF= aqueous fraction of hydroalcoholic extract of *Cassia fistula* L. pods. BHT= butylated hydroxyl toluene

### 3.4 Superoxide scavenging activity

The relationship with reducing power and anti-oxidant activity has long been established [13, 20]. Among all the fractions examined in our study, the EAF showed a higher reducing power than the rest of the fractions. The lower anti-oxidant

activity of AQ could be attributed to the presence of reducing sugars [21] as reducing sugars have been shown to generate prooxidants and that may nullify the anti-oxidant activity of other phytoconstituents [22].

Table 2: Superoxide scavenging activity of various fractions of *Cassia fistula* L. pods

S.No	EAF			NHF			AQF		
	Conc (µg/ml)	% Inhibition	IC <sub>50</sub>	Conc (µg/ml)	% Inhibition	IC <sub>50</sub>	Conc (µg/ml)	% Inhibition	IC <sub>50</sub>
1	20	17.46	51.38	20	14.25	53.23	100	5.99	440.52
2	40	33.46		40	28.92		200	18.79	
3	60	53.46		60	51.33		300	28.93	
4	80	69.46		80	62.53		400	44.93	
5	100	89.19		100	81.46		500	60.39	

‡ Data expressed as average of three observations EAF= Ethyl acetate fraction, NHF= n-hexane fraction, AQF= aqueous fraction of hydroalcoholic extract of *Cassia fistula* L. pods. BHT= butylated hydroxyl toluene

### 4. Conclusion

The Present study clearly states the significant anti-oxidant activity of EAF and NHF and among these the EAF showed the superior and AQF showed the inferior anti-oxidant potential. This study further reveals that the presence of

polyphenols and flavonoids could play a major role in the anti-oxidant activity of the EAF and AQF. The inferior anti-oxidant potential of AQF may be due to the presence of negligible amount of polyphenols and flavonoids or may be due to the presence of pro-oxidants such as reducing sugars. Hence, the

findings of the present study clearly reveal the anti-oxidant activity of *Cassia fistula* L. pods. However, further study focused on the isolation of active anti-oxidant molecule and *in vivo* studies could be useful in understanding the mechanism of action of anti-oxidants.

### 5. Acknowledgement

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### 6. Conflicts of interest

The authors declare no conflicts of interest.

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