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Antioxidant potential of aqueous extract of *Cassia fistula* L. leaves

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Abstract

The aqueous extract of *Cassia fistula* L. leaves showed an antioxidant activity as evaluated by studying the inhibition of lipid peroxidation, estimation of glutathione, scavenging effects on DPPH and H_2O_2, metal chelating ability and reducing power assay. Total phenol and flavonoid content of the extracts were also determined.

Key words: *C. fistula* L., Antioxidant activity, Scavenging activity

1. Introduction

Cassia fistula L. (Leguminosae) is medium size deciduous tree bearing yellow blossoms, cultivated throughout India, West Indies, Ceylon, Egypt, China and many other countries. Besides being ornamental plant, leaves has been used traditionally in India in jaundice, piles, rheumatism, ulcer and externally, in skin eruption, ringworm, eczema, prurigo and pruritis. It also has laxative and antiperiodic property [1, 2]. The leaves contain various phytoconstituents like, anthraquinone glycosides and polyphenolic compounds such as, tannin, flavanol, flavonol glycoside and phenolic acid [1, 3].

2. Materials and methods

2.1. Collection of plant material

The leaves of Cassia fistula were collected in the month of May - June 2006, from Herbal Garden, Jamia Hamdard, New Delhi. Plant sample was identified by Taxonomist, Department of Botany of the University and voucher specimen was deposited in Phytochemistry Research Laboratory.

Animals: Male Wistar rats (100-150 g) were procured from Central Animal House of Hamdard University. The animals were group-housed in PVC cages maintained under standard conditions. The overnight fasted animals were used for the experiment.

Sample preparation: Aqueous extract was obtained by extraction of powdered raw material

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Table 1. Extracts yields, total phenol and flavonoid content

Extract	Nature	Yield, %	Total phenol mg/g	Total flavonoid mg/g
Aqueous	Brown semisolid	12.8	12.99 ± 0.38	5.24 ± 0.36

Values are means ± SD of triplicate analyses

Table 2. Reducing power, free radical scavenging and metal chelating activity of aqueous extract of *C. fistula* leaves

Extracts (mg/ml)	Aqueous extract			Standard		
	Absorbance*	% inhibition*	IC_{50} value**	Absorbance*	% inhibition*	IC_{50} value**
Reducing power (absorbance at 700 nm) Standard as Ascorbic acid, Absorbance of control (0.081 ± 0.001)						
2	0.212 ± 0.009			0.511 ± 0.011		
4	0.464 ± 0.004		4.61 ± 0.0008	0.645 ± 0.005		4.05 ± 0.008
8	0.877 ± 0.013			0.742 ± 0.012		
10	1.062 ± 0.069			1.316 ± 0.005		
DPPH-Free radical scavenging activity (absorbance at 517 nm) Standard as Ascorbic acid, Absorbance of control (0.515 ± 0.002)[a]						
2	0.307 ± 0.008	40.38		0.310 ± 0.005	39.80	
4	0.268 ± 0.005	47.96	0.070 ± 0.785	0.298 ± 0.002	42.13	0.066 ± 0.643
8	0.228 ± 0.005	55.72		0.212 ± 0.002	58.83	
10	0.217 ± 0.012	57.86		0.172 ± 0.007	66.60	
Metal chelating activity (absorbance at 562 nm) Standard as Na_2EDTA***, Absorbance of control (0.451 ± 0.005)[a]						
2	0.322 ± 0.012	28.60		0.114 ± 0.005	74.72	
4	0.304 ± 0.005	32.59	0.073 ± 0.39	0.085 ± 0.007	81.15	0.042 ± 1.465
8	0.219 ± 0.005	51.44		0.042 ± 0.009	90.68	
10	0.157 ± 0.006	65.18		0.026 ± 0.011	94.23	

*Values are means ± SD of triplicate analyses; ** IC_{50} (mg/ml) ± SE was calculated by linear regression; ***Na_2EDTA-Ethylenediamine tetraacetic acid disodium salt; a inhibition was calculated using formula $[(A_C - A_S) / A_C]$ X 100 Where: $\boldsymbol{A_C}$ - absorbance of control sample; $\boldsymbol{A_S}$ - absorbance of tested extract solution;

Table 3. Quantities of malonyldialdehyde, reduced glutathione and H_2O_2 scavenging effect of aqueous extract of *C. fistula* leaves

Extracts (mg/ml)	MDA nmol / mg protein (Control 4.49 ± 18.09) (n = 6)	GSH mg/gm tissue (Control 0.21 ± 0.005) 50 min (n = 6)	H_2O_2 scavenging effect (%)[b]	
			Standard (Ascorbic acid)	Aqueous extract
			(Volume of control 52.8 ml)	
2	3.86 ± 9.429	0.17 ± 0.006	46.3 ± 0.165	14.07 ± 0.063
4	2.92 ± 10.442	0.15 ± 0.011	55.17 ± 0.060	20.91 ± 0.183
8	1.41 ± 18.08	0.23 ± 0.0001	73.16 ± 0.140	25.67 ± 0.176
10	1.18 ± 9.397	0.30 ± 0.015	84.16 ± 0.008	31.67 ± 0.011
IC_{50} (mg/ml) ± SE	2.16 ± 0.09	15.92 ± 0.002	0.05 ± 0.758	0.14 ± 0.237

Values are means ± SD of triplicate analyses; [b]Scavenging rate = $(V_0\text{-}V_1)/ V_0 \times 100$ %, Where V_0 was volume of $Na_2S_2O_3$ solution used to titrate the control sample in presence of hydrogen peroxide, V_1 was the volume of $Na_2S_2O_3$ solution used in presence of extracts.

with eight parts of distilled water (70-80°C) for 3-4 h on orbital shaker.

Evaluation of antioxidant activity: The antioxidant potential of the extract was determined by using free radical scavenging capacity by the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) [4], reducing power assay [5], metal chelating ability [6] and ability to scavenge hydrogen peroxide [7]. The inhibition of lipid per oxidation was determined using thiobarbituric acid (TBA) method [8], values were given as equivalent amounts of malonyldialdehyde (MDA). Protein concentration was measured by Lowry method using Bovine serum albumin as standard [9]. The amount of glutathione was determined according to Tripathi and Pandey [10]. The total phenol [11] and flavonoid contents were also estimated [12].

2. Result and discussion

The results are presented in Table 1-3. The aqueous extract of *C. fistula* leaves possess a significant antioxidant activity in all test performed.

Table 1: Extracts yields, total phenol and flavonoid content

Table 2: Reducing power, free radical scavenging and metal chelating activity of aqueous extract of *C. fistula* leaves

Table 3: Quantities of malonyldialdehyde, reduced glutathione and H_2O_2 scavenging effect of aqueous extract of *C. fistula* leaves

The activity is in good correlation with extract concentrations. According to literature survey, leaves are rich in polyphenols and polyphenols are natural antioxidant [13, 14]. Thus, we can assume that antioxidant activity of analyzed *C. fistula* extract is due to presence of polyphenols. Further work is necessary to isolate the active constituents.

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