

COMPARATIVE ANTIOXIDANT STUDIES OF *CASSIA AURICULATA* PLANT PARTS

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ABSTRACT: Comparative antioxidant studies were carried out for methanolic extract of *Cassia auriculata* flowers, leaves and roots for proving its utility in inflammation and healing mechanism. The methanolic extracts were screened for antioxidant activity by nitric oxide radical scavenging, lipid peroxidation inhibition and DPPH methods at different concentrations. Throughout the studies flowers extract showed marked antioxidant activity compared to leaves and roots extract. The antioxidant activity of the flower flowers extract may be due to stabilization of plasma membrane, thereby lowering the elevated levels of serum lysosomal enzymes. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of high flavanoids and bioflavonoids content in the flowers of *Cassia auriculata*.

Key words: Free radicals, 1, 1- diphenyl-2-picrylhydrazyl, Spectrophotometer, *In-vitro*, nitric oxide.

INTRODUCTION

Oxidative stress has been associated with the pathogenesis of many human diseases; the use of antioxidants in therapeutics is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Antioxidants are widely used as ingredients in dietary supplements maintaining health and preventing diseases such as cancer and coronary heart disease¹.

Cassia auriculata, Linn (Caesalpiniaceae) commonly known as Tanners *Cassia* [**Avaram**] is a shrub with large bright yellow flowers, growing wild in Central Provinces and Western peninsula parts of India² It is one of the main constituents of '**Kalpa herbal tea**' and has proven antidiabetic action³. The five parts of the plant roots, bark, leaves, flowers, and unripe fruits taken in equal quantity, dried and powdered known as '**Avarai panchaga choornam**' has beneficial effect in diabetes⁴. The flowers are used in urinary discharges, nocturnal emissions, diabetes, throat troubles. The roots are alexeteric, useful in urinary discharges, tumors, skin diseases, asthma. The leaves are used as anthelmintic, in ulcers, leprosy, skin diseases⁵. The plant has been reported to possess hepatoprotective⁶, antiperoxidative⁷, antihyperglycemic activity⁸ microbicidal activity⁹.

Since antioxidant and membrane stabilizing activity have not been systematically studied in the plant parts, therefore *in vitro* studies were undertaken which could be the major mechanism involved in the protective effect.

MATERIALS AND METHODS

Collection, authentication and extraction

Fresh flowers, leaves and roots of *Cassia auriculata* were collected from Tamil-Nadu, India in month of August-September and authenticated at Agharkar Research Institute, Pune. The plant parts were dried in an oven below 60°C for 2 hrs. The dried parts of plant were finely powdered and extracted with 70% aqueous methanol using Soxhlet apparatus at 50°C. The soluble part was concentrated over water bath maintained below 60°C and dried in a vacuum oven to obtain free flowing reddish brown powder.

Experimental model

Albino mice of Swiss strain (20-25kg) were purchased from Bharat Serum and Vaccines, Thane. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light/12 h dark cycle; $25 \pm 3^\circ\text{C}$; humidity 35-60 %). They were fed with Amrut brand pelleted standard diet manufactured by Nav Maharashtra Chakan oils, Ltd., Maharashtra and drinking water *ad libitum*. The animals had free access to water all the time. They were allowed to adapt to the animal house conditions by keeping them for a period of 8-10 days prior to using them for the experiments. The study was conducted after seeking clearance from the Institutional animal ethical committee.

Chemicals and reagents

Acetic acid, Ascorbic acid, Ferrous sulphate, Potassium chloride, Sodium bicarbonate, Sodium carboxy methyl cellulose, Sodium hydroxide, Sodium lauryl sulphate, Sodium nitroprusside, Sulphuric acid and Tris-HCl buffer were procured from Sd Fine Chem Ltd, Mumbai.

Glacial Acetic Acid, n-Butanol, Pyridine were obtained from Sisco Labs., Mumbai.

Sodium Chloride, Ethanol were procured from Merck Ltd., Mumbai. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Sulphanilamide, Phosphoric acid, 0.1% naphthylethylenediamine dihydrochloride from Hi-Media, Mumbai.

Instruments:

UV-Visible Spectrophotometer (Shimadzu) for carrying antioxidant studies

Homogenizer to homogenize liver for *in-vitro* lipid peroxidation studies.

Antioxidant activity evaluation:

Nitric oxide scavenging activity

1.5 ml, 10 mM sodium nitroprusside in phosphate buffer saline pH 7.4 was mixed with 0.5ml various concentrations (5mM to 50mM) of MECA and the mixture was incubated at 25°C for 150 min. During which sodium nitroprusside spontaneously generates nitric oxide. After the incubation 1.5 ml Griess reagent (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride) was added. The reaction mixture incubated at room temperature for 30 min. The absorbance was measured with UV-spectrophotometer at 540 nm^{10} .

The activity of MECA was compared with ascorbic acid which was used as a standard antioxidant. The nitric oxide scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = \frac{(A_0 - A_1)}{(A_0)} \times 100$$

Where

A_0 is the absorbance of the control (blank, without MECA)

A_1 is the absorbance in the presence of the MECA or the standard ascorbic acid.

In vitro lipid peroxidation

Preparation of liver homogenate:

The liver that was excised from the mice was weighed and chilled in ice cold saline. After washing with ice cold saline tissue homogenates was prepared in a ratio of 1g of wet tissue to 9ml of KCl.

Assay Procedure:

The reaction mixture contained 0.2 ml, 10% w/v mice liver homogenate in 0.2 ml, 0.15 M Potassium Chloride, 0.4 ml Tris buffer pH 7.5 and various concentrations (5-100 $\mu\text{g/ml}$) of MECA. Lipid peroxidation was initiated by addition of 0.1 ml, 10 μM Ferrous Sulphate and 0.1 ml, 100 μM Ascorbic Acid. The reaction mixture was incubated at 37°C for 1h. After the incubation, reaction was terminated by adding 2 ml 0.8% w/v thiobarbituric acid. The contents were heated at 95°C for 15 min for development of colored complex. The tubes were cooled and centrifuged at 4000 rpm for 10 min and supernatant were removed and its color intensity was measured at 532 nm^{11} . The % inhibition of lipid peroxidation was determined by comparing the result of MECA with those of controls not treated with MECA. The results were expressed as IC_{50} values that are the concentration of MECA required for 50% inhibition of lipid peroxide.

The activity of MECA was compared with curcumin which was used as a standard in lipid peroxidation. The inhibition of lipid peroxidation was calculated according to the following equation:

$$\% \text{ inhibition} = \frac{(A_0 - A_1)}{(A_0)} \times 100$$

Where

A_0 is the absorbance of the control (blank, without MECA)

A_1 is the absorbance in the presence of the MECA or standard curcumin.

DPPH Radical method

The reaction mixture consisted of 1 ml of 0.1mM DPPH in ethanol, 0.95 ml of 0.05 M Tris-HCl buffer (pH 7.4), 1 ml of ethanol and 0.05 ml of MECA at various concentrations (10mg/ml to 100mg/ml). The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding MECA. The % of scavenging activity was determined by comparing the result of MECA with those of standard antioxidant ascorbic acid. The results were expressed as IC_{50} values that are the concentration of MECA required for 50% inhibition¹².

$$\% \text{ of scavenging activity} = 100 - (100 / \text{blank absorbance} \times \text{Standard} / \text{sample absorbance})$$

Where

Blank absorbance is the absorbance that is without the MECA.

Sample absorbance is the absorbance of MECA.

Statistical analysis

The data was analyzed using SPSS packages and IC_{50} values representing the concentration required to induce 50 % inhibition were calculated.

RESULTS

Plant extract evaluation

Preliminary phytochemical screening revealed presence of saponins, carbohydrates, flavanoids, steroids and tannins.

Antioxidant activity evaluation

Nitric oxide scavenging activity

MECA flowers showed a significant free radical scavenging action against nitric oxide (NO) induced release of free radicals in comparison to leaves and roots. The IC_{50} value was found to be 43.38 mM. (Table No.1)

Table No.1 Results of nitric oxide radical scavenging activity of MECA

Effective concentration (mM)	% of nitric oxide scavenging activity (Flowers)	% of nitric oxide scavenging activity (Leaves)	% of nitric oxide scavenging activity (Roots)	IC50 Value of the flowers
5	28.69	28.33	28.09	43.38 mM
10	30.08	29.96	29.58	
15	31.69	31.33	31.04	
20	33.33	33.15	33.02	
25	35.03	35.00	34.96	
30	36.41	36.28	36.11	
35	38.03	37.99	37.88	
40	39.70	39.57	39.43	
45	41.73	41.61	41.38	
50	44.00	43.78	43.57	

In vitro lipid peroxidation

MECA extract elicited concentration dependent inhibition of FeSO₄ induced lipid peroxidation in mice liver homogenate. The IC₅₀ value was found to be 43.99 µg/ml. (Table No.2).

Table No.2 Results of Lipid Peroxidation Inhibitory Activity of MECA

Effective concentration (µg/ml)	% Inhibition in TBARs formation (flowers)	% Inhibition in TBARs formation (Leaves)	% Inhibition in TBARs formation (Roots)	IC ₅₀ value
5	26.44	26.32	25.93	43.99µg/ml
10	27.75	27.57	27.31	
20	32.42	32.21	31.99	
40	38.70	38.45	38.33	
60	49.27	49.22	49.08	
80	56.71	56.34	56.07	
100	62.77	62.55	62.44	

DPPH Radical method

MECA extract showed promising free radical scavenging effect of DPPH in concentration dependent manner upto 100 mg/ml. The IC₅₀ value was found to be 39.17mg/ml. (Table No.3).

Table No.3 Results of DPPH Radical scavenging Activity of MECA

Effective concentration (mg/ml)	% inhibition of DPPH radical scavenging activity (Flowers)	% inhibition of DPPH radical scavenging activity (Leaves)	% inhibition of DPPH radical scavenging activity (Roots)	IC ₅₀ value
10	30.17	30.00	29.83	39.17mg/ml
20	32.50	32.22	32.01	
30	34.00	33.75	33.22	
40	36.20	36.08	35.88	
50	38.00	37.54	37.33	
60	41.92	41.55	41.11	
70	44.12	44.02	43.77	
80	46.40	46.14	45.95	
90	48.90	48.56	48.21	
100	51.50	51.32	51.10	

DISCUSSION

Sodium nitroprusside serves as a chief source of free radicals. Scavengers of nitric oxide compete with oxygen leading to reduced formation of Nitric Oxide (NO). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine is used as the marker for NO scavenging activity¹⁰. The chromophore formation was not complete in the presence of MECA, which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the MECA extract increases in a dose dependent manner¹².

Lipid peroxidation has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. The MECA extract exhibited protection against lipid peroxidation induced by FeSO₄. Initiation of lipid peroxidation by ferrous sulphate takes place through Ferryl perferryl complex¹³. The MECA inhibited the FeSO₄ induced lipid peroxidation in a dose dependent manner. The inhibition could be caused by the inhibition of formation of Ferryl perferryl complex. The presence of flavonoids in *Cassia auriculata* flowers may be responsible for antioxidant activities. The DPPH system is a stable radical generating procedure¹⁴. It is well known that the DPPH has ability to capture free radicals is due to the delocalization of the unpaired electron all over the molecule. DPPH is a potent scavenger for many other radicals, due to the easiness in following the procedure – violet colour of DPPH faints into the yellow colour of its reduced congener (DPPH-H), with a high shift in the visible spectra (from 520 nm to 330nm)^{10,15}.

Cassia auriculata is widely used in number of pharmacological actions with high content of flavanoids and bioflavonoid seems to have a high potential for antioxidant activity. Inhibition of lipid peroxides can be explained as one of the important biochemical paradigm in understanding of the mechanism(s) of the action of MECA flowers.

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