

In vitro study on antioxidant activity of methanolic leaf extract of *Cassia fistula* Linn.

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ABSTRACT

Background: The use of medicinal plants for maintaining good health is getting attention worldwide. Antioxidants play an important role to protect damage caused by oxidative stress. In the present study methanolic extracts of *Cassia fistula* was determined using DPPH for its antioxidant activity. Phytochemical investigation confirmed the presence of bioactive ingredients in the extract.

Methods: The antioxidant activity of methanolic extract of *Cassia fistula* was evaluated using DPPH free radical assay. DPPH (1,1-diphenyl-2-picrylhydrazine) free radical analysis is one of the accurate and frequently employed method for evaluating antioxidant activity.

Results: The methanolic extracts showed increase in radical scavenging activity as concentration increases. The IC₅₀ values were calculated for the methanolic extract. Ascorbic acid was used as control. *Cassia fistula* exhibited IC₅₀ of 79.42 µg/ml.

Conclusions: Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease and infectious diseases. Further evaluation of pharmacological activities of *Cassia fistula* may prove useful in treatment of cancer and heart diseases.

Keywords: Antioxidant activity, *Cassia fistula*, DPPH, IC₅₀

INTRODUCTION

Over the last few years, researchers have aimed at identifying and validating plant-derived substances for the treatment of various diseases. Interestingly it is estimated that nearly 25% of the modern medicines are directly or indirectly derived from plants. It is worth mentioning that Indian medicinal plants are considered as a vast source of several pharmacological principles and compounds that are commonly used as home remedies against multiple ailments.¹

With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential.¹

Cassia fistula Linn. (leguminosae), Indian Laburnum (Golden shower tree) is a very common plant known for its medicinal properties. Antioxidant activity power was in the decreasing order of stem bark, leaves, flowers and pulp and was well correlated with the total polyphenolic content of the extracts.¹

Aragvadha or *Cassia fistula* is one of the four hundred and odd species comprising the genus *Cassia* and is widely distributed all over.² Extract of *Cassia fistula* showed anti-inflammatory, hypoglycaemic, hepatoprotective, anti-fertility and antioxidant activity.³ In the Indian literature this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands.⁴ The lipid oxidation process is involved in oxidative damage at cellular level leading to ageing.⁵ *Cassia fistula* is widely

used for its antitumor, hepatoprotective, antifertility, antioxidant properties as well as its actions on central nervous system, wound healing and inhibitory effect on leukotriene biosynthesis.⁶ There is overwhelming evidence showing that natural antioxidants play a role in wellness, health maintenance and prevention of chronic degenerative disease.⁷ Natural antioxidants can protect a human body from radicals and retard the process of many chronic diseases as well as lipid oxidative rancidity in foods. Hence the studies on natural antioxidants have greater importance.⁸

The plant has been reported to possess antioxidant properties as well as antimicrobial properties by different studies conducted in India as well as other countries. Plants growing in different climatic and seasonal conditions do not have identical constituents or therapeutic effects.⁹ Thus active components may differ in nature or concentration. Phytochemical analysis of the crude extracts can indicate the presence of active constituents in the plant.

Antioxidants that can trap free radicals are our crucial defence against free radical induced damages and are crucial for maintaining optimum health. They can reduce the risk for chronic diseases including cancer and heart disease.¹⁰

The search for plant derived antioxidants has been received much attention and effort in order to identify the compounds that have high capacity in scavenging free radicals related to various diseases. Generally, antioxidant compounds like Phenolic acids, poly phenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanism that lead to degenerative diseases.¹¹

METHODS

Collection of plant materials

Fresh leaves of *Cassia fistula* were collected from GMC, Ernakulam campus in month of May- June. Leaves were shade dried and crushed into fine powder with electric blender. The powdered sample were sealed in polythene box and stored.

Preparation of methanolic extracts: Sample preparation

Preparation of methanol extract: Dried and powdered leaves (25g) were used. The extract is prepared using Soxhlet apparatus. Powdered leaves with 200ml of methanol was subjected for extraction for about 10h in a water bath. The crude plant extract in solvent was removed from the Soxhlet apparatus and concentrated to dryness in water bath and is stored in refrigerator.

Phytochemical screening

Preliminary phytochemical screening was performed by using standard tests.

Test for alkaloids

Dragendroff's test: 1ml of extract+ 1ml of mayers reagent. Yellow precipitate

Glycosides

1ml of extract+ 1ml of water+ Aq. NaOH. Yellowish brown precipitate

Flavanoids

1ml extract+ 10% lead acetate. Yellowish green precipitate

Saponins

1ml extract + boiled with 1ml of distilled water. Froth

Tannins

1ml extract+ 0.1% FeCl₃. Brownish green precipitate

Terpenoids

1ml extract+ 1ml of CHCl₃ + 2ml conc. H₂SO₄. Brown precipitate

Proteins

1ml extract+ 1ml 40% NaOH+ 2 drps of 1% CuSO₄. Pink color

Carbohydrates

Benedicts test: 1ml extract+ 5ml benedicts reagent. Boil 2 minutes and cool. Slight red precipitate

DPPH radical scavenging activity

DPPH free radical scavenging of *Cassia fistula* methanolic extracts was estimated according to the method of Blois et al.¹² Free radical scavenging ability of the extracts was tested using DPPH radical scavenging assay. Stock solution of the whole plant extract was prepared to concentration of 1mg/ml. A solution of 0.1mM DPPH was prepared. To 1ml this solution 3ml. of different concentration extracts in methanol were added. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, absorbance was measured at 517 m. by using spectrophotometer. Ascorbic acid was used as reference standard. Lower absorbance of the reaction mixture indicates higher free radical activity. All the tests were performed in triplicate.

The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of extracts/standard

The % of inhibition was plotted against concentration, and from the graph IC50 was calculated.

Values are the average of triplicate experiments and are represented as mean (n=3).

Statistical analysis

Results are expressed as mean value (n=3). Regression analysis was performed to calculate the dose response relationship of the extract.

RESULTS

The radical scavenging activity of methanolic leaf extracts was determined on the basis of the radical scavenging effect on the DPPH (2, 2-diphenyl-1-picrylhydrazyl). DPPH is a stable free radical and accepts hydrogen radical to become diamagnetic molecule. The solution appears a deep violet colour and changes to yellow after accepting hydrogen. The reduction capability of DPPH radical was determined by decrease in its absorbance at 517 nm which is induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and DPPH which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. *Cassia fistula* methanolic extracts exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentrations tested. There was a dose dependent increase in the percentage antioxidant activity for all concentrations tested. All the tests were done in triplicate and the values were plotted as mean ± standard deviation. The IC50 values of methanolic leaf extract of *Cassia fistula* by DPPH method is obtained as 79.42 µg/L.

Table 1: Phytochemical screening of methanolic extract of *Cassia fistula* leaf.

Phytochemical constituent	Methanolic extract <i>Cassia fistula</i>
Alkaloids	+
Glycosides	+
Flavanoids	+
Saponins	+
Tannins	+
Terpenoids	+
Proteins	+
Carbohydrates	+

Phytochemical composition of plant extract
(+) Indicate Present and (-) Indicate Absent

Table 1 represents phytochemical screening of *Cassia fistula* leaf extract. It shows presence of alkaloids,

glycosides, flavonoids, saponins, tannins, terpenoids, proteins and carbohydrates.

Table 2 Shows percentage inhibition of methanolic leaf extract of *Cassia fistula* at concentrations (µg/ml) in DPPH scavenging model.

Table 2: Percentage inhibition of methanolic leaf extract of *Cassia fistula* at concentrations (µg/ml) in DPPH scavenging model.

Concentration (µg/ml)	% Inhibition
8.6	3.99
17.2	8.92
25.8	15.22

Values are mean of three measurements

Increasing the concentration of *Cassia fistula* (8.6 µg, 17.2 µg, 25.8 µg) shows a linear increase in its antioxidant activity. The mean value (n=3) of antioxidant activity at each concentration is 3.99, 8.92 and 15.72 respectively.

Table 3: Percentage inhibition of standard Ascorbic Acid at concentrations (µg/ml) in DPPH scavenging model Concentration.

Concentration (µg/ml)	% Inhibition
0.143	0.82
0.28	16.69
0.42	27.34
0.56	40.57

Values are mean of three measurements

Increasing the concentration of standard Ascorbic acid (0.143 µg, 0.28 µg, 0.42 µg and 0.56 µg) shows a linear increase in its antioxidant activity. The mean value (n=3) of antioxidant activity at each concentration is 0.82, 16.69, 27.34 and 40.57 respectively

Table 4 shows IC50 values of Ascorbic acid which is 0.67 µg/ml and the sample methanolic extract of *Cassia fistula* leaf which is 79.4 µg/ml. IC50 represent a concentration that will scavenge 50% of the free radicals.

Table 4: IC50 Values of ascorbic acid and methanolic extract of *Cassia fistula* Linn leaf.

Specification	IC 50
Ascorbic acid	0.67 µg/ml
Methanolic extract of <i>Cassia fistula</i> Linn leaf	79.42 µg/ml

A straight line graph is obtained by plotting between different concentration and % inhibition. R² is the regression coefficient.

Ascorbic acid is a proven antioxidant, which is used as a standard in Antioxidant assays. *Cassia fistula* shows good antioxidant activity with an IC50 value of 79.4 µg/ml.

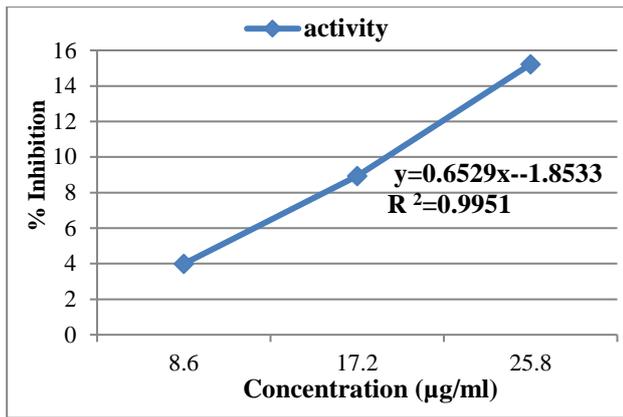


Figure 1: DPPH free radical scavenging activity of methanolic leaf extract of *Cassia fistula*.

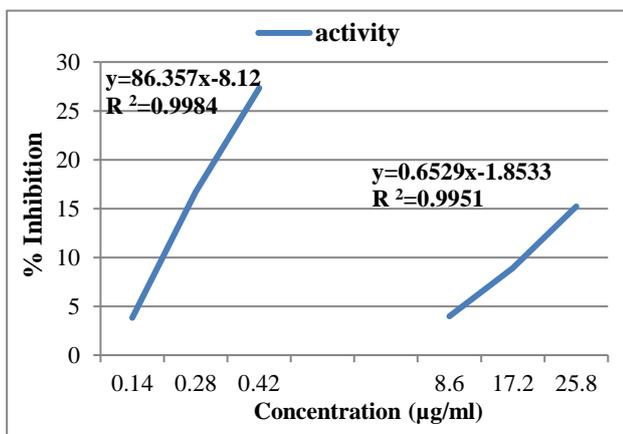


Figure 2: DPPH free radical scavenging activity of Std ascorbic acid and methanolic leaf extract.

DISCUSSION

DPPH analysis is the best known accurate and frequently employed methods for evaluating antioxidant activity.¹³ In this present study *Cassia fistula* methanolic leaf extract demonstrated significant antioxidant activity. This study is comparable to the study conducted by Md. Irshad et al, which showed significant antioxidant potential of various extracts *Cassia fistula* determined by DPPH, FRAP, Fe₃₊ reducing power and hydrogen peroxide scavenging assay.¹⁴ Bhalodia et al, recently reported the antioxidant activity of *Cassia fistula* flower in DPPH assays.¹⁵

Antioxidants are the compounds that neutralise the attack of free radicals and therefore reduce the risk of disorders.¹⁶ Abid R et al, in his study demonstrated antioxidant and protective effect of *Cassia fistula* Linn fruit extracts.¹⁷ A study was performed by Lia TK et al, which showed *Cassia fistula* bark extracts have high antioxidant activity.¹⁸ In another study done by Evans R et al, to investigate the antioxidant activities of *Cassia fistula* with 90 % ethanol extracts of leaves and 90% methanol extracts of stem bark.¹⁹ In a study done by Siddhuraju et al, the stem bark of *Cassia fistula* demonstrated high antioxidant

activity.²⁰ Pulp and flowers results confirmed that stem bark showed high antioxidant activity.²⁰ Teeshan Bahuron et al, revealed significant antioxidant from the aqueous extracts of *Cassia fistula* linn.⁴

Manomani et al, evaluated the antioxidant effect of aqueous extract of fistula (Linn.) flowers and results revealed the reversal back of antioxidant enzymes to near normal levels.²¹

CONCLUSION

From this study it is clear that crude extracts of *Cassia fistula* leaf contain medicinal active components. The preliminary Phytochemical investigation confirms the presence of bioactive components in the extract. The result of this study on *Cassia fistula* methanolic extract demonstrates significant antioxidant activity of *Cassia fistula* which can be used as easily accessible source of natural antioxidant. However, the components responsible for the antioxidant activity of methanolic extract are currently unclear. Therefore, further works have to be performed on the isolation and identification of the antioxidant compounds present in the plant extract.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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