Research Article

In-vitro antioxidant activity of *Cassia auriculata* Leaves

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ABSTRACT

Objective: Assessment of Antioxidant activity of *Cassia auriculata* leaves by using In vitro models. Method: In the present study, various extracts of *Cassia auriculata* leaves were evaluated by Hydrogen peroxide scavenging activity, Reducing power method, Nitric-oxide scavenging Activity and DPPH method. IC_{50} value was calculated and compared with standard Ascorbic acid. Results: Ethyl acetate extract was found to be extremely effective in scavenging of nitric-oxide, hydrogen peroxide (IC_{50} 232.56 $\mu g/ml$, 229.88µg/ml). In inhibition of DPPH Radical Scavenging activity (IC_{50}222.2 $\mu g/ml$) and by using reducing method (IC_{50} 212.76µg/ml).Ethyl acetate extract showed different levels of antioxidant activities in all tested models.

Keywords: *Cassia auriculata,* antioxidant activity, *in vitro* models.

INTRODUCTION

Reactive oxygen species (ROS), which consist of free radicals such as superoxide anion (O^2-) and hydroxyl (HO·) radicals and non-free radical species such as H_2 O_2 and singled oxygen (O_2), are different forms of activated oxygen. ROS are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA. Thus, ample generation of ROS proceed to a variety of pathophysiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity. Therefore,living organisms possess a number of protective mechanisms against the oxidative stress and toxic effects of ROS. Antioxidants regulate various oxidative reactions naturally occurring in tissues. Antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors.

Cassia auriculata (family:caesalpinaceae) is found throughout central and southern India,also cultivated in Punjab, Haryana, Uttar Pradesh and West Bengal.The shrub usually occurs on roadsides, waste line, and railway bankments(Kirtikar KR,19995). Leaves are anthelmintic and also used to treat ulcers,skin diseases and leprosy. An aqueous extract of leaves possesses hypoglycemic activity(Latha M,2002).The leaves are eaten as a vegetable in times of scarcity, the infusion of leaves possesses a slight purgative activity.Aqueous extract of leaves also possesses antioxidant activity(Sabu MC,2002).The present study is an attempt to evaluate the antioxidant activity of *Cassia auriculata* Linn.

EXPERIMENTAL

Plant material collection

The leaves of *C. auriculata* were collected in the month of August from chandragiri,chittoor district, Andhra Pradesh. The plant material was taxonomically identified by Dr. B. Sitaram, S.V. Ayurvedic College, Tirupathi. The herbarium was prepared and deposited in Sri Padmavathi School of Pharmacy for future reference.

Preparation of various extracts

The plant material was washed, shade dried coarsely powdered and extracted with petroleum ether to remove the oily and fatty substances present in the drug. Then the drug was dried and was extracted with chloroform, ethyl acetate and methanol. The extract was filtered and the filtrate was concentrated over water bath and dried in vacuum desiccators for further use.

Phytochemical Investigation of Extracts
Different chemical constituents present in the various extracts were performed by using standard procedures (Kokate, 2004).

***In-Vitro* Antioxidant Assays:**
DPPH Radical Scavenging Activity
DPPH scavenging activity was assessed using the method of Hatano *et. al.,* (1998). In this, different vials contain 1ml of extract solution and standard were taken. To these solutions, 5 ml of methanolic solution of DPPH was added, shaken well and the mixture was incubated at 37^0C for 20 min. The absorbance was measured against methanol as a blank at 517 nm. The absorbance of DPPH taken as a control was measured. All tests were carried out in triplicate. Percentage anti-radical activity can be calculated by using following formula

$$\% \text{ Anti-radical activity} = \frac{\text{Control Absorbance - Sample Absorbance} \times 100}{\text{Control Absorbance}}$$

Hydrogen peroxide scavenging Activity
The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch *et al.(*1989). A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50mMpH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (25- 400µg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

% Scavenged (H_2O_2) = ($A_0 - A_1$ / A0) x 100

Where; A_0 is the absorbance of control and A_1 is the absorbance of test.
Ascorbic acid can be used as a positive control. All tests were carried out in triplicate.

Nitric oxide radical scavenging (NO) assay
Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent (Green et al., 1982). 3.0 ml of 10mM sodium nitroprusside in phosphate buffer is added to2.0 ml of extract and reference compound in different concentrations (25 - 400 µg/ml). The resulting solutions are then incubated at 25°C for 60 min. A similar procedure is repeated with methanol as blank, which serves as control. To 5.0 ml of the incubated sample, 5.0ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylenediamine dihydrochloride in 2% H_3PO_3) is added and absorbance of the chromophore formed is measured at 540 nm. Percent inhibition of the nitrite oxide generated is measured by comparing the absorbance values of control and test preparations. Ascorbic acid can be used as a positive control. All tests were carried out in triplicate.

Reducing power assay
Reducing power of the extracts was determined by the method of Yildirm *et al*., (2003).Various concentrations of the extracts (25-100µg) in 1 ml of distilled water were mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 ml (10 g/L) potassium ferricyanide. The mixture was incubated at 50 C for 30 min followed by addition of 2.5 ml trichloroaceticacid (100 g/l) and centrifugation at 1650 rpm for 10 min. From the upper layer solution 2.5 mL was taken and mixed with 2.5 mL ferric chloride (1 g/L). The absorbance was read at 700 nm against reagent blank. Higher absorbance indicates higher reducing power. Ascorbic acid was used as reference compound. All tests were carried out in triplicate..

RESULTS AND DISCUSSION
Preliminary phytochemical screening of different extracts of *Cassia auriculata* revealed the presence of Flavonoids, Tannins, Saponins, Phenolic compounds and proteins,glycosides etc. The plant extracts used for the experiment possessed a significant dose dependent (25µg-400µg), anti-oxidant activity on all reactive species when compared with ascorbic acid as a standard. Among these extracts, ethylacetate extract of *Cassia auriculata* leaves had shown prominent anti-oxidant potential than other extracts were studied.

Inhibition of DPPH radical
The potential decrease in the concentration of DPPH radical due to the scavenging ability of *Cassia auriculata* and Ascorbic acid (reference

standard) showed significant free radical scavenging activity. The IC_{50} values of different extract (the inhibitory concentration at which there is 50% reduction of free radical) of *Cassia auriculata* was shown in Table - 1**.**

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of *Cassia auriculata.* The results of DPPH radical Scavenging Activity was indicated in Fig.1.The IC_{50} values were found to be 222.2µg/ml and 243.9 µg/ml for ethyl acetate extract and ascorbic acid respectively.

Nitric oxide scavenging activity

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 napthylethylene diamine is used as the marker for NO scavenging activity. The chromophore formation was not complete in the presence of CALEA, which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the CALEA extract increases in a dose dependent manner. The results of nitric oxide Scavenging Activity was shown in Fig.2 .The concentration of extract needed for 50% inhibition was found to be 232.56 µg/ml whereas 217.39 µg/ml for ascorbic acid.

Reducing power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of eachcompound.Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Pearl's Prussian blue at 700nm, it is possible to determine the concentration of Fe3+ ion. The results of reducing power assay were represented in Fig.4. The IC_{50} values were found to be 212.7 µg/ml and 229.9 µg/ml for ethyl acetate extract and ascorbic acid respectively.

Hydrogen peroxide scavenging activity

Hydrogen peroxide has strong oxidizing properties. It can be formed *in vivo* by many oxidizing enzymes, such as superoxide dismutase and can cross cellular membranes and may slowly oxidize a number of intracellular compounds. The ability of crude extract to scavenge hydrogen peroxide when compared with Ascorbic acid was observed.

Hydrogen peroxide scavenging activity of the different extracts (25-400 µg/ml) and on the other hand, standard ascorbic acid taken at the same concentrations, respectively. The results of hydrogen peroxide scavenging activity were represented in Fig.3. The concentration of extract needed for 50% inhibition was found to be 229.88 µg/ml whereas 263.15 µg/ml for ascorbic acid. Hydrogen peroxide itself is not very reactive; however, it can sometimes be toxic to cell, because it may give rise to hydroxyl radical in the cells.

Table 1: IC_{50} values of different extracts of *Cassia auriculata* leaves (µg/ml)

S. No.	GROUP	DPPH	H_2O_2	R.P	NO
1	Ascorbic acid	243.9	263.15	229.88	217.39
2	CALPE	322.58	312.5	281.69	294.12
3	CALCE	294.117	238.09	246.91	270.27
4	CALEA	222.22	229.88	212.76	232.56
5	CALME	263.16	263.15	285.71	246.91

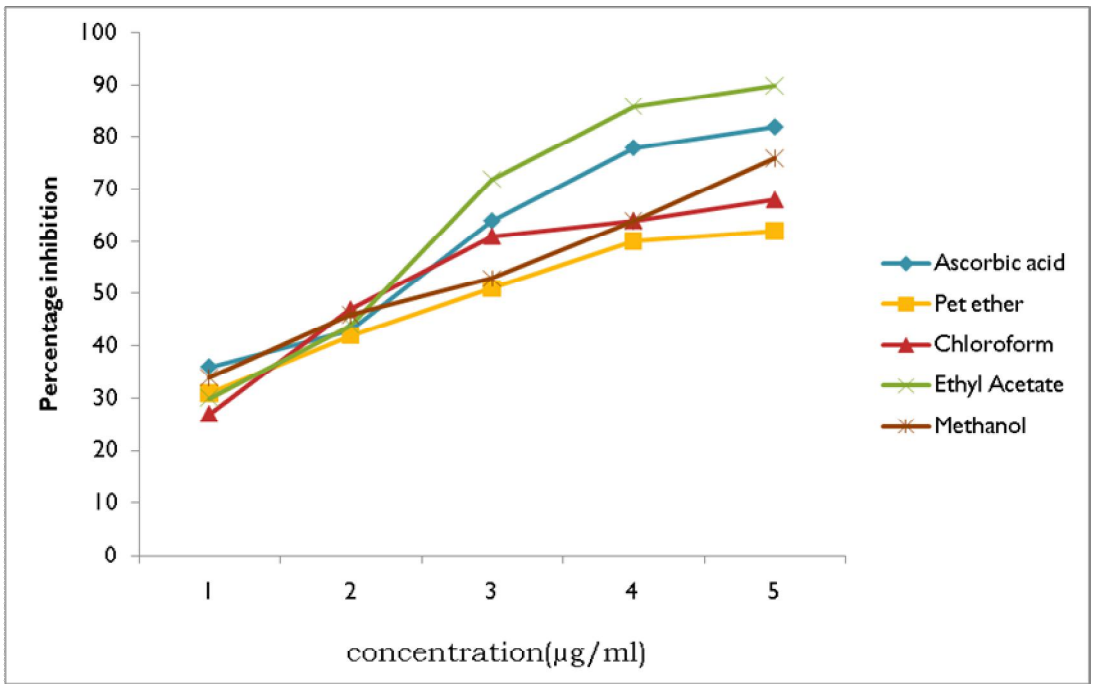

Fig.1: Effect of plant extracts on DPPH scavenging activity

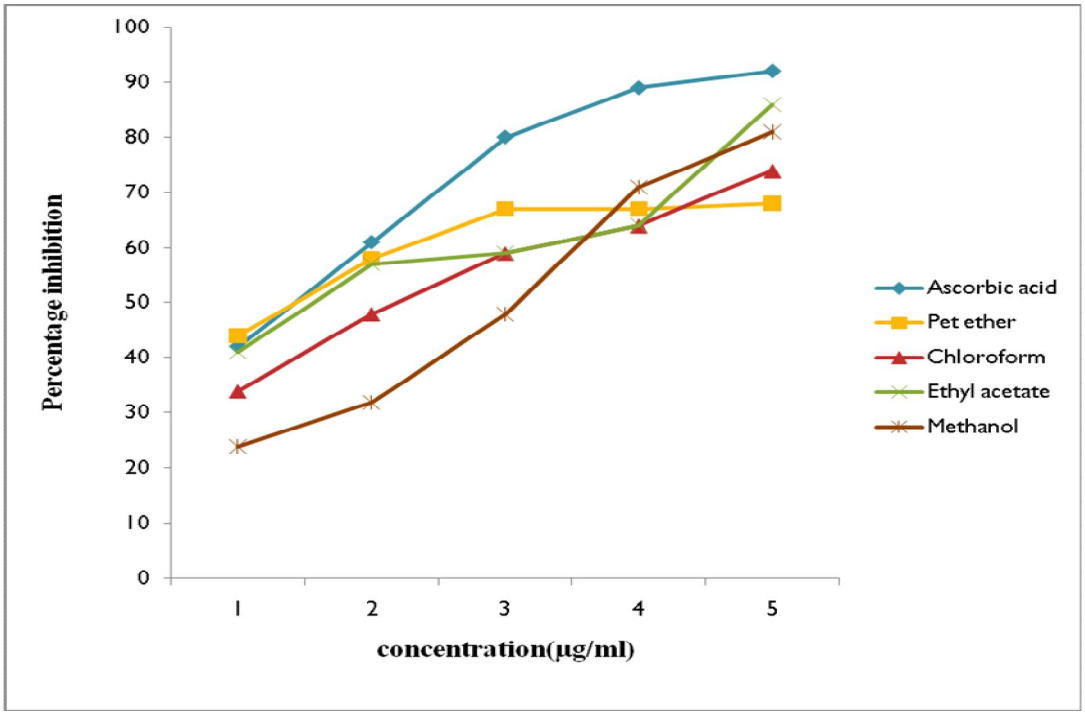

Fig. 2: Effect of plant extracts on Nitric oxide scavenging activity

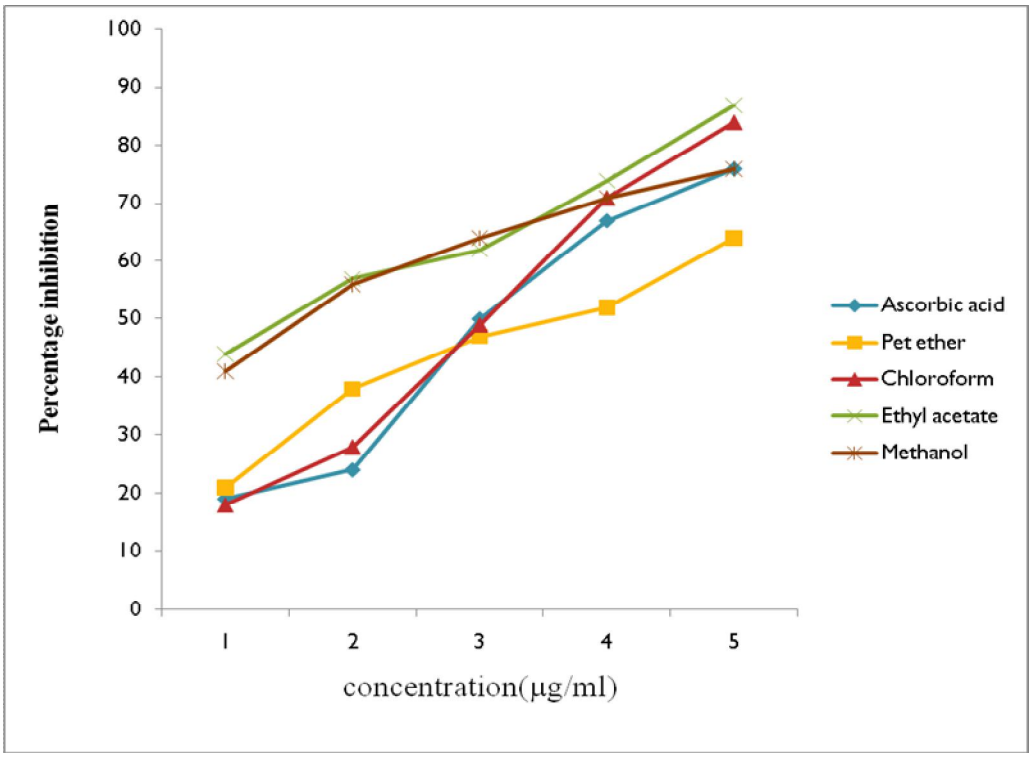

Fig. 3: Effect of plant extracts on H_2O_2 scavenging activity

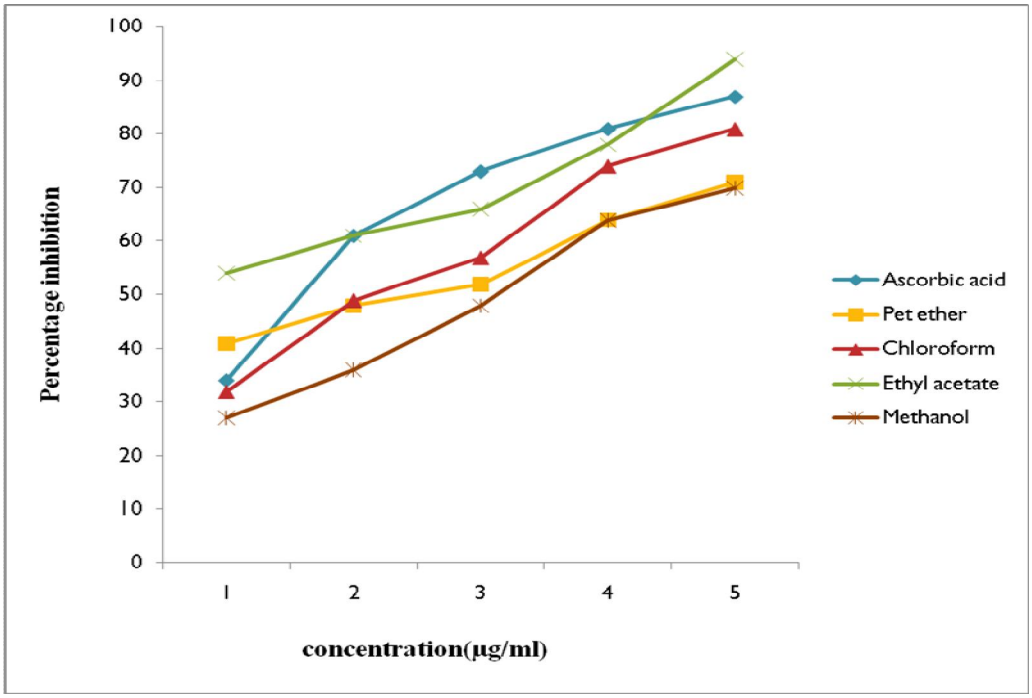

Fig. 4: Effect of plant extracts on reducing power assay

CONCLUSION

From the results, we infer that anti-oxidant property of CALPE, CALCE, CALME and CALEA may be due to the presence of flavanoids, saponins and tannins in extracts. These results clearly indicate that CALEA is effective against free radical mediated diseases. Ethyl acetate has possessed prominent significant **scavenging** activity in removing the free radicals like DPPH, H_2O_2 hydroxyl and nitric oxide when compared to the reference standard ascorbic acid. Ascorbic acid also has shown significantly increase in the scavenging activity. The *Cassia auriculata* has potent antioxidant and free radical scavenging effects in different *in vitro* antioxidant models.

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