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In *vitro* Antioxidant Activity of *Cassia* tora Lin.

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Abstract

Free radicals are toxic byproducts of natural cell metabolism and are responsible for causing a wide number of health problems. The use and search of drugs and dietary supplements from plants with antioxidant activity are needed now a day. In this study methanolic leaves extract of Cassia tora was evaluated invitro by experimentral parameters such as DPPH scavenging activity, total antioxidant assay activity, scavenging superoxide anion radicals, nitric oxide radical, hydrogen peroxide scavenging capacity and total phenolic content. In the present study, reduction of the DPPH radicals was found in concentration- dependent manner. The results showed that Cassia tora displayed potent invitro antioxidant activity that may be attributed to the phenolics present in methanolic extract of the leaves of Cassia tora.

Keywords: *Cassia tora*, antioxidant activity, flavonoid, DPPH, hydroxyl radical.

Introduction

The use and search of drugs and dietary supplements from plants have been intensified in recent year [1]. Medicinal plants are a source of great economic value in the Indian sub continent. For most of the disease, plant materials are used as drugs because of its active compounds. In many disorders the free radical mediated damage may play an important role. Free radicals are responsible for causing a wide number of health problems which include cancer, aging, heart diseases and gastric problems etc. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age[2,3] Antioxidant compounds can decrease oxidative stress and minimize the incidence of these diseases. The mechanism of the action of these antioxidant compounds include suppression of reactive oxygen species formation either by inhibition of the enzymes or by chelating of trace elements involved in free radical production, scavenging of reactive species and up- regulating or protecting antioxidant defense[4].

Cassia tora Linn. (Caesalpiniaceae) is a shrub widely used as traditional medicine in Africa and India for the treatment of ulcers. The fermented leaves are pounded and added to food or local gin and taken orally for antihelmintic and purgative effects[5]. *Cassia tora* Linn is an annual herb (30-90) is widely found in the tropical regions of India as wasteland rainy season weed. The leaf are pinnate and is flowers are pale yellow in colour with five patals. The Preliminary phytochemical screening of leaf showed the presence of polyphenols which prompted researchers to evaluate its antioxidant and antiproliferative potential[6]. Presence of emodin, kaempferol-2-diglucoside is reported in the leaves. Leaves also contain chrysophanol, aloe-emodin, rhein, glucose, 1-stachydnine, amino acids, fatty acids, d-mannitol, β-sitosterol, myricyl alcohol, trigonelline, choline. Ononitol monohydrate, structurally similar toglycoside was isolated from *Cassia tora* Linn. Leaves[7]. *Cassia tora* is used in treatment of various diseases like ageing, atherosclerosis[8], Cancer[9], Inflammatory joint disease[10], asthma[11], diabetes[12] and degenerative eye diseases[13] and the origin is deleterious free radical reactions[14].

Material and Methods

Chemicals: Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloro acetic acid (TCA), thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and potassium hexa cyano ferrate [$K_3Fe(CN)_6$] were purchased from SISCO Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade.

Extract Preparation: The leaves of *Cassia tora* were first collected from Thirumangalakkottai Village, Thanjavur district, Tamilnadu, India, in January 2012. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The different concentrations (20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml respectively) of plant extract were used to determine in*vitro* activity.

Total polyphenolic compounds were determined according to a protocol similar to that of[15] *Cassia tora* (1 ml) was mixed with 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of 50% Folin–Ciocalteu reagent. The mixture was allowed to react for 5 min and 1 ml of 5% Na_2CO_3 was added. Thereafter, it was thoroughly mixed and placed in the dark for 1 h and the

absorbance was measured at 725 nm using UV–vis spectrophotometer. A gallic acid standard curve was obtained for the calculation of polyphenolic content. The concentration of polyphenols was expressed in terms of mg/100ml of sample. Total flavonoids of aluminum chloride colorimetric method was used for flavonoids determination[16]. 1 ml of sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/100ml of sample.

The DPPH radical scavenging activity of *Cassia tora* methanolic extract was measured in terms of hydrogen donating or radical scavenging ability using the method of[17] different concentrations (20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml respectively).of samples BHT were taken. To about 5ml of 0.1Mm methanolic solution DPPH was added and shaken vigorously. After incubation at 27^0C for 20 min, the absorbance was measured at 517 nm. The radical inhibition percentage was calculated using the following formula: DPPH radical scavenging activity (%) = (Absorbance $_{control}$ - Absorbence $_{sample}$ Absorbance $_{control}$) × 100. Ascorbic acid was used as reference standard.

Superoxide anion scavenging activity of the *Cassia tora* towards superoxide anion radicals was measured by the method of[18] Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMSNADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation: % Inhibition = (A0-A1) / A0 × 100) Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

Nitric oxide radical scavenging activity of *Cassia tora* methanolic extract was determined according to the method reported by[19]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations (20-80 µg / ml) and the mixture incubated at 25oC for 150 min. From the incubated mixture 0.5 ml was taken out and added in to 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. Hydrogen peroxide scavenging activity of *Cassia tora* methanolic extract was estimated by replacement titration[20]. Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts (20-80 µg / ml) were mixed, followed by 2 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM NaS_2O3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as: % Inhibition = (A0-A1) / A0 × 100).

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract. The total antioxidant capacity of the *Cassia tora* methanolic extract was evaluated by the phosphomolybdenum method according to the procedure of[21]. The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. Percentage of scavenging of total antioxidant capacity was calculated as % Inhibition = (A0-A1) / A0 × 100).

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

Statistical analysis: Tests were carried out in triplicate for 3–5 separate experiments. The free radical scavenging activity of *Cassia tora* expressed in percentage (%).

Results and Discussion

Free radical scavenging activity of *Cassia tora* expressed in table 1. Polyphenolic compounds present in *Cassia tora* contributes significantly to the total antioxidant capacity of the fruits. Flavonoids play some important pharmacological roles against diseases, such as cardiovascular diseases, cancer, inflammation and allergy. Epidemiological studies have indicated the relationship between flavonoid intake and reduced risk of certain cancer[22]. In the present study, reduction of the DPPH radicals was found in concentration- dependent manner (figure 1). The *Cassia tora* methanolic extract reduced the stable DPPH radical to yellow colored unstable compound, with and IC_{50} value of (35.59 µg/ml). However, ascorbic acid displays significant scavenging activity over the *Cassia tora* methanolic extract. This might to due to the presence of methoxy group which increases the accessibility of radical center of DPPH to ascorbic acid[23].

Table-1
Invitro* antioxidant activity of *Cassia tora

S. No	Particulars	20 µl	40 µl	60 µl	80 µl
1	DPPH	6.3 ± 0.44	31.42 ± 2.19	52.38 ±3.66	71.18 ± 4.98
	Ascorbic acid (Standard)	10.23 ± 0.71	39.45 ± 2.76	63.63 ± 4.45	82.63 ± 5.78
2	Nitric oxide	8.36 ± 0.58	32.44 ± 2.27	73.58 ± 5.15	98.72 ± 6.91
	Ascorbic acid (Standard)	16.23 ± 1.13	41.23 ± 2.88	82.56 ± 5.77	99.23 ± 6.94
3	Superoxide	12 ± 0.84	35 ± 2.45	59 ± 4.13	85 ± 5.95
	Ascorbic acid (Standard)	23.43 ± 2.9	42.52± 2.97	72.34 ± 5.06	94.23 ± 6.59
4	Hydrogen peroxide	14.68± 1.021	45.55 ± 3.18	72.22 ± 5.05	80.98 ± 4.04
	Ascorbic acid (Standard)	21.32 ± 1.49	52.63 ± 5.68	81.23 ± 5.68	92.34 ± 6.46
5	Total antioxidant	10.42 ± 0.72	37.45 ± 2.62	61.09 ± 4.27	76.38 ± 5.34
	Ascorbic acid (Standard)	48.42 ± 4.96	48.42 ± 3.38	70.12 ± 4.90	93.45 ± 6.54

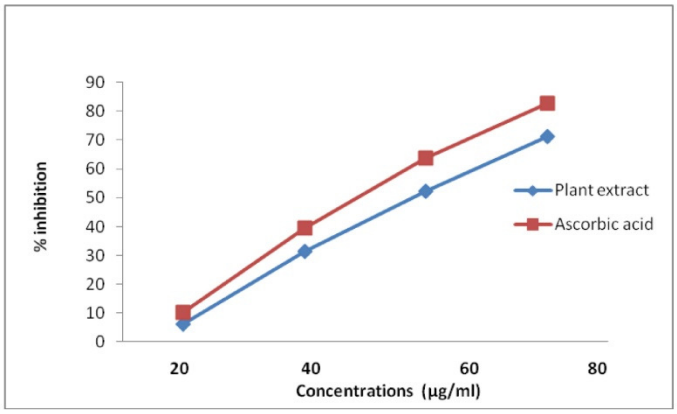

Figure-1
DPPH Scavenging activity of *Cassia tora* methanolic extract at different concentrations

Cassia tora methanolic extract (98.72 ± 6.91 mg/ml) moderately inhibited nitric oxide in dose dependent manner (figure 2) with the IC_5 of (49.36 µg/m). Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. Methanolic extract with *Cassia tore* of (80.98 ± 5.66 mg/ml) demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner with an IC_{50} of (40.49 µg/ml).Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects[24]. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed[25] to accumulate. As shown in (figure 3).

The superoxide scavenging activity of *Cassia tora* was increased markedly with the increase of concentrations (figure 4). These results suggested that *Cassia tora* had important superoxide radical scavenging effect. Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system[26]. The antioxidant activity is expressed as the IC_{50} values of (38.19).

The study revealed that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract (figure 5).

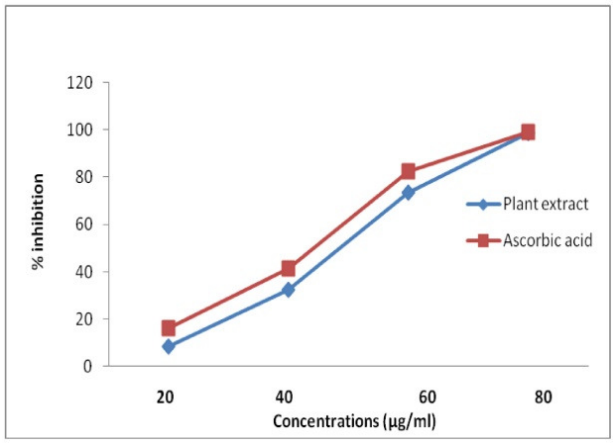

Figure-2
Nitric oxide scavenging activity of *Cassia tora* methanolic extract at different concentrations

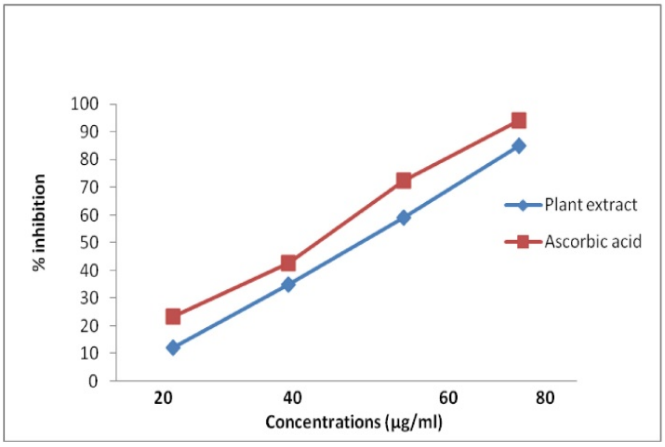

Figure-3
Superoxide anion Scavenging activity of *Cassia tora* methanolic extract at different concentrations

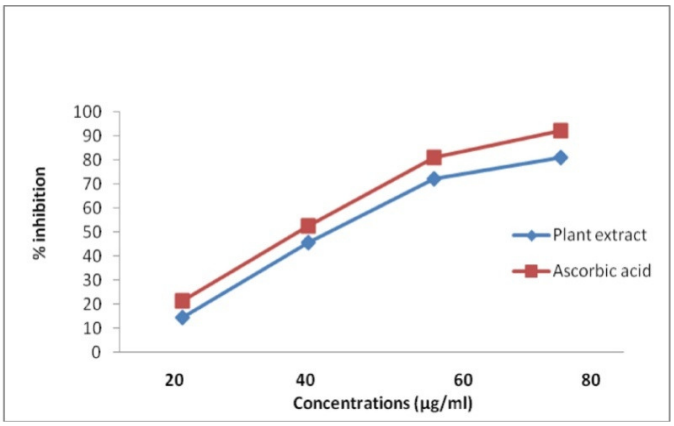

Figure-4
Hydrogen peroxide Scavenging activity of *Cassia tora* methanolic extract at different concentrations

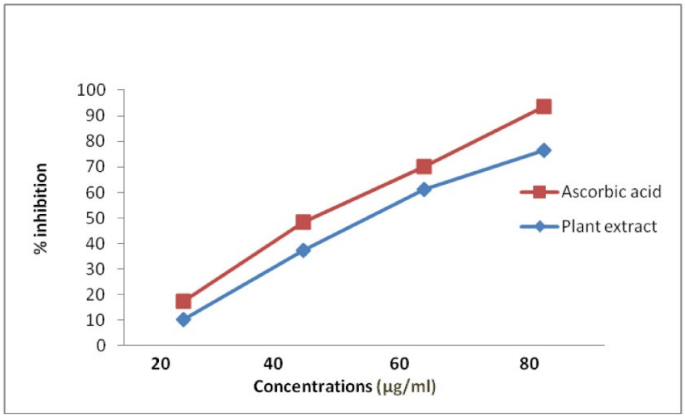

Figure-5
Total antioxidant assay activity of *Cassia tora* methanolic extract at different concentrations

This study suggested that methanolic extract of *Cassia tora* have antioxidant activity which may be helpful in preventing or slowing progress of various oxidative stress related diseases. Thus, antioxidant potential of methanolic extract of *Cassia tora* may be attributed to the presence of flavanoids, terpenoids, tannins, alkaloids, steroids and phenolic content. Overall *Cassia tora* can be consider as a model herbal drug for experimental studies including free radical induced disorders like cancer, diabetics, atherosclerosis, etc.

Conclusion

The leaves would be useful as an antioxidant and free radical scavenging agent and it helps in treatment of many diseases that was mediated by reactive oxygen species. Accordingly in this study, a significant and linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to antioxidant activity. Thus, it can be concluded that methanolic extract of *Cassia tora* leaves can be used as an accessible source of natural antioxidants with consequent health benefits. Futher studies should be undertaken to elucidate the mechanism of action through which the extract exert the antioxidant activity.

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