IN-VITRO ANTIOXIDANT PROPERTIES OF LEAVES OF *CASSIA GRANDIS* LINN

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The *in-vitro* antioxidant activity of various extracts of *Cassia grandis* leaves was investigated. The extracts and the reference standard, butylated hydroxyl toluene (BHT) were evaluated for DPPH, nitric oxide, superoxide and hydroxyl radical scavenging activity. The methanolic extract exhibited significant antioxidant activity but petroleum ether and chloroform extracts of *Cassia grandis* did not show any significant antioxidant activity in comparison with standard (BHT).

Keywords : DPPH, Nitric oxide, butylated hydroxyl toluene, superoxide and hydroxyl radical scavenging activity.

INTRODUCTION

Oxygen is essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer. In spite of comprehensive network of cellular defensive antioxidants, many ROS still escape this surveillance inflicting serious anomalies favouring such diseases states[1-3]. Though synthetic antioxidants, BHT, BHA and radioprotector, Warfarin are being used widely, however, due to their potential health hazards, they are under strict regulation[4-5]. Antioxidant principles from natural resources are multifaceted in their multitude/ magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits[6-10]. Phytoantioxidants, commonly available, less toxic, serving food and medicinal components have been suggested to reduce threat of wide range of ROS[8-10].

In view of this and the present understanding about ROS-induced multiple diseases, we have selected one of such ayurvedic herb, *Cassia grandis* L. (Family: *Leguminosae*) is a deciduous or semi deciduous spreading tree. It is well known as a Pink shower. The phytochemical studies revealed the presence of flavonoids, anthraquinones and sterols. Several studies on the various parts of this plant have been reported as analgesic, anti-inflammatory, purgative and in treatment of skin disorders etc. The pulp from the pods is very strong smelling with a bitter and astringent taste, which has laxative properties. It is sometimes used in veterinary practices also hence known as Horse Cassia. The juice from the pods is reported to strengthen the blood[11-17].

MATERIAL AND METHODS

The chemicals used DPPH (1,1-diphenyl-2-100 µg/ml) were dissolved in methanol and incubated at 25°C for 30 min. After 30 min, To 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylenediamine dichloride). The absorbance of the chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylene diamine dihydrochloride was measured at 546 nm.

Superoxide scavenging

Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method[19]. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hrs and the solution was filtered immediately before use; the filtrate (200 µl) was added to 2.8 ml of an aquous solution containing nitroblue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 µM, pH 7.4). Test solutions at different concentrations (5-100 µg/ml) were added and absorbances were recorded at 560 nm against the control.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was determined according to the modified method[20]. The assay was performed by adding 0.1 ml of EDTA, 0.01 ml of ferric chloride, 0.1 ml of hydrogen peroxide, 0.36 ml of deoxyribose, 1.0 ml of test solutions (5-100 µg/ml) in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid were dissolved in sequence. The mixture was then incubated at 37^{0}C for 1 hr and 1.0 ml portion of the incubated mixture was mixed with

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10 % TCA and 1.0 ml of 0.5 % TBA to develop the pink chromogen and measured at 532 nm.

Statistical analysis

The results are presented as mean ± SEM. All parameters were analysed using Student's *t*-test. P <0.05 was considered as significant.

PEECG and CECG (Table-1).

Superoxide radical scavenging

The MECG and BHT showed a moderate inhibition of the superoxide radical 76.50% and 82.87% respectively at 100µg/ml. There was no significant inhibition of superoxide radical by PEECG and CECG (Table 2).

TABLE- 1 Free radical scavenging activity of various extracts of *Cassia grandis*

Drugs	Concentration (µg/ml)	DPPH radical inhibition (%)	Nitric oxide
Petroleum ether extract of *Cassia grandis* (PEECG)	5	03.27±0.142	05.12±0.544
	10	05.65±0.331	12.78±0.313
	25	11.07±0.004	33.10±0.004
	50	15.62±0.009	39.14±0.009
	100	48.14±0.007	48.14±0.007
Chloroform extract of *Cassia grandis* (CECG)	5	05.47±0.941	02.67±0.125
	10	11.59±0.215	05.88±0.318
	25	20.26±0.002	14.17±0.003
	50	22.26±0.009	25.09±0.006
	100	32.59±0.004	44.81±0.005
Methanolic extract of *Cassia grandis* (MECG)	5	12.24±0.517	10.89±0.812
	10	23.36±0.753	21.62±0.851
	25	52.28±0.008*	51.14±0.009*
	50	73.75±0.006**	72.92±0.001**
	100	88.75±0.004***	75.72±0.001***
Butylated hydroxyl toluene (BHT)	25	84.23±0.412	76.69±0.054
	50	87.12±0.132	80.12±1.215
	100	92.87±0.246	83.54±0.512

Values are mean± SEM, 6 independent analysis, P<0.05*, P<0.01**, P<0.001*** as compared to standard (Student's *t*-test)

RESULTS

Inhibition of DPPH radical

The potential decrease in the concentration of DPPH radial due to scavenging property of MECG and BHT showed significant free radical scavenging activity viz. 88.75% and 92.87% respectively at 100µg/ml. The IC_{50} of MECG was found to be 24.62µg/ml whereas PEECG and CECG did not show any significant activity (Table-1).

Nitric oxide scavenging activity

The scavenging of nitric oxide by MECG and BHT was concentration dependent. There was a moderate inhibition of nitric oxide formation, with the maximum inhibition being 75.72 % and 83.54% respectively at 100µg/ml MECG and BHT. The IC_{50} of MECG was found to be 24.56µg/ml. Similar results were not found in case of

Hydroxyl radical activity

The effect of MECG and BHT on hydroxyl radical and iron (II)-dependent deoxyribose damage was protected significantly at all concentrations; the percentage of inhibition of hydroxyl radical being 69.84% and 74.56% respectively at 100µg/ml. no significant inhibition of superoxide radical by PEECG and CECG (Table-2).

DISCUSSION

Oxidative stress, in which large quantities of reactive oxygen species (ROS) like hydrogen peroxide, superoxide, hydrogen radical, singlet oxygen and nitrogen species are generated, one of the earliest responses to stress. These ROS have a role in disease and aging in animals [21]. The antioxidative system protects the organism against ROS-induced oxidative damage. There are restrictions on the use of synthetic antioxidants such as BHT, as they are

TABLE- 2 Free radical scavenging activity of various extracts of *Cassia grandis*

Drugs	Concentration (µg/ml)	Superoxide inhibition (%)	Hydroxyl radical inhibition (%)
Petroleum ether extract of *Cassia grandis* (PEECG)	5	02.55±0.154	06.82±0.125
	10	04.96±0.574	13.44±0.879
	25	13.23±0.888	36.23±0.457
	50	16.54±0.042	38.99±0.849
	100	47.56±0.189	47.87±0.717
Chloroform extract of *Cassia grandis* (CECG)	5	03.98±0.946	03.11±0.526
	10	09.65±0.532	06.96±0.548
	25	21.12±0.941	18.36±0.543
	50	23.66±0.784	28.35±0.951
	100	35.36±0.654	46.56±0.356
Methanolic extract of *Cassia grandis* (MECG)	5	11.87±0.254	10.74±0.125
	10	25.69±0.592	23.58±0.876
	25	69.46±0.558*	53.63±0.745*
	50	70.43±0.364**	62.33±0.984**
	100	76.50±0.654***	69.84±0.647***
Butylated hydroxyl toluene (BHT)	25	75.48±0.784	68.65±0.386
	50	76.02±0.887	71.88±0.423
	100	82.87±1.246	74.56±0.368

Values are mean± SEM, 6 independent analysis, P<0.05*, P<0.01, P<0.001*** as compared to standard (Student's *t*-test)**

suspected to be carcinogenic.[22] Natural antioxidants therefore have gained importance.

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form 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 DPPH radical is due to the scavenging ability of MECG.

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrate ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide complete with the oxygen, leading to reduced production of nitric oxide[23]. The MECG was shown significant scavenging activity.

The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins, the effect of MECG and BHT on the inhibition of free radical-mediated deoxyribose damage was assessed by means of iron (II)-dependent DNA damage assay, which showed significant results.[24]

The MECG has potent antioxidant and free radical scavenging effects in different *in-vitro* systems, but PEECG and CECG showed no significant effects as compared to standard BHT. Further work is necessary to elucidate the mechanism involved in the antioxidant activity MECG.

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