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ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *CASSIA TORA* L

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ABSTRACT
Development of a potential antioxidant molecule is gaining importance in the recent years as it plays an important role, in preventing or delaying the onset of certain pathological consequences such as hepatotoxicity, heart diseases and cancer. *Cassia tora* Linn. (Family :Fabaceae) is an annual herb growing in Asian countries. The plant is reported to have medicinal value as an antimicrobial, antidiuretic, antidiarrheal and antihepatotoxic. In the present study, antioxidant activity of ethanolic extract of *Cassia tora* leaves was investigated using three *in vitro* assays, viz., total antioxidant capacity by phosphomolybdenum method, DPPH free radical scavenging assay and ferric ion reducing assay. L-Ascorbic acid was used as a reference antioxidant. The extract showed strong antioxidant activities in all the three assays, indicating that *Cassia tora* ethanolic extract functions as an efficient antioxidant to scavenge free radicals and reduces free radical induced cellular damage.
KEYWORDS: *Cassia tora* extract, total antioxidant activity, DPPH, reducing power.

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INTRODUCTION

Oxidation is one of the most important processes, which produce free radicals in food, chemicals and even in living systems. The importance of reactive oxygen species (ROS) has attracted increasing attention worldwide over the last decade. ROS are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA. Evidence indicate that harmful free radicals play an important role in most major health forms such as cancer, cardiovascular diseases, rheumatoid arthritis, cataract, alzheimers disease and other degenerative diseases associated with aging[1].

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, by reacting with ROS and neutralizing them. This natural antioxidant system includes enzymes like catalase, superoxide dismutase and glutathione which protect the body from free radical species and prevent oxidative stress. Synthetic antioxidants such as butylated hydroxyl toluene and Butylated hydroxyl anisole, are carcinogenic in nature[2]. In the past few years, addition of synthetic antioxidants to food is restricted because of their health risks and toxicity.

Interest in the role of antioxidants in human health has prompted research in the fields of food science and medicinal herbs to assess the role of herbs as antioxidants. Besides, traditionally well known natural antioxidants from teas, wines, fruits, vegetables and spices, some natural antioxidants like rosemary and sage are already exploited commercially either as antioxidant additives or as nutritional supplements.

Cassia tora Linn. (Family: Fabaceae) is an annual herb growing in Asian countries. It is a well known Ayurvedic medicinal plant as a laxative, antiperiodic and is useful for leprosy, ringworm, bronchitis and cardiac disorders, ophthalmic, skin diseases, cough, hepatic disorder, liver tonic and haemorroids[3]. It is a well known ayurvedic plant as an laxative, antiperiodic and is useful for leprosy, ringworm, bronchitis and cardiac disorders, hepatic disorders and haemorrhoids[4]. The present study was carried out to evaluate the *in vitro* antioxidant activity of ethanolic leaf extract of *Cassia tora*.

MATERIALS AND METHODS

Preparation of the plant extract

Plant material was collected from the surroundings of Mangalore, Karnataka. Leaves were washed, shade dried and powdered using a kitchen blender. Leaf powder was extracted with ethanol in a soxhlet apparatus. Solvent was evaporated and the resultant extract was stored at -20^{0}C until use.

Chemicals and reagents

DPPH was procured from Sigma-Aldrich. Ammonium molybdate, Sodium phosphate, Potassium ferricyanide, Trichloroacetic acid and ferric chloride were purchased from SRL, Mumbai. Phosphate buffer was procured from Himedia.

Estimation of total antioxidant activity by phosphomolybdenum method

This assay is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) complex at acidic pH[5]. Different concentrations of *Cassia tora* extract prepared in ethanol ranging from 200-1000ug/ml were pipetted out into a series of test tubes and combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28mM Sodium phosphate and 4mM Ammonium molybdate). The tubes were capped, incubated at 95^{0}C for 90 minutes, cooled to room temperature and absorbance was recorded at 695 nm against blank. L-Ascorbic acid was used as a reference antioxidant. Total antioxidant capacities of the extract were expressed as equivalents of ascorbic acid (µ moles/g of the sample).

DPPH free radical scavenging activity

Disappearance of the DPPH free radical absorption at a characteristic wavelength is monitored by decrease in optical density[6]. Different concentrations of the extract in ethanol were taken in a series of test tubes and volume was made up to 1 ml with ethanol. Four ml of 0.1mM methanolic solution of DPPH was added to all the tubes. The contents were shaken well and allowed to react for 20 minutes at room temperature. Absorbance of samples was recorded at 517nm against control. Free radical scavenging activity was expressed as percentage inhibition which was calculated as follows:

$$\% \text{ free radical scavenging activity} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Reducing power assay

The formation of ferrous form from Ferric/ferricyanide complex is monitored by measuring the formation of Pearl's Prussian blue at 700 nm[7]. Different concentrations of the extract in 1 ml ethanol were mixed with 2.5 ml of 0.2M phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The contents were allowed to react at 50^{0}C for 20 minutes. 2.5 ml of 10% TCA was added and centrifuged at 3000 rpm for 10 minutes. Supernatent was mixed with distilled water and ferric chloride. Absorbance was measured at 700 nm.

RESULTS

Total antioxidant capacity

Cassis tora extract showed significant total antioxidant activity. Results of antioxidant capacity of the extract are represented graphically in **Fig.1**. An increasing trend in the activity was observed with increasing concentration. Values ranged from 283.5 to 1476.1 µ mole equivalents of ascorbic acid/g of the sample. Phosphomolybdenum method is widely used to study the antioxidant capacity of plant extracts, wherein the intensity of green phosphomolybdenum complex gives the measure of total antioxidants present in the sample.

DPPH free radical scavenging activity

In DPPH free radical scavenging activity, it was observed that *Cassia tora* extract in dose of 200, 400, 600, 800 and 1000ug/ml significantly scavenged DPPH free radical to an extent of 22.04, 41.71, 57.92, 66.84 and 72.13% respectively, in a concentration dependent manner. The results of DPPH radical reduction are represented in **Fig.2**.

Reducing power assay

Fig 3 shows the reducing power of *Cassia tora* extract. Reducing power of the extract displayed an increasing trend with the increasing concentrations, as indicated by the increase in the absorbance of the reaction mixture. Absorbance of the reaction mixture was found to be 0.178, 0.310, 0.453, 0.580 and 0.666 for 200, 400, 600, 800 and 1000 ug/ml concentration of the extract respectively.

DISCUSSION

Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species in the cells. These reactive oxygen species can cause lipid peroxidation, DNA damage, protein peroxidation and cellular degeneration in the cells and can induce numerous diseases[8].Certain phytoconstituents are commonly found in both edible and non edible plants, and have multiple biological effects, including antioxidant activity.

There are restrictions on the use of synthetic antioxidants, such as Ascorbic acid, BHT, as they are suspected to be carcinogenic. Natural antioxidants, therefore, have gained importance in the recent years. The reduction capability of DPPH free radicals was determined by the decrease in its absorbance at 517 nm,

which is induced by antioxidants present in the extract. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of the phytoconstituents present in the extract.

The reducing capacity of the extract may serve as a significant indicator of its potential antioxidant activity. Natural antioxidants have some advantages over synthetic ones in that they can be obtained easily, has lesser side effects and cheaply available Antioxidant activity may be probably due to phenolic compounds present in the extract. It had been reported that the antioxidant activity of plant extract is correlated with the amount of their phenolic compounds[9]. Preliminary phytochemical screening *Cassia tora* ethanolic extract indicated the presence of alkaloids, phenolics, glycosides, fatty alcohols and triterpenoids. These compounds may be responsible for the antioxidant activity of the extract and the leaf extract of *Cassia tora* may serve as a substitute for synthetic antioxidants and can be used to boost the antioxidant status of the body.

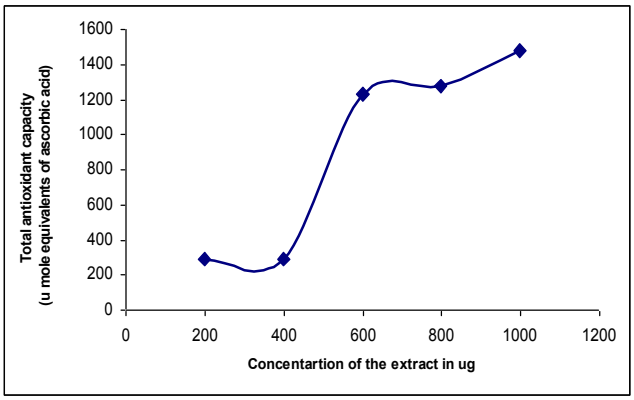

Fig. 1: Total antioxidant capacity of *Cassia tora* extract

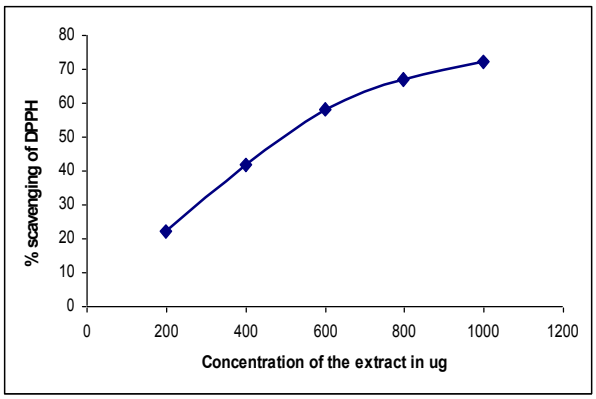

Fig .2: DPPH free radical scavenging activity of *Cassia tora* extract

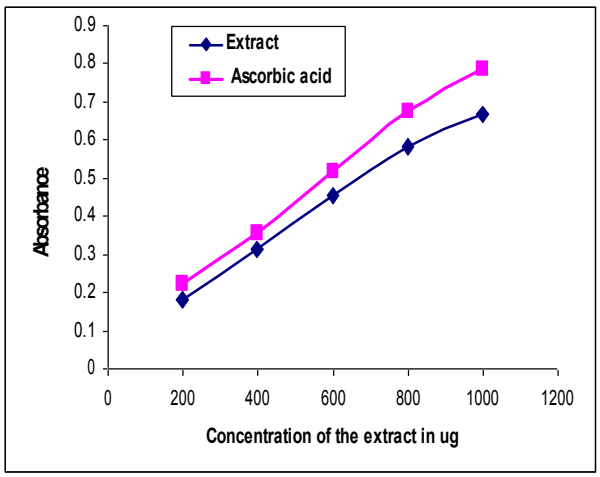

Fig. 3: Reducing power of *Cassia tora* extract

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