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## A study on the free radical scavenging and anti oxidant activity of *Cassia fistula* from Karimnagar region, Telangana

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### Abstract

In the present study, phytochemicals and anti oxidant activity of *Cassia fistula* from Karimnagar region of Telangana was studied. DPPH, Nitric oxide, Super oxide, Hydrogen peroxide scavenging activity of methanol bark extracts at different concentrations were studied (ascorbic acid used as standard). The present study revealed the presence of some phyto chemicals viz, tannins, poly phenols, flavonoids, quinines, terpenoids and saponins. The anti oxidant activity assay revealed significant anti oxidant activity.

**Keywords:** *Cassia fistula*, anti oxidant, free radicals

### Introduction

Ayurveda is being practiced in many parts of the world since the Vedic period. Medicinal plants are connecting links between the traditional and modern systems. Government of India officially recognized and established AYUSH ministry.

*Cassia fistula*, has potential therapeutic value. It is anti pyretic, analgesic, anti inflammatory and hypoglycemic. There is an increased exploration of medicinal plants for pharmacological activity. Free radicals contain one or more unpaired electrons. In excess they cause damage to the cells and system of the body and subsequently results in pathological conditions (Madhavi, 1996) <sup>[1]</sup>. The singlet oxygen quenches, chelates metals. Super oxide, hydrogen peroxide, peroxy, singlet oxygen, nitric oxide, peroxy nitrite, hypochlorous acid (Upadhye, 2009; Mishra, 2007) <sup>[2,3]</sup> is considered as free radicals.

Free radicals prevent lipid peroxidation, capture reactive oxygen species and repair the damage induced by ROS. Initiation and propagation of lipid oxidation is interrupted by anti oxidants by scavenging free radicals. Peroxidation of lipid membranes, oxidative DNA damage is believed to be caused by free radicals. Anti oxidants also needed in food as they enhance the shelf life, helps in improving nutritional quality. Natural anti oxidants are cheap, non toxic, easily available. They scavenge the free radical in the body. Phytochemical analysis revealed the presence of phenolic compounds in anti oxidants.

The antioxidant, antimicrobial and antipyretic activity of the plants is due to some phytochemicals (Larson, 1998; Vani *et al* reported the antioxidant properties of the Ayurvedic formulation Triphala and its constituents. (1997) <sup>[4,5]</sup>. Plants are the sources of drugs and should be screened for their safety and efficacy (Nascimento *et al.*, 2000) <sup>[6]</sup>. Malaya Gupta *et al* (2004) <sup>[7]</sup> studied the antioxidant and free radical scavenging activities of *Ervatamia coronaria*. Patel Rajesh (2011) <sup>[8]</sup> reported the antioxidant activity of coumarin compounds. Pooja *et al* (2010) <sup>[9]</sup> evaluated the scavenging activity of *Dalbergia sissoo* roots. Rajaiah *et al.*, (2022) <sup>[10]</sup> documented the antioxidant potential of some selected medicinal plants.

### Materials and Methods

#### DPPH radical scavenging activity

The total anti-oxidant potential was determined by Brand1 Williams *et al.*, and Parejo *et al.*, Various concentrations of test sample were prepared by serial dilution and 0.1mL of each

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dilution was added to 3.9 mg DPPH was dissolved in 3 mL of methanol solution, followed by vortexing. The reaction was allowed to take place in the dark at room temperature to reach a plateau. The decrease in the absorbance was measured at 517 nm was determined by using a Shimadzu spectrophotometer. The concentration of remaining DPPH in the reaction medium was calculated from the calibration curve as follows:

Bark was dried in shade after thorough wash. These dried materials are pulverized into powdered form. By intermittent shaking the extract was collected in methanol and was concentrated in an evaporator. The dried residue was stored in a desiccator. This extract was used for in vitro antioxidant activity screening.

The total anti-oxidant potential was determined by Brand-Williams *et al.*, and Parejo *et al.*, Various concentrations of test sample were prepared by serial dilution and 0.1mL of each dilution was added to 3.9 mL of methanol solution of DPPH, followed by vortexing. The reaction was allowed to take place in the dark at room temperature to reach a plateau.

The decrease in the absorbance was measured at 517 nm was determined by using a Shimadzu spectrophotometer. The concentration of remaining DPPH in the reaction medium was calculated from the calibration curve as follows:

$$\text{Scavenging effect (\%)} = \frac{(1 - A_{\text{Sample (517nm)}})}{A_{\text{Control (517nm)}}} \times 100$$

#### Super oxide free radical scavenging activity

Different concentrations of 50, 100, and 150 µg/mL (10, 20, 30 µL) of plant extracts were taken and the volume was made up to 150 µL with methanol, to each of this, 100 µL of riboflavin, 200 µL EDTA, 200 µL methanol and 100 µL NBT was mixed in test tubes and further diluted up to 3mL with phosphate buffer and absorbance was measured after illumination for 5 min, at 590 nm on UV visible spectrophotometer (Shimadzu, UV-1601), Japan and results

were compared with ascorbic acid (10 µg/mL as standard).

#### Scavenging of nitric oxide

Sodium nitroprusside (5 µM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 h. After 5 h, 0.5 mL of incubation solution was removed and diluted with 0.5 mL Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthyl ethylene di amine dihydro chloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid.

#### Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1mL of the extracts or standards in methanol were added to 2 mL of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide. IC<sub>50</sub> value is the concentration of the sample required to scavenge 50% free radical. The percentage inhibition was calculated by using the following formula.

$$\text{Scavenging activity (\%)} = \frac{\text{OD of control} - \text{OD sample}}{\text{OD of control}} \times 100$$

#### Results

Pairing of unpaired electrons results in neutralization and converts it into 1-1 di phenyl-2- picryl hydrazine and becomes colorless from purple color. DPPH, Superoxide, Nitric oxide, Hydrogen Peroxide scavenging activity of methanol bark extracts at different concentrations were measured (ascorbic acid (10 µg/ml) was used as standard). The anti oxidant activity was presented in tabular form 2 and histogram 1.

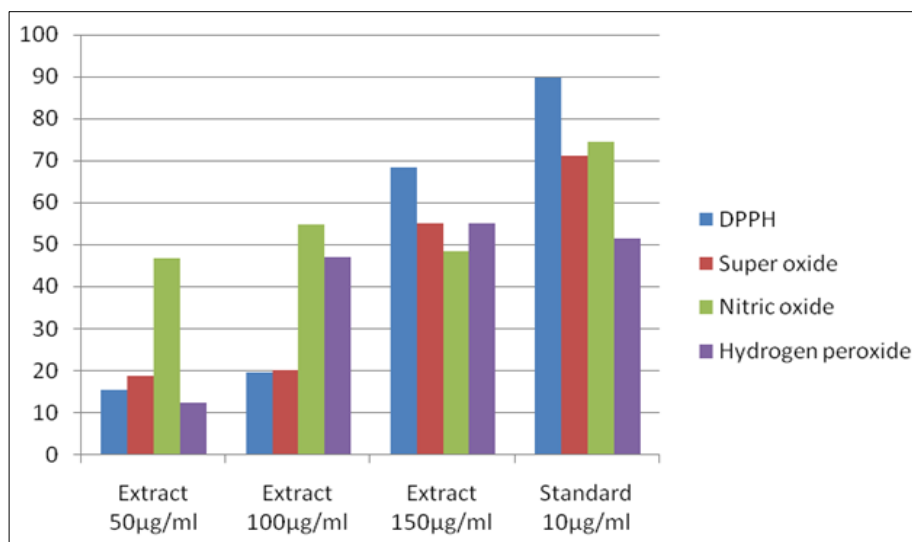
The present study revealed the presence of some phytochemicals *viz.* tannins, poly phenols, flavonoids, quinines, terpenoids and saponins.

**Table 1:** Phytochemicals present in *Cassia*

| Phyto chemical |   |
|----------------|---|
| Alkaloid       | - |
| Tannin         | + |
| Polyphenol     | + |
| Flavonoids     | + |
| Quinines       | + |
| Steroids       | - |
| Terpenoids     | + |
| Saponins       | + |

**Table 2:** Anti oxidant activity of *Cassia fistula*

|                   | Extract 50µg/ml | Extract 100µg/ml | Extract 150µg/ml | Standard 10µg/ml |
|-------------------|-----------------|------------------|------------------|------------------|
| DPPH              | 15.32           | 19.54            | 68.47            | 89.67            |
| Super oxide       | 18.57           | 19.87            | 54.91            | 71.24            |
| Nitric oxide      | 46.61           | 54.65            | 48.34            | 74.54            |
| Hydrogen peroxide | 12.34           | 46.84            | 54.87            | 51.42            |



**Histogram 1:** Anti oxidant activity of *Cassia fistula*

The bark of *Cassia fistula* was rich in flavonoids and phenolic compounds and therefore it was exhibited high antioxidant and free radical scavenging activities. The present study demonstrated that *Cassia* is a better antioxidant source that may prevent the oxidative stress. Present findings revealed the antioxidant nature of methanol extract. It supports the intake of diet enriched with herbs and plants can possibly reduce oxidation and may prevent some free radical induced disorders. *Cassia fistula* may be a potential source of anti oxidant activity, therefore they can be considered as a drug candidate for the same, but it requires further detailed study.

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