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# *In-vitro* antioxidant properties of *Sterculia foetida* Linn.

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## ABSTRACT

The purpose of this work is to assess the in vitro antioxidant activity of methanolic extract of Sterculia foetida by using the following analytical methods: 1, 1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) free radical scavenging, nitric oxide radical inhibition, superoxide anion ($O2^{\bullet-}$) radical scavenging, inhibition of xanthine oxidase activity and the inhibition of oxidation of β-carotene. Results of methanolic extract of leaves of Sterculia foetida were compared with the activity of respective standard drugs and the extract was found to be equipotent with the standards in all the tested methods. The flavonoids present in the Sterculia foetida may believed to be responsible for its antioxidant activity.

**Keywords:** Sterculia foetida; Antioxidant; β-carotene oxidation; DPPH; Nitric oxide; superoxide anion; xanthine oxidase.

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## INTRODUCTION

Oxidative damage in the human body plays an important causative role in disease initiation and progression [1]. Damage from reactive oxygen species (ROS) including free radicals has been linked to some neurodegenerative disorders (Alzheimer's disease and Parkinson's) [2] and cancers [3]. ROS include free radicals such as superoxide anion radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$) and non-free-radical species such as $H_2O_2$ and singled oxygen ($^1O_2$). These molecules are exacerbating factors in cellular injury and aging process [4, 5]. Recently, ROS have also been shown to play a critical role in the development of acute experimental gastric lesions induced by stress, ethanol and NSAIDs [6-8]. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents and pesticides [9]. ROS also induce some oxidative damage to biomolecules such as lipids, nucleic acids, proteins and carbohydrates. Damage of these biomolecules causes aging, cancer, and many other diseases [9]. As a result, ROS have been implicated in more than one hundred diseases, including malaria, heart disease, stroke, arteriosclerosis, diabetes, cancer and gastric ulcers [10-12].

The human body has inherent mechanisms to reduce free radical injury by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, and catalase. Exogenous free radical scavengers include vitamin E and vitamin C [13, 14]. These vitamins protect the human body and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [15-17]. Antioxidant defense systems, including antioxidant enzymes which are found in foods and drugs are important in the prevention of the toxic ROS effects [14, 18, 19]. Hence, the search for exogenous antioxidants is continued.

The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and tert-butylhydroquinone [20]. However, BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis [20, 21]. Therefore, the development and utilization of more effective antioxidants is desired [5, 22]. Much attention has been focused on the antioxidative compounds present in plants because of safety concerns associated with synthetic antioxidants [23].

Sterculia foetida Linn., a soft wooded tree belonging to the family Sterculiaceae, grows up to 35 m high. Originally from East Africa and North Australia, it grows freely down the west peninsular, in Burma and Ceylon. It is commonly called as Wild Indian almond and contains edible seeds when roasted. The leaves of Sterculia foetida Linn. have been traditionally known for its various therapeutic uses: laxative, carminative, astringent, anti-inflammatory [24], antifungal [25], analgesic, anti-ulcer, liver disease, nausea and vertigo. The present study focused to evaluate in vitro antioxidant activity of methanolic extract of leaves of S. foetida Linn (MSF).

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## MATERIALS AND METHODS

### Plant material

The leaves of the plant were collected in the in the month of December 2005 from the foot hills of Tirumala located at Tirupathi in Andra Pradesh and cleaned to remove debris. The plant was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Deptartment of Botany, Sri Venkateswara University, Tirupathi and voucher specimen (984) was lodged in the department herbarium of Sri Venkateswara University, Tirupathi (AP.) India. The leaves were dried in the shade at room temperature for 10 days then coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

### Preparation of the extract

The powder of leaves of S. foetida was extracted separately by a continuous hot percolation process using a soxhlet apparatus with different solvents in increasing order of polarity from n-hexane, chloroform, methanol, to water. After extraction, the extracts were dried under reduced pressure at 40$^{o}$ C by using a rotary evaporator via tared vessel and extractive values were calculated with reference to the air-dried leaves. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

### Chemicals

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) was obtained from Sigma–Aldrich Co., St. Louis, USA. Rutin was obtained from Acros Organics, NJ, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was from Roch-Light Ltd., Suffolk, UK. Allopurinol was from GlaxoSmithKline, Philadelphia, USA. Superoxide dismutase (SOD) was from Shenzhen Jenlong Biotechnology Co., Ltd, China. Butylated hydroxy anisole (BHA) was from SD Fine Chemicals Ltd., Mumbai, India. Sodium nitroprusside was from Ranbaxy Laboratories Ltd., Mohali, India. All chemicals used were of analytical grade.

### 1,1-Diphenyl-2-picryl-hydrazil (DPPH) freeradical-scavenging activity

The stable free radical scavenging effect of MSF was assessed by the decoloration of a methanolic solution of DPPH according to the Blois' method [26]. Test samples (20 -100 µg/ml) were dissolved in 0.1 ml DMSO and then added to 0.1 ml of 0.1 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand for 10 minutes at room temperature (30$^{o}$ C) in the dark. The absorbance at 517 nm by DPPH was measured spectrophotometrically (SPECTRA$_{MAX}$ PLUS$^{384}$, Molecular Devices, USA) at 517 nm. Ascorbic acid was used as a positive control. The degree of discoloration indicates the scavenging efficacy of the extract. The capability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH^{\bullet} \text{ scavenging effect (\%)} = (A0 - A1/ A0) \times 100$$

wherein A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of MSF [22, 27].

**Nitric oxide radical inhibition assay**

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. These ions can be estimated by the use of Griess Illosvoy reaction [28]. In the present investigation, Griess Illosvoy reagent is modified by using naphthyl ethylene diamine dihydrochloride (0.1 %, w/v) instead of 1-napthylamine (5 %). Nitric oxide scavengers compete with oxygen leading to reduce the production of nitric oxide [29]. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and extract (MSF) or standard (Rutin) solution (0.5 ml) was incubated at 25$^{o}$ C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 minutes for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed, and allowed to stand for 30 minutes at 25$^{o}$ C. A pink colored chromophore was formed in diffused light. The absorbance of these solutions were measured spectrophotometrically (SPECTRA$_{MAX}$ PLUS$^{384}$, Molecular Devices, USA) at 540 nm against the corresponding blank solutions. $IC_{50}$ value is the concentration of sample required to inhibit 50 % of the nitric oxide radical.

**Scavenger effect on superoxide anion**

Superoxide anion was generated in vitro using the method described by Paoletti et al [30]. The assay mixture contained in a total volume of 1 ml, 100 mM triethanolamine-diethanolamine buffer, pH 7.4, 3 mM NADH, 25 mM/12.5 mM EDTA/MnCl2, 10 mM β-mercapto-ethanol and MSF at different concentrations (0.15, 0.3, 0.6, 1.2, 2.4 μg/ml). After a 20 min incubation at 25$^{o}$ C, the decrease in absorbance was measured spectrophotometrically (SPECTRA$_{MAX}$ PLUS$^{384}$, Molecular Devices, USA) at λ = 340 nm. SOD (80 mU/ml) was used as a standard to compare the activity of MSF.

**Xanthine oxidase activity inhibition**

Xanthine oxidase activity was evaluated spectrophotometrically as previously reported by following the formation of uric acid at λ = 292 nm (εM = 9.2×103) [31]. The assay mixture contained, in a final volume of 1 ml, 50 mM phosphate buffer pH 7.8, 25 μM solution of xanthine and 24 mU xanthine oxidase (specific activity 1 U/mg of protein). Different concentrations of MSF (25, 50, 100, 200 μg/ml) were added to samples before the enzyme and their effect on the generation of uric acid was used to calculate regression lines. The results were expressed as a percentage of inhibited enzyme activity with allopurinol (30 μM) was used as a standard.

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**β-Carotene oxidation**

The assay system used to evaluate the potential of MSF to suppress β-carotene oxidation was the coupled oxidation of β-carotene and linoleic acid described by Vaya et al [32] with two minor modifications. The plant powder was dissolved in water instead of alcohol to a final concentration of 100 µg/mL and spectrophotometric readings were taken (SPECTRA$_{MAX}$ PLUS$^{384}$, Molecular Devices, USA) at 454 nm. The assay was performed at 50$^{o}$ C. The readings of the rate of β-carotene bleaching were recorded at 20 minutes intervals for 2 hours against water blank. BHA (100 µg/mL) was served as a positive control. This method allows evaluation of the antioxidative activity of a preparation over a time scale. This assay was repeated three times.

## RESULTS

Since the DPPH test can accommodate a large number of samples in a short period of time and it is sensitive enough to detect natural compounds at low concentrations, it was used in the present study for a primary screening of MSF for its free radical-scavenging activity. This assay provides information on the reactivity of test compounds with a stable free radical. Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, resulting in stoichiometric decolorization with respect to the number of electrons taken up [26].

The MSF exhibited DPPH free radical scavenging activity in a concentration-dependent manner (Fig. 1). Increasing concentrations of MSF were taken and the following percent inhibition was observed subsequently at 20 µg/ml (64.74 ± 1.99), 40 µg/ml (70.01 ± 2.37), 60 µg/ml (76.42 ± 1.25), 80 µg/ml (85.83 ± 2.89) and 100 µg/ml (94.61 ± 3.13) with Ascorbic acid equaling 100 µM (95.33 ± 1.19). Significant DPPH radical scavenging activity was evident at all tested concentrations of the extract.

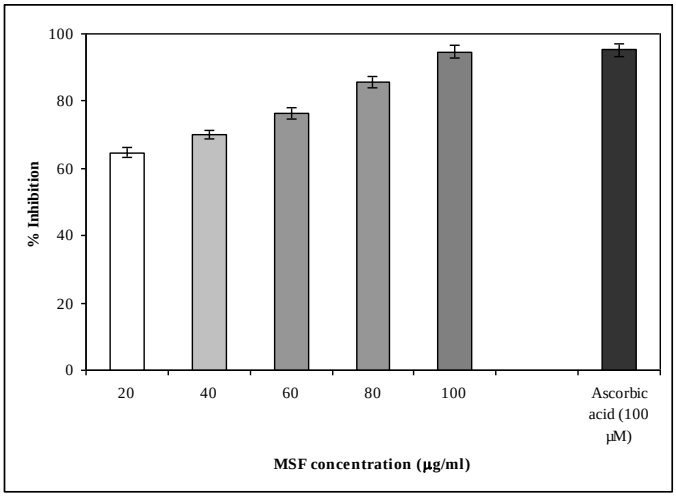

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FIG. 1: Scavenger effect of MSF, at different concentrations (20, 40, 60, 80 and 100 µg/ml), on the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Results are expressed as percentage decrement of absorbance at 517 nm with respect to control. Ascorbic acid (100µM) was used as a standard. Each value represents the mean±S.D. of four experiments, performed in duplicate.

Among the extract and standard tested for antioxidant activity using the nitric oxide radical inhibition method, the MSF showed better activity. It showed the $IC_{50}$ value of 422.85±8.79 µg/ml, where as the standard, Rutin showed 470.90±10.99 µg/ml (Table 1).

**TABLE 1: In vitro antioxidant activity of Sterculia foetida against Nitric oxide method**

| S. No | Test compounds | $IC_{50}$ values ± S.E.* (µg/ml) |
|---|---|---|
| 1 | MSF | 422.85±8.79 |
| 2 | Rutin | 470.90±10.99 |

*. Average of 10 determinations.

As DPPH is a synthetic radical, we therefore also investigated the superoxide anion scavenging capacity of this extract using the method of Paoletti et al, which excludes the Fenton-type reaction and the xanthine/xanthine oxidase system [30]. In this assay, MSF showed a dose-dependent superoxide scavenging effect (Fig. 2).

The effect of MSF on xanthine oxidase activity is shown in Fig. 3. Results are expressed as a percentage of inhibition of xanthine oxidase activity with respect to control. The extract showed a dose-dependent inhibition of uric acid formation. Under our experimental conditions, 200 µg/ml of extract was corresponded with 30 µM of allopurinol activity.

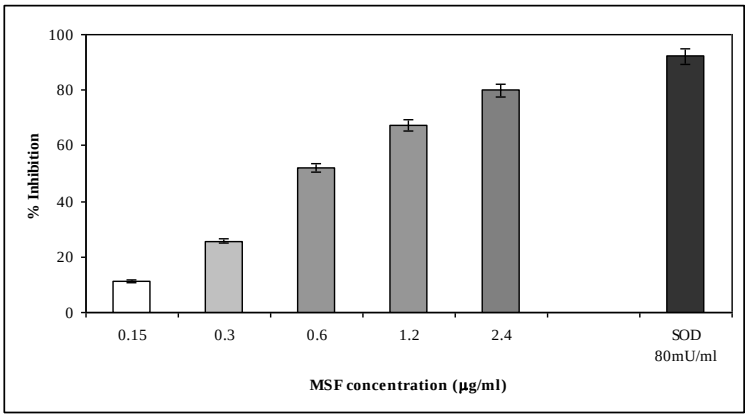


**FIG. 2:** Scavenger effect of MSF, at different concentrations (0.15, 0.3, 0.6, 1.2, 2.4 µg/ml), on $O_2^{\bullet -}$ expressed as percentage of inhibition of NADH oxidation. Superoxide dismutase (SOD) (80 mU/ml) was used as a standard. Each value represents the mean±S.D. of four experiments, performed in duplicate.

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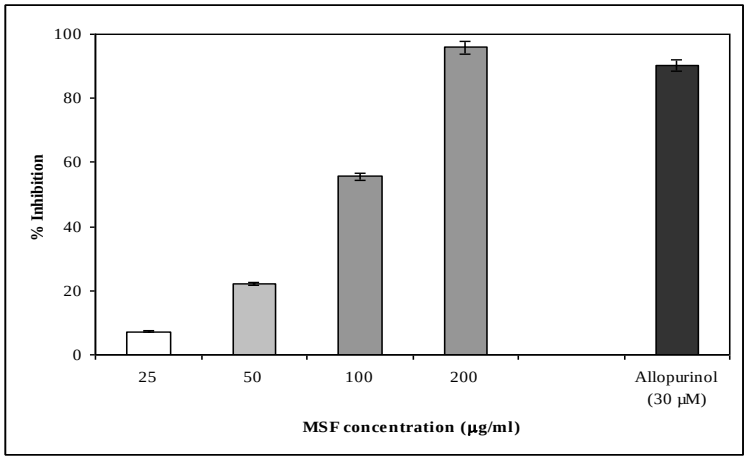


**FIG. 3:** Effect of methanolic extract from MSF, at different concentrations (25, 50, 100, 200 μg/ml), on xanthine oxidase activity. Results are expressed as percentage of inhibition of xanthine oxidase activity with respect to control. Allopurinol (30 μM) was used as a standard. Each value represents the mean±S.D. of four experiments, performed in duplicate.

The antioxidant potential of the extract was evaluated by determining the ability of 100 μg/mL extract to inhibit β-carotene oxidation. The rate of β-carotene oxidation was inhibited up to 85.57 %, whereas BHA almost completely inhibited the rate of oxidation (99.08 %). Typical curves showing the rate of β-carotene oxidation (bleaching) are presented in Fig. 4.

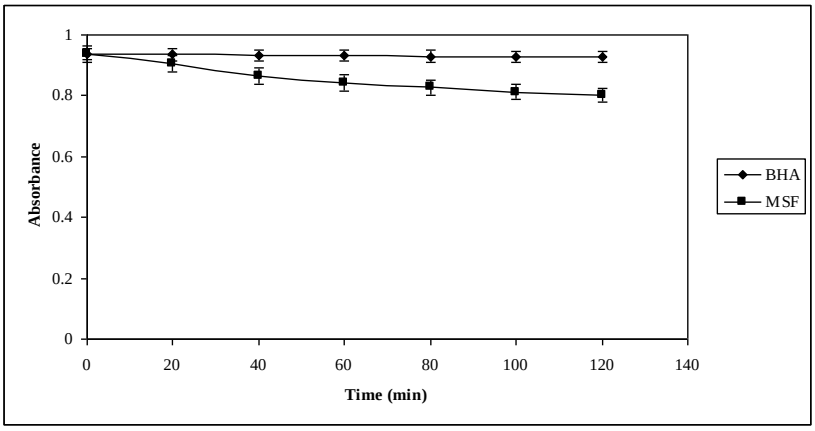


**FIG. 4:** Effect of 100 μg/mL *Sterculia foetida* extract on the oxidation of β-carotene. Data are shown as mean±S.D.

## DISCUSSION

Oxidants are involved in many human diseases and the aging processes. Chronic damage associated with the development of aging can generate destructive oxidants and oxygen free radicals, which are very toxic to tissues and may result in further tissue necrosis and the cellular damage. Cellular mechanisms and external factors involved in the production of oxidative stress include the inflammatory response, free radical leak from mitochondria, auto-oxidation of catecholamines, xanthine oxidase activation, prooxidant activities of toxins such as $CCl_4$ and

exposure to ionizing radiation [33]. Aerobic cells are endowed with extensive antioxidant defense mechanisms including both low molecular weight scavengers, such as α-tocopherol, cysteine, β-carotene, reduced glutathione, ascorbic acid and enzymatic systems, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Red) and glucose-6-phosphate dehydrogenase (G6PD), which counteract the damaging effects of reactive oxygen species [34]. However, when the balance between these reactive species and antioxidants are altered, a state of oxidative stress results, possibly leading to permanent cellular damage. Our results demonstrate that MSF exhibited an interesting antioxidant activity in cell-free systems. It was able to quench the synthetic DPPH radical and exhibited a SOD-like effect,inhibiting $O_2^{\bullet-}$ formation in a dose-dependent manner [33].

It is reported that green tea tannins are able to protect renal cells against ischemia reperfusion injury [35], characterized by an overproduction of $O_2^{\bullet-}$ due to both an electron leak in the mitochondrial respiratory chain and the conversion of xanthine dehydrogenase to xanthine oxidase [36], which produces $O_2^{\bullet-}$ when it oxidizes xanthine into uric acid. This study, therefore, also considered a possible inhibitory action of the extract on the primary function of this enzyme. The results obtained showed that MSF may determine a dose-dependent inhibition of xanthine oxidase activity.

The $IC_{50}$ values observed were found to be slightly lower than that of standard rutin in nitric oxide radical inhibition assay. The results clearly indicated that the extract was found to be more effective in scavenging the DPPH free radical when compared to the nitric oxide radical. We also observed the extract inhibited the β-carotene oxidation at 100 μg/mL concentration.

The first step in investigating the antioxidant potential of MSF involved defining the relationship between concentration and effect by evaluating its ability to inhibit oxidative processes. Initially, we used a single concentration of extract against all the methods. Having demonstrated that the extract was effective at inhibiting this oxidative process, we then proceeded to confirm this finding with various concentrations of the extract that inhibited the oxidation.

On the basis of these experiments, it could be concluded that the methanolic extract of Sterculia foetida Linn. can act as primary and secondary antioxidant. It scavenges free radicals and therefore inhibits the lipid peroxidation and may have beneficial effect on prevention of diseases, where reactive oxygen species are involved. Antioxidant properties of its flavonoid compounds can be at the origin of these effects, but further in vivo experiments are planned to verify the relationship between chemical composition and antioxidant activity.

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