# FREE RADICAL SCAVENGING ACTIVITY OF *Stereospermum Suaveolens* DC: AN *IN-VITRO* EVALUATION

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## Summary

Free radicals are highly reactive species produced in the body during normal metabolic functions or introduced from the environment, which are capable of causing tissue injury and have been implicated in the pathology of various human diseases. Antioxidants act as a major defense against radical-mediated toxicity by protecting against the damages caused by free radicals. A number of plants and plant isolates have been reported to protect free radical-induced damage in various experimental models. The *Stereospermum suaveolens* DC commonly known as 'Patala'. Traditionally it is mainly used as analgesic, wound healing, antidyspeptic, astringent and liver stimulant. The different concentrations of methanolic extracts of plant was studied for its *in vitro* free radical scavenging activity with different methods *viz* DPPH radical scavenging, lipid peroxidation assay, hydroxyl radical and nitric oxide scavenging assay. Antioxidant activity was estimated by IC50 values. The maximum 91.44% DPPH radical scavenging, 72.54% lipid peroxidation, 74.13% hydroxyl radical scavenging and 79.55% nitric oxide scavenging activity were found at concentrations 125µg/ml, 62.5µg/ml, 62.25ppm and 125µg/ml respectively.

**Key words:** 1, 1-diphenyl-2-picrylhydrazyl, Free radical, hydroxyl radical, lipid peroxidation, nitric oxide, *Stereospermum suaveolens*.

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## Introduction

Reactive oxygen species (ROS) are the intermediate products resulting from univalent reduction of molecular oxygen, including the superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radicals ($^{\bullet}OH$). These oxygen intermediates differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission[1], modification of polypeptides[2] and lipid peroxidation[3]. Many of these free radicals have been also implicated in the pathology of various human diseases including atherosclerosis, ischemic heart disease, the aging process, inflammation, diabetes, immunodepression, neurodegenerative and other disease conditions[4]. However antioxidant enzymes like superoxide dismutase (SOD)[5], Catalase[6], and glutathione peroxides (GPX)[7] as well as smaller molecules such as Vitamin E[8] are mainly responsible for the primary defense mechanism against oxidative damage.Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants.[9] In Indian system of medicine, *Stereospermum suaveolens* DC (Bignoniaceae) commonly known as 'Patala' is widely available in India. It mainly contains lapachol, dinatin, β-sitosterol, saponin and palmitic, stearic and oleic acids[10]. Traditionally it is mainly used as analgesic, wound healing, antidyspeptic, astringent and liver stimulant. Root bark is constituent of an Ayurvedic compound, Dasamoola. It is also useful in vomiting, diarrhoea, in asthma and flowers are used in semen debility[11]. Thus based on above literature, different concentrations of methanolic extract of stem bark of *Stereospermum suaveolens* was evaluated for *in vitro* antioxidant activity.

## Materials and Methods

1, 1-diphenyl-2-picrylhydrazyl (DPPH), Griess reagent, 2-Thiobarbituric acid (TBA), and Trichloroacetic acid (TCA) were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). Dimethyl sulphoxide (DMSO) (Nice chem. Pvt. Ltd, Cochin), Sodium nitroprusside (Qualigens Fine Chem., Mumbai), Ascorbic acid (Burgoyne Burbidges and Co, Mumbai), Ferrous sulphate ($FeSO_4$) (SD Fine Chem., Mumbai) and Acetyl acetone was obtained from Loba Chem. Pvt. Ltd., Mumbai. All other reagents and solvents used in the experiment were of analytical grade. UV- Spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan). Homogenizer and Centrifuge were obtained from Remi instruments Mumbai.

### *Plant material and preparation of extract*

The fresh stem barks were collected from Kolhapur district of Maharashtra. The specimen is further identified and authenticated by Shri Jadimath, Prof. Ayurveda College, Bagalkot, Karnataka. The bark was cleaned with 10% $KMnO_4$, air dried and subjected to coarse powdered (#: 44). The powder was further defatted with petroleum ether followed by 24 hrs methanolic extraction using Soxhlet apparatus, which yields 0.34% brownish solid mass. The different concentrations of extract *viz* 7.81, 15.62, 31.25, 62.5, 125, 250µg/ml were prepared in methanol and used for *in vitro* antioxidant studies. Similar concentrations of extract were prepared in ppm for hydroxyl radical scavenging assay.

***Preliminary Phytochemical screening***

The extract was subjected to preliminary qualitative chemical tests[12] which revealed the presence of alkaloids, phenols, saponins, flavonoids, tannins and carbohydrates. The preliminary chromatographic studies (TLC) were also carried out to determine the presence of phytoconstituents[13].

***DPPH radical scavenging assay***[14]

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DDPH. A 0.1mM solution of DDPH in methanol was prepared and 1.0ml of this solution was added to 3.0ml of control (without the test compound, but an equivalent amount of methanol) and test solutions at different concentrations (7.81-250µg/ml) in different test tubes. Thirty minutes latter, the absorbance was measured at 517nm. The concentration of extract at which maximum percentage inhibition of DPPH radical and $IC_{50}$ values were determined with the help of standard graph. The percentage hydroxyl radical scavenging is calculated by the formula: % DPPH radical scavenging activity = 1- (absorbance of sample/absorbance of blank) x 100.

***In vitro lipid peroxidation activity***[15,16]

The degree lipid peroxidation was evaluated by estimating the thiobarbituric acid-reactive substances (TBARS). Briefly, different concentrations of the extract (7.81-250µg/ml) were added to the liver homogenate. Lipid peroxidation was initiated by adding 100µl of 15mmol $FeSO_4$ solution to 3ml of liver homogenate. After 30min, 100µl of this reaction mixture was placed in test tube containing 1.5ml of 10% trichloroacetic acid (TCA) and centrifuged after 10min. the Supernant was separated and mixed with 1.5ml of 0.67% thiobarbituric acid (TBA) in 50%acetic acid. The mixture was heated in water bath at 85°C for 30min to complete the reaction. The intensity of the pink colored complex was measured at 535nm with spectrophotometer.

***Nitric oxide scavenging activity***[15,17]

Nitric oxide scavenging activity was measured by the spectrophotometry method. Sodium nitropruside (5mMol) in phosphate-buffered saline (pH 7.4) was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (7.81-250µg/ml) were dissolved in methanol and incubated at 25°C for 30 min. After 30 min, 1.5ml of the incubated solution was removed and diluted with 1.5ml of Griess reagent. The absorbance of the chromophore formed during the diazotization of the nitrite with sulphalinamide and the subsequent coupling with napthyethylene diamine dihydrochloride was measured at 546nm. Maximum percentage inhibition of nitric oxide scavenging activity of extract and $IC_{50}$ values were determined with the help of standard graph.

***In vitro* hydroxyl radical scavenging activity**[18,19]

Various concentrations (7.51-250ppm) of extracts were taken in different test tubes and evaporated to dryness. 1ml of Iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA (0.018%) and 1ml of DMSO (0.85%v/v in 0.1M phosphate buffer, pH 7.4) were added to these tubes and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on water bath at 80-90 °C for 15 minutes. The reaction was terminated by the addition of 1ml ice-cold TCA (17.5%w/v), 3 ml of Nash reagent (75.0 g ammonium acetate, 3 ml glacial acetic acid and 2ml acetyl acetone were mixed and raised to one liter with

distilled water) was added to all the tubes and left at room temperature for 15 minutes for color development. Intensity of yellow color formed was measured spectrophotometrically at 412nm against reagent blank. Maximum percentage inhibition of hydroxyl radical scavenging activity of extract and $IC_{50}$ values were determined. The percentage hydroxyl radical scavenging is calculated by the formula, % hydroxyl radical scavenging activity = 1-(absorbance of sample/absorbance of blank) x 100.

## Results

Different concentrations ranging from 7.81-250 µg/ml of methanolic extract of *Stereospermum suaveolens* were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner upto the given concentration in all the models. The maximum percentage inhibition with different concentrations of extract for different antioxidant models have been given in Table 1.

Table: 1 Maximum percentage inhibition and $IC_{50}$ values of different concentrations of extract in different *in vitro* models

| Conc. of extract (µg/ml)/ppm | DPPH radical scavenging (%) | Lipid peroxidation inhibition (%) | Nitric oxide scavenging (%) | Hydroxyl radical scavenging ( %) |
|---|---|---|---|---|
| 7.81 | 46.91 | 48.19 | 49.53 | 48.74 |
| 15.62 | 75.18 | 59.71 | 58.99 | 55.95 |
| 31.25 | 86.56 | 68.83 | 67.52 | 62.69 |
| 62.5 | 89.49 | 72.54 | 77.92 | 74.13 |
| 125 | 91.44 | 71.54 | 79.55 | 73.19 |
| 250 | 87.97 | 69.33 | 76.51 | 72.10 |

The potential decrease in the concentration of DPPH radical is due to the scavenging ability of methanolic extract of *S. suaveolens*. A maximum 91.44 % DPPH radical scavenging activity of extract was found to be at concentration 125µg/ml and an $IC_{50}$ (an inhibitory concentration of extract at which there is 50% reduction of free radical) is at 8.59µg/ml. The maximum 74.13% inhibition of lipid peroxidation activity of extract was showed at concentration 62.5µg/ml and an $IC_{50}$ is at 8.51µg/ml. Similarly, The maximum 79.55% nitric oxide and 72.54% hydroxyl radical scavenging activity was found at concentrations 125µg/ml and 62.25ppm respectively. The $IC_{50}$ values were found at concentrations 5.92µg/ml and 9.37ppm for nitric oxide and hydroxyl radical scavenging activity respectively.

## Discussion

Free radicals and other reactive oxygen species (ROS) are formed constantly in human body during normal metabolic processes. They help to destroy micro-organisms and fight against infections. However accumulation of ROS is toxic.

Increased ROS results in oxidative stress, which may lead to extensive cellular damage through covalent binding and lipid peroxidation[20]. Antioxidants act as a major defense against radical-mediated toxicity by protecting against the damages caused by free radicals[21]. The free radical scavenging activity of different concentrations of methanolic extract of *Stewreospermum suaveolens* was studied by different *in vitro* models. DPPH is a stable free radical at room temperature and accept an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm. DPPH radicals react with suitable reducing agents, the electrons be come paired off and the solution loses color stoichiometrically depending on the number of electrons taken up[14,22]. Thus, the significant decrease in the concentration of the DPPH radical is may be due to the scavenging ability of *Stereospermum suaveolens* (Fig. 1)

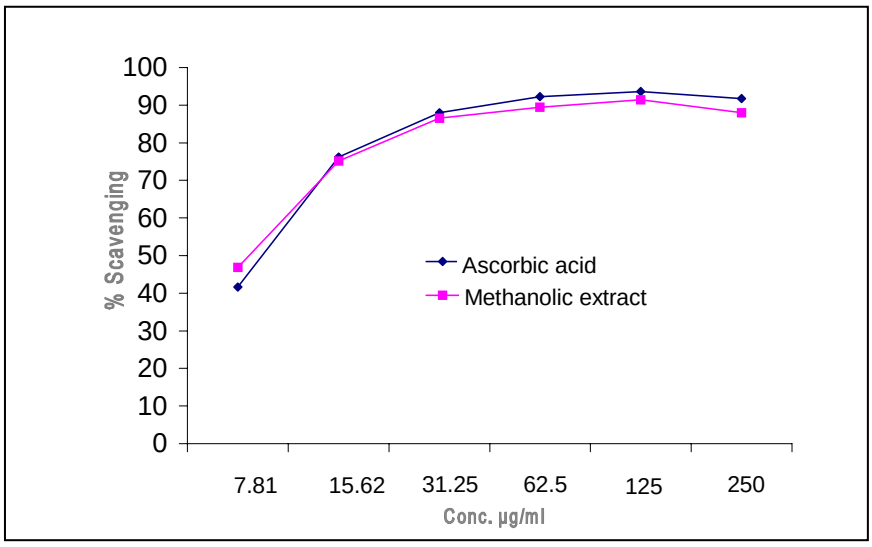


Fig. 1 Antioxidant activity of different concentrations of methanolic extract and ascorbic acid in DPPH radical scavenging assay.

The methanolic extract also shows a maximum (74.13%) protection against lipid peroxidation. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex[23] or through $^{\bullet}OH$ radical[24]. Ferryl-perferryl complex can also initiate lipid peroxidation on its own in similar manner as $^{\bullet}OH$, though it is less reactive than $^{\bullet}OH$[25]. The inhibition could be caused by the absence of ferryl-perferryl complex or by scavenging of $^{\bullet}OH$ radical or changing the Fe3+/Fe2+ ratio or by reducing the rate of conversion of ferrous to ferric ion or by chelating the iron itself[26].

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 compete with the oxygen, leading to reduced production of nitric oxide[14,17]. Similar activity was also found by different concentrations of extract, showing nitric oxide scavenging activity.

Similarly, hydroxyl radical is an extremely reactive species formed in biological systems and have been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells[27,28]. In addition, this species is considered to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids[29]. The hydroxyl radicals formed by the oxidation react with DMSO to yield formaldehyde, which provides a convenient method for their detection by treating with Nash reagent. The ability of the *Stereospermum suaveolens* methanolic extract to quench hydroxyl radicals seems to directly relate to the prevention of propagation of the process of lipid peroxidation and to be good scavenger of active oxygen species, thus reducing the rate of chain reaction.

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