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In Vitro Antioxidant Activity of *Senna auriculata* Leaves Extract

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Abstract: Oxidative stress is linked to the development of numerous human diseases. Theuse of antioxidants in medical treatment is extensively researched, especially for stroke and neurodegenerative conditions. Antioxidants are commonly included in dietary supplements to promote health and prevent diseases like cancer and heart disease. Antioxidants are essential for protecting cells from damage caused by free radicals. *Senna auriculata,* also known as Tanner tree. *Senna* is a plant historically used for medicinal purposes. Analyzing the antioxidant activity of its leaf extract *in vitro* could offer valuable insights into its health benefits. This study aims to assess the *in vitro* antioxidant activity of *Senna auriculata* leaf extract. *Senna auriculata* leaves were collected and extracted using a suitable solvent (ethanol). The antioxidant activity of the extract was assessed through *in vitro* assays, including the DPPH radical scavenging assay. The results were compared with those of standard antioxidants for reference. The *Senna auriculata* leaves extract exhibited significant antioxidant activity in all the assays conducted. DPPH activity was found on parcomparable to standard antioxidants, indicating the potential of *Senna auriculata* as a natural source of antioxidants. The study demonstrates the promising antioxidant activity of *Senna auriculata* leaves extract, suggesting its potential application in the food and pharmaceutical industries as a natural antioxidant source.

Keywords: *Senna auriculata*, Antioxidant activity, *In vitro* assay, DPPH

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Introduction

Medicinal plants are part and parcel of human society from the dawn of civilization to combat diseases and have been considered valuable and cheap source of unique phytoconstituents which are used extensively in the development of drugs for various diseases. Variety of plants are

used medicinally mainly as herbal preparations in the indigenous systems of medicine in different countries which have stood the test of time, and therefore, modern medicines have not been able to replace most of them. This is due to the fact that herbal medicines are economic, natural origin with higher safety margins and lesser or no side effects (Dey *et al*., 2012).

India has a rich heritage of traditional medicine and the traditional health care system has been flourishing in many countries. Traditional medicine is an important part of healthcare. During the last decade, the use of herbal medicine has been increased. Consequently, an increase in traditional tread in herbal medicines and other type of traditional medicines has occurred. Proper use of these different types of medicines has therefore become a concern. In recent years, the use of herbal medicines worldwide has provided an excellent opportunity to India to look for therapeutic lead compounds from an ancient system of therapy, i.e. Ayurveda, which can be utilized for development of new drug (Dey *et al*., 2012). Over 50% of all modern drugs are of natural product origin and they play an important role in drug development programs of the pharmaceutical industry **(**Baker *et al*., 1995).

The potentially imprudent derivatives of oxygen, endorsed as ROS such as O_2−, H_2O_2 and OH radical are incessantly generated within the human body as consequences of revelation to a superfluity of exogenous chemicals in our ambient milieu and/or a number of endogenous metabolic processes linking redox enzymes and bioenergetics electron transmit. Under normal state of affairs, the ROS generated are detoxified by the antioxidants nearby in the body and there is symmetry between the ROS generated and the antioxidants present. However, due to ROS overproduction and/or derisory antioxidant argument, this equilibrium is hindered favouring the ROS gain that culminates in oxidative hassle. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Farber *et al*., 1994). This oxidative damage is a decisive etiological factor concerned in quite a lot of chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also in the ageing course (Hogg, 1998).

Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the expediency of antioxidants in protection against these diseases is defensible. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive. Therefore, in current years, substantial attention has been directed towards credentials of plants with antioxidant ability that may be used for human expenditure.

Several plant extracts and different lessons of phytochemicals have been shown to have antioxidant activity (Larson, 1998). *Senna auriculata* (L.) Roxb. is found in wooden grasslands up to a height of 500 m. It breeds wild in dry regions with annual precipitation of 300 mm. It grows well in areas with an annual temperature range of 15–28° C and needs full sun for its growth. It is a branched shrub with height of 1.5–5 m, trunk diameter of 20 cm and brown lenticellate bark (Nille *et al*., 2021). Avartaki (*Senna auriculata* (L.) Roxb.syn. *Cassia auriculata* L.; Family- Fabaceae) is a traditional medicinal plant, widely used for the treatment of various ailments in Ayurveda and Siddha system of medicine in India. Almost all the parts of the plant, such as flowers, leaves, seeds, barks, and roots have been reported for their medicinal uses (Zipora *et al*., 2024). It has been used in the treatment of diabetes, asthma, rheumatism, dysentery, skin disease, and metabolic disorders.

The principal phytochemicals in *Senna auriculata* (L.) Roxb. are alkaloids, anthraquinone, flavone glycosides, sugar, saponins, phenols, terpenoids, flavonoids, tannins, steroids, palmitic acid, linoleic acid, benzoic acid 2-hydroxyl methyl ester, 1-methyl butyl ester, resorcinol, α-tocopherol-β-D-mannosidase, epicatechin, ferulic

acid, quercetin-3-O-rutinoside, quercetin, proanthocyanidin B1. The extracts from its different parts and their isolated compounds possess a wide range of pharmacological activities such as antidiabetic, antioxidant, anti-inflammatory, anti-hyperlipidemic, hepato-protective, nephro-protective, cardio-protective, anti-atherosclerotic, anticancer, antimutagenic, antimicrobial, antiulcer, antipyretic, anthelmintic, immunomodulatory, antifertility, anti-venom, and anti-melanogenesis.

The current study is an attempt to detect the phytochemicals present and screening of *in vitro* antioxidant activity of ethanolic leaves extract of *Senna auriculata* (EESA) with the objectives-- (i) To prepare ethanolic extract of *Senna auriculata* leaves; (ii) To detect the presence of phytoconstituents in crude extract of *Senna auriculata* leaves; and (iii) To evaluate *in vitro* antioxidant activity of ethanolic extract of *Senna auriculata* leaves by DPPH radical method.

Materials and Methods

Plant material

The plant sample was identified and authenticated by Dr. Ramachandra Naik. Department of Botany, S.B Arts and KCP Science College, Vijayapur, Karnataka, India. Then sufficient amount of *Senna auriculata* were collected and dried at room temperature.

Preparation of sample

The leaves of the collected plants were separated. Each part was cleaned and dried at room temperature. The dried plant parts were crushed, using a pestle and mortar and stored in airtight containers until analysis.

Preparation of extract

The crushed plant material was mixed with the solvent (250 ml of ethanol) in a round bottom flask, which was attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material was loaded into the thimble, which was placed inside the Soxhlet extractor. The side arm was lagged with glass wool. The solvent was heated using the isomantle and evaporated, moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reaches the siphon it was poured back into the flask and the cycle begins again. The powder was extracted with 250 ml of solvent at 40°C for 24 h. The solvent containing active constituents was transferred to rotavapour to evaporate the solvent and to get solid extract. The extract was kept in deep freeze at -4°C, to be used for further study.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on test extract for the detection of phytoconstituents by following methods of Kokate *et al*. (1996).

Test for Carbohydrates

Molisch's Test: 2-3 drops of beta-naphthol solution were added to test extract. Very gently added 1 ml of conc. H_2SO_4 along the side of the test tube. A deep violet coloration was produced at the junction of two layers showed presence of carbohydrates.

Tests for alkaloids

Mayer's test: To test extract, added a few drops of Mayer's reagent. Formation of precipitate indicated presence of alkaloids.

Tests for Tannins

Goldbeater's skin test

A small piece of goldbeater skin (membrane prepared from the intestine of an ox) is soaked in 20% hydrochloric acid, ringed with distilled water and placed in a solution of tannin for 5 min. The skin piece is washed with distilled water and kept in a solution of ferrous sulphate. A brown or black color was produced on the skin due to presence of tannins which showed presence of tannins.

Test for Proteins

Biuret test: Took a small quantity of the dispersion of the sample in a test tube and added 2 ml of NaOH solution into it. Now added 4-5 drops of 1% $CuSO_4$ solution and warm the mixture for about 5

min. Bluish violet color was formed which confirmed presence of proteins.

Test for Steroids

Libermann Burchard`s Test: The extract was treated with conc. sulphuric acid and glacial acetic acid followed by acetic anhydride, a violet ring appeared at the junction of the liquids and appearance of green colour in the aqueous layer indicated presence of steroids.

Test for Sterols

The extract was treated with 5% KOH solution, appearance of pink colour indicated the presence of sterols.

Test for Phenols

The extract was treated with neutral Ferric chloride solution, appearance of violet colour indicated presence of phenols. The extract was treated with 10% sodium chloride solution, appearance of cream colour indicated presence of phenols.

Test for Flavanoids

5 ml of the extract solution was hydrolyzed with 10% Sulphuric acid and cooled. It was then extracted with diethyl ether and divided in to 3 portions in three separate test tubes.1 ml of diluted sodium carbonate, 1 ml of 0.1 N sodium hydroxide and 1 ml of diluted ammonia solutions were added to the first, second and third test tube, respectively. Development of yellow colour in each test tube indicated presence of flavanoids.

Test for Glycosides

A pinch of the extract was dissolved in Glacial acetic acid and few drops of Ferric chloride solution was added followed by the addition of conc. sulphuric acid, formation of red ring at the junction of the two liquids indicated presence of glycosides.

Test for Saponins

Foam test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes showed presence of saponins.

Test for Terpenes

The extract was treated with tin and thionyl chloride, appearance of pink colour indicated presence of terpenes

In vitro total antioxidant activity

DPPH radical scavenging activity

DPPH scavenging activity was assessed by using the method of Hatano *et al.* (1998). In this, different vials contain 1 ml of extract solution and standard were taken. To these solutions, 5 ml of methanolic solution of DPPH was added, shaken well and the mixture was incubated at 37°C for 20 min (Ecobichon,1997). The absorbance was measured against methanol as a blank at 517 nm. The absorbance of DPPH taken as a control was measured. Percentage anti-radical activity can be calculated by using following formula,

$$\% \text{ Anti-radical activity} = \frac{\text{Control Absorbance - Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Statistical analysis:

All data are expressed as mean ± standard deviation of five replicate (n=5). The significance of the experimental observation was checked by Student's t-test and $P < 0.05$ was considered as statistically significant when compared to relevant controls.

Results and Discussion

The phytochemical analysis of the extract of *Senna auriculata* Linn. leaf is illustrated in Table 1. The *in vitro* antioxidant activity of *Senna auriculata* leaf extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay typically measured the ability of the extract to scavenge free radicals, expressed as a percentage of inhibition or IC_{50} value (the concentration required to inhibit 50% of DPPH free radicals). The antioxidant activity of *Senna auriculata* leaf extract is attributed to bioactive compounds such as flavonoids, phenolics, and tannins. Results exhibited a dose-dependent increase in % DPPH scavenging

Table 1: Preliminary phytochemical analysis of the extract of *Senna auriculata* Linn. leaf

S. No.	Constituents	Ethanolic Extract
1	Alkaloids	++
2	Carbohydrates and glycosides	+
3	Proteins and amino acids	++
4	Tannin and phenolic compounds	+
5	Fixed oils and fats	-
6	Saponins	++
7	Gums and mucilage	-
8	Flavanoids	+++
9	Phenols	+
10	Glycosides	+
11	Steroids	+

Table 2: DPPH Radical scavenging activity

Effective concentration (mg/ml)	% inhibition of DPPH radical scavenging activity of EESA	% inhibition of DPPH radical scavenging activity of Ascorbic acid
10	30.17	19.07
20	32.50	20.40
30	34.00	21.84
40	36.20	23.50
50	38.00	24.59

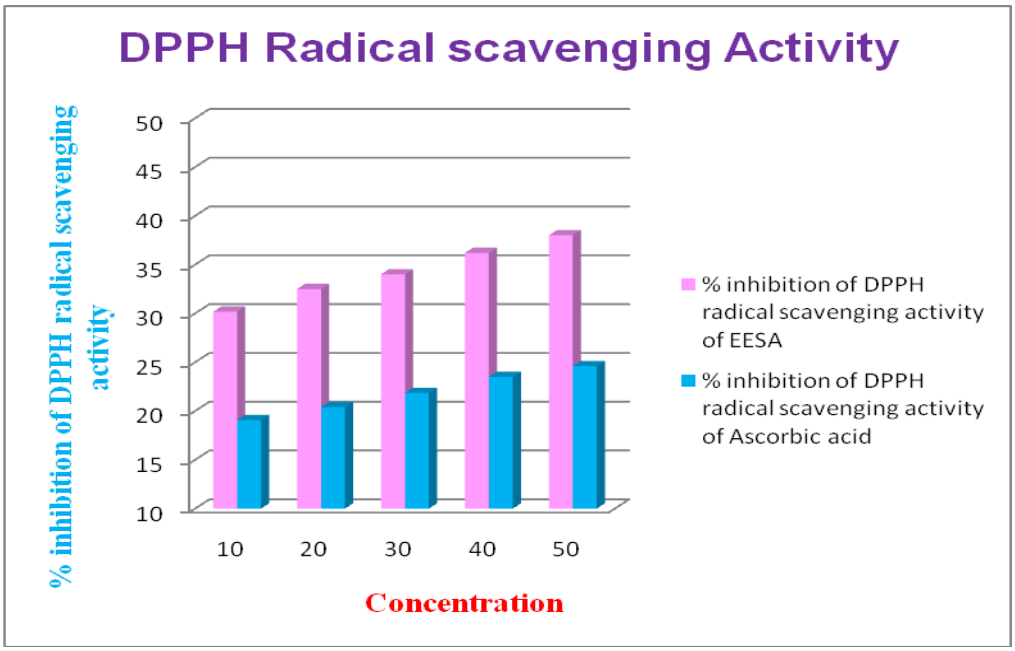

Fig. 1: DPPH Radical scavenging activity.

activity, where higher concentrations of the extract yield higher radical scavenging effects.

The *Senna auriculata* leaf extract showed better antioxidant activity in comparison with standard antioxidant ascorbic acid. Standard drug Ascorbic acid typically exhibited lower IC_{50} values as compared to ethanolic extract of *Senna auriculata* leaves. Higher phenolic and flavonoid content correlates with stronger DPPH radical scavenging ability.

Plants have diverse groups of phenolic compounds, such as simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavonoids. All these phenolic classes have gained extensive attention because of their physiological functions, including free radical scavenging, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects.

The antioxidant activity of phenolics is largely due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers as well as potential metal chelators. In this study, a considerable high level of phenolics was observed in the ethanol extracts of the leaf of *Senna auriculata.* This may explain the widespread folklore use of the plant.

The DPPH system is a stable radical generating procedure. It is well known that the DPPH ability to capture free radicals is due to the delocalization of the unpaired electron all over the molecule. DPPH is a potent scavenger for many other radicals, due to the easiness in following the procedure – violet colour of DPPH faints into the yellow colour of its reduced congener (DPPH-H), with a high shift in the visible spectra (from 520 nm to 330 nm).

Senna auriculata is widely used in number of pharmacological actions with high content of flavanoids and bioflavonoid seems to have a high potential for antioxidant activity. Extracts of the leaf of *Senna auriculata* showed promising free radical scavenging effect of DPPH in concentration dependent manner up to 100 mg/ml.

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