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Comparing of antioxidant and DPPH induced free radical scavenging activity of Sesbania grandiflora and Acacia nilotica plants

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Abstract: Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body causing them to lose their structure and function. It is essential in many living organism for the production of energy to fuel biological processes. The present study aimed to compare the antioxidant activity of dried leaf and bark extract (methanolic) of *Sesbania grandiflora* leaves and *Acacia nilotica.* The obtained results for DPPH radical scavenging were (132.91 µg/ml) for Sesbania grandiflora whereas (184.55 µg/ml) for *Acacia nilotica*.

Keywords: DPPH, *Sesbania grandiflora*, *Acacia nilotica*, Antioxidant, Ascorbic Acid

Introduction:

Free Radicals are molecules with an unpaired electron. They contain an odd number of electrons. Due to the presence of a free electron, these molecules are highly reactive. They are very unstable, react quickly with other compounds and try to capture the needed electron to gain stability.[1, 2] They are important intermediates in natural processes involved in cytotoxicity, control of vascular tone, and neurotransmission. Radiolysis is a powerful method to generate specific free radicals and measure their reactivity.[1, 3]

At low or moderate concentration some of the free radicals plays beneficial physiological role in vivo this include defence against infectious agents by phagocytosis, energy production, cell growth, function in different cellular signaling systems and the induction of a mitogenic response at low concentrations.[4]

Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules are O^{-}_{2} (superoxide radical), HO (hydroxyl radical) and H_2O_2 oxygen. The nitrogen derived molecules are NO_2 (nitrogen dioxide) and N_2O_3 (dinitrogen trioxide).[5]

They are well documented for playing a dual role in our body as both deleterious and beneficial species. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress. It is a harmful process that can be mediates damage to cell structures, including lipids, proteins, RNA and DNA which leads to number of diseases.[4]

Sesbania grandiflora L. is an Indian medicinal plant which belongs to family Leguminosae. It is cultivated in south or west India in the ganga valley and in Bengal. The plant contains rich in tanins, flavonoides, coumarins, steroids and triterpens. The plant used in colic disorder, jaundice, poisoning condition, small-pox, eruptive fever, epilepsy, as a Antioxidant etc.[2, 6-8] The tanins, flavonoides, coumarins, steroids and triterpens were present on all organ tested, with more or less important contents according to the intensity of coloring obtained. The alkaloids are generally found in the form of traces.

All parts of *Sesbania grandiflora L.* are utilized for medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers, and fruit. In Folk Medicine it is resorted to be aperient, diuretic, emetic, emmenagogue, febrifuge, laxative, and tonic.[9-11]

Material and Method:

Collection of Plant material-

The leaves of *Sesbania grandiflora* were collected near Kanichikudi temple, Samalpatti to Oothngrai main road, Krishnagiri district, Tamil Nadu state in the

month of October and was authenticated by Dr. K. Ravikumar, Assistant Director, Foundation of revitalisation of Local Health Traditions, Bangalore.

The bark of *Acacia nilotica* was collected in the month of October near Thiruvadhigai Anaicut, panruti Taluk, cuddalore district, Tamil Nadu state and was authenticated by Dr. K. Ravikumar, Assistant Director, Foundation of revitalisation of Local Health Traditions, Bangalore.

Preparation of plant extracts-

The freshly collected leaves and bark of *Sesbania grandiflora* and *Acacia nilotica* respectively were dried in shade under the control conditions and powdered. The powered leaves and bark of the plants (500g) were extracted in solvents with increasing polarity from petroleum ether, chloroform, ethyl acetate, methanol and water for 24 hours with each solvent, by successive extraction method (Soxhlet apparatus) at a temperature of 30º to 35º C. The extracts were concentrated by evaporating the solvent on water bath until it got reduced to a semisolid mass obtain methanolic extract of *Sesbania grandiflora* and *Acacia nilotica* plants.

DPPH assay: [12, 13]

The DPPH assay of leaf and bark methanolic extract was determined by the method as reported by **Tanwar M. et.al. (2011).**[14] It is a non enzymatic method. The procedure involved UV- Spectrophotometric determination. Three solutions i.e. standard, Test and Control were prepared.

Preparation of Standard Ascorbic acid solution:-

Different solutions of the ascorbic acid were prepared in ethanol to give concentrations (25 - 250µg/ml). 3 ml of each solution of ascorbic acid were mixed with 1 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of each solution was taken after 30 min against ethanol (as blank) at 517 nm.

Preparation of Test solutions:-

Different solutions of the leaf and bark methanolic extract was prepared in ethanol to give concentrations (25 - 250µg/ml). 3 ml of each solution of leaf and bark methanolic extract was mixed with 0.1mM DPPH solution and incubated for 30 min at room temperature in dark conditions. Absorbance of each solution of leaf and bark methanolic

extract was taken after 30 min against ethanol (as blank) at 517 nm.

Preparation of Control solution:-

For control, 3 ml of ethanol was mixed with 1ml 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken after 30 min against ethanol (as blank) at 517 nm.

Percentage antioxidant activity of leaf and bark methanolic extract and ascorbic acid was calculated by using the formula:

$$I\% = [\{A_o - A_1/A_o\}] \times 100$$

Where,

- I% = Percentage inhibition
- A_o = Absorbance of control (ethanol and 0.1mM DPPH)

A_1 = Absorbance of ascorbic acid / plant drug extract with 0.1mM DPPH solution after 30 min

Statistical Analysis:-

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni Multiple Comparisons Test. The values were expressed as mean ± SEM and $P<0.05$ were considered significant.

Result:

DPPH Assay:-

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay of methanolic extracts of *S. grandiflora* leaves and *A. nilotica* bark was estimated by using Ascorbic acid solution as standard. The absorbance data (Table no. 1, 2 and 3) were recorded against the selected concentration (25, 50, 100, 150, 200, 250 µg/ml).

Table no. 1: % Inhibition data for DPPH assay of Ascorbic acid

Conc. of Ascorbic acid (µg/ml)	% Inhibition
25	17.36
50	30.41
100	42.66
150	56.23
200	70.54
250	77.45

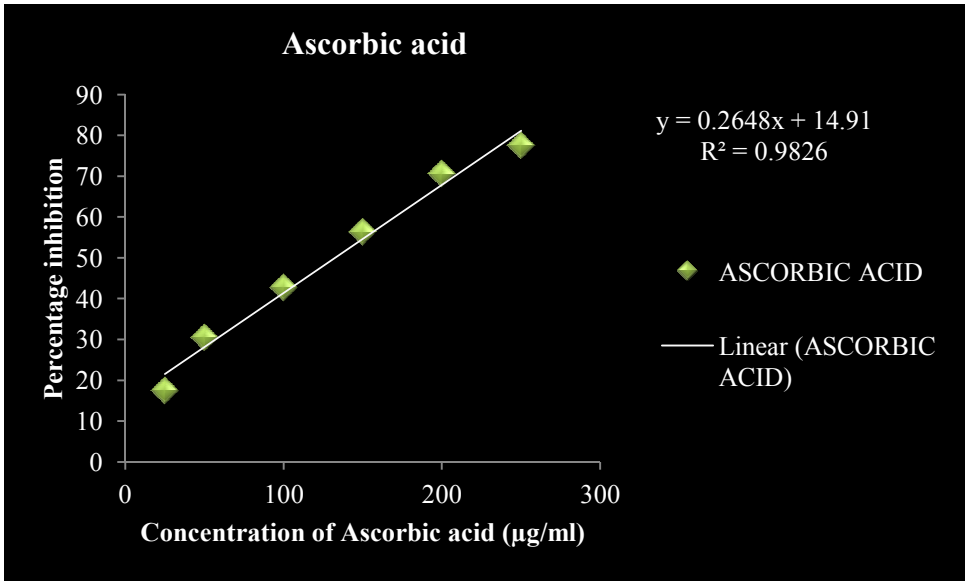

Fig no. 1: % Inhibition DPPH assay curve of Ascorbic acid

Table no. 2: % Inhibition data for DPPH assay of *S. grandiflora* methanolic leaves extract

Conc. of *S. grandiflora* extract (µg/ml)	% Inhibition
25	10.31
50	18.91
100	37.37
150	44.50
200	51.74
250	62.81

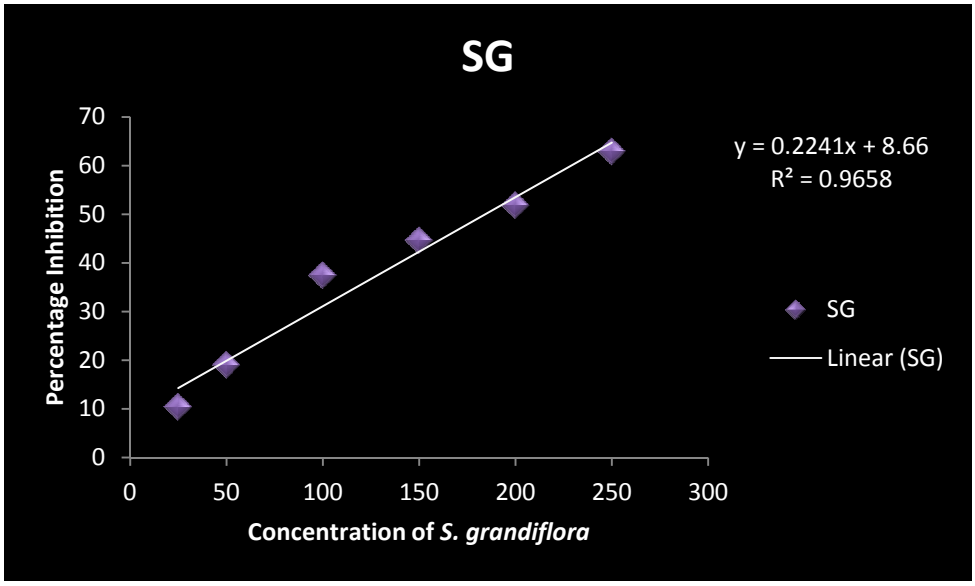

Fig no. 2: % Inhibition DPPH assay curve of *S. grandiflora* leaves methanolic extract

Table no. 3: % Inhibition data for DPPH assay of *A. nilotica* bark extract

Conc. of *A. nilotica* extract (µg/ml)	% Inhibition
25	4.18
50	8.33
100	19.33
150	39.32
200	43.94
250	58.41

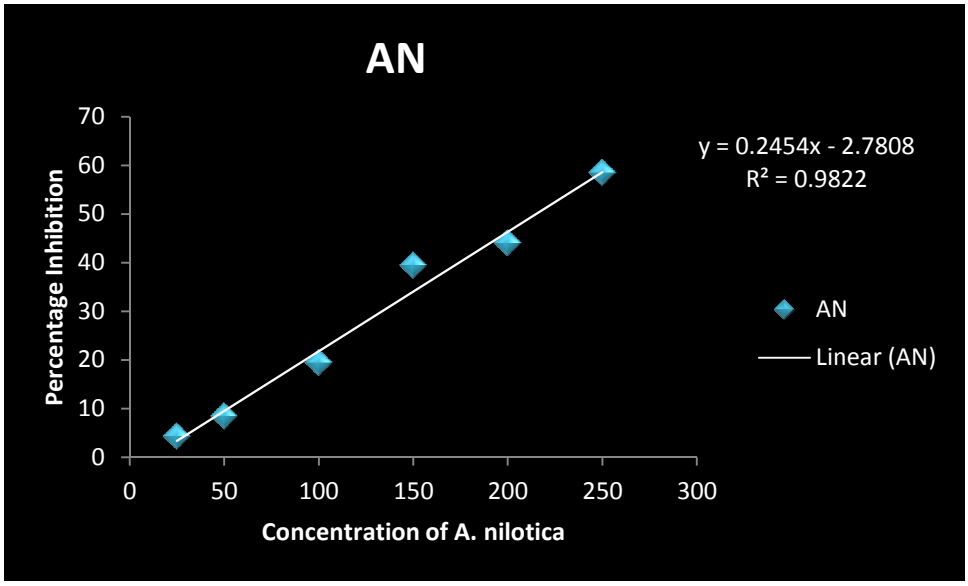

Fig no. 3: % Inhibition DPPH assay curve of *A.* nilotica bark methanolic extract

Discussion:

On the basis of IC_{50} value for standard (ascorbic acid) and the methanolic leaves and bark extracts of *S. grandiflora* and *A. nilotica* extracts were 132.91 µg/ml, 184.55 µg/ml and 154.69 µg/ml respectively, represents the antioxidant potential of the extract and the standard.

The antioxidant potential of *S. grandiflora* and *A. nilotica* was assessed by DPPH assay. The DPPH assay is based on the measurement of scavenging activity of antioxidant toward the stable radical $DPPH^{\cdot}$. antioxidants reduce the radical to the corresponding hydrazine donor in the antioxidant principle. $DPPH^{\cdot}$ radical react with suitable reducing agent, the electrons become paired off and the solution losses colour stoichimetrically depending on the number of electron taken up.[15] The plant extract show reduction in absorption value in DPPH assay is due to radical scavenging activity of ascorbic acid and methanolic leaves and bark extract of *S. grandiflora* and *A. nilotica.*

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