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In-vitro antioxidant activity of *Sida spinosa* Linn

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ABSTRACT

The present study is to evaluate a systemic record of the relative antioxidant activity of Sida spinosa. The ethanolic extract of Sida spinosa was screened for their free radical, hydroxy radical, superoxide & nitric oxide scavenging activity. Total antioxidant activities of ethanolic extract were compared with standard antioxidants ascorbic acid, copper sulphate 2, 6- di-ter-butyl-p-hydroxytoluene (BHT). Results indicate the ethanolic extract exhibited antioxidant potential of in-vitro screening methods. The results indicate that ethanolic extract showed moderate activity against standard drugs.

Key Words: *Sida spinosa*, *in-vitro* antioxidant activity, DPHH, free radical scavenging activity.

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons and involved in many pathological conditions [1]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants [2]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic [3]. Plant polyphenolic compounds, such as flavonoids are described

as scavengers of reactive oxygen species [4]. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated [5]. Most sources of natural antioxidants originate from plant materials, but the content of polyphenolic compounds in the roots and pericarp of tropical and sub-tropical flora have sparsely reported [6].

Sida spinosa is an erect annual hairy herbs having height up to 70 cm; Leaves ovate-oblong or lanceolate, obtuse or acute, 1-4 cm long, serrate, leaving a distinctive, spine like scar on falling; Flowers are axillary, solitary or 2-5 together; Corolla yellow; Mericarps 5, trigonous, strongly reticulately veined, hairy at apex; seed ovoid. Flowering and fruiting timing are in October-December.

Leaves are used in demulcent, refrigerant and are useful in gonorrhoea, gleet and scalding urine. Decoction of the root-bark and root is used in mild cases of debility and fever. Leaves are bruised in water, strained through cloth and administered in the form of a draught. Root is used in decoction.

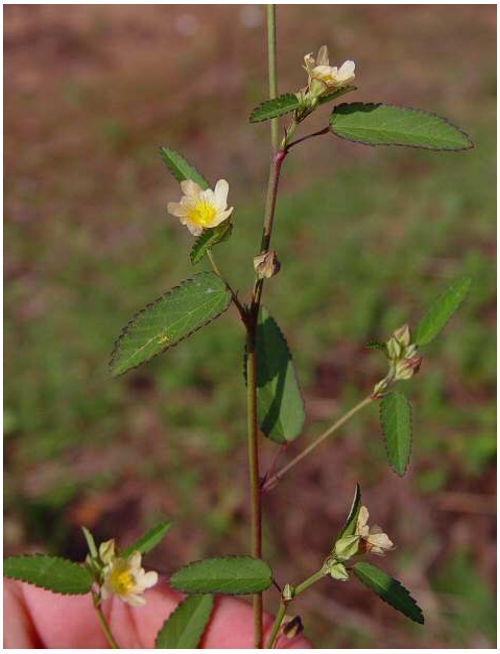

Fig 1: *Sida spinosa* Linn

MATERIALS AND METHODS

Collection of plant:

The dried plant of *Sida spinosa Linn* was collected from Tirupathi hills, Andhra Pradesh, India in the month of May 2011 and authenticated by Dr. P. Jayaraman, Plant Anatomical Research Centre (PARC), Tambaram, Chennai and the voucher specimen was kept in the Department of Pharmacognosy, Safa College of Pharmacy, B.Tandrapadu, Kurnool. Andhra Pradesh, India. The whole plant was cleaned, air dried and grounded into powdered separately. The dried powdered plant material was passed through sieve 60 and stored in air tight containers.

Preparation of extract:
The dried powder of *Sida spinosa* plant (200gms) was successively extracted with Ethanol (80%) at room temperature by Soxhlet extraction process**.** Each time before extracting with the solvent, dry the powdered material in oven below 50^0c. Concentrate the extract at reduced pressure by Rotary Flash Vacuum Evaporator. Weigh the extract obtained with the solvent and calculate its percentage in terms of the air-dried weight of the plant material. Further the concentrated extract was dried in desicator and stored in vacuum sealed air tight containers.

Free radical scavenging activity by DPPH:
The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extract. Different concentrations of each extract were added, at an equal volume, to ethanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C was used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. The ethanolic solution of ascorbic acid was taken as standard. The percent reduction in absorbance was calculated from the initial and final absorbance at each level [7].

Calculation of percentage inhibition
The percentage inhibition of radical production by the test sample was calculated using the formula:

$$\text{Inhibitory ratio} = (Ao-A1)\ x100/Ao$$

Where Ao is the absorbance of control
A1 is the absorbance with addition of test sample.

Nitric oxide scavenging activity:
Aqueous solution of sodium nitroprusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitric oxide which was measured calorimetrically. Ethanolic extract of whole plant were served as sample (0.5ml from 20mg/ml). Three ml of reaction mixture containing sodium nitroprusside (100mM in PBS) and 0.5ml extracts (20mg/ml) were incubated at 25° C for 150 minutes. Controls were kept without test sample in an identical manner. After incubation, 0.5 ml of reaction mixture was removed and 0.5ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4, 0.1% naphthalene diamine dihydrochloride was added. The absorbance of the chromophore formed was read at 546nm.The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test samples [8-9].

Superoxide radical scavenging activity:
The extent of superoxide generation was studied on the basis of inhibition in the production of nitroblue tetrazolium formazon of the superoxide ion by the test area, where they received uniform illumination for 30 minutes. Again the optical density was measured at 560nm. The difference in optical density before and after illumination is the quantum of superoxide production and the percentage of inhibition by the test sample was calculated by comparing with the optical density of the control [10].

Hydroxyl radical scavenging activity:

Deoxy ribose assay was used to detect hydroxyl radical scavenging activity and iron binding ability of them test sample. Hydroxyl radicals are generated in a reaction mixture containing ascorbate, H_2O_2 and iron–III-EDTA at pH 7.4 and measured by their ability to degrade the sugar deoxyribose. The extent of inhibition is dependent on the concentration of the scavenger and its rate constant for reaction with hydroxyl radicals. A mixture of 0.5ml of 3mM deoxyribose solution, 0.1mM ferric chloride solution, 0.1mM solution of EDTA, 0.1mM solution of ascorbic acid and 2 mM solution of phosphate buffer was taken in a series of test tube and final volume was made up to 3ml. To this 0.5ml of Trichloro Acetic Acid (TCA) and Thio Barbituric Acid (TBA), 10-50mg/ml of various concentrations of the test samples were added to the above mixture and absorbance was measured at 532nm. Concentration of extracts required for 50 per cent reduction in absorbance was calculated from the calibration curve of concentration of the extracts Vs per cent reduction in absorbance. 0.1mM solution of $CuSO_4$ was prepared and used as reference standard [11].

RESULTS AND DISCUSSION

Table 1: Free radicals and Hydroxyl radicals scavenging activity of *Sida spinosa*

S. No	Free radicals scavenging activity		Standard (Ascorbic Acid)	Hydroxyl radicals scavenging activity		Standard (CuSO4)
	Conc µg/ml	Mean ± SD	Mean ± SD	Conc µg/ml	Mean ± SD	
1	10	41.23±0.4132	20.52±0.52	10	14.54±0.03	32.24±0.003
2	20	46.87±0.4951	34.67±0.56	20	17.91±0.03	46.84±0.003
3	30	50.45±0.7412	38.15±0.20	30	18.54±0.04	58.96±0.004
4	40	54.45±1.2456	41.57±0.61	40	22.73±0.04	65.89±0.004
5	50	58.21±0.9854	63.87±0.57	50	25.46±0.04	72.64±0.04

Values are represented in Mean ±SD of six replicates performed using graph pad prism software using Kolmogorov-smirnov test

Table 2: Nitric oxide and superoxide radicals scavenging activity of *Sida spinosa*

S. No	Super oxide radical % inhibition		Standard (Butylated Hydroxy toluene)	Nitric oxide % inhibition	
	Conc µg/ml	Mean ± SD	Mean ± SD	Conc µg/ml	Mean ± SD
1	10	36.54±0.004	8.52±0.461	10	17.82±0.004
2	20	43.61±0.021	12.57±0.460	20	19.87 ±0.005
3	50	46.67 ±0.462	15.49±1.182	50	22.73±0.004
4	100	63.62 ±0.005	20.71±0.402	100	27.59±0.519
5	150	67.34±0.042	25.69±0.840	150	38.14±0.004

Values are represented in Mean ±SD of six replicates performed using graph pad prism software using Kolmogorov-smirnov test.

The crude ethanolic extract of whole plant of *Sida spinosa* exhibited significant inhibition of nitric oxide & superoxide scavenging activity. The ethanolic extract has shown moderate activity against standard drugs. The presence of flavonoids and tannins in the ethanolic extract is likely to be responsible for the in-vitro antioxidant against different in-vitro screening methods. Flavanoids and tannins are phenolic compounds and plant phenolic compounds are major group of compounds that act as primary antioxidants. Scavenging activity for free radicals of 1.1-

diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free cells and oxidizing agents. In biochemical systems, superoxide radical can be converted into hydrogen peroxide by the action of superoxide dismutase and the H_2O_2 can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions. Hydroxyl radicals can attack DNA molecules to cause strand scission .The IC_{50} values are represented in Table 1 & 2 against standard drugs. The study reveals in-vitro antioxidant activity of *Sida spinosa*.

CONCLUSION

Reactive oxygen species contribute to various pathophysiological conditions and endogenous defense mechanisms have evolved to offer protection in these conditions. Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy. The overall antioxidant activity of *Sida spinosa* is due to flavanoids, tannins and other phytochemical constituents. The active chemical constituents responsible for in-vitro antioxidant should be isolated and the results should be compared with in vivo results.

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