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IN-VITRO ANTIOXIDANT ACTIVITY OF *SOLANUM SISYMBRIFOLIUM* AERIAL PARTS

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Keywords:

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ABSTRACT:

Objective: Recently, natural plants have received much attention as sources of biological active substances including antioxidants. In the present study we investigated *In-vitro* antioxidant activity of hexane, ethyl acetate and ethanol extracts of *Solanum sisymbrifolium* aerial parts. **Methods:** *In-vitro* antioxidant activity was evaluated for extracts by using free radicals Superoxide (Riboflavin photo reduction method) and DPPH (The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca and Sanja). **Results:** The selected plant extracts produced concentration dependent percentage inhibition of different free radicals and produced maximum activity at a concentration of 800µg and there after the percentage inhibition was raised gradually to its maximum level with higher concentrations. **Conclusion**: In the present study we found that the extracts of *Solanum sisymbrifolium* showed good antioxidant activity. Among the three extracts, hexane extract showed better activity than the other extracts on the tested super oxide free radical. The order of activity is in the following manner: Ascorbic acid >hexane extract > ethyl acetate extract> ethanol extract. Ethanol extract showed better activity than the other extracts on the tested DPPH free radical. The order of activity is in the following manner: ethanol extract >Ascorbic acid > ethyl acetate extract > hexane extract.

INTRODUCTION: Free radicals are defined as molecules having an unpaired electron in the outer orbit [1]. They are generally unstable and very reactive. Examples of oxygen free radicals are superoxide, hydroxyl, peroxyl (RO_2•), alkoxyl (RO•) and hydroperoxyl (HO_2•) radicals. Nitric oxide and nitrogen dioxide (•NO_2) are two nitrogen free radicals.

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Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide, hypochlorous acid (HOCl), hypobromous acid (HOBr), and peroxynitrite (ONOO_).

Reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species are produced in animals and humans under physiologic and pathologic conditions [2]. The reactive oxygen species play an important role related to the degenerative or pathological processes of various serious diseases, such as aging [3], cancer, coronary heart disease, Alzheimer's disease [4, 5], neuro-degenerative disorders, atherosclerosis, cataracts, and inflammation [6].

Over the past three decades, the free radical theory has greatly stimulated interest in the role of dietary antioxidants in preventing many human diseases including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes [7-10].

The most commonly used synthetic antioxidants in the food industry are butylated 4-hydroxytoluene (BHT) and butylated 4-hydroxyanisole (BHA). However, the use of synthetic antioxidants in the health industry has been fraught with concerns about the toxicity associated with synthetic compounds [11]. Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs with less toxicity than the synthetic compounds.

In the search for sources of natural antioxidants and compounds with radical scavenging activity during recent years, some have been found, such as echinacoside in *Echinaceae* root, anthocyanins , phenolic compounds , water extracts of roasted *Cassia tora*, whey proteins [12], and thioredoxin *h* protein from sweet potato [13].

In the present study, different extracts of *Solanum sisymbrifolium* were investigated for their *In-vitro* antioxidant activity.

MATERIALS AND METHODS:

Preparation of extract from of *Solanum sisymbrifolium*: The plant *S. sisymbriolium* was collected in the month of November, 2011 from Hanumadwake area, Visakhapatnam, Andhra Pradesh, India. The plant material was taxonomically identified by Dr. M. Venkaiah, Botanist, Andhra University. Freshly collected plant material was dried under shade and milled to obtain a coarse powder. To the coarse powder (500gms) four liters of ethanol (70%) was added and macerated for 5 days at room temperature (30°C).

The macerated extract was obtained and concentrated under vacuum at temperature of 45°C by using rotary evaporator (Buchi, Switzerland), dried completely and stored in desiccator. The 70%v/v ethanolic extract was then fractionated into hexane and ethyl acetate fractions.

Chemicals and Drugs: All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, Mumbai.

***In-vitro* anti-oxidant activity:** For the assessment of free radicals scavenging activity, the hexane, ethyl acetate and ethanol extracts were dissolved in water and 5% dimethyl sulphoxide (DMSO) respectively.

Superoxide radical Scavenging activity [14, 15]: Superoxide scavenging activity of the plant extract was determined by McCord and Fridovich method, 1969, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. 0.1 ml of different concentrations of plant extract and 0.1 ml of 6 µM ethylenediamine tetraacetic acid containing NaCN, 0.1 ml of 50 µM nitroblue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube, and final volume was made up to 3 ml using phosphate buffer.

Then, the assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 minutes and thereafter the optical densities were measured at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes.

DPPH radical Scavenging activity: The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca [16] and Sanja [17]. In DPPH assay, method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non-radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity [18]. An aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid.

Calculation of percentage inhibition: The percentage inhibition was calculated using the formula:

Formula 1: Inhibitory ratio = $\frac{(A_0 - A_1)}{A_0} \times 100$

Where, A_0 is the absorbance of control; A_1 is the absorbance with addition of plant extract/ ascorbic acid.

Calculation of 50% inhibition concentration: The optical density value obtained with each concentration of the extract/ ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

RESULTS:

In-vitro Antioxidant activity:

Superoxide radical: In the present study, ethanol, ethyl acetate and hexane extracts of *S. sisymbrifolium* aerial parts were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin and the results are given in **Table 1** and **Fig. 1**.

TABLE 1: CONCENTRATION DEPENDENT PERCENT INHIBITION OF SUPER OXIDE RADICAL BY DIFFERENT EXTRACTS OF *SOLANUM SISYMBRIFOLIUM* AND ASCORBIC ACID IN *IN-VITRO* STUDIES

Name of the extract of *S. sisymbrifolium*	Percentage inhibition of Superoxide radical				
	50µg/ml	100µg/ml	200µg/ml	400µg/ml	800µg/ml
Hexane	48.26	51.11	53.34	56.57	58.31
Ethyl acetate	7.25	15.01	35.73	55.7	79.9
Ethanol	5.38	8.06	31.26	53.1	62.13
Ascorbic acid	84.66	91.52	93.98	95.13	96.61

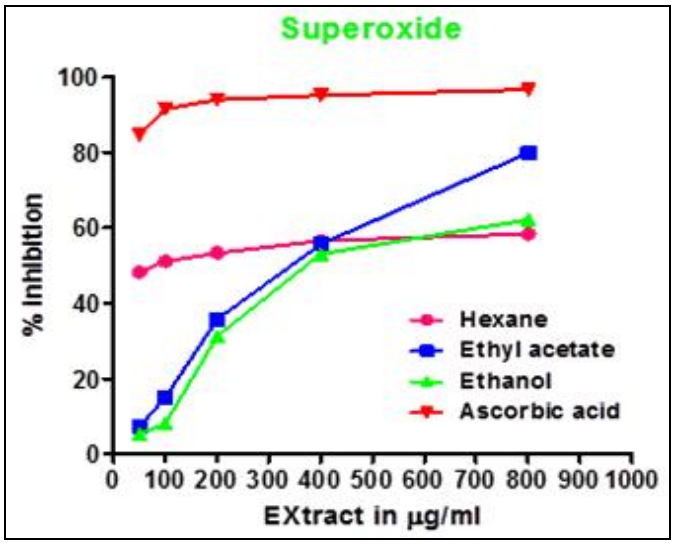

FIGURE 1: CONCENTRATION DEPENDENT PERCENT INHIBITION OF SUPEROXIDE RADICAL BY DIFFERENT EXTRACTS OF *S. SISYMBRIFOLIUM* AND ASCORBIC ACID IN *IN-VITRO* STUDIES

The mean IC_{50} values for superoxide radical of ethanol, ethyl acetate and hexane extracts of *S. sisymbrifolium* aerial parts were found to be 73.7µg, 340µg and 372µg respectively. The mean IC_{50} value of ascorbic acid was found to be 46.7µg. The results were given in **Table 3** and **Fig. 3**.

DPPH radical: The ethanol, ethyl acetate and hexane extracts of *S. sisymbrifolium* aerial parts were found to possess concentration dependent scavenging activity on DPPH radicals and the results were given in **Table 2** and **Fig. 2**. The mean IC_{50} values for DPPH radical of ethanol, ethyl acetate and hexane extracts of *S. sisymbrifolium* aerial parts were found to be 669µg, 197µg, and 50µg respectively. The mean IC_{50} value of ascorbic acid was found to be 93.3µg. The results were given in **Table 3** and **Fig. 3**.

TABLE 2: CONCENTRATION DEPENDENT PERCENT INHIBITION OF DPPH RADICAL BY DIFFERENT EXTRACTS OF *SOLANUM SISYMBRIFOLIUM* AND ASCORBIC ACID IN *IN-VITRO* STUDIES

Name of the extract of *S. sisymbrifolium*	Percentage inhibition of DPPH radical				
	50µg/ml	100µg/ml	200µg/ml	400µg/ml	800µg/ml
Hexane	13.02	16.47	37.16	40.61	45.21
Ethyl acetate	28.01	42.41	50.3	78.14	81.62
Ethanol	51.3	53.25	57.05	58.62	66.66
Ascorbic acid	84.66	91.52	93.98	95.13	96.61

TABLE 3: IC_{50} VALUES OF DIFFERENT EXTRACTS OF *SOLANUM SISYMBRIFOLIUM*

Name of the extract of *S. sisymbrifolium*	50% inhibition Concentration (IC50) (µg)	
	Superoxide radical	DPPH radical
Hexane	73.7	669
Ethyl acetate	340	197
Ethanol	372	50
Ascorbic acid	46.7	93.3

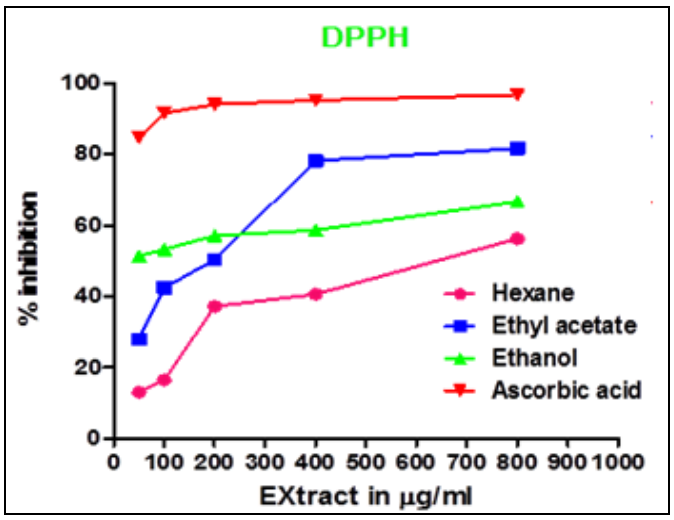

FIGURE 2: CONCENTRATION DEPENDENT PERCENT INHIBITION OF DPPH RADICAL BY DIFFERENT EXTRACTS OF *S. SISYMBRIFOLIUM* AND ASCORBIC ACID IN *IN-VITRO* STUDIES

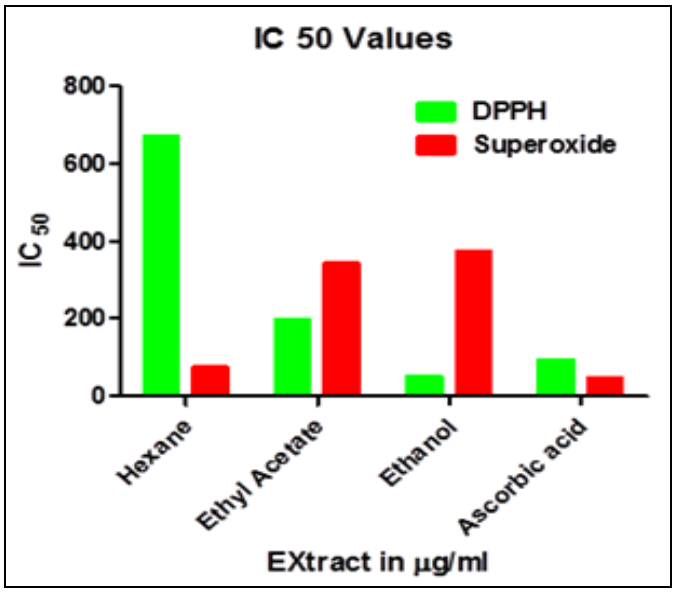

FIGURE 3: *IN VITRO* 50% INHIBITION CONCENTRATION (IC_{50}) OF DIFFERENT EXTRACTS OF *S. SISYMBRIFOLIUM* ON DPPH AND SUPEROXIDE RADICALS

DISCUSSION: Phytochemicals, especially phenolics in fruits and vegetables, are suggested to be the major bioactive compounds for health benefits. Phenols have been found to be useful in the preparation of some antimicrobial and antioxidant compounds [19, 20].

The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals [21, 22]. The phytochemical analysis conducted on *S. sisymbrifolium* extracts revealed the presence of carbohydrates, alkaloids, steroids, phenols, Glycosides etc.

The presence of phenolic compounds in this plant may contribute to its antioxidative properties and thus the usefulness of these plants in herbal medicament.

The result of scavenging activity assay in this study indicates that the plant is potently active. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The plant extracts were capable of scavenging super oxide and DPPH in a concentration dependent manner.

CONCLUSION: Among the three extracts of *S. sisymbrifolium*, the hexane extract showed better activity than other extracts on the tested super oxide free radical. The order of activity is in the following manner: Ascorbic acid >hexane extract > ethyl acetate extract> ethanol extract.

Among the three extracts of *S. sisymbrifolium*, the ethanol extract showed better activity than other extracts on the tested DPPH free radical. The order of activity is in the following manner: ethanol extract >Ascorbic acid > ethyl acetate extract > hexane extract.

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