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In-vitro antioxidant activity of *Triumfetta pilosa* Roth

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ABSTRACT

The present study is to evaluate a systemic record of the relative antioxidant activity of Triumfetta pilosa .The ethanolic extract of Triumfetta pilosa 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 invitro screening methods .The results indicate that ethanolic extract showed moderate activity against standard drugs.

Key Words: Triumfetta Pilosa, invitro antioxidant activity, DPHH, free radical scavenging activity.

INTRODUCTION

Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging. Free radicals due to environmental pollutants, radiation, Chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins [1]. Oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to nonradical forms and function as natural antioxidants in human body [2-3]. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary. Currently available synthetic antioxidants like butylated polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-

inflammatory action [4]. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases. Several studies have demonstrated that plants produce potent antioxidants and represent important sources of natural antioxidants. Several studies from literature revealed that plants produce potent antioxidants and represent important source of antioxidants [5-7]. The present study is an attempt to investigate antioxidant activity of ethanolic extract of plant *Triumfetta pilosa* Roth to provide a scientific proof for the activity. Antioxidant activity was examined for ethanol extract using different antioxidant assays such as DPHH free radical scavenging activity, Nitric oxide & superoxide scavenging activity and hydroxyl radical scavenging activity.

MATERIALS AND METHODS

Collection of plant:
The dried plant of *Triumfetta pilosa* Roth. was collected from Herbal garden Tirupathi, Andhra Pradesh, India in the month of January 2009 and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Dept. of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh. 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 *Triumfetta pilosa* 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 methanolic 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 [8].

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

$$\text{Inhibitory ratio} = (A_o - A_1) \times 100 / A_o$$

Where A_0 is the absorbance of control
A_1 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 colorimetrically.Methanolic 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^{O} 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 [9-10].

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 [11].

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 concentration 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 [12].

Table 1: Free radicals and Hydroxy radicals scavenging activity of Triumfetta pilosa

S.No	Free radicals scavenging activity		Standard (Ascorbic Acid)	Hydroxy radicals scavenging activity		Standard ($CuSO_4$)
	Conc µg/ml	Mean ± SD	Mean ± SD	Conc µg/ml	Mean ± SD	
1	10	40.33 ± 0.5164	19.67 ± 0.81	10	13.60±0.05	31.81±0.005
2	20	45.33 ± 0.5164	33.50 ± 0.83	20	16.66±0.05	45.83±0.005
3	30	49.50 ±0.8367	37.58 ± 0.20	30	19.23±0.04	57.69±0.004
4	40	53.83 ± 1.169	40.50 ± 0.83	40	21.42±0.04	64.28±0.004
5	50	57.17 ± 0.9832	62.33 ± 0.40	50	24.83±0.04	71.87±0.004

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 Triumfetta pilosa

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	35.29±0.004	7.167±0.408	10	16.66±0.004
2	20	42.84±0.012	11.17±0.408	20	19.04±0.005
3	50	44.17±0.408	14.00±1.265	50	21.73±0.004
4	100	62.69±0.004	20.17±0.408	100	27.50±0.547
5	150	65.52±0.036	24.50±0.836	150	37.03±0.004

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

RESULTS AND DISCUSSION

The crude ethanolic extract of whole plant of *Triumfetta pilosa* exhibited significant inhibition of nitric oxide & superoxide scavenging activity. The ethanolic extract has shown moderate activity against standard drugs. The presence of flavanoids and tannins in the ethanolic extract is likely to be responsible for the invitro antioxidant against different invitro 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 invitro antioxidant activity of Triumfetta pilosa.

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 *Triumfetta pilosa* is due to flavanoids, tannins and other phytchemical constituents. The active chemical constituents responsible for invitro antioxidant should be isolated and the results should be compared with in vivo results.

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