INVITRO FREE RADICAL SCAVENGING ACTIVITY OF *BAUHINIA TOMENTOSA* L

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Abstract

The present study was aimed at investigating the antioxidant activities of ethanolic extract of flowers of *Bauhinia tomentosa* L. (Caecalpinaceae). The antioxidant activities of the extract have been evaluated by using four invitro assays and were compared to standard antioxidants such as ascorbic acid and α-tocopherol. The antioxidant property depends upon concentration of the extract and it was increased with tretment of the extract. *Bauhinia tomentosa* L. showed effective hydrogen donor activity, free radical scavenging activity, peroxy radical scavenging activity and antioxidant activity in this study .

Key words: *Bauhinia tomentosa L*, antioxidant, free radical scavenging activity, invitro study, peroxy radical.

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INTRODUCTION

There is extensive evidence to implicate free radical in the development of degenerative diseases (Cross, 1987). Free radicals have been implicated in causation of ailments such as diabetes, liver cirrhosis, nephrotoxicity etc (Marx, 2002). Together with other derivatives of oxygen they are inevitable byproducts of biological redox reactions (Ajay arora *et al.,* 2002). Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical (·OH) and nitric oxide (NO), inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation (Geesin *et al.,* 1990). The increased production of toxic oxygen derivatives is considered to be a universal feature of stress conditions. Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecule and enzymes.

The atmospheric oxygen ($^3O_2$), once inhaled, it undergoes a gradual reduction process that ultimately gets metabolized into water. During this process, some amounts of reactive intermediate i.e. free radicals are formed. The role of free radical reaction in disease pathology is well established. Diseases caused by free radical reactions are atherosclerosis, ischaemic heart disease, ageing, inflammation, diabetes, immunosuppression, neurogenerative diseases and others (Harman, 1988; Maxwell, 1995).

Bauhinia tomentosa Linn. (Caecalpinaceae) is a scrambling, many stemmed shrub or small tree upto 4m in height and distributed all over India. This plant is traditionally used for the treatment of dysentry, diahorea, inflamed glands, liver troubles, abdominal troubles and is also recommended in combination with other drugs for the treatment of snake bite and scorpion sting (The wealth of India, 1988). Some of the important chemical constituents of flowers include isoquercitin and rutin (Rama Roa *et al.,* 1994). A detailed review of literature afforded no information on the invitro antioxidant potential of the plant. It was therefore thought worthwhile to investigate the antioxidant potential of ethanolic flower extract of *Bauhinia tomentosa* L. in this study.

MATERIALS AND METHODS

All chemicals and solvents were of analytical grade and were obtained from Sigma chemicals. The chemicals used were 1,1-diphenyl 1-2- picryl hydrazyl (DPPH), hydrogen

peroxide, ammonium molybdate, sulphuric acid, sodium phosphate, ammonium thiocyanate, ferric chloride, hydrochloric acid, linoleic acid, α-tocopherol and ascorbic acid.

Plant material

The fresh flowers (1kg) of *Bauhinia tomentosa* L. collected in and around mannargudi, during April 2007. The petals of the fresh flowers of *Bauhnia tomentosa* L. were shade dried and powdered. The powdered materials were extracted with 70% ethanol using soxhlet apparatus. This ethanolic extract was then concentrated and dried under reduced pressure. The ethanol free semisolid thus obtained was stored in dessicator and until further use.

DPPH radical scavenging activity

DPPH radical scavenging activity was measured by spectrophotometric method (Mensor et al., 2001). To 1ml of ethanolic solution of DPPH (0.3mM), 3ml of the fraction dissolved in ethanol were added at different concentration (20-100μg/ml). 2.4ml of ethanol in 0.1ml of DPPH was used as a control. The mixture was shaken and allowed to stand at room temperature for 20 minutes and the decrease in absorbance was measured at 517nm. The percentage inhibition at different concentrations was determined and IC_{50} values of the extract were compared with that of ascorbic acid, which was used as standard.

Hydrogen peroxide scavenging assay

Different concentrations of the extract (20-100μg/ml) in distilled water were added to 0.6ml of hydrogen peroxide (0.2mM) in phosphate buffer (pH 7.4). After 10 minutes, the absorbance was determined and the IC_{50} values were compared with the standard α-tocopherol. 2.4ml of phosphate buffer in 0.1ml of hydrogen peroxide was used as a control (Oktay et al., 2003).

Total antioxidant capacity

The total antioxidant capacity of the extract was determined by phosphomolybdate method using α-tocopherol as the standard (Jayaprakasha et al., 2002). An aliquot of 0.1ml of the extract (20-100μg/ml) solution was combined with 0.1ml of the reagent (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the sample had cooled to room temperature, the absorbance was measured at 695nm against blank using an UV

spectrophotometer. 1.0ml of the reagent solution and the appropriate volume of the same solvent used for the sample act as a blank.

Peroxy radical scavenging activity

The peroxy radical scavenging activity was determined by thiocyanate method using α-tocopherol (20-100µg/ml) as standard (Yildrim *et al.,* 2001). Increasing concentration of the extracts (20-100µg/ml) in 0.5ml of distilled water was mixed with 2.5ml of 0.02M linoleic acid emulsion (in 0.4M phosphate buffer pH 7.0) and 2ml phosphate buffer (0.04M, pH 7.0) in a test tube and incubated in darkness 37°C. At intervals (15, 30, 45) during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 300nm by the addition of 150ml of 30% ammonium thiocyanate solution and 0.1ml of 20mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture.The percentage of scavenging activity was calculated and the IC_{50} valuues of the fractions were compared with the standard α-tocopherol. Additionally a reference cuvette filled with 2ml of phosphate buffer, 0.1ml of 30% ammounium thiocyanate solution and 0.1ml of ferrous chloride solution was used.

RESULTS

The concentration ranging from (20-100µg/ml) of the ethanolic extract of *Bauhinia tomentosa* L. were tested for their antioxidant activity in different models and using ascorbic acid and α-tocopherol as standards.

The ethanolic extract of *Bauhinia tomentosa* L. demonstrated hydrogen donar activity. The maximum inhibitory percentage in DPPH assay was detected in 100 µg/ml concentration (93.4 µg/ml). It was less than that of ascorbic acid (Table 1).

The ethanolic extract of *Bauhinia tomentosa* L. scavenged hydrogen peroxide in a concentration dependent manner. The extracts exhibit inhibitory percentage of about 43.5, 52.3, 64.7, 73.4, 80.3 (µg/ml) at various concentrations of 20, 40, 60, 80, 100 (µg/ml) respectively (Table I). These values are greater than that of standard, α-tocopherol.

The total antioxidant capacity of ethanolic extract of *Bauhinia tomentosa* L. was determined by phosphomolybdate method. The inhibitory percentage of 14.10, 30.02, 47.70, 64.02, 80.41 (µg/ml)was observed at various concentrations of 20, 40, 60, 80, 100 (µg/ml) respectively when compared to α-tocopherol 12.10, 27.12, 49.20, 62.12, 77.14 (µg/ml) respectively (Table1).

The peroxy radical scavenging activity of the ethanolic extract of *Bauhinia tomentosa* was determined by the thiocyanate method and compared with α-tocopherol. The absorbance decreased with increasing concentration of the extract, which indicates the extract could effectively decrease the amount of formed peroxides. The ethanolic extract of *Bauhinia tomentosa L.* showed increased values of inhibitory percentage in various concentrations of various intervals (15, 30, 45 min) when compared to α-tocopherol (Table 2).

DISCUSSION

In spite oxygen is essential for life; its transformation to reactive oxygen (ROS) may provoke uncontrolled reactions. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals and by many other mechanisms (Gillman et al., 1977).

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidant towards the stable radical DPPH. From the present results it may be postulated that ethanolic extract of *Bauhinia tomentosa* L. reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donars in the antioxidant principles (Sanchez-Moreno, 2002) and represents the good antioxidant property.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is an intracellular precursor of hydroxyl radicals, which is very toxic to the cell (Halliwell, 1991). Thus, scavenging of H_2O_2 is a measure of the antioxidant activity of the extract. The ethanolic extract of *Bauhinia tomentosa* L. scavenged hydrogen peroxide which may attributed to the presence of phenolic compounds.

The phosphomolybdate method has been routinely used to evaluate the total antioxidant capacity of the extracts (Prieto *et al.,* 1999). In the presence of the extract, the Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum V complex that allows maximum absorbance at 695nm and the extract of *Bauhinia tomentosa* possessed good antioxidant activity.

The amount of peroxide, which is formed, was measured by incubating the extract with linoleic emulsion in dark at 37°C and the amount of peroxides was determined spectrophotometrically by the thiocyanate method (Yen *et al.,* 1993). A decrease in absorbance indicated the antioxidant activity of the ethanolic extract of *Bauhinia tomentosa* L. that might be due to the inactivation of the free radicals.

In conclusion, the results of this study demonstrated that using several invitro models, *Bauhinia tomentosa* was found to have potent antioxidant, and act as scavenger of hydrogen peroxide radical. This activity was found due to the presence of flavanoid and total phenols in the flowers of the plan that quench the DPPH radical. Over all, *Bauhinia tomentosa* L. *may* be considered as a model drug for experimental studies including free radical induced disorders like cancer, diabetes, atherosclerosis etc. Thus the present study supports the view that *Bauhinia tomentosa* L., the traditionally used Indian medicinal plant, is a promising source of potential antioxidants in various diseases.

ACKNOWLEDGEMENT

We acknowledge Dr. V. Dhivakaran for providing facilities to carry out the work.

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Table 1: DPPH radical scavenging assay, H_2O_2 scavenging assay and total antioxidant activity of ethanolic extract of *Bauhinia tomentosa* L.

S.No.	Concentration of the extract and standard (µg/ml)	Inhibitory percentage (µg/ml)					
		DPPH scavenging assay		H_2O_2 scavenging assay		Total antioxidant activity	
		Extract	Standard (Ascorbic acid)	Extract	Standard (α-tocopherol)	Extract	Standard (α-tocopherol)
1.	20	52.2 ± 1.09	58.4 ± 1.21	43.5 ± 0.9	41.3 ± 1.0	14.10 ± 0.09	12.10 ± 0.07
2.	40	66.4 ± 1.03	69.7 ± 1.15	52.3 ± 1.3	50.5 ± 1.3	30.02 ± 0.02	27.12 ± 0.02
3.	60	74.8 ± 0.9	77.3 ± 1.0	64.7 ± 0.8	61.9 ± 0.7	47.30 ± 0.21	44.20 ± 0.02
4.	80	85.6 ± 1.006	86.7 ± 1.09	73.4 ± 1.3	71.2 ± 1.1	64.02 ± 0.12	62.12 ± 0.1
5.	100	93.4 ± 0.92	95.3 ± 0.95	80.3 ± 1.9	77.5 ± 1.8	80.41 ± 0.03	77.14 ± 0.02

Values were expressed as mean ± SD

Table2: Peroxy radical scavenging activity of *Bauhinia tomentosa* L.

S.No	Concentration of the sample and standard (α-tocopherol) (µg/ml)	Inhibitory percentage (µg/ml)					
		15 (min)		30 (min)		45 (min)	
		Extract	Standard	Extract	Standard	Extract	Standard

1.	20	10.3 ± 0.02	11.1± 0.03	21.1 ± 1.0	22.3 ± 1.0	31.1± 1.2	32.1 ± 1.5
2.	40	15.5 ± 0.1	16.3 ± 0.1	25.3 ± 0.7	26.7 ± 0.8	40.2 ± 1.1	41.3 ± 1.2
3.	60	27.1 ± 1.0	28.5 ± 0.9	35.3 ± 1.23	36.5 ± 1.25	53.1 ± 1.4	54.1 ± 1.5
4.	80	29.8 ± 1.08	30.5 ± 1.7	41.0 ± 1.3	42.5 ± 1.4	57.0 ± 2.1	58.0 ± 2.1
5.	100	34.3 ± 0.3	36.2 ± 0.3	47.0 ± 1.4	48.5 ± 01.5	63.3 ± 2.2	64.3 ± 2.3

Values were expressed as mean ± SD