IJBPAS, November, 2013, 2(11): 2130-2136

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC FLOWER EXTRACT OF *SPATHODEA CAMPANULATA P. BEAUV*

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ABSTRACT

The aim of the study is to investigate the antioxidant activity of ethanol extract of flowers of *Spathodea campanulata* was evaluated through total antioxidant, nitric oxide scavenging activity, reducing power assay and hydrogen peroxide scavenging activity. Ascorbic acid was kept as standard. IC_{50} values observed for total antioxidant, nitric oxide scavenging activity, reducing power assay and hydrogen peroxide scavenging activity were 280, 150, 220 and 250µg/ml respectively. The extract showed significant activity in the entire assay when compared to the standard antioxidants. The results clearly indicate that the flower extract of the study species is effective in scavenging free radicals and has the potential to be powerful antioxidant.

Keywords: *Spathodea campanulata*, Nitric Oxide, Hydrogen Peroxide, Ascorbic Acid

INTRODUCTION

Plant and plant products are being used as a source of medicine since long due to their potent antioxidant activities, no side effects and economic viability. Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti inflammatory, anti carcinogenic **[1]**.

Antioxidant is a molecule, which terminate the chain reaction by removing free radical intermediates. Plants and animals maintain complex system of multiple type of antioxidant; the natural plant based antioxidants are playing an important role in the maintenance of human playing an important role in the maintenance of human health for the past decades **[2]**.

Synthetic antioxidants like butylated hydroxytoluene (BHT) have been used as food additives; but recent reports have expressed safety concerns allowing natural antioxidant to be the focus of intense interest. Plants are rich sources for natural antioxidants, the best known are tocopherols, flavonoids, vitamin C and other phenolic compounds **[3]**. Other contributors to the antioxidant activity include alkaloids, proteins, minerals and other vitamins such as the carotenoids and vitamin B_6, B_{12} and K **[4]**. Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities **[5]**.

Spathodea campanulata P. Beauv. species belonging to the Bignoniaceae family. It is a native of West Africa. This spectacular flowering tree is abundantly planted throughout the tropics and has naturalized in many parts of the Pacific. It is a large tree that can reach 50ft in height. The flared, funnel shaped flowers appear in 8 to 10cm long racemes on the tips of the branches**;** leaves grow to 40cm long**;** fruit is a long pod**;** very small seeds with transparent wings. This species has many uses in folk medicine such as; the flowers are employed as diuretic and anti inflammatory, while the leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomach aches and diarrhoea **[6]**.

MATERIALS AND METHODS

Plant Collection and Identification

The Plant species namely *Spathodea campanulata* flowers were collected in Mannargudi and around Thiruvarur (Dt), Tamil Nadu. The plant was identified and authenticated by Dr. Soosairaj, Department of botany, St. Joseph's college, Trichirappalli. [Voucher number of the specimen: SJCBOT 1563/2013].

Preparation of Plant Powder

The flowers were air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder mater was used further *in vitro* Antioxidant analysis.

Extraction of Plant Material

Ethanol and aqueous extracts were prepared according to the methodology of Indian pharmacopoeia. The coarse powder material was subjected to soxhlet extraction separately and successively with Ethanol and distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40°C-50°C) the ethanol and aqueous extracts put in air tight container stored in a refrigerator.

***In vitro* Antioxidant Activity**

- The total antioxidant capacity was evaluated by the phosphomolybdenum method **[2]**.
- Nitric oxide scavenging assay was carried out using sodium nitroprusside **[7]**.
- Assay of reducing power was carried out by potassium ferric cyanide method **[8]**.
- Hydrogen peroxide scavenging activity by the method of **[9]**.

RESULTS AND DISCUSSION

***In vitro* Antioxidant Activity**

In vitro antioxidant activity of the ethanolic flower extract of *Spathodea campanulata* was investigated in the present study by Total antioxidant, Nitric oxide radical scavenging activity, Reducing power assay and Hydrogen peroxide scavenging activity. It is probably due to the presence of respective phytochemicals like flavonoids and phenolics etc in these species **[10, 11]**.

Total Antioxidant Capacity

Total antioxidant activity of ethanolic extract of *Spathodea campanulata* and ascorbic acid values were presented in the **Table 1**. The half maximal inhibitory concentration (IC_{50}) of ethanolic extract and ascorbic acid were found to be 280µg/ml and 250µg/ml respectively. The ethanolic extract has potent total antioxidant activity more than ascorbic acid. The total antioxidant activity of extract of *Spathodea campanulata* was estimated from their ability and being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts **[12]**. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species **[13]**. The statement has been justified in the current study where the methanol extract of *Spathodea campanulata* showed maximum antioxidant capacity.

Nitric Oxide Radical Scavenging Activity

Nitric Oxide Radical Scavenging Activity of ethanolic extract of *Spathodea campanulata* and ascorbic acid values were presented in the **Table 2**. NO is a very unstable species and reacting with oxygen molecule producing stable nitrate and nitrite which can be estimated by using Griess reagent. The half maximal inhibitory concentration (IC_{50}) of ethanolic extract and ascorbic acid were found to be 150µg/ml and 100µg/ml respectively. The ethanolic extract has potent nitric oxide scavenging activity more than ascorbic acid. NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation,

neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and anti tumour activities **[14]**. The increased nitric oxide radical scavenging activity was observed in ethanolic extract of tested plant. Of these plant *Spathodea campanulata* showed better scavenging capacity more than ascorbic acid. The nitric oxide scavenging potentiality may be due to antioxidant principle in the extract which competes with oxygen to react with nitric oxide and thus inhibit the generation of nitrites.

Reducing Power Assay

Reducing power assay of ethanolic extract of *Spathodea campanulata* and ascorbic acid values were presented in the **Table 3**. Reducing power of the extract was assessed using ferric to ferrous reducing activity. The half maximal inhibitory concentration (IC_{50}) of ethanolic extract and ascorbic acid were found to be 220µg/ml and 120µg/ml respectively. The ethanolic extract has potent reducing activity.The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity **[15]**. However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging. For the measurement of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of the ethanolic extract of *Spathodea campanulata* using the method of **[2]**.

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity of ethanolic extract of *Spathodea campanulata* and ascorbic acid values were presented in the **Table 4**. The half maximal inhibitory concentration (IC_{50}) of ethanolic extract and ascorbic acid were found to be 250µg/ml and 210µg/ml respectively. The ethanolic extract has potent scavenging activity. Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of hydrogen peroxide systems may be important **[16]**. H_2O_2 is reactive oxygen metabolic by-product that serves as a key regulator for a number oxidative stresses related states. Functioning through NF kappa B and other factors, Hydrogen peroxide-mediated pathways have been linked to inflammatory, asthma, arthritis, diabetic vasculopathy, atherosclerosis, osteoporosis, and a number of neuro degenerative disease.

Hydrogen peroxide only initiates lipid peroxidation weakly. The ability of plant extract to scavenge H_2O_2 reflects its ability in the prevention and management on asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, and a number of neurodegenerative diseases. A wide variety of phenolic substances derived from edible plants have been reported to retain marked antioxidant and anti-inflammatory activities, which contribute to their chemo preventive potential. Hydrogen peroxide scavenging activity of test plant may be due to their free radical scavenging properties which play an important role in decomposing hydrogen peroxide radicals. In this study, it is evident that the extract of the study species, *Spathodea campanulata* possess effective antioxidant activity.

From the study it is concluded that the ethanolic extract of flowers of *Spathodea campanulata* has a potent antioxidant activity in different *in vitro* models system. Further investigation of individual phytocompounds, their isolation, identification of active compounds and its efficacy needs to be done.

Table 1: Total Antioxidant Activity of Flowers of *Spathodea campanulata*

S. No	Concentration (µg/ml)	Ethanolic Extract		Standard (Ascorbic Acid)	
		% of Inhibition	IC_{50} (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
1.	100	28.86±14.29		38.32±12.08	
2.	200	32.87±15.35	280	45.34±15.10	250
3.	300	52.18±12.21		57.26±15.01	

NOTE: Values are Expressed as Mean ± SD. (P<0.001)

Table 2: Nitric Oxide Radical Scavenging Activity of Flowers of *Spathodea campanulata*

S. No	Concentration (µg/ml)	Ethanolic Extract		Standard (Ascorbic Acid)	
		% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	100	45.62±5.57	150	50.65±8.98	100
2.	200	52.56±4.41		53.92±6.45	
3.	300	59.47±4.13		62.75±9.89	

NOTE: Values are Expressed as Mean ± SD (P<0.001)

Table 3: Reducing Power Assay of Flowers of *Spathodea campanulata*

S. No	Concentration (µg/ml)	Methanolic Extract		Standard (Ascorbic Acid)	
		% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	100	46.65±10.97		49.20±20.41	
2.	200	49.64±13.91	220	54.58±16.48	120
3.	300	53.34±7.30		60.50±13.44	

NOTE: Values are Expressed as Mean ± SD (P<0.001)

Table 4: Hydrogen Peroxide Scavenging Activity of Flowers of *Spathodea campanulata*

S. No	Concentration (µg/ml)	Methanolic Extract		Standard (Ascorbic Acid)	
		% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	100	41.08±11.20	250	43.06±15.49	210
2.	200	44.78±11.21		49.37±11.93	
3.	300	57.91±18.54		58.77±18.58	

NOTE: Values are Expressed as Mean ± SD (P<0.001)

ACKNOWLEDGEMENT

The authors are thankful to the correspondent, Sengamalathayaar Educational Trust Women's College, Sundarakkottai, Mannargudi, for providing the laboratory facilities for research work.

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