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Evaluation of *In Vitro* Antioxidant Activity of *Sateria verticillata* Leaves by Using DPPH Scavenging Assay



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ABSTRACT

Drugs from the plant origin are easily available, less expensive, safe, efficient and rarely have side effects. The compounds such as alkaloids, tannins, flavanoids and phenols have a major role in preventing a number of chronic diseases by a definite physiological action on the human body like antiinflammatory, anti-thrombotic, anti-oxidant, hepatoprotective and anticarcinogenic activities. The aim of present study was to evaluate the undertaken to analyze the antioxidant activity of various extract of *Sateria verticillata* plant. Standard methods were adopted to assess antioxidant activity of the plant materials. The extent of radical scavenging was determined by calculated IC_{50} value. The results revealed that aqueous, ethyl acetate and ethanol extracts showed good antioxidant activity when compared to Ascorbic acid standard.

INTRODUCTION

Oxygen is an essential element for life to perform biological functions such as catabolic and anabolic process of fats, proteins and carbohydrates in order to generate energy for growth and other activities of the cell. Although oxygen is not dangerous by itself, but it is involved in the generation of various kinds of "reactive oxygen species" (ROS). ROS can interact with biomolecules and ultimately lead to free radical chain reactions. Free radical chain reactions are produced in the mitochondrial respiratory chain, liver mixed function oxidase, xanthine oxidase activity, atmospheric pollutants and for transition metal catalysts, drugs and xenobiotics [1, 2]. ROS attacks the unsaturated fatty acids present in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane protein leading to cell inactivation [3], mutation leading to cancers [4]. ROS also leads to pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegenration, Parkinson's diseases, mongolism, ageing process and dementia. Antioxidants are used in the treatment of diseases caused by ROS. Antioxidants are composed of a group of compounds and enzymes potent enough to scavenge free radicals before they cause tissue damage [5]. Vitamin E, vitamin C, carotenoids, natural flavanoids etc. are the natural antioxidants produced in the body while others must be sequestered from the diet or through supplementation. Most antioxidants were found in citrus and dried fruits, cruciferous vegetables, garlic, onions, carrots, tomatoes, sweet potatoes, sesame and olive oil. There are thousands of naturally occurring and synthetic antioxidants known; these antioxidants belong to different classes of compounds and may cause some side effects [6]. Plant secondary metabolites such as phenolic compounds, carotenoid, ascorbic acid, thiols and tocopherols have shown antioxidant activity that includes scavenging free radical species, inhibiting the production of reactive species, inhibiting the production of reactive species resulting from normal cell metabolism. Thereby prevent the damage to lipids, proteins, nucleic acids and subsequent cellular damage and death [7].

Sateria verticillata (family: poacae) commonly known as bristly foxtail and hoocked bristle grass. Parts of the plant are also being used for many disorders like rheumatism, psoriasis and chronic eruptions.

MATERIALS AND METHODS

Collection and authentication

Sateria verticillata were collected from Narsapur, Medak District and authenticated by D. Venkaeswara Rao, Deputy Director, A. P. Forest Academy, Dullapally, Hyderabad, Ranga Reddy Dist.

Processing of plant materials

Each part of the plant was washed under running water to make it free from dust and foreign particles. The plant parts were powdered and kept in air tight container before analysis.

Preparation of Extracts

Powdered plant material (200 gm) was extracted with water, ethanol, and ethyl acetate using cold maceration method. All the extracts were filtered with a muslin cloth and the filtrate was concentrated in vacuum evaporator. Dried extracts were used for further studies [8].

Preparation of standard solution

Required quantity of Ascorbic acid was dissolved in ethanol to give the concentration of 20, $30, 40, 50 \mu \text{g/ml}$.

Preparation of test solution

Stock solutions of samples were prepared by dissolving 10 mg of dried plant extract in 10 ml of ethanol to give concentration of 1 mg/ml. Then prepared sample concentrations of 20, 30, 40, 50 μ g/ml.

Preparation of DPPH solution

3.9 mg of DPPH was dissolved in 3.0 ml ethanol; it was protected from light by covering the test tubes with aluminum foil.

In-vitro antioxidant assay

A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. α,α -diphenyl-6 picrylhydrazyl radical Scavenging assay (DPPH), Ferric reducing antioxidant power (FRAP), Nitric oxide

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Scavenging assay, Superoxide anion radical Scavenging assay, ABTS radical Scavenging assay, hydroxyl radical Scavenging assay, are the *in vitro* antioxidant assay methods used to assess the antioxidant activity of the leaves extracts of *Sateria verticillata*. The absorption maximum of a stable DPPH radical in method was at 517 nm. The decrease in absorption of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecule and radical progresses, results in the Scavenging of the radical by hydrogen donation.

Statistical analysis

All data were expressed as mean \pm SD. Statistical analysis was performed by One-way ANOVA using Origin version 6.0 software and p<0.05 and p<0.001 was considered as statistically significant.

RESULTS AND DISCUSSION

These diverse groups of compounds have potential of natural antioxidant and have ability to act as both efficient radical scavengers. The antioxidant activity of phenols is due to their redox properties, hydrogen donors and singlet oxygen quenchers [13]. The antioxidative characteristics might be attributed to the presence of phytochemical such as flavanoids and other phenolic compounds. Polyphenols have been known to show medicinal activity as well as exhibiting physiological activity. The compounds such as flavanoids; which contain hydroxyls are responsible for the radical scavenging activity in plant [10, 9, 2,]. The reducing capacity of a compound may be used as a significant indicator of its potential antioxidant activity [11]. Reducing power is to the measure of the reductive ability of antioxidant and it is judged by the transformation of Fe^{3+} to Fe^{2+} in the presence of extracts [12]. The reduction power of aqueous and other extracts was summarized in Table1. The data showed that reducing power of the extracts increased with increased concentration of extracts. The extracts showed potent ferric reducing power.

The result of DPPH scavenging activity assay in this study indicates the ethyl acetate extract was potentially active. The aqueous and other extracts produced more or less similar DPPH anion scavenging power of $44.36\pm2.09\%$ ethyl acetate & $40.12\pm5.36\%$ ethanol extract at 50 µg/ml concentration with 92.648 ± 30.68 µg/ml of IC₅₀ for ethyl acetate extract & 106.15 ±25.33 µg/ml of IC₅₀ value for ethanol extract and 63.99 ± 25.24 µg/ml for Ascorbic acid (Table 1). The scavenging activity of ethyl acetate soluble fractions compared with the standard drug ascorbic acid suggests that the plant phytochemicals are a potent scavenger of

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free radicals. However further study aimed at characterization of active constituents responsible for antioxidant activity. Overall, the ethyl acetate extract of *Sateria verticiilata* Linn leaves have most potent antioxidant activity.

Table 1.	In	vitro	free	radical	scavenging	effect	of	Sateria	verticillata	leaves	by	DPPH
method:												

Extracts		IC ₅₀ µg/ml					
	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml			
Aqueous	31.23±4.26**	33.32±4.81*	34.62±3.04*	37.73±4.46*	84.54±4.62		
Ethanol	21.33±2.08**	25.08±1.00*	30±4.35**	44.36±2.09**	92.648±30.368		
Ethyl acetate	13.40±4.01**	22.48±2.04**	28.35±8.95**	29.25±9.47**	106.158±25.332		
Standard	4.95±6.85**	12.77±6.96**	27.96±1.41*	27.72±3.77**	63.997±25.244		

Significant at *p=<0.05; **p=<0.001

CONCLUSION

On the basis of the results it is concluded that the extracts contain higher quantities of phenolic compounds, which exhibit antioxidant and free radical scavenging activity. It also chelates iron and possesses reducing power. *In vitro* assay systems confirm *Sateria verticillata* leaves as natural antioxidants. Further *in vivo* assessment also needed to confirm the antioxidant nature of *Sateria verticillata* leaves.

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