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Phytochemical Screening and *In Vitro* Anti-Oxidant Activity of *Alternanthera Pungens*

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ABSTRACT

The aim of this article is to evaluate antioxidant activity of leaf extract of *Alternanthera pungens* by using *in vitro* assay. Extraction was carried out with ethanol extract by using Soxhlet apparatus. The *in vitro* antioxidant activity ethanol extract has been investigated by 1, 1-diphenyl, 2-picryl-hydrazyl free radical (DPPH) method. The ethanol extract exhibited maximum antioxidant activity. The results have been compared with the standard ascorbic acid.

KEYWORDS

Antioxidant activity, DPPH, Free radicals, Ethanol extract

INTRODUCTION

Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [1]. Human body has an inherent antioxidative mechanism and many of the biological functions such as the anti-mutagenic, anti-carcinogenic and anti-aging responses originate from this property [2,3]. The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immune suppression and neurodegeneration [4].

Antioxidant activities increase proportionally with the polyphenol content, primarily because of their redox properties [5]. Among the diverse roles of polyphenols, they protect cell constituents against destructive oxidative damage, thus limiting the risk of various degenerative diseases associated with oxidative stress by acting as potent free radical scavengers. The polyphenol antioxidant activity is due to the chemical structure and ability to donate/accept electrons, thereby delocalizing the unpaired electron within the aromatic structure [6]. In addition to plant extracts, numerous naturally occurring compounds are useful as antioxidants, ranging from α-tocopherol and β-carotene to plant antioxidants such as phenolic compounds, alkaloids, and organic sulfur compounds [7]. Thus, antioxidant activity of *Alternanthera pungens* was evaluated.

Plant collection and authentication

The leaves of the plant *Alternanthera pungens* were collected in the month of March in Narsapur, Medak District, Telangana, India. The plant was authenticated by M. Malla Reddy (M.Sc, M.Phil in Botany), Retired lecturer in Botany, Vikarabad, Telangana.

Material used

In the present study 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and alcohol used during investigation of antioxidant activity. All the material was used in lab oratory grade.

Preparation of plant extract

The leaves of *Alternanthera pungens* were shade dried and crushed into powder and sieved to get a coarse powder. The powder was subjected to Soxhlet extract ion using ethanol for 72 h. The solvent was evaporated using rotary evaporator then the extract was used for the evaluation of antioxidant activity.

Preliminary phytochemicalanalysis

The ethanol extract were screened for the phytochemical constituents using the standard method (Table 1).

Table 1: Phytochemical screening of ethanolic extract of *Alternanthera pungens*

S. No.	Name of phytochemical	Alternanthera pungens
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponin	+
5	Phenolic compounds	+
6	Steroids	+
7	Glycosides	+
8	Quinone	+
9	Acid	-
10	Coumorin	-
11	Triterpenoid	-
12	Carbohydrate	+
13	Protein	+
14	Amino acid	+
15	Fat	+

+ indicates present; - indicates absent

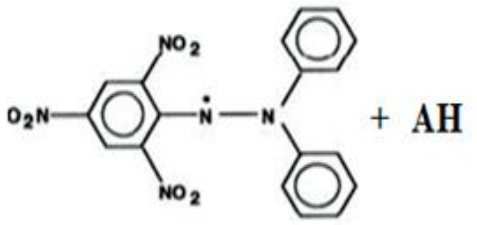
ANTIOXIDANT ACTIVITY

DPPH Scavenging activity

The molecule 1,1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

Procedure

The free radical scavenging activity of all the samples was evaluated by DPPH according to the previously reported method by Shen et al., 2010. Briefly, a 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of the solution of all samples in ethanol at different concentration (1, 2, 3, 4, 5 & 10 µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.



Diphenylpicrylhydrazyl (Free radical)

DPPH scavenging effect (% inhibition) = {(A0 – A1)/A0)*100}

Where, A0 is the absorbance of the control reaction, A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged [8].

Determination of IC_{50}

The IC_{50} value (The concentration of sample required to scavenge 50% of DPPH free radicals) was determined by interpolation from the calibration curve plotted between percentage inhibition and sample concentration and expressed as µ/ml.

RESULTS AND DISCUSSION

Antiradical activity assay is based on the reduction of DPPH. Due to the presence of an odd electron it gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured. The decomposition of DPPH free radicals by *Alternanthera pungens* may at least partly result from its antioxidant and free radical scavenging activity (Table 2).

Table 2: DPPH free radical scavenging activity

Concentrations (µg/ml)	Standard percent scavenging (%)	Ethanol extract percent scavenging (%)
20	91	84
40	92	86
60	94	88
80	96	89
100	97	91

CONCLUSION

The results obtained in the present study indicate that *Alternanthera pungens* ethanolic extract exhibit significant free radical scavenging and antioxidant activity. The overall antioxidant activity might be attributed to its phytochemical constituents. The findings of the present study suggest that, this *Alternanthera pungens* could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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