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IN-VITRO ANTIOXIDANT STUDIES AND TOTAL PHENOLIC CONTENT OF ETHANOLIC EXTRACT OF *ALTERNANTHERA SESSILIS* LINN. AERIAL PARTS

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Abstract:

Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, ageing etc. In the treatment of these diseases, antioxidant therapy has gained utmost importance. Ethanolic extract of Alternanthera sessilis (EEAS) was studied for its in vitro antioxidant activity using different models viz. Free radical scavenging activity assay (DPPH method), Reducing power assay, Nitric oxide scavenging activity, Superoxide radical scavenging activity and Hydroxyl radical scavenging activity. Total phenolic content was determined by using Pyrocatechol as a standard. The results were analyzed statistically by the regression method. Its antioxidant activity was estimated by IC_{50} value and the values are 74.05 ± 1.44 µg/ml (Superoxide radical scavenging), 31.50 ± 1.04 µg/ml (Hydroxyl radical scavenging activity), 28.75 ± 0.07 µg/ml (Nitric oxide scavenging) and 86.83 ± 1.09 µg/ml (DPPH radical scavenging). In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. These results clearly indicate that Alternanthera sessilis is effective against free radical mediated disease.

Keywords: *Alternanthera Sessilis, DPPH method, Total Phenolic Content, Nitric Oxide, Reducing Power, Hydroxyl Radical.*

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INTRODUCTION

Oxidative stress has been implicated in the pathology of many diseases such as inflammatory conditions, cancer, diabetes and aging [1]. Free radicals induced by peroxidation have gained much importance because of their involvement in several pathological conditions such as atherosclerosis, ischemia, liver disorder, neural disorder, metal toxicity and pesticide toxicity [2]. Together with other derivatives of oxygen, they are inevitable byproducts of biological redox reactions. Reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydroxyl radical (OH^-) and nitric oxide (NO) inactivate enzymes and damage important cellular components causing injury through covalent binding and lipid peroxidation [3]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent diseases [4]. Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases, cancer, neurodegenerative diseases, inflammation and problems caused by cell and cutaneous aging [5].

Alternanthera sessilis Linn. (Amaranthaceae) is an annual or perennial prostate herb with several spreading branches, bearing short petioled simple leaves and small white flowers, found throughout the hotter part of India, ascending to an altitude of 1200m. The plant spreads by seeds, which are wind and water-dispersed and by rooting at stem nodes. Young shoots and leaves are eaten as a vegetable in Southeast Asia[6]. It is a weed of rice throughout tropical regions and of other cereal crops, sugarcane and bananas. Although it is a weed, it has many utilities. The leaves were used in eye infections, cuts, antidote to snake bite and skin diseases [7]. It was also reported that the plant *Alternanthera sessilis* shown wound healing property [8]. The degenerative and necrotic changes in the liver and kidney in Swiss mice, caused by oral administration of water extract of *A. sessilis* in high doses through histopathological test were revealed [9].

The aim of this study was to investigate the antioxidant properties of ethanolic extracts of aerial parts of *Alternanthera sessilis* against the free radicals.

MATERIALS AND METHODS

Plant Material

The plant was identified by the botanists of the VR College, Nellore, Andhra Pradesh. After authentication, fresh aerial parts of the young and matured plants were collected in bulk from the rural belt of Jangalakandriga, Nellore, Andhra Pradesh, India during early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder.

Preparation of Plant Extracts (EEAS)

The powdered plant material (400gm) was defatted with petroleum ether (60-80^{0}C) and then extracted with 1.5 litre of ethanol (95%) in a Soxhlet apparatus. The solvent was removed under reduced pressure, leaving a greenish-black sticky residue (yield: 14.8% w/w with respect to dried plant material). The dried extract (EEAS) was stored in a desiccators till needed.

Chemicals Used

All the chemicals and reagents used in the study were of analytical grade.

Estimation of Total Phenolic Content

0.1ml of 10mg/ml aqueous solution of the extract was diluted with 46 ml of distilled water in an Erlen meyer flask. Afterwards, 1 ml of Folin-Ciocalteu Reactive (FCR) was added into this mixture followed by addition of 3 ml of Na_2CO_3 (2%) after 3 min. Subsequently, mixture was shaken on a shaker for 2 h at room temperature and then absorbance was measured at 760nm. The concentration of total phenolic compounds was determined as micrograms of pyrocatechol equivalent by using the equation that was obtained from the standard pyrocatechol graph [10].

In-Vitro Antioxidant Activity Study

- ***Reducing Power assay***

The reducing power of the ethanolic extract of *Alternanthera sessilis* was determined according to the method of Oyaizu. Accurately weighed 10 mg of the extract in 1 ml of distilled water were mixed in to the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50^{0}C for 20 min. Following incubation, 2.5 ml of 10% trichloro acetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power [11].

- ***Assay for Superoxide Radical Scavenging Activity***

The assay for superoxide radical scavenging activity was performed as per standard procedure. The reaction mixture contained 50 mM phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA and 0.1 mg/ml of NBT (Nitro blue tetrazolium), all added in chronological sequence. Reaction was started by

illuminating the reaction mixture containing different concentrations of the sample extract for 90 sec and then measuring the absorbance at 590nm. Ascorbic acid was taken as the positive standard [12].

- ***Hydroxyl Radical Scavenging Activity***

The scavenging capacity for hydroxyl radical was measured according to the modified method of [13]. The assay was performed by adding 0.1 ml of 1mM EDTA, 0.01 ml of 10 mM $FeCl_3$, 0.1 ml of 10 mM H_2O_2, 0.36 ml of 10 mM deoxyribose, 1.0 ml of different dilutions of the extract (10-100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation and is calculated as,

OH- scavenged (%) = [Abs (control) – Abs (standard) / Abs (control)] × 100.

Where, Abs (control): Absorbance of the control reaction and

Abs (standard): Absorbance of the extract/standard.

- ***Assay for Nitric Oxide Scavenging Activity***

For this study, Sodium Nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations of the extract dissolved in methanol and incubated at room temperature for 2½ h. The same reaction without the sample but equivalent amount of methanol served as control. After incubation period, 0.5 ml of Griess reagent was added. Absorbance of the chromophore formed was measured at 546 nm. Ascorbic acid was used as positive control. The procedure is based on the principle that, Sodium nitroprusside solution spontaneously generates nitric oxides, which reacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxides compete with oxygen leading to reduce production of nitrite ions [14].

Free Radical Scavenging Activity (DPPH method)

The antioxidant activity of the plant extracts and standard were assessed on the basis of the radical scavenging effect of the stable DPPH free radical [15]. About 10-100 µg/ml of extract and standard was added to 2 ml of DPPH in methanol (0.33%) in a test tube. After incubation at 37°C for 30 minutes the absorbance of each solution was determined at 517 nm using spectrophotometer [16]. The corresponding blank reading were also taken and the remaining DPPH was calculated by using the following formula,

DPPH radical scavenging activity (%) = [Abs (control) – Abs (standard)] × 100.

Where, Abs (control): Absorbance of DPPH radical + methanol

Abs (standard): Absorbance of DPPH radical + extract/standard.

IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

RESULTS

Total Phenolic Content

The study revealed that 1mg of ethanolic extract of *Alternanthera sessilis* contains 42.5 µg of pyrocatechol equivalent.

Reducing power assay

The result in Table 1 shows the reductive capabilities of ethanolic extracts of *Alternanthera sessilis* aerial parts when compared to standard Ascorbic acid. The reducing power increased significantly with increasing concentration. However, the activity of the extract was less than the standard.

Assay for Superoxide Radical Scavenging Activity

It has been observed from the result of Table 2 that the extract reduced the absorbance in a dose dependent manner and the IC_{50} value of extract and standard were calculated as 74.05 ± 1.44 µg/ml and 34.24 ± 0.35 µg/ml from the regressional line, which is comparable with the reference drug.

Hydroxyl Radical Scavenging Activity

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins [17]. This assay shows the abilities of the extract and standard to inhibit hydroxyl radical-mediated deoxyribose degradation in Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture. The result in Table 3 shows that the extract has hydroxyl radical scavenging activity (H_2O_2) when compared with Ascorbic acid. The IC_{50} values of the extract and standard in this assay were 31.50 ± 1.04 µg/ml and 27.92 ± 0.12 µg/ml.

Assay for Nitric Oxide Scavenging Activity

Table 4 shows that the ethanolic extract of *Alternanthera sessilis* significantly decreased with IC_{50} value 28.75 ± 0.07 µg/ml, in a dose dependent manner, the concentration of nitrite after spontaneous decomposition of sodium nitroprusside, indicating that the ethanolic extract may contain compounds that are able to scavenge nitric oxide.

Free Radical Scavenging Activity (DPPH method)

Results of the DPPH radical scavenging activity was shown in Table 5 can be used to determine free radical scavenging activity as it forms a stable molecule on accepting an electron or hydrogen atom. The extract and standard reduced DPPH to yellow in concentration dependent manner. The IC_{50} values of extract and standard was found to be 86.83 ± 1.09 µg/ml and 26.80 ± 0.71 µg/ml. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [18]. The study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

DISCUSSION

Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structures and functions. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, and immune system decline and brain dysfunction [19]. Overall, free radicals have been implicated in the pathogenesis of various diseases. Fortunately free radical formation is controlled naturally by various beneficial compounds known as antioxidants.

Antioxidants fights against free radicals by protecting from various diseases and scavenges of reactive oxygen radicals or protects the antioxidant defense mechanism. Reactive oxygen species (ROS) are capable of damaging biological macromolecules such as DNA, Carbohydrates, and Proteins etc. ROS is a collective term, which includes not only oxygen radical (O_2 and OH) but also some non-radical derivatives of oxygen like H_2O_2, HOCl and ozone (O_3). If human disease is believed to be due to the imbalance between oxidative stress and antioxidative defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements [20]. Phenols are very important plant constituents because of their free radical scavenging ability due to their hydroxyl groups [21]. The phenolic compounds may contribute directly to antioxidant action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in human beings [22]. Ethanolic extract of *Alternanthera sessilis* is found to contain phenolic compound in significant amount, which attributes to its rationality of possessing antioxidant activity.

The transformation of Fe^{3+} to Fe^{2+} in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductones (Antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating hydrogen [23]. The antioxidant principle present in the extract caused the reduction of Fe^{3+}/ferricyanide complex to the ferrous form and thus proved the reducing power ability.

The *in vitro* superoxide radical scavenging activity is measured by riboflavin/ light/ NBT (Nitroblue tetrazoline) system reduction. The method is based on generation of superoxide radicals by auto oxidation of riboflavin in the presence of light. The superoxide radical reduces NBT to a blue colored formazone that can be measured at 560 nm. Superoxide radical is known to be very harmful to the cellular components as a precursor of more ROS [24]. The extract has been found to have significant superoxide radical scavenging activity, which ultimately adds to its antioxidant potential.

Hydroxyl radical is the most deleterious and reactive among the ROS and it bears the shortest half-life compared with other free radicals. The oxygen derived hydroxyl radicals along with the added transition metal on (Fe^{2+}) causes the degradation of deoxyribose into Malondialdehyde which produces a pink chromogen with thiobarbituric acid [25]. The extract when added to the reaction mixture, scavenges the hydroxyl radicals and prevented the degradation of deoxyribose.

Nitric oxide (NO) exhibits numerous physiological properties and it is also implicated in several pathological states [26]. However, the specificity of this assay has been questioned since nitrite is one final product of the reaction of nitric oxide with oxygen, through intermediates such as NO_3, N_2O_4 and N_2O_3 [27]. Therefore the decrease in the nitrite production could also be due to interaction of the extract with other nitrogen oxides [28].

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extract [29]. DPPH (2,2´-diphenyl-1-picrylhydrazyl) is a stable, nitrogen centered free radical which produces violet color in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl group. The extract showed significantly higher inhibition percentage and positively correlates with total phenolic content.

On the basis of the results obtained in the present study, it is concluded that an ethanolic extract of *Alternanthera sessilis* Linn. aerial parts, which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. It also has high reducing power ability. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

CONCLUSION

The results of the present study show that the extract of *Alternanthera sessilis* contains the highest amount of polyphenol compounds and exhibits the greatest antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing.

RESULTS

Table: 1. Reducing Power ability of Ethanolic Extract of *a. Sessilis* linn. Aerial parts

Sl. No.	Sample	% inhibition			
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
1.	Ethanolic Extract	0.017 ± 0.03	0.120 ± 0.029	0.159 ± 0.042	0.231 ± 0.047
2.	Ascorbic Acid	0.713 ± 0.005	0.839 ± 0.003	0.911 ± 0.008	1.190 ± 0.110

Values are expressed as mean ± SEM of three parallel measurements,
p<0.001 when compared with the standard Ascorbic acid.

Table: 2. *In-Vitro* Superoxide Radical Scavenging Activity of Ethanolic Extract of *A. Sessilis* Linn. Aerial Parts

Sl. No.	Sample	Concentration (µg/ml)	% inhibition	IC_{50}
1.	Ethanolic Extract	12.50	12.03 ± 2.06	74.05 ± 1.44
2.		25.00	19.09 ± 1.69	
3.		50.00	32.25 ± 2.15	
4.		100.00	51.09 ± 4.25	
5.	Ascorbic Acid			34.24 ± 0.35

Values are expressed as mean ± SEM of three parallel measurements,
p<0.001 when compared with the standard Ascorbic acid.

Table: 3 *In-Vitro* Hydroxyl Scavenging Activity Ethanolic Extract of *a. Sessilis* Linn. Aerial Parts

Sl. No.	Sample	Concentration (µg/ml)	% inhibition	IC_{50}
1.	Ethanolic Extract	10.00	24.13 ± 0.13	31.50 ± 1.04
2.		20.00	45.10 ± 0.07	
3.		40.00	55.61 ± 0.06	
4.		80.00	64.41 ± 0.07	
5.		100.00	76.59 ± 0.08	
6.	Ascorbic Acid			27.92 ± 0.12

Values are expressed as mean ± SEM of three parallel measurements,
p<0.001 when compared with the standard Ascorbic acid.

Table: 4. *In-Vitro* Nitric Oxide Scavenging Activity of Ethanolic Extract of *a. Sessilis* Linn. Aerial Parts

Sl. No.	Sample	Concentration (µg/ml)	% inhibition	IC_{50}
1.	Ethanolic Extract	10.00	12.92 ± 0.09	28.75 ± 0.07
2.		20.00	20.15 ± 0.07	
3.		40.00	38.28 ± 0.04	
4.		80.00	52.94 ± 0.06	
5.		100.00	72.32 ± 0.05	
6.	Ascorbic Acid			15.02 ± 0.11

Values are expressed as mean ± SEM of three parallel measurements,
p<0.001 when compared with the standard Ascorbic acid.

Table: 5. *In-Vitro* DPPH Scavenging Activity Ethanolic Extract of *A. Sessilis* Linn. Aerial Parts

Sl. No.	Sample	Concentration (µg/ml)	% inhibition	IC_{50}
1.	Ethanolic Extract	10.00	16.66 ± 0.45	86.83 ± 1.09
2.		20.00	26.47 ± 0.36	
3.		40.00	37.56 ± 0.17	
4.		80.00	46.98 ± 0.16	
5.		100.00	60.98 ± 0.46	
6.	Ascorbic Acid			26.80 ± 0.71

Values are expressed as mean ± SEM of three parallel measurements,
p<0.001 when compared with the standard Ascorbic acid.

REFERENCES

1. Marx JL. Oxygen free radicals linked to many diseases. Sciences, 1987; 235:529-531.
2. Pandey S, Sharma M, Chaturvedi P, Tripathi B. Protective effects of *Rubia cordifolia* on lipid peroxide formation in isolated rat. Indian J Exp Biol, 1994; 32:180-183.
3. Arora A, Sairam RK, Srivastava GC. Oxidative stress and antioxidant system in plants. Curr Sci, 2002; 82:1227-1238.
4. Geesin JG, Gordon JS, Berg RA. Retinoid affect collagen synthesis through inhibition of ascorbate induced lipid peroxidation in cultured human dermal fibroblast. Arch Biochem Biophys, 1990; 278:352-357.
5. Youdim KA, Joseph JA. A possible emerging role of phytochemicals in improving age related neurological dysfunctions - a multiplicity of effects. Free Rad Biol Med, 2001; 30:583-594.
6. Scher J. Federal Noxious Weed disseminates of the U.S. Center for Plant Health Science and Technology, Plant Protection and Quarantine, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, 2005; 54(1):291-300.
7. Gupta A. 2004. Indian Medicinal Plants. ICMR, New Delhi CBS Publisher and Distributors.
8. Paridhavi PK, Sunil SJ, Nitin A, Patil MB, Chimkode R, Tripathi A. Wound Healing of *Alternanthera sessilis*. International Journal of Green Pharmacy, 2008; 2:141-144.
9. Gayathri BM, Balasuriya K, Gunawardena GSPS, Rajapakse RPVJ, Dharmaratne HRW. Anti-diuretic activity study of *Alternanthera sessilis* on Swiss mice. Research Communications Current Science, 2006; 91:1517-1520.
10. Naczk M, Shahidi F. The effect of methanol-ammonia- water treatment on the content of phenolic acids of Canola. Food Chem, 1989; 31:159-164.
11. Oyaizu M. Studies on products of browning reaction: Antioxidative activities of products

of browning reaction prepared from glucosamine. Jpn J Nutr, 1986; 44:307-315.
12. Beuchamp C, Fridovich I. Superoxide dismutase: Improved assays and assay applicable to acrylamide gels. Anal Biochem, 1971; 44:276-277.
13. Halliwell B, Gutteridge JMC. The deoxyribose method: a simple "test-tube" assay for determination of rate constant for reactions of hydroxyl radicals. Anal Biochem, 1987; 165:215- 219.
14. Sreejayan R, Rao MNA. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol, 1958; 49(6):105-107.
15. Blois MS. Antioxidant determinations by the use of stable free radical. Nature, 1958; 181:1199-1200.
16. Hwang BY, Kim HS, Lee JH, Hong YS, Ro JS, Lee KS. Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. J Nat Prod, 2001; 64:82-84.
17. Spencer JP, Jenner A, Aruoma OI, Evans PJ, Kaur H, Dexter DT. Intense oxidative DNA damage promoted by L-dopa and its metabolites: Implications for neurodegenerative disease. FEBS Lett, 1984; 353:246-250.
18. Shirwaikar A, Prabhu KS, Punitha ISR. *In-vitro* antioxidant studies of *Sphaeranthus indicus* (Linn). Indian J Exp Biol, 2005; 44(3):993-996.
19. Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress and aging. Free Radical Biol and Med, 2000; 29:222-230.
20. Govindarajan R, Vijaykumar M, Rao CV, Shirwaikar A, Rawat AKS, Malhotra S. Antioxidant potential of *Anogeissus latifolia*. Biol Pharm Bullet, 2004; 27:1266-1269.
21. Hatano T, Edamaysu R, Mori A, Fujita Y, Yasuhara E. Phenols and its free radical scavenging ability. Chem Pharm Bull, 1989; 37:2016-2021.
22. Tsao R, Akhtar MH. Nutraceuticals and functional foods: Current trend in phytochemical antioxidant research. J Food Agri Environ, 2005; 3:10-17.
23. Meir S, Kanner J, Akiri B, Haldar SP. Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves. J Agri Food Chem, 1995; 43:1813-1817.
24. Halliwell B, Gutteridge JMC. 1991. Free radicals, ageing and disease. Free radicals in Biology and Medicine. Clarendron Press; Oxford.
25. Halliwell B, Gutteridge JMC. 1999. Free radicals in Biology and Medicine, University Press; Oxford.
26. Marletta MA. Nitric oxide: Biosynthesis and biological significance. Trends Biol Sci, 1989; 14:488-492.
27. Marcocci I, Poacker L, Droy Lefaix M, Guardes S, Albert M. Reaction of nitric oxide with oxygen. Meth Enzymol, 1994; 5:234-242.
28. Baheti JR, Kumar V, Shah GB, Goyal RK. Nitrite production and interaction of the extract with nitrogen oxides. J Nat Rem, 2005; 5:15-21.
29. Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picryl hydrazyl radical. Free Radical Biol and Med, 1996; 21(5):895-902.