Research Article

Antioxidant activity of ethanolic and aqueous extracts of *Alternanthera pungens* Kunth

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Abstract

Objective: The objective of present work to evaluates in-vitro antioxidant activities of the ethanolic and aqueous extracts of *Alternanthera pungens* Kunth. **Material and methods:** The antioxidant activity and phenolic contents of the whole plant were determined by the DPPH and ABTS scavenging method. The DPPH and ABTS scavenging activity were compared with standard Ascorbic acid and BHT. **Results:** The DPPH assay revealed that at a conc. of 100 mg/ml, the scavenging activity of ethanolic extract reached 61.92 % while at the same conc., that the aqueous extract was 30.90 %. The effect of antioxidants on DPPH is due to their hydrogen donating ability. Though the DPPH radical scavenging abilities of the extracts were less than those of ascorbic (77.27 %) and BHT (96.12 %) at 100 mg/ml, the ethanolic extracts have the proton- donating ability and could serve as free radical inhibitors or scavengers. **Conclusion:** The ethanolic extract demonstrates potent antioxidant activity in different concentration. The total phenolic content in plant may play a major role as an antioxidant. The result of this study shows that the ethanolic extract can be used as easily accessible source of natural antioxidants and as possible food supplement.

***Keywords*:** Antioxidant, *Alternanthera pungens* Kunth, DPPH and ABTS scavenging method, ascorbic acid

Introduction

Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism which is called a Reactive oxygen species (ROS). which include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH), as well as non-free radicals species (H_2O_2) and the singled oxygen ($^1O_2$) (Yildrim et al., 2001; Gulcin et al., 2002). Also excessive generation of ROS, induced to variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer (Kourounakis et al., 1999). Exogenous sources for generation of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Yildrim et al., 2001; Davies et al., 1994). Therefore, ROS can cause lipid peroxidation in foods, leading to their deterioration. In addition, these ROS can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxidation. The peroxidation products and their secondary oxidation products such as malondialdehyde and 4-hydroxyinonenal can react with biological substrates such as protein, amines, and deoxyribonucleic acid (Kehrer et al., 1994). As a result of this, much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals. A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties. Sources of natural antioxidant are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Mathew et al., 2006; Charde et al., 2011).

Alternanthera pungens Kunth (family-Amarathaceae, Syn. *Achyranthes repens* L, *Alternanthera repens* L.) A spiny prostrate branched weed of roadside which form mat like structure on waste land and arid open regions. Branches, 25-50 cm long. Leaves of the same pair unequal, obliquely elliptic to orbicular, with flowers and fruits through the year. The whole plant contains choline, oleanolic acid and β-spinasterol (Wealth of India). The flower on steam distillation, yielded (0.6 %) a pale yellow volatile oil having the following physicochemical constituent α-pinene, β-pinene, camphene, myrcene, π-cymene limonene, β-ocimene, cineole etc. (Gupta et al., 1987). The whole plant is used in gastric, hepatic and intestinal disturbances (such as dyspepsia, sensitive, secretory and motor symptoms). The aqueous extract is found to exhibit spasmogenic properties. It

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was found that some species of Alternanthera was used as anti-diabetic agent in remote area by local healer the results of present study of antioxidant of ethanolic and aqueous extract of *Alternanthera pungens* Kunth were presented. The findings of present work may add to the overall value of the medicinal potential of the plant (Kritikar et al., 1994).

Material and methods

Plant collection and preparation

The plant was collected from Jabalpur district, Madhya Pradesh, India during August- September 2018. This species forms dense mats of stems and leaves in rainy season. It was collected freshly and authenticated by the Dept. of Pharmacognosy of Oriental University; Indore M.P. Voucher samples were deposited in the herbarium for reference. It was dried under shade and pulverized into coarse powder with mechanical grinder .The powder was passed through sieve no.30 and kept in polythene bags at room temperature (25^0 C) for further extraction Process.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl(DPPH), 2,2'-azinobis-3-ethylbenzothiazoline -6-sulfonic acid (ABTS), potassium ferricyanide, catechin, butylated hydroxytoluene (BHT) and ascorbic acid were purchased from Sigma Aldrich Chemicals Pvt Ltd, Mumbai, India. Folin-Ciocalteus's phenol reagent and sodium carbonate were purchased from Merck Chemical. All the chemicals used including solvents were of analytical grade.

Extraction of plant material

The dried coarse powder was defatted with petroleum ether (60-80˚c) in a Soxhlet apparatus by continuous hot Soxhlet apparatus. The defatted powder material thus obtained was further extracted with ethanol (95% v/v) with same method and fresh powder was used for aqueous extraction by Cold maceration method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by rotary flash evaporator. Qualitative analysis of extracts was carried out to find out the presence of various phytoconstituents (Mourya et al., 2017).

Determination of total phenolic content

The total phenolic content in ethanolic and aqueous extracts was determined by colorimetric method with Folin–Ciocalteu reagent (Wolfe et al., 2003). A reaction mixture contain 500 mL of 0.1% aqueous dilution of both extracts, 2.5 mL of freshly prepared 0.2M FC reagent and 2 mL of sodium carbonate solution. The mixture was kept in the dark under ambient conditions for 30 min to completion of reaction. Absorbance of the resulting solution was measured at 760 nm in a UV–Vis spectrophotometer (Shimadzu, USA). The total phenolic content was expressed as mg of gallic acid equivalents per gram of extracts, using a standard curve of gallic acid.

Total flavonoids content

Total flavonoids content from ethanol and aqueous extract was determined by aluminum chloride colorimetric assay method (Ordon et al., 2006). A test tube containing 0.3 mL of extract, 3.4 mL of 30% methanol, 0.15 mL of NaNO2 (0.5 M) and 0.15 mL of AlCl3.6H2O (0.3 M) was shake up to complete mixing. One milliliter of NaOH (1 M) was added after 5 min, with mixing well and the absorbance was measured at 510 nm. The standard curve of quercetin (Sigma Aldrich Chemicals Pvt Ltd) was made and the total flavonoids content was expressed as milligrams of quercetin equivalents per 100 gm of dried extract.

Antioxidant activity

ABTS (2, 2-azinobis-3-ethybenzothiazoline-6-sulfonic acid) radical scavenging assay

The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then dilute by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) is allow to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer (Re et al., 1999). The ABTS scavenging capacity of the extract was compare with that of BHT and percentage inhibition calculate as:

ABTS radical scavenging activity (%)

$= [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

Where $Abs_{control}$ is the absorbance of ABTS radical + Ethanol; Abs_{sample} is the absorbance of ABTS radical + sample extract /standard.

DPPH Radical scavenging assay

The effect of the extracts on DPPH radical was estimated using the method of Liyana- Pathiranan and Shahidi (Liyana et al., 2005; Shukla et al., 2014). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in ethanol containing 0.02–0.1 mg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and BHT were used as references. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%)

$= [(Abs_{control} - Abs_{sample})]/ (Abs_{control})] \times 100$

Where, $Abs_{control}$ is the absorbance of DPPH radical + Ethanol; Abs_{sample} is the absorbance of DPPH radical + sample extract /standard.

Statistical analysis

The experimental results were expressed as mean± standard deviation (SD) of three replicates i.e. n =3. Where applicable, the data were subjected to one way analysis of variance (ANOVA).

Results and discussion

Results obtained in the present study revealed that the level of these phenolic compounds in the ethanolic and aqueous extracts of whole of Alternanthera Pungens were considerable (Table 1). Polyphenolic compounds (Okudu et al., 1994, Tepe et al., 2006). This activity idea believed to be mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng et al., 2001). In fact, many medicinal plants contain large amounts of antioxidants such as polyphemols. Many of these phytochemical posses' significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson et al., 2001, Djeridane et al., 2006). The results strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

Table 1. Polyphenol contents of the ethanolic and aqueous extract of whole plant of *Alternanthera pungens* Kunth.

Phenolic	Ethanolic extract	Aqueous extract
Total polyphenol	16.43± 0.32	14.04±0.12
Flavonoids	2.09 ± 0.06	0.91± 0.07

ABTS (2, 2-azinobis-3-ethybenzothiazoline-6-sulfonic acid) scavenging activity

The ethanolic and aqueous extract of *Alternanthera pungens* Kunth were fast and effective scavengers of the ABTS radical (Table 2) and this activity was comparable to that of BHT. At 100 mg/ml, the extracts exhibited good activity but when compare to BHT it has less activity. The Percentage inhibition was found to be a graded manner as the concentration of extracts increase the percentage inhibition also increase. At conc. 20 mg/ml the percentage inhibition of ethanolic and aqueous extract was 36.30 and 25.53 respectively and at 100 mg/ml the percent inhibition was 64.42 and 47.18. The result revealed that the ethanolic extract bear a good scavenging activity as compare to aqueous extract but on comparing to Standard BHT it has less activity as BHT at 100 mg/ml it has 97.65 inhibition percentage.

Reduction of 1, 1-diphenyl-2picrylhydrazyl (DPPH)

Figure shows the dose response curve of DPPH radical scavenging activity of the ethanolic and aqueous extract of *Alternanthera pungens* Kunth, compared with Ascorbic acid and BHT. It was observed that the ethanolic extract had higher activity than that of aqueous extract at different concentration. At a conc. of 100 mg/ml, the scavenging activity of ethanolic extract reached 61.92 % while at the

Table 2. Antiradical activity of BHT and plant extracts of *Alternanthera pungens* Kunth

Sample	Concentration mg/ml	Mean±SEM	Percentage (%)
1 ml ethanolic ABTS+ 1 ml BHT	20	0.0416±0.0038	95.53
	40	0.0341±0.0019	96.34
	60	0.0321±0.0048	96.55
	80	0.0236±0.0052	97.46
	100	0.0219±0.0036	97.65
1 ml ethanolic ABTS + 1 ml Ethanolic Extract	20	0.5938±0.00021	36.30
	40	0.5845±0.00026	37.30
	60	0.4823±0.00033	48.26
	80	0.3656±0.00042	60.78
	100	0.3317±0.00024	64.42
1 ml ethanolic ABTS + 1 ml Aqueous Extract	20	0.6942±0.00052	25.53
	40	0.5942±0.00037	36.26
	60	0.5213±0.00028	44.08
	80	0.5156±0.00045	44.69
	100	0.4924±0.00524	47.18

Data expressed as mean± SD, n =3; Absorbance of Blank = 0.9323

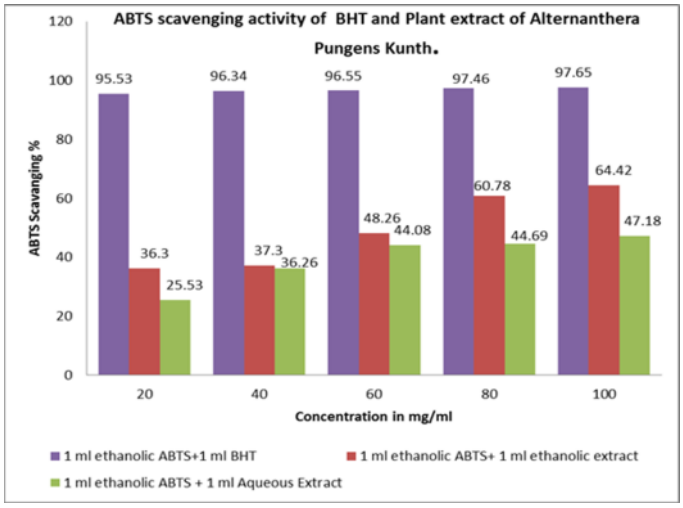

Figure 1. ABTS scavenging activities of the ethanolic and aqueous extracts of *Alternanthera pungens* Kunth

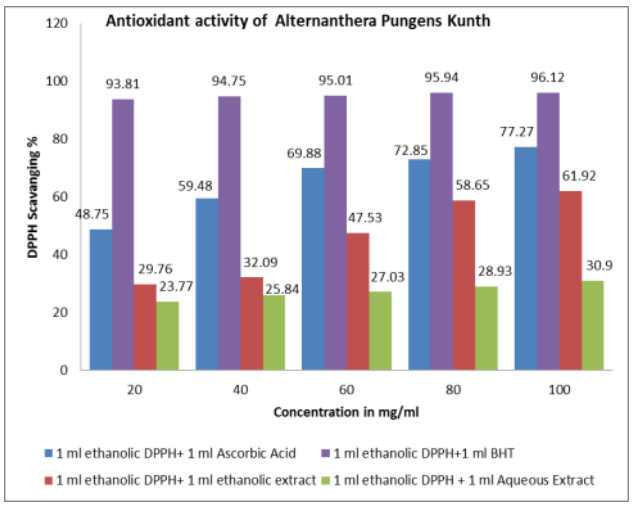

Figure 2. DPPH scavenging activities of the ethanolic and aqueous extracts of *Alternanthera pungens* Kunth

same conc., that the aqueous extract was 30.90 %. The effect of antioxidants on DPPH is due to their hydrogen donating ability. Though the DPPH radical scavenging abilities of the extracts were less than those of ascorbic (77.27 %) and BHT (96.12 %) at 100 mg/ml, the study revealed that the ethanolic extracts have the proton- donating ability and could serve as free radical inhibitors or scavengers. The ethanolic extract demonstrates potent antioxidant activity in different

Table 3. Antiradical activity of Ascorbic acid, BHT and plant extracts of *Alternanthera pungens* Kunth

Sample	Concentration mg/ml	Mean±SEM	Percentage (%)
1 ml ethanolic DPPH+ 1 ml Ascorbic Acid	20	0.4311±0.00034	48.75
	40	0.3408±0.00012	59.48
	60	0.2534±0.00037	69.88
	80	0.2284±0.00011	72.85
	100	0.1912±0.00430	77.27
1 ml ethanolic DPPH+ 1 ml BHT	20	0.0516±0.0018	93.18
	40	0.0441±0.0012	94.75
	60	0.0419±0.0032	95.01
	80	0.0341±0.0047	95.94
	100	0.0326±0.0142	96.12
1 ml ethanolic DPPH + 1 ml Ethanolic Extract	20	0.5908±0.00011	29.76
	40	0.5712±0.00038	32.09
	60	0.4413±0.00043	47.53
	80	0.3478±0.00044	58.65
	100	0.3203±0.00012	61.92
1 ml ethanolic DPPH + 1 ml Aqueous Extract	20	0.6412±0.00032	23.77
	40	0.6238±0.00048	25.84
	60	0.6107±0.00017	27.03
	80	0.5978±0.00031	28.93
	100	0.5812±0.00480	30.90

Data expressed as mean± SD, n =3; Absorbance of Blank = 0.8412

concentration. The Ethanolic extract found to contain a noticeable amount of total phenol. The total phenolic content in plant may play a major role as an antioxidant. The result of this study shows that the ethanolic extract can be used as easily accessible source of natural antioxidants and as possible food supplement or in pharmaceutical industry (Garg et al., 2018).

Conclusion

The ethanolic extract exhibited the potent antioxidant activity which confirmed through their free radical scavenging properties (i.e. ABTS and DPPH radical scavenging activity). It is an indication that the solvent is capable of extracting the active constituents from *Alternanthera pungens* Kunth. The antioxidant effect of these extracts on both ABTS and DPPH may be due to presence of Phenolic and flavonoid content. This may be beneficial for the development of new antioxidant agents which help in management of problem associated with the free radicals like Diabetes. Hence, a further investigation was needed to explore the possible mechanism of action.

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Conflict Of Interest

The authors have declared that there is no conflict of interest.

References

Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM. 2001. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Biochemical and molecular action of nutrients. The Journal of Nutrition 131; 2837- 2842.

Charde MS, Shukla A, Bukhariya V, Mehta J, Chakole R. 2011. Herbal remedies as antioxidants: An overview. International Journal of Pharmacological Research, 1(2):25-34.

Davies KJA. 1994. Oxidative stress: the paradox of aerobic life. Biochemical Society Symposia 61; 1-34.

Djeridane A, Yousfi M, Nadjemi B, Boutassouma D, Stocker P, Vidal N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry 97; 654-660.

Garg S, Garg A , Shukla A , Dev SK , Bishnoi RS , Kumar M. Review on antioxidant evaluation methods: In- vitro and in-vivo models. Asian Journal of Pharmacy and Pharmacology 2018; 4(2): 147-154.

Gulcin I, Oktay M, Kufrevioglu IO, Aslan A. 2002. Determination of antioxidant activity of Lichen Cetraria islandica (L) Ach. Journal of Ethnopharmacology 79; 325-329.

Gupta RK, Saxena VK. 1987. Volatile constituents from the flowers of Athernanthera pungens HBK (Amaranthaceae). Indian Perfume 31(4):366-369.

Kehrer JP. 1993. Free radicals as mediators of tissue injury and disease. CRC Critical Reviews in Toxicology 23; 21-48.

Kourounakis AP, Galanakis D, Tsiakitzis K. 1999. Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities. Drug Development Research 47: 9-16.

Kritikar KR, Basu BD. 1994. Indian medicinal plants, Vol II, 2nd edition. (Bishan singh and Mahendrapal Singh, Dehradum.

Liyana-Pathiranan CM, Shahidi F. 2005. Antioxidant activity of commercial soft and hard wheat (Triticum aestivum L) as affected by gastric pH conditions. Journal of Agriculure and Food Chemistry 53, 2433-2440.

Mathew S, Abraham ET. 2006. In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food Chemistry and Toxicology 44: 198-206.

Mourya P, Shukla A, Rai G, Lodhi S. 2017. Hypoglycemic and hypolipidemic effects of ethanolic and aqueous extract from Ziziphus oenoplia (L) Mill on alloxan-induced diabetic rats. Beni- suef university Journal of Basic and Applied Sciences 6(1): 1-9.

Okudu T, Yoshida T, Hatano T. 1994. Food phytochemicals for cancer prevention II. In C. T. Ho, T. Osawa, M.T. Huang and R.T. Rosen (Eds.), Chemistry and antioxidative effects of phenolic compounds from licorice, tea and Compositae and Labiateae herbs Washington, DC: American Chemical Society 132-143.

Ordon Ez AAL, Gomez JD, Vattuone MA, Isla MI. 2006. Antioxidant activities of Sechium edule (Jacq.) Swart extracts. Food Chemistry 97: 452-458.

Pratt DE, Hudson BJF. 1990. Natural antioxidants not exploited commercially. In: Hudson BJF (Ed.), Food Antioxidants. Elsevier, Amsterdam 171-192.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-EVANS C, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26, 1231–1237.

Shukla A, Shukla R, Pandey V, Golhani D. 2014. In-Vitro antioxidant activity of garcina cambogia fruits. Journal of Medical Pharmaceutical and Allied Sciences, 3:67-73.

Tepe B, Sokmen M, Akpulat H A, Sokmen A. 2006. Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chemistry 95: 200- 204.

Wealth of India, First Supplement Series (Raw Mate: The rial) Volume –I: 51.

Wolfe K, Wu X, Liu RH. 2003. Antioxidant activity of apple peels. J. Agriculture and Food Chemistry 51: 609-614.

Yildrim A, Oktay M, Bilaloglu V. 2001. The antioxidant activity of the leaves of Cydonia vulgaris. Turkish Journal of Medical Science 31: 23-27.

Zheng W, Wang SY. 2001. Antioxidant activity and phenolic compounds in selected herbs. Journal Agriculture and Food Chemistry 49: 5165–5170.