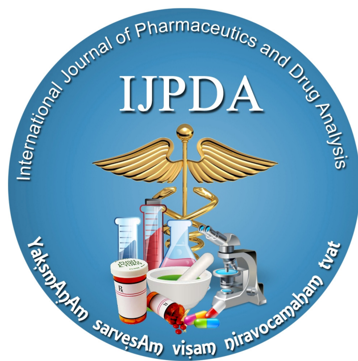
ISSN: 2348-8948
Vol: 3; Issue: 7

RESEARCH ARTICLE



Antioxidant activities of *Amaranthus retroflexus* leaves

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Date Received:	Date of Accepted:	Date Published:
28-May-2015	18-Jun-2015	08-Jul-2015

Abstract:
Amaranthus retroflexus is a medicinal plant. Our aim is to investigate its invitro antioxidant property. Hence 70% ethanolic extract of *Amaranthus retroflexus* leaves (AREE) was taken and the parameters studied were reducing power and DPPH (1,1-Diphenyl-2-Picryl-hydrazyl) free radical scavenging activity *in-vitro* models were carried out to evaluate its antioxidant activity. Therefore these results concluded that, the ethanolic extract afford significant antioxidant activities which may be attributed due to polyphenols.

Keywords: *AREE*. reducing power, DPPH and polyphenols.

Introduction

Amaranthusretroflexus is a edible plant used as vegetable which is being used by native practitioner as hepato protective in treating various types of jaundice. The leaves of this plant contain polyphenolic compounds like tannins and flavonoids. These polyphenolic compounds have antioxidant property and anti-oxidants have been known to possess hepato protective activity. Keeping the native knowledge and the above mentioned literature information[1], this plant was selected for present study to screen the leaves of this edible plant for the presence of phyto constituents, and antioxidant activities. This study was carried out by using AREE as antioxidant.

MATERIALS AND METHOD

Collection and identification of plant: The plant was collected from Kusnoor village (Gulbarga district), in the month of March and was authenticated by Dr. Srinath Rao, chairman, P.G. Department of Studies and Research in Botany, Gulbarga University, Gulbarga, Karnataka. The plant was thoroughly cleaned and the leaves were shade dried and made into a coarse powder by rubbing in the palms.

Extraction

250 gms of shade dried leaf powder of *Amaranthus retroflexus* was extracted in Soxhlet's apparatus using petroleum ether for defatting and then it was extracted with 70% ethanol. This solvent was evaporated on a water bath at a low temperature (50°C) and finally the residue was obtained.

Materials used
All chemicals and reagents used were of analytical grade.

I. *In-vitro*antioxidant activities
The Reducing power and DPPH (1,1-Diphenyl-2-Picryl-hydrazyl) free radical scavenging activity *in-vitro* models were carried out to evaluate antioxidant activity.

a. Reducing power
The reducing power of AREE were determined according to the method of Oyaizu (Oyaizu, 1986)[2].

Procedure
Different doses of AREE were mixed in 1 ml of distilled water so as to get 5 µg, 10µg, 25µg, 50µg and 100µg concentrations. This was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%) and the absorbance (OD) was measured at 700 nm in double beam spectrophotometer. Increased absorbance of the reaction mixture indicates increase in reducing power. The % reducing power was calculated by using the formula.

% increase in absorbance =

$$\frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

25 µg of sodium metabisulphate was used as standard compound and the results are complied in Table No. 1.

b.DPPH (1,1-Diphenyl-2-Picryl-hydrazyl) free radical scavenging activity[3].
DPPH radical scavenging activity of AREE was measured by the method described by Sabiret. al. Different concentrations of this extract (5, 10, 25, 50, 100 µg) was added to a 0.5 ml solution of DPPH (0.25 mM in 95% ethanol). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm in a double beam spectrophotometer using DPPH solution as blank. Percentage inhibition was calculated from the control. Vitamin C (25 µg) was used as a standard compound in the DPPH assay. The results are complied in Table No. 2.

Statistical analysis
The data presented in Table No. 1 and 2 (n=3) were expressed as mean ± SEM. Significant difference among the mean were calculated at the level of $p < 0.001$ and analyzed by one-way analysis of variance by Dunnet's 't' test. A value of $p < 0.05$ was defined as significant.

RESULTS
It was observed that AREE have demonstrated dose dependent increase in the reducing property. Whereas, 25 µg of sodium metabisulphate (standard) has 240% reducing property. But this extract at 50 µg has more reducing property than compared to standard. However 100 µg of this extract has shown maximum reducing power i.e., 400%. The results are shown in Table No. 1.

DPPH is an unstable nitrogen centered free radical that accepts an electron or hydrogen radical from suitable antioxidants and gets reduced to stable diamagnetic molecule along with stiochiometric loss of colour. This phenomenon has been widely used by researchers as a quick and reliable parameter to assess the *in-vitro* antioxidant activity of crude extracts. From the DPPH radical scavenging activity of this extract is shown in Table No. 2. It is clear that this extract has shown a dose dependent activity and this extract claims that it is more efficient in scavenging DPPH scavenging radical.

DISCUSSIONS
The invitro-antioxidation offered by AREE may be attributed due to the presence of antioxidant phytoconstituents like flavonoids, phytosterols and other polyphenolic constituents, by the virtue of which the extract showed a very good antioxidant activity. These findings adds strength to our claim.

CONCLUSION
AREE has a good *in-vitro* antioxidant properties which are attributed due to the presence of antioxidant phytoconstituents. Therefore the above findings revels that the use of *Amaranthus retroflexusus* leaves in our food protects our vital organs from various types of diseases.

SCOPE FOR FUTURE STUDY
As it is a medicinal plant, hence isolation of its phyto constituents are needed to screen various organ protective potentials.

ACKNOWLEDGEMENTS
We are thankful to the Management and Principal of Luqmancollege of Pharmacy, Gulbarga for providing all the necessary facilities to carry out this research work.

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*vivo*hepatoprotective activity against paracetamol induced liver damage in mice. Food chemistry 111 (2008) 845-851.

Table No. 1

Reducing power activity of 70 % ethanolic extract of *Amaranthusretroflexus*(AREE) leaves

Groups	Absorbance Mean ± SE	% Increase
Control	0.056±0.003	---
Control + Standard 25 µg	0.176±0.003***	240
Control + 70 % ethanolic extract 5 µg	0.130±0.005***	160
Control + 70 % ethanolic extract 10 µg	0.160±0.005	220
Control + 70 % ethanolic extract 25 µg	0.173±0.008	240
Control + 70 % ethanolic extract 50 µg	0.223±0.008	340
Control + 70 % ethanolic extract 100 µg	0.250±0.011**	400

Values are the mean ± S.E.., n=3; Significance ***P<0.001, **P<0.01compared to standard. Std: sodium metabisulphate

Table No. 2

DPPH Radical scavenging activity of 70 % ethanolic extract of *Amaranthusretroflexus*(AREE) leaves

Groups	Absorbance Mean ± SE	% Inhibition
Control	0.540±0.010	---
Control + Standard 25 µg	0.390±0.005***	27.777
Control + 70 % ethanolic extract 5 µg	0.263±0.006***	33.333
Control + 70 % ethanolic extract 10 µg	0.216±0.003***	46.153
Control + 70 % ethanolic extract 25 µg	0.183±0.003***	53.846
Control + 70 % ethanolic extract 50 µg	0.153±0.003***	61.538
Control + 70 % ethanolic extract 100 µg	0.106±0.003***	74.358

Values are the mean ± S.E.., n=3; Significance ***P<0.001compared to standard.Std: Ascorbic acid (Vitamin C)