

Original Article

## Superoxide and Nitric Oxide Activities of *Amaranthus Retroflexus* Leaves

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### ABSTRACT

*Amaranthus retroflexus* is a medicinal plant. Our aim is to investigate its in-vitro antioxidant properties. Therefore 70% ethanolic extract of *Amaranthus retroflexus* leaves (AREE) was taken and the parameter studied was superoxide and nitricoxide free radical scavenging activities. *In-vitro* models were carried out to evaluate its antioxidant activities. Hence these results concluded that, the ethanolic extract afford significant antioxidant activities which may be attributed due to polyphenols.

## 1. INTRODUCTION

*Amaranthus retroflexus* is an edible plant which is used as vegetable and also used by native practitioner as hepatoprotective in treating various types of jaundice. The leaves of this plant contain polyphenolic compounds like tannins and flavonoids. These polyphenolic compounds have antioxidant property. Therefore antioxidants have been known to possess hepatoprotective 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 phytoconstituents, and antioxidant activity. This study was carried out by using AREE as antioxidant.

## 2. MATERIALS AND METHODS

### 2.1 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.

### 2.2 Extraction

100 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.

### 2.3 Materials used

All chemicals and reagents used were of analytical grade.

### 2.4 *In-vitro* antioxidant activity

#### 2.4.1 Superoxide radical scavenging activity

Superoxide radical scavenging activity of 70% ethanolic extract of *Amaranthus retroflexus* (AREE) was studied by using the method explained by and modified by Ilhami et. al.

Oxygen is essential for the survival of aerobic cells, but it is toxic to them if it is supplied at greater concentration than those in normal air. The biochemical mechanisms responsible for oxygen toxicity include lipid peroxidation and the generation of  $H_2O_2^+$  the superoxide radical,  $O_2^-$ . This superoxide radical can inhibit or propagate the process of lipid peroxidation. Measurement

of superoxide anion scavenging activity of AREE was done by using the method explained and modified by Ilhami et. al.[2].

#### 2.4.1.1 Procedure

Various concentrations of AREE were prepared such that each 0.1ml contains 10, 20, 25, 50 and 100 µg. About 1 ml of Nitroblue tetrazolium (NBT) solution (156 µM NBT in 100mM phosphate buffer, pH 7.4) and 1 ml of Nicotinamide adenine dinucleotide (NADH) solution (468 µM Phenazine Methosulphate in 100 mM phosphate buffer, pH 7.4) were mixed with 0.1 ml of various concentrations of AREE and standard in water was mixed. The reaction was initiated by adding 100 µl of Phenazine Methosulphate (PMS) solution (60µM PMS in 100 mM phosphate buffer, pH 7.4) to the above mixture and was incubated at 25°C for 5 minutes. The absorbance was measured at 560 nm. against blank by using UV- double beam spectrophotometer.

Decrease in the absorbance of the reaction mixture indicates the increase in the superoxide anion scavenging activity. % inhibition of reducing power was calculated by using the formula:

$$\% \text{ Superoxide scavenging activity} = \frac{\text{Control Abs} - \frac{\text{Sample Abs}}{\text{Control Abs}} \times 100}{\text{Control Abs}}$$

The results are compiled in Table No.1

#### 2.4.2 Nitric oxide radical scavenging activity

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc and it is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals. This forms the basis of this experiment. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions and peroxynitrite anions, which act as free radicals. This can be determined by the use of the Griess Illosvoy reaction. [3]

##### 2.4.2.1 Procedure

1 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 1 ml of different concentrations of AREE (10, 20, 25, 50 and 100 µg/ml) and the mixture was incubated at 50°C for 150 minutes. From the incubated mixture 1 ml was pipetted out and added to 1ml of Griess's reagent (1% sulphanilamide, 2% orthophoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the reaction mixture was read at 546 nm using double beam spectrophotometer. The percentage of inhibition of OD i.e. percentage radical scavenging activity was calculated by using the formula:

$$\% \text{ Antioxidant activity} = \frac{\text{Control Abs} - \frac{\text{Sample Abs}}{\text{Control Abs}} \times 100}{\text{Control Abs}}$$

All the estimations were performed in triplicate and results were averaged, tabulated as shown in Table No. 2.

## 2.5 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.

**Table 1** Superoxide Radical scavenging activity of 70% ethanolic extract of *Amaranthus retroflexus* leaves (AREE)

Groups	Absorbance Mean ± SEM	% Increase
Control	0.483±0.003	---
Control + Standard 25 µg	0.193±0.003***	60.041
Control + 70% ethanolic extract 10µg	0.456±0.003***	5.590
Control + 70% ethanolic extract 20 µg	0.423±0.003***	12.422
Control + 70% ethanolic extract 25 µg	0.343±0.003***	28.985
Control + 70% ethanolic extract 50 µg	0.293±0.003***	39.337
Control + 70% ethanolic extract 100 µg	0.196±0.006	59.420

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

**Table 2** Nitric oxide Radical scavenging activity of 70 % ethanolic extract of *Amaranthus retroflexus* leaves (AREE)

Groups	Absorbance Mean ± SEM	% Inhibition
Control	0.304±0.144	---
Control + Standard 25 µg	0.105±0.064***	65.46
Control + 70 % ethanolic extract 10µg	0.280±0.087**	7.895
Control + 70 % ethanolic extract 20 µg	0.256±0.046 ***	15.789
Control + 70 % ethanolic extract 25 µg	0.254±0.069 **	16.447
Control + 70 % ethanolic extract 50 µg	0.233±0.109***	23.355
Control + 70 % ethanolic extract 100 µg	0.187±0.086**	38.487

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

## 3. RESULTS

It was observed that this extract have demonstrated concentration dependent increase in the superoxide anion activity. Whereas, the standard i.e. sodium metabisulphate (25mg) showed 60.041% of superoxide radical scavenging activity. AREE at 100mg showed 59.420%. AREE was more efficient in scavenging this radical. The results are summarized in Table No. 1. This extract has also shown concentration dependent inhibition in the nitric oxide anion activity. Whereas, the standard i.e. sodium metabisulphate

(25mg) showed 65.46% of nitric oxide radical scavenging activity. However, AREE at 100mg showed 38.487%. AREE was efficient in scavenging this radical. The results are summarized in Table No. 2

#### 4. DISCUSSIONS

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

#### 5. CONCLUSION

AREE has a good *in-vitro* antioxidant property which is attributed due to the presence of antioxidant phytoconstituents. Therefore the above findings reveals that the use of *Amaranthus retroflexus* leaves in our food, protects our vital organs, from various types of diseases.

#### 6. SCOPE FOR FUTURE STUDY

As it is a medicinal plant, hence isolation of its phytoconstituents are needed to screen various organ protective potentials.

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