ORIGINAL RESEARCH PAPER

Volume-7 | Issue-6 | June-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SUPEROXIDE AND NITRICOXIDE ACTIVITIES OF AMARANTHUS GANGETICUS LEAVES

Pharmacology

G. Jyoti Jain* Prof. & H.O.D. Dept. of Pharmacology, Luqman College of Pharmacy, Gulbarga (Karnataka) *Corresponding Author

S. Ramachandra Setty Prof. & H.O.D. Dept. of Pharmacology, Govt. college of Pharmacy, Bangalore (Karnataka)

ABSTRACT

Amaranthus gangeticus is a very good medicinal plant. Our aim is to investigate its invitro antioxidant properties. Hence 70% ethanolic extract of *Amaranthus gangeticus* leaves (AGEE) 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. Therefore these results concluded that, the ethanolic extract afford significant antioxidant activities which may be attributed due to polyphenols.

KEYWORDS

AGEE. Superoxide, Nitricoxide and Polyphenols.

INTRODUCTION

Amaranthus gangeticus is a edible plant 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 AGEE 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

200 gms of shade dried leaf powder of *Amaranthus gangeticus* 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 (50C) and finally the residue was obtained.

Materials used

. All chemicals and reagents used were of analytical grade.

. In-vitro antioxidant activity

Superoxide radical scavenging activity:

Superoxide radical scavenging activity of 70% ethanolic extract of *Amaranthus gangeticus* (AGEE) was studied by using the method explained by Nishimiki (Nishimiki et.al, 1972)[2] 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 inhibt or propagate the process of lipid peroxidation. Measurement of superoxide anion scavenging activity of AGEE was done by using the method explained by Nishimiki et.al, 1972[2] and modified by Ilhami et. al.

Procedure:

Various concentrations of AGEE 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 AGEE 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^{0}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:

% Superoxide scavenging activity = control Abs-sample Abs / Control Abs x 100

The results are compiled in Table No.1.

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].

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 AGEE (10, 20, 25, 50 and 100 µg/ml) and the mixture and was incubated at 50^{0}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:

% Antioxidant activity = control Abs-sample Abs / Control Abs x 100

All the estimations were performed in triplicate and results were averaged, tabulated as shown 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.

Table No. 1 Superoxide Radical scavenging activity of 70 % ethanolic extract of *Amaranthus gangeticus* leaves (AGEE)

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	0.416±0.003***	13.871
Control + 70 % ethanolic extract 20 µg	0.366±0.006***	24.223
Control + 70 % ethanolic extract 25 µg	0.300±0.005***	37.888
Control + 70 % ethanolic extract 50 µg	0.256±0.003***	46.997
Control + 70 % ethanolic extract 100 µg	0.170±0.005**	64.803

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

Table No. 2 Nitric oxide Radical scavenging activity of 70 % ethanolic extract of Amaranthus gangeticus leaves (AGEE)

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	0.273±0.080**	10.197
Control + 70 % ethanolic extract 20 µg	0.251±0.064***	17.434
Control + 70 % ethanolic extract 25 µg	0.244±0.057**	19.737
Control + 70 % ethanolic extract 50 µg	0.222±0.086***	26.974
Control + 70 % ethanolic extract 100 µg	0.174±0.092**	42.763

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

RESULTS

It was observed that this extract have demonstrated concentration dependent increase in the superoxide anion activity. Whereas, the standard i.e. sodium metabisulphate (25g) showed 60.041% of superoxide radical scavenging activity. AGEE at 100g showed 64.803% . AGEE 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 (25g) showed 65.46% of nitric oxide radical scavenging activity. However, AGEE at 100g showed 42.763% . AGEE was efficient in scavenging this radical. The results are summarized in Table No. 2.

DISCUSSIONS

The invitro-antioxidation offered by AGEE 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.

CONCLUSION

AGEE has a good *in-vitro* antioxidant property which is attributed due to the presence of antioxidant phytoconstituents. Therefore the above findings revels that the use of *Amaranthus gangeticus* 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 phytoconstituents are needed to screen various organ protective potentials.

ACKNOWLEDGEMENTS

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

REFERENCES

1. www.google.co.in – wikipedia, the free encyclopedia : navigation search
2. Ilhams Gulcin, Munir Oktay, Irfan Kufre Vioglu O, Ali Aslan, Determination of antioxidant activity of lichen Cetraria islandica (L) Ach. J. Ethanopharmacol 2002;79: 325-29.
3. Sangameswaran B. Balakrishnan BR, Chumbhale Deshraj, Jayakar B. Invitro antioxidant activity of roots of Thespesia lampas dalz and gibs. Pak. J. Pharm. Sci. 2009: 22(4): 368-72.