

## *In vitro* antioxidant potential of stem of *Amaranthus viridis* - A medicine used in the Ayurvedic system of medicine

Nadhirah Faiz<sup>1</sup>, V. Vishnu Priya<sup>1</sup>, R. Ponnulakshmi<sup>2</sup>, R. Gayathri<sup>1</sup>, B. Shyamaladevi<sup>1</sup>, K. Madhan<sup>1</sup>, M. Manikannan<sup>3</sup>, J. Selvaraj<sup>1\*</sup>

### ABSTRACT

**Aim:** The study was aimed to assess the antioxidant potentials of *Amaranthus viridis* stem extract by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, nitric oxide (NO) radical, and superoxide anion scavenging activity. **Materials and Methods:** *A. viridis* plant was collected from local market and stem was separated and shade dried. Aqueous extract of the stem of the plant was done as per the standard methods. Then, the antioxidant properties of *A. viridis* such as DPPH radical, NO anion, superoxide anion, and free radical-mediated DNA damage assays were analyzed spectrophotometrically by previously published methods. The data were analyzed using computer-based software by one-way Analysis Of Variance. In this, the significance is considered at the level of  $P < 0.05$ . **Results:** DPPH radical, NO radical, and superoxide anion radical scavenging activity was found to be increased in a dose-dependent manner. **Conclusion:** The present findings indicate that *A. viridis* can be used as a potential antioxidant for the treatment of various diseases.

**KEY WORDS:** 2, 2-Diphenyl-1-picrylhydrazyl, *Amaranthus viridis*, Antioxidant potential, Nitric oxide, Stem extract, Super oxide anion

### INTRODUCTION

*Amaranthus viridis* L, also known as Chilaka ThotakuralIn Telugu, has been used in Nepalese and Indian traditional system to reduce labor pain and act an antipyretic.<sup>[1,2]</sup> The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis, rashes, etc.<sup>[3]</sup> Other traditional uses range from an anti-inflammatory agent of the urinary tract, diuretic, antirheumatic, venereal diseases vermifuge, antiulcer, analgesic, antiemetic, laxative, improvement of appetite, antileprotic, treatment of asthma, to treatment of respiratory, and eye problems.<sup>[1,4-11]</sup>

The plant possesses antiproliferative, antifungal lactic acid properties, ribosome inactivating protein - t-carotene,<sup>[12-14]</sup> and antiviral activities.<sup>[15]</sup> In addition, the whole plants possess antipyretic and analgesic properties and are used for the treatment of pain and fever, respectively, in traditional systems of medicine.<sup>[16]</sup> However, there are

not enough scientific reports to support these supposed antioxidant properties. This prompted us to conduct this study.

### MATERIALS AND METHODS

#### Collection of Plant Material

The whole plants of *A. viridis* stem were collected in fresh condition from Chennai region, Tamil Nadu. The voucher specimens of the plants were authenticated by the Department of Plant Biology and Plant Biotechnology, Presidency College, Chennai, Tamil Nadu, India. The plant was dried under shade then ground into a uniform powder using a blender and stored in polyethylene bags at room temperature.

#### Preparation of Ethanolic Extract From the Stem of *A. Viridis*

The plant seed powder was loaded into Soxhlet extractor and subjected to extraction with methanol. After extraction, the solvent was distilled off, and the extracts were concentrated on a water bath to a dry residue and kept in a desiccator.

#### Access this article online

Website: [jprsolutions.info](http://jprsolutions.info)

ISSN: 0975-7619

<sup>1</sup>Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, <sup>2</sup>Department of Central Research Laboratory, Meenakshi Academy of Higher Education and Research, Chennai, India, <sup>3</sup>Centre for Drug Discovery and Development, Col. Dr. Jeppiaar Research Park, Sathyabama Institute of Science and Technology, Chennai, India

\*Corresponding author: Dr. J. Selvaraj, Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai - 600 077, India. E-mail: [jselvaendo@gmail.com](mailto:jselvaendo@gmail.com)

Received on: 04-11-2018; Revised on: 06-12-2018; Accepted on: 02-01-2019

## Assessment of *In Vitro* Antioxidant Potential Activity of *A. Viridis*

### DPPH free radical scavenging activity of *A. viridis*

Scavenging of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the method of Ponnulakshmi *et al.* (1989). Briefly, DPPH solution (1.0 ml) was added to 1.0 ml of different extracts of *A. viridis* in methanol at different concentrations (100, 200, 300, 400, and 500 µg/ml). The mixture was kept at room temperature for 50 min, and the activity was measured at 517 nm. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg/ml) was used as a standard. The capability to scavenge the DPPH radical was calculated using the following formula.

$$\text{DPPH radicals scavenged (\%)} = \frac{\text{Control OD} - \text{sample OD}}{\text{control OD}} \times 100.$$

### Nitric Oxide (NO) Radical Scavenging Activity of *A. Viridis*

Scavenging of NO radical was assayed by the method of Garrat, (1964). Briefly, the reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml), and different concentrations (100, 200, 300, 400, and 500 µg/ml) of extracts of *Abutilon indicum* varieties (0.5 ml) were incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted out and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink colored chromophore is formed in diffused light. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg/ml) was used as a standard. The activity was measured at 550 nm and the results were expressed as percentage of scavenging using the following formula:

$$\text{NO radical scavenged (\%)} = \frac{\text{Control OD} - \text{sample OD}}{\text{control OD}} \times 100.$$

### Superoxide Anion Scavenging Activity of *A. Viridis*

Superoxide anions were chemically generated in a mixture of phenazine methosulfate and NADH. The reaction was quantified by coupling superoxide generation to the reduction of nitroblue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 µM), 1 ml of NADH (78 mM), and 1 ml of various concentrations (100, 200, 300, 400, and 500 µg/ml) of *Allium cepa* varieties extracts. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg) was used as a standard. The reaction mixture was incubated at 25°C for 5 min, and the activity was measured at 560 nm. Results

were expressed as percentage of scavenging using the following formula.

$$\text{Superoxide anion scavenged (\%)} = \frac{\text{Control OD} - \text{sample OD}}{\text{control OD}} \times 100.$$

### Statistical Analysis

Results are expressed as Mean ± standard deviation (SD). The difference between experimental groups was compared by one-way analysis of variance followed by Dunnett's Multiple comparison test (control vs. test) using the GraphPad Prism software.

## RESULTS

### Effect of *A. Viridis* Stem Extract on DPPH Radical Scavenging Activity

In the present study, *A. viridis* stem ethanolic extract showed a significant increased the DPPH radical scavenging activity. However, the maximum inhibition was observed at 500 µg/ml. Ascorbic acid, a standard drug used in this study [Figure 1].

### Effect of *A. Viridis* Stem Extract on NO Radical Scavenging Activity

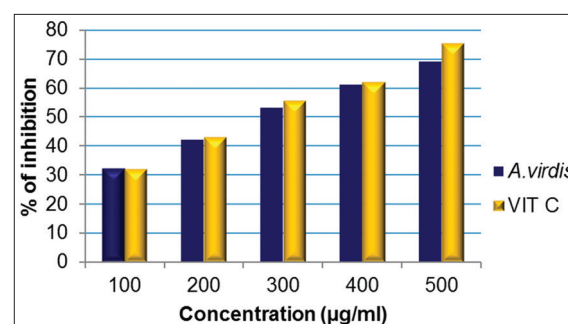
In the present study, *A. viridis* stem ethanolic extract showed a significant increased the NO radical scavenging activity. However, the maximum inhibition was observed at 500 µg/ml. Ascorbic acid, a standard drug used in this study [Figure 2].

### Effect of *A. Viridis* Stem Extract on Superoxide Anion Scavenging Activity

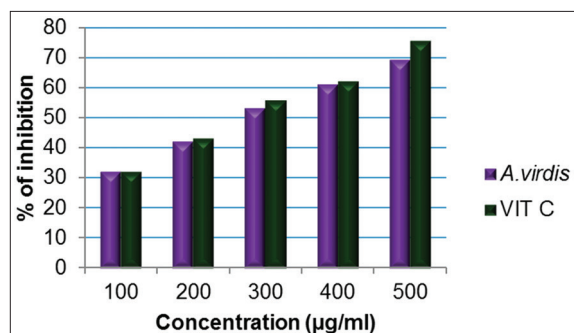
In the present study, *A. viridis* stem extract showed a significant increased the superoxide anion radical scavenging activity. However, the maximum inhibition was observed at 500 µg/ml. Ascorbic acid, a standard drug used in this study [Figure 3].

## DISCUSSION

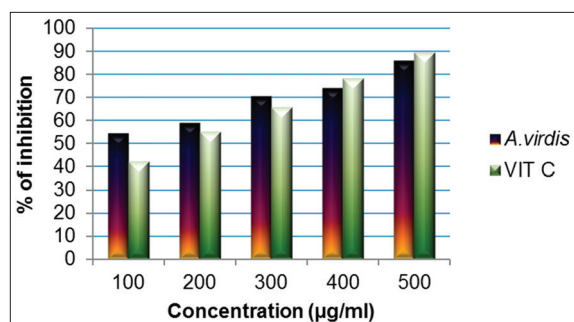
Medicinal plants represent one of the richest sources of therapeutic properties and natural phenolic



**Figure 1:** Effect of *Amaranthus viridis* stem extract on 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity. Each bar represents mean ± standard deviation of five independent observations



**Figure 2:** Effect of *Amaranthus viridis* stem extract on nitric oxide radical scavenging activity. Each bar represents mean $\pm$ standard deviation of five independent observations



**Fig 3:** Effect of *Amaranthus viridis* stem extract on superoxide anion scavenging activity. Each bar represents mean $\pm$ standard deviation of five independent observations

compounds, which provide the advantageous roles in the prevention or treatment of different diseases such as cancer, diabetes, chronic inflammatory disorders, and tumorigenesis disorders.<sup>[17]</sup> Nowadays, many scientific studies have convincingly demonstrated that medicinal plants constitute a significant source of substances with strong biological properties, having anticancer, antibacterial, and immunosuppressive as well as anticoagulant activities.<sup>[18]</sup> Taken together, indigenous drugs and in the utilization of therapeutic plant-based medicine for the treatment of various diseases have been in use since ancient times and will continue to spare humankind with new remedies.<sup>[19]</sup>

Phenolic compounds represent one of the important families of antioxidants due to their free radical scavenging activity.<sup>[20]</sup> Nevertheless, the presence of phytochemical compounds such as phenolics, at a lower concentration, has shown significant beneficial pharmacological properties, such as antiviral, anti-inflammatory, antioxidant, antimicrobial, antimutagenic, and chemopreventive activity.<sup>[21]</sup> Many reports also suggested that with an elevated concentration of polyphenols present in the plant extracts may contribute directly to their antioxidant properties. DPPH radical, NO radical, and superoxide anion radical scavenging activity recorded by the *A. viridis* which may be attributed to the presence of polyphenols.<sup>[22]</sup>

## CONCLUSIONS

As per your suggestion we reviewed the sentence, there is no changes in the paragraph and we missed one sentence as follows (extract) kindly add to this sentence in the paragraph.

The *in vitro* studies on DPPH, NO radical, and superoxide anion radical scavenging study showed *A. viridis* stem ethanolic extract has a strong dose-dependent free radical scavenging ability may be due present of active principles present in the extract

Hence, the present findings indicate that *A. viridis* can be used as potential antioxidant for the treatment of various diseases.

## REFERENCES

1. Kirtikar KR, Basu BD. In: Kirtikar KR, Basu BD, editors. Indian Medicinal Plants. 2<sup>nd</sup> ed., Vol. 3. India, Dehra Dun: International Book Distributors; 1987. p. 2061-2.
2. Turin M. Ethnobotanical notes on Thangmi plant names and their medicinal and ritual uses. CNAS 2003;30:19-52.
3. Quisumbing E. Manila: Bureau of Printing. Medicinal Plants of the Philippines, PA: Department of Agriculture and Natural Resources; 1951. p. 298-351.
4. Council of Scientific and Industrial Research (CSIR) Publications and Information Directorate. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. New Delhi, India: Council of Scientific and Industrial Research; 1988. p. 221.
5. Agra MF, Baracho GS, Nurit K, Basilio IJ, Coelho VP. Medicinal and poisonous diversity of the flora of "Cariri paraibano", Brazil. J Ethnopharmacol 2007;111:383-95.
6. Agra MF, Silva KN, Basilio IJ, De Freitas PF, Filho JM. Survey of medicinal plants used in the region northeast of Brazil. Braz J Pharmacogn 2008;18:472-508.
7. Sher H, Khan ZD. Resource utilization foreconomic development and folk medicine among the tribal people. Observation from Northern part of Pakistan. Pak J Plant Sci 2006;12: 149-62.
8. Quershi SJ, Khan MA, Ahmed M. A survey of useful medicinal plants of Abbottabad, in Northern Pakistan. Trakia J Sci 2008;6: 39-51.
9. Dar ME. Ethnobotanical uses of plants of Lawat district Muzaffarabad Azad Jammu and Kashmir. Asian J Plant Sci 2003; 2:680-2.
10. Arshad M, Khan QU. Ethnobotanical study of some medicinal plants of Rawal Town. Pak J Biol Sci 2000;3:1245-6.
11. MuhammadS, AmusaNA. The important food crops and medicinal plants of north-western Nigeria. Res J Agric Biol Sci 2005; 1:254-60.
12. Kaur N, Dhuna V, Kamboja SS, Agrewala JN, Singh J. A novel antiproliferative and antifungal lactic acid from *Amaranthus viridis* Linn seeds. Protein Pept Lett 2006;13:897-905.
13. Kwon SY, An CS, Liu JR, Pack KH. Aribosome inactivating protein from *Amaranthus viridis*. Biosci Biotechnol Biochem 1997; 61:1613-4.
14. Sena LP, Vanderjagt DJ, Rivera C, Tsin AT, Muhamadu I, Mahamadou O, *et al.* Analysis of nutritional components of eight famine foods of the Republic of Nigeria. Plant Foods Hum Nutr 1998;52:17-30.
15. Obi RK, Iroagba II, Ojiako OA. Virucidal potential of some edible Nigerian vegetables. Afr J Biotechnol 2006;5:1785-8.
16. Yusuf M, Chowdhury JU, Wahab MA, Begum J. Chittagong Bangladesh Council for Science and Industrial Research (BCSIR) 1994. Medicinal Plants of Bangladesh. São Paulo:

- Sadia.
17. Shamala S, Gunasekaran B, Shukor MY, Bakar B MZ, Ahmad SA. Phytochemical investigation, hypocholesterolemic and anti-atherosclerotic effects of *Amaranthus viridis* leaf extract in hypercholesterolemia-induced rabbits. RSC Adv 2016;6:32685-96.
  18. Kumari S, Deori M, Elancheran R, Kotoky J, Devi R. *In vitro* and *in vivo* antioxidant, anti-hyperlipidemic properties and chemical characterization of *Centella asiatica* (L.) extract. Front Pharmacol 2016;7:400.
  19. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016;79:629-61.
  20. Siger A, Czubiński J, Kachlicki P, Dwiecki K, Lampart-Szczapa E, Nogala-Kalucka M. Antioxidant activity and phenolic content in three lupin species. J Food Compos Anal 2012;25:190-7.
  21. Sarma R, Kumari S, Elancheran R, Deori M, Devi R. Polyphenol rich extract of *Garcinia pedunculata* fruit attenuates the hyperlipidemia induced by high fat diet. Front Pharmacol 2016;7:294.
  22. Kumari S, Elancheran R, Devi R. Phytochemical screening, antioxidant, antityrosinase, and antigenotoxic potential of *Amaranthus viridis* extract. Indian J Pharmacol 2018;50:130-8.

Source of support: Nil; Conflict of interest: None Declared