REVIEW ARTICLE

A Study of Antioxidant Activity of *Amaranthus viridis*

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ABSTRACT

Plants possess good antioxidant capacity to get rid of free radicals that cause many lifestyle diseases, especially cancer. *Amaranthus viridis* (Amaranthaceae) widely distributed all over the world, growing under a wide range of climatic conditions, and has been utilized as a medicinal herb in traditional *Ayurveda* medicine as antipyretic agents, also for the treatment of inflammation, ulcer, diabetic, asthma, and hyperlipidemia. The aim of the study was designed to evaluate the antioxidant and biological properties of *A. viridis*. In the present study, evaluation of aqueous extract of plant with the help of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity, reducing power, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The study concluded that the promising antioxidant capacities of *A. viridis* extract can further be utilized in various agricultural, pharmaceutical, and food applications.

Keywords: ABTS, *Amaranthus viridis*, Antioxidants, Aqueous extract, Cancer, DPPH.

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INTRODUCTION

Medicinal plants represent one of the richest sources of therapeutic properties and natural phenolic compounds, which provide the advantageous roles in the prevention or treatment of different diseases, such as, cancer, diabetes, chronic inflammatory disorders, and tumor genesis disorders.[1] The utilization of therapeutic plant-based medicine for the treatment of various diseases has been in use since ancient times and will continue to spare mankind with new remedies.[2] Recently, several research groups have demonstrated that natural antioxidants are the promising therapeutic agents for reactive oxygen species, leading to the prevention of oxidative stress-related diseases.[3] The earlier study also demonstrated that polyphenol is the major secondary metabolite that is extensively distributed in the medicinal plants, vegetables, and dietary fruits and considered as potent oxygen and nitrogen radical scavenger.[4] In addition, high polyphenol intake has also been directly associated with lowering the risk of cardiovascular diseases and many other degenerative diseases.[5] Hence, attention has been escalated considerably in finding naturally occurring antioxidants.

The leaves of *A. viridis* have a long history of indigenous utilized as a medicinal herb in the traditional *Ayurveda* medicine as antipyretic agents and also eaten conventionally as a vegetable among tribal and non-tribal people of northeast India.[6] To the best of our knowledge, limited studies have been reported on the chemical characterization of different fractions and antioxidant activities of *A. viridis* plant. Therefore, this current study was designed to investigate the antioxidant potential of *A. viridis* extract, and their phytochemical properties were determined.

MATERIALS AND METHODS

Collection of Samples

Fresh plant leaves of *A. viridis* were collected from Chennai, Tamil Nadu, India, and was authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. A voucher specimen (reg. no. PARC/2019/4054). The leaves are thoroughly washed through tap water and dried under shade for 3 to 5 days. The dried leaves are ground to fine powder and stored in bags for further use.

Preparation of Extracts

Fifty grams of dried powder of *A. viridis* leaves were packed in a separate round bottom flask for sample extraction using 500 mL water. The extraction was conducted with 20 mL of water for a period of 24 hours. At the end of the extraction, the crude extract was stored in refrigerator.

Screening for the Antioxidant Activity

DPPH Radical Scavenging Assay

The percentage of antioxidant activity (AA%) of each substance was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Different concentrations (100–500 µg) of the sample were added to all the tubes except blank, then made up to 3 mL using ethanol and 1 mL of 0.1 mM

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DPPH radical solution in ethanol was added. The control solution was prepared by mixing ethanol (3 mL) and DPPH radical solution (1 mL). Ascorbic acid was used as standard. Absorbance was read at 517 nm after 30 minutes of reaction. The scavenging activity percentage (AA%) was calculated using the below formula.

% antioxidant activity = {(absorbance at blank) – (absorbance at test)/(absorbance at blank)} × 100

ABTS Radical Scavenging Assay

Take 50 to 250 µL of sample and add 0.9 mL of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) solution. The stock solutions included 7 mM ABTS solution and 2.45 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 4 to 16 hours at room temperature in the dark. The resulting solution was then diluted with ethanol by mixing 1 mL of freshly prepared ABTS solution to obtain an absorbance of 0.706 ± 0.001 units at 734 nm (using the spectrophotometer.) Fresh ABTS solution was prepared for each assay. The absorbance was taken at 734 nm after 15 minutes using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of butylated hydroxytoluene (BHT), and percentage inhibition was calculated

ABTS radical scavenging activity (%) = (OD of control - OD of test/OD of control) × 100

Total Antioxidant Activity

Take different concentrations of sample and standard, make up to 3 mL using distilled water and add 1 mL of 4 mM ammonium molybdate, and 28 mM sodium phosphate dissolved in 0.6 M sulphuric acid reagent. Incubate at 95ºC for 90 minutes. Check the OD at 695 nm.

Reducing Power Activity

Take 20 to 100 µL concentrations of the plant extracts in corresponding were taken and mixed with phosphate buffer (2.5 mL) and 1% potassium ferricyanide (2.5 mL). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 3,000 rpm for 10 minutes. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared 0.1% ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. Control was prepared in similar manner, excluding samples. Ascorbic acid at various concentrations was used as standard. Increase in absorbance of the reaction mixture directly proportional to increase in reducing power.

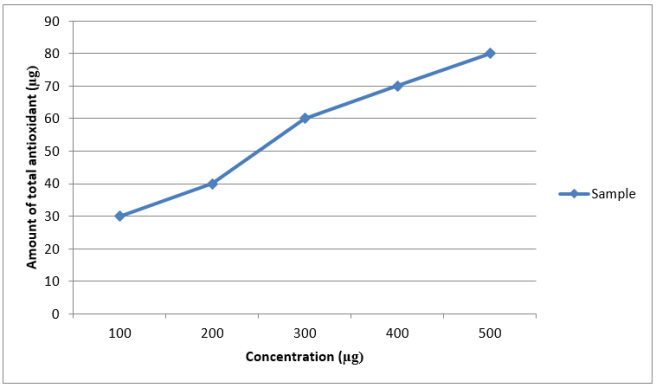
Table 1: Percentage of inhibition of DPPH free radical activity

Concentration (µg/mL)	*% inhibition*
100	90.76 ± 0.4
200	95.76 ± 0.7
400	96.44 ± 0.8
600	97.57 ± 0.3
800	99.69 ± 0.2
IC_{50}	29.25

RESULTS AND DISCUSSION

Antioxidants compounds can fight with free radicals and protect our body against various diseases. Various techniques have been delineated to determine the *in vitro* antioxidant activity for rapid screening of compounds if substances that have low *in vitro* antioxidant activity will definitely exhibit low *in vivo* antioxidant activity. It has been reported that phenolic compounds can manifest antioxidant activity due to redox properties so they can scavenge free radicals either by donating H+ atom with electron or delocalized unpaired electron by reason of having extended conjugated aromatic system; in addition, they have metal chelators potential.[7] Table 1 demonstrates the percentage increase of DPPH scavenging activity of aqueous extract of *A. viridis*. As the DPPH radical scavenging activity is recorded in terms of % inhibition, it is observed that the extract has demonstrated dose-dependent increase in the DPPH scavenging activity. The 800 µg of ascorbic acid (as standard) has 99.69% DPPH scavenging property. The IC50 value of aqueous extract of *A. viridis* was perceived to be 29.25 µg/mL compared to the IC50 value of ascorbic acid as standard. Table 2 displays the percentage increase of ABTS scavenging activity of aqueous extract of *A. viridis* exhibited good antioxidant activity with IC50 value of 21.83 µg/mL compared to IC50 value of BHT as standard 15.47 µg/mL. Table 2 indicates the significant ($p < 0.001$) increase of reducing power of *A. viridis* as compared to the standard. The percentage of increase in reducing power was found to be 101.09% in 800 µg of aqueous extract, respectively.

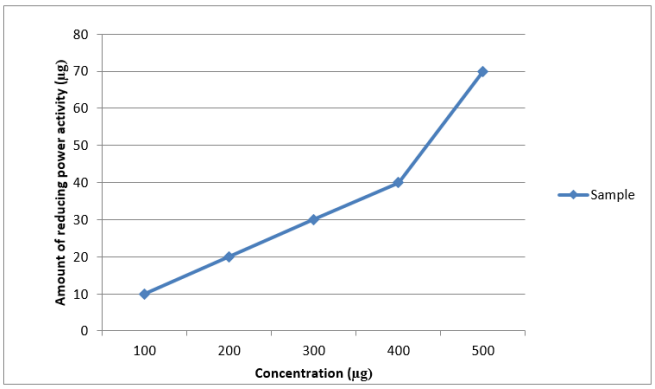
Graph 1 reveals the percentage increase of total antioxidant activity of the sample demonstrated dose-dependent increase in the antioxidant scavenging activity. The 500 µg of the extract has shown maximum scavenging activity (80%). Graph 2



Graph 1: Total antioxidant activity

Table 2: Percentage of inhibition of ABTS radical activity

Concentration (µg/mL)	*% inhibition*
100	92.29 ± 0.13
200	96.81 ± 0.42
400	97.85 ± 0.29
600	99.97 ± 0.02
800	101.09 ± 0.08
IC_{50}	21.83



Graph 2: Reducing power activity

demonstrates the percentage increase of reducing power activity of the extract of *A. viridis*. As the reducing power scavenging activity is recorded in terms of % inhibition, it is observed that the extract have demonstrated dose-dependent increase in the scavenging activity of *A. viridis* was perceived to be 70 µg/mL at a concentration of 500 µg.

CONCLUSION

The present study validates that *A. viridis* has excellent phytochemical activity and might be a promising ingredient for food with potential health and nutritional benefits. The results of *in vitro* evaluations suggest that aqueous extract of *A. viridis* may be useful in defense against illness, such as, cancer, obstruction of arteries (atherosclerosis), central nervous system (CNS) disorder, neurodegenerative disorder, etc., due to antioxidant properties. However, further investigations on the *in vivo* antioxidant activity and establish the mechanism of action of antioxidant activity of *A. viridis* can be warranted.

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