Phytochemical screening, antioxidant, antityrosinase, and antigenotoxic potential of *Amaranthus viridis* extract

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Abstract:

**OBJECTIVE:** *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 Ayurvedic 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 chemical composition and antioxidant and biological properties of different fractions obtained from *A. viridis*.

**MATERIALS AND METHODS:** Four different extracts of *A. viridis* were prepared using aqueous, methanol, chloroform, and hexane and investigated their antioxidant potential using free radical scavenging activities such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and nitric oxide (NO) radical scavenging activity, as well as metal chelating activity. In addition, antityrosinase and antigenotoxicity properties were also evaluated by the standard *in vitro* methods. Finally, the active methanolic extract (ME) was investigated for identifying the phenolic compounds using UPLC-MS/MS.

**RESULTS:** In the present study, chlorogenic acid, gulonic acid, and kaempferol were found to be the major components responsible for the antioxidant activity of *A. viridis* extract as evidenced from UPLC-MS/MS. Furthermore, the ME of *A. viridis* revealed excellent antioxidant activities such as DPPH radical scavenging activity (IC₅₀ = 47.23 ± 0.66 μg/mL), NO radical scavenging activity (IC₅₀ = 64.33 ± 2.01 μg/mL), hydrogen peroxide (H₂O₂) radical scavenging activity (IC₅₀ = 33.21 ± 3.3 μg/mL), ABTS radical scavenging activity (IC₅₀ = 47.61 ± 1.31 μg/mL), metal chelating activity (IC₅₀ = 32.1 ± 1.11 μg/mL), as well as lipid peroxidation inhibiting activity (IC₅₀ = 112 ± 1.21 μg/mL). Furthermore, ME revealed that the protective effects of extract were observed on H₂O₂-induced DNA damages with alkaline comet assay.

**CONCLUSIONS:** Taken together, the study concluded that the promising antioxidant capacities of *A. viridis* extract can further be utilized in various agricultural, pharmaceutical, and food applications.

**Keywords:** *Amaranthus viridis*, antioxidant, medicinal plants, polyphenols, ultra-high pressure liquid chromatography-tandem mass spectrometer

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 tumorigenesis disorders.[¹] 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.[²] Taken together, indigenous drugs and in 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.[3]

Overproduction of reactive oxygen species (ROS) positively correlates with various pathological degenerative diseases such as diabetes, cardiovascular dysfunction, atherosclerosis, inflammation, cancer, and neurodegenerative disease.[4] Nowadays, many reports confirm that food as well as pharmaceutical and nutraceutical industries commonly utilized many synthetic antioxidant compounds such as tert-butyl hydroquinone, butyl hydroxyanisole, and butyl hydroxytoluene. However, the commonly utilized synthetic antioxidant due to their toxic effects or undesirable effects on human health.[5] Recently, several research groups have demonstrated that natural antioxidants are the promising therapeutic agents for ROS, leading to the prevention of oxidative stress-related diseases.[6,7] The earlier study also demonstrated that polyphenol is the major secondary metabolite which is extensively distributed in the medicinal plants, vegetables, and dietary fruits and considered as potent oxygen and nitrogen radical scavenger.[8] In addition, high polyphenol intake has also been directly associated with lowering the risk of cardiovascular diseases (CVDs) and many other degenerative diseases.[2,3] Hence, attention has been escalated considerably in finding naturally occurring antioxidants.

*Amaranthus viridis* (*Amaranthaceae*) is a spread glabrous herb, normally called as Chauraiya widely distributed throughout the world, grown throughout the world and has been using in folk medicine for the treatment of hypercholesterolemia and diabetic. The leaves of *Amaranthus viridis* have a long history of indigenous utilized as a medicinal herb in the traditional Ayurvedic medicine as antipyretic agents and also eaten conventionally as a vegetable among tribal and nontribal people of Northeast India.[9,10] Most of the research on *A. viridis* has indicated that it has folkloric uses for the treatment of inflammation, ulcer, asthma, hyperlipidemic, diuretic, rheumatic, and analgesic.[10-12] In our earlier report, we have characterized and identified phenolic compounds present in *A. viridis* using ultra performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS).[12] To the best of our knowledge, limited studies have been reported on the chemical characterization of different fractions and antioxidant activities of *A. viridis* plants from northeast region. Therefore, this current study was designed to investigate the antioxidant, antityrosinase and antigenotoxicity potential of *Amaranthus viridis* extracts and their phytochemical properties were also determined.

**Materials and Methods**

**Chemicals and reagents**

All the standard were obtained from Sigma-Aldrich such as 2,2’-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), quercetin, 2-2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, ascorbic acid, and catechin. Sodium nitroprusside (SNP), ferrous chloride, sulfanilamide, ferrozine, and naphthyl ethylene diamine dichloride were procured from HiMedia Laboratory, India. All reagents and chemicals were obtained from certified suppliers and were of the highest analytical standard.

**Sample collection**

*A. viridis* were obtained from Six Mile, Guwahati, Kamrup district of Assam (situated in between 25° 43’ and 26° 53’ North latitude and 90° 39’ and 92° 11’ East latitude) in June–August 2016. It was authenticated in the Department of Botany, Gauhati University, Assam. Herbarium *A. viridis* (IASST/BCCS/HNO22/2013) were voucher specimens deposited for future references.

**Sample preparation**

*Amaranthus viridis* leaves were separated and washed under distilled water and dried at room temperature, powdered and utilized for extraction. Therefore, known amounts of dried plant powder (100 g) were dissolved in 1000 mL of methanol, distilled water, chloroform, and hexane for 72 h. The resulting extract was filtered through Whatman No. 1 filter paper and solvent filtrate was concentrated using a Rotary Evaporator and a BenchTop Lyophilizer. The extracts were stored at 4°C until further utilization in the designated experiment.

**Qualitative phytochemical analysis**

*A. viridis* leaf extracts were screened for the presence of secondary metabolites such as flavonoids, alkaloids, saponins, steroids, cardiac glycosides, phenolics, and tannins as described by previous method.[13]

**Quantitative phytochemical analysis**

**Determination of total phenolic, flavonoid, and tannin content**

Total content of polyphenols, flavonoids, and tannin in the extracts was determined as previously reported.[14-16] The amount of total polyphenol was calculated as a catechin equivalent from the calibration curve of catechin as a reference standard and expressed as mg catechin/g dry weight of the sample. Flavonoid concentration was expressed as quercetin equivalent, i.e., expressed as mg quercetin/g dry weight of the sample. In addition, total tannin concentration was expressed as mg catechin/g dry weight of the sample.
The identification and screening of the phenolic compounds present in the *A. viridis* extract were determined using mass spectrum and its unique fragmentation spectrum. The comparison of the observed MS/MS spectra with those found in the literature and mass bank database was the main tool for putative identification of the phenolic compounds.

**In vitro measurement of antioxidant properties**

*Diphenyl‑1‑picrylhydrazyl and 2,2’‑azinobis‑3‑ethylbenzothiazoline‑6‑sulfonic acid* radical scavenging activity

The antioxidant ability of the different fractions of *A. viridis* extracts was determined by the improved DPPH and ABTS' radical cation scavenging capacity methods as previously reported. For both free radical methods, ascorbic acid was utilized as a standard. The concentration range of different extract used for the antioxidant tests was 4–64 μg/mL. The percentage inhibition of DPPH and ABTS radical scavenging ability was calculated by applying the formula given below.

\[
\text{Scavenging effect (\%)} = \left( \frac{A_0 - A_s}{A_0} \right) \times 100
\]

**Total reduction capability**

Total reduction powers of different fractions of *A. viridis* extract were measured by the method. Briefly, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferric cyanide were added to 1 mL of samples in different concentrations (4–64 μg/mL), followed by gentle mixing. The mixture was incubated at 50°C in a water bath for 20 min. The reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid, and the mixture was centrifuged at 4000 rpm for 10 min. From the top layer, 2.5 mL was transferred into the tube containing 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride (FeCl₃·6H₂O) and mixed thoroughly. After 5 min, the absorbance was measured at 700 nm against blank. Ascorbic acid was taken as a reference standard.

**Hydrogen peroxide radical scavenging activity**

The radical scavenging activity of the extracts against hydrogen peroxide (H₂O₂) was measured by utilizing the method. Briefly, H₂O₂ (43 mM) was prepared in 0.1 M phosphate buffer solution (PBS, pH 7.4). Different fractions of *A. viridis* extract (1 mL) were mixed with H₂O₂ solution (43 mM). After the incubation, the reaction mixture absorbance was determined at 230 nm. The phosphate buffer without H₂O₂ was utilized as blank. Ascorbic acid was utilized as a reference standard. The percentage of scavenging was calculated based on the formula: percentage (%) of inhibition = (Aᵢ − A₂)/Aᵢ × 100.

**Nitric oxide radical scavenging assay**

Nitric oxide (NO) radical scavenging activity was measured according to the method. NO radical was generated from SNP solution. One milliliter of SNP (10 mM) was mixed with 1 mL of different fractions of *A. viridis* in different concentration extracts in phosphate buffer (0.2 M, pH 7.4). The mixture was incubated at 25°C for 150 min followed by mixing with 1.0 mL of pre-prepared Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dichloride and 2% phosphoric acid), and the absorbance was measured at 546 nm.

**Metal chelating activity**

The metal chelating capacity has been performed as described from the method. Briefly, 2 mL extract (A. viridis) solutions prepared in different concentrations were mixed with 0.2 mL of 5 mM ferrozine and 0.05 mL of 2 mM of FeCl₃. After mixing, 5 mL of total volume was adjusted by adding methanol. The mixture was incubated at room temperature for 10 min. Ethylenediaminetetaacetic acid (EDTA) was utilized as a reference standard. The absorbance was measured at 562 nm, and percentage of metal chelating activity was calculated in the formula as shown below:

\[
\text{Metal chelating ability (\%)} = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100
\]

where A_{sample} was absorbance of control (the control contained FeCl₃ and ferrozine) and A_{control} was absorbance of the extract.

**In vitro lipid peroxidation assay**

In this assay, lipid peroxidation (LPO) induced by Fe²⁺ ascorbate system in rat liver homogenate was determined as thiobarbituric acid reactive substance (TBARS) by the method. Liver homogenate (0.25 mL) was mixed with 0.1 mL Tris HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL (4 mM) FeCl₂ solution and 0.05 mL of different fractions of *A. viridis* extract. The mixture was incubated at 37°C for 1 h and followed by the addition of 1.5 mL 0.8% (w/v) 2-thiobarbituric acid, 1.5 mL 20% acetic acid, and 0.2 mL 8.1% (w/v) sodium dodecyl sulfate to the reaction.
mixture. The mixture was made up to 4.0 mL of distilled water and heated at 95°C for 60 min. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added. The mixture was shaken vigorously and was measured at 532 nm in a spectrophotometer (Beckman, UK). The results were compared with the reference standards of ascorbic acid as positive control. All tests were conducted in triplicate.

**Antityrosinase assay**

Tyrosinase activity was measured by the method. Briefly, 400 μL tyrosinase mushroom solution and PBS at pH 6.5 (300 μL) were mixed with or without *A. viridis*. The mixture was then preincubated at 25°C for 5 min before adding 400 μL of 1.25 mM DOPA solution. The reaction was monitored at 475 nm. Kojic acid was utilized as a positive control. The concentration range of different extract used for the antioxidant tests was 4–64 μg/mL. Each measurement was made in triplicate.

**Antigenotoxicity assay**

Antigenotoxicity assay was carried out according to the method. Leukocytes (blood collected from the human volunteer with proper consent) were isolated as a fraction of mononuclear cells (containing lymphocytes and monocytes) from anonymous buffy coat preserved by gradient centrifugation with Histopaque–1077 (Sigma, St. Louis, MO, USA). Leukocytes were incubated with different fractions of *A. viridis* extracts in DMSO (100 μg/mL) for 30 min at 37°C in a dark incubator. For oxidative stimulus, they were then re-suspended in PBS with 200 mM H₂O₂ for 5 min in ice. After each treatment, samples were centrifuged at 5000 rpm for 5 min and washed with PBS. 1% of DMSO without oxidative stimulus was treated for negative control. The leukocytes were then mixed with 75 μL of 0.7% low melting agarose and added to the slides precoated with 0.5% agarose. The slides were covered with coverslips and were refrigerated for 10 min to solidify the agarose. Next, the coverslips were removed, and the slides were immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, and 1% sodium lauryl sarcosinate; NaOH) for 1 h at 4°C. The slides were then placed in an electrophoresis tank containing 300 mM NaOH and 1 mM EDTA (pH >13.0) for 40 min to unwind DNA strands and expose the alkali-labile sites (alkaline unwinding). For electrophoresis of the DNA, an electric current of 24 V/300 ± 3 mA was applied for 20 min at 4°C. The slides were washed three times with a neutralizing buffer (0.4M Tris, pH 7.5) for 5 min at 4°C, stained with 65 μL of ethidium bromide (2 μg/mL) leave for 5 min, then dipped in chilled distilled water to remove excess stain, and then treated with cold 100% ethanol for another 20 min for dehydration. The slides were then air-dried and placed in an oven at 50°C for 30 min before covering with coverslips. Measurements were carried out by a fluorescence microscope (Leica DMLB, Germany) to determine the percentage of fluorescence in the tail (tail intensity, 50 cells from each of two replicate with slides). Measurements were carried out by visual scoring of the degree of DNA damage from 0 to 3 according to comet appearance.

**Statistical analysis**

The statistical analysis was carried out using the OriginPro 9.0 software packages (OriginLab Corporation, Northampton, MA, USA) and the statistical Pearson’s correlation coefficients using the OriginPro 6.0 software packages (OriginLab Corporation, Northampton, MA, USA). To determine the Student’s *t*-test, statistical significance was used and *P* < 0.05 was set as significant. IC₅₀ determinations were calculated by utilizing GraphPad Prism (GraphPad software, version 6.03 for Windows, La Jolla California USA) for Windows. All the measurements were performed in triplicates (*n* = 3). Mean values ± standard deviation were calculated.

**Results**

**Identification of phenolic compounds**

The screening and identification of *A. viridis* extract were performed by UPLC–ESI-MS/MS. The chromatographic runs are demonstrated in Figure 1. It reveals the observation of intense peaks at 0–10 min. The peak identification was performed by comparison of the retention time (RT), mass spectra, and λmax of the *A. viridis* with the reference standard compounds and the previous reports. Peaks with RTs (min) of 1.54, 1.69, 1.91, and 2.24 (peaks 1–5) were identified as the following: ferulic acid (RT 1.54, [M−H]⁻ ion at m/z 193.81, λmax 275 nm), chlorogenic acid (RT 1.91, 296 [M−H]⁻ ion at m/z 353.09, λmax 275 nm), gulonic acid (RT 1.62, [M−H]⁻ ion at m/z 195.81, λmax 275 nm), and kaempferol (RT 1.69, [M+H]⁺ ion at m/z 287.89, λmax 275 nm).

![Figure 1: Ultra-high pressure liquid chromatography chromatogram at 354 nm, 190–400 nm range of Amaranthus viridis extract](image-url)
Phytochemical content
In the present investigation, the phytochemical analysis of the extracts of *A. viridis* revealed the presence of major phytochemical compounds, including flavonoids, alkaloids, phenolics, steroids, terpenoids, saponins, cardiac glycosides, and tannins. In addition, total phenolic content (TPC), total flavonoids content, and total tannin content were found to be 16.4 ± 0.45 mg catechin/g of dry weight, 5.28 ± 0.33 mg quercetin/g of dry weight, and 1.76 ± 0.21 mg catechin/g of dry weight in *A. viridis*, respectively.

In vitro antioxidant activities

**Diphenyl‑1‑picrylhydrazyl radical scavenging activity**
As shown in Figure 2a, the photometric evaluations of the antioxidant capacities of aqueous extract (AE), methanolic extract (ME), chloroform extract (CE), and hexane extract (HE) of *A. viridis* were determined. The results from the DPPH radical scavenging ability was found to be high in ME of *A. viridis* (65.2% ± 1.41%, IC$_{50}$ = 47.23 ± 0.66 µg/mL) compared to AE (53.31% ± 1.08%, IC$_{50}$ = 57.32 ± 1.1 µg/mL), CE (60.33% ± 1.08%, IC$_{50}$ = 60.31 µg/mL), and HE (53.66% ± 1.08%, IC$_{50}$ = 56.31 µg/mL), respectively. Ascorbic acid showed 83.03% ± 1.83%, IC$_{50}$ = 3.21 ± 0.53 µg/mL at a concentration of 64 µg/mL.

**Total reduction capability**
The reduction capabilities of all the tested plant extracts were increased with increasing concentration in ascorbic acid equivalent as demonstrated in Figure 2b. From the present study, the absorbance of the reduction capability of the different fractions of AE, ME, CE, and HE of *A. viridis* and ascorbic acid at 64 µg/mL was 0.09, 0.096, 0.08, 0.28, respectively. The results revealed a concentration‑dependent significant elevation (*P* < 0.05) in the reductive capability of the test samples.

**2,2‑Azinobis‑3‑ethylbenzothiazoline‑6‑sulfonic acid ‑ radical cation decolourization assay**
*A. viridis* exhibited dose‑dependent effective antioxidant properties [Figure 2c]. The results from the ABTS' radical scavenging activity were found to be high in ME of *A. viridis* (60.2% ± 1.17%, IC$_{50}$ = 47.61 ± 1.31 µg/mL) at the concentration of 64 µg/mL followed by AE (56.83% ± 1.02%, IC$_{50}$ = 42.76 µg/mL), CE (52.26% ± 3.81%, IC$_{50}$ = 118 ± 2.63 µg/mL), and HE (57.66% ± 2.11%, IC$_{50}$ = 127 ± 0.63 µg/mL) at the concentration of 64 µg/mL, respectively.
Hydrogen peroxide radical scavenging assay

The scavenging ability of AE, ME, CE, and HE of A. viridis and ascorbic acid is demonstrated in Figure 2d. H₂O₂ radical scavenging activity of different fractions (AE, ME, CE, and HE) of A. viridis (51.54% ± 1.77%, IC₅₀ = 56.89 ± 2.51 μg/mL), (53.43% ± 1.07%, IC₅₀ = 33.21 ± 3.3 μg/mL), (41.54% ± 2.03%, IC₅₀ = 84.23 ± 2.1 μg/mL), (40.01% ± 1.03%, IC₅₀ = 34.23 ± 2.1 μg/mL), respectively. Ascorbic acid showed 13.21 ± 1.61 μg/mL at the concentration of 64 μg/mL. The IC₅₀ value revealed that the extract is an effective hydroxyl radical scavenger.

Nitric oxide radical scavenging activity

In the present investigation, ME of A. viridis revealed the highest inhibitory effect with the concentration of 64 μg/mL as (69.87% ± 1.09%, IC₅₀ = 34.23 ± 2.1 μg/mL), (52.06% ± 2.19%, IC₅₀ = 67.70 ± 2.56 μg/mL) at the concentration of 64 μg/mL. In contrast, reference standard ascorbic acid showed potent inhibitory effect of 87.14 ± 2.41% (IC₅₀ = 12.06 ± 0.78 μg/mL), respectively [Figure 2e]. Herein, A. viridis and other standards showed prominent NO scavenging activity which was quite comparable to reference standard at the concentration of 64 μg/mL.

Metal chelating activity

The ion chelating ability of A. viridis with positive control EDTA is summarized in Figure 2f. AE, ME, CE, and HE extracts of A. viridis showed the potent chelating ability at 64 μg/mL as (69.87% ± 1.09%, IC₅₀ = 33.06 ± 1.11 μg/mL), (58.03% ± 0.92%, IC₅₀ = 32.21 ± 3.3 μg/mL), (52.06% ± 2.19%, IC₅₀ = 58.16 ± 2.13 μg/mL), (44.06% ± 2.09%, IC₅₀ = 53.06 ± 1.11 μg/mL). On the other hand, chelating activity of synthetic chelator EDTA showed an excellent chelating ability of 76.03% ± 2.39% (IC₅₀ = 5.12 ± 1.1 μg/mL). The results obtained from the present investigation revealed that ME of A. viridis has an effective ability for iron binding, suggesting that its action as an antioxidant may be related to its iron binding capacity.

Inhibition of lipid peroxidation

LPO was significantly inhibited by all the extracts of A. viridis in a dose-dependent manner [Figure 3]. The ME of A. viridis revealed the highest inhibition of LPO with (45.66% ± 1.41%, IC₅₀ = 112 ± 1.21 μg/mL), whereas AE, CE, and HE of A. viridis showed inhibition of LPO with (40.86% ± 1.31%, IC₅₀ = 84.30 ± 3.31), (47.33% ± 3.24%, IC₅₀ = 73.32 ± 1.13 μg/mL), and (33.12% ± 4.24%, IC₅₀ = 143.32 ± 1.13 μg/mL), respectively.

Antityrosinase activity

Figure 4 displays the inhibition of tyrosinase at different concentrations (4–64 μg/mL) of AE, ME, CE, and HE extracts of A. viridis. The MEs of A. viridis showed higher antityrosinase activity (64.2% ± 1.12%, IC₅₀ = 36.31 ± 1.02 μg/mL) followed by AE (56.2% ± 2.2%, IC₅₀ = 62.32 ± 3.11 μg/mL), CE (51.1% ± 2.4%, IC₅₀ = 57.12 ± 2.41 μg/mL), and HE (48.2% ± 1.7%, IC₅₀ = 87.14 ± 2.41 μg/mL). Kojic acid was utilized as a reference standard, and it showed inhibition of 88.2% ± 1.7% (IC₅₀ = 6.32 ± 0.77 μg/mL). The results had distinctly indicated the efficacy of all the extracts as a significant and promising source of inhibiting tyrosinase.

Antigenotoxic activity

At the concentration of 64 μg/mL, the AE, ME, CE, and HE of A. viridis revealed the protective effects on H₂O₂-induced DNA damages with visual scoring 1. A. viridis extract at concentration 100 μg/mL showed the protective effects on H₂O₂-induced DNA damages with visual scoring 2 [Figure 5].

Discussions

Phenolic compounds represent one of the important families of antioxidants due to their free radical scavenging activity. Nevertheless, the presence of phytochemical compounds such as phenolics, at lower concentration, has shown significant beneficial pharmacological properties, such as antiviral, anti-inflammatory, antioxidant, antimicrobial, antimutagenic, and chemopreventive activity. Many reports also suggested that with an elevated concentration of polyphenols present in the plant extracts may...
contribute directly to their antioxidant properties.\textsuperscript{[11,13,17]} The previous report already suggested that the TPCs of \textit{A. viridis} were in the range of 2.81–3.61 gallic acid equivalent, g/100 g.\textsuperscript{[19]} Nevertheless, in the present study, the TPC of \textit{A. viridis} was significantly high in comparison with the earlier study.\textsuperscript{[12,19]} An earlier report also demonstrated that the presence of phenolic compounds such as chlorogenic acid, apigenin, kaempferol, asiaticosides, brahmic acid, asiatic acid, steroids, glycosides, and rosmarinic acid in different medicinal plants might be responsible for scavenging the free radical as well as enhanced the antioxidant activities.\textsuperscript{[2,12,16,18,20]}

In addition, the reduction ability of DPPH radicals was determined by the decrease in its absorbance at 517 nm enhanced by different fractions having antioxidant potential.\textsuperscript{[12,21]} The earlier report also suggested that high polyphenols intake from natural sources such as vegetables and fruits are associated with decreased risk of many degenerative diseases and CVD by attenuated free radicals from the human body.\textsuperscript{[2,12,15,21]} It was already reported that lower the IC\textsubscript{50} value, the higher is the free radical scavenging capability and vice versa.\textsuperscript{[2,12]} Herein, from this study, it is revealed that MEs of \textit{A. viridis} have excellent antioxidant potential as compared to AE, CE, and HE, which was quite in similar with the previous reports. In addition, the reducing ability of a compound may serve as a remarkable indicator of its potent antioxidant properties.\textsuperscript{[2,12,15,16,21]} In our previous report, the phenolic compounds of \textit{A. viridis} revealed significantly minimized Fe\textsuperscript{3+}/ferricyanide complex into the ferrous form (Fe\textsuperscript{2+}); the yellow color of test sample changes to blue and green colors depending on the reducing ability of antioxidant activity.\textsuperscript{[21]} However, in this study, the reducing capability of the tested different fractions of \textit{A. viridis} was quite corroborated with earlier studies.\textsuperscript{[2]} These findings indicated that the \textit{A. viridis} extract had noticed the difference in ferric ions (Fe\textsuperscript{3+}) reducing power.

It is believed that ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm, which decreases with the scavenging capacity.\textsuperscript{[2,12,15]} Earlier studies have already demonstrated that the high molecular weight phenolics have potent capacity to quench free radicals such as ABTS\textsuperscript{+}.\textsuperscript{[16]} Herein, from this

![Figure 4: Effect of different extracts of \textit{A. viridis} on antityrosinase activity. Each point represents the mean ± SD (n = 3). ***, **, and * show statistical significant differences at p < 0.001, p < 0.01, and p < 0.05](image)

![Figure 5: Protective effects of different extracts \textit{Amaranthus viridis} on oxidative DNA damage in human leukocytes. (a) Negative control without oxidative stimulus (visual scoring 0), (b) positive control with oxidative stimulus (visual scoring 3), (c) aqueous extract with oxidative stimulus (visual scoring 1), (d) methanolic extract with oxidative stimulus (visual scoring 2), (e) chloroform extract with oxidative stimulus (visual scoring 1), (f) hexane extract with oxidative stimulus (visual scoring 1)](image)
In the present study, NO was remarkably inhibited by all the fractions of *A. viridis* extracts in a dose-dependent manner. In addition, we further confirm that different fractions of *A. viridis* showed an excellent metal chelating ability with low IC<sub>50</sub> value. It was already showed that the chelating properties revealed the presence of the potential antioxidant activity of the pure phenolic compounds or the plant extracts. In addition, it was already reported that LPO is one of the vital causes of cancer and CVD generating malondialdehyde (MDA)-like product during through a set of chemical reaction. Similarly, the result of the present study reports that LPO was remarkably inhibited by all the fractions of *A. viridis* extracts a dose-dependent manner. Hence, there is an expanding enthusiasm in the exploration on the oxidative degradation level produced by MDA and the protection by antioxidants. In our previous study, it was already reported that polyphenolic compounds have revealed more potent to prevent LPO and the associated degenerative diseases.

Tyrosinase is an essential enzyme in melanin biosynthesis, which involved determining the color of hair and skin, and its abnormal expression is responsible for the various skin disorders such as melasma, neuromelanin in the human brain, age spots, and Parkinson’s disease. However, many reports revealed a positive relationship between antioxidant and antityrosinase activity. Herein, in this study, the results were quite in convincingly agreement with the previous reports. In addition, several reports also demonstrate that H<sub>2</sub>O<sub>2</sub> is a common intermediate in a variety of oxidative stress and has also been revealed to induce DNA damage, leading to mutagenesis. Our results clearly indicated that compared to all the fractions, ME of *A. viridis* is a potent inhibitor of OH radical in a dose-dependent manner. Earlier reports have demonstrated that the nutraceuticals that protect DNA from oxidant challenge or that encourage DNA repair may have potential health benefits and help lower risk of age-related disease.

**Conclusions**

The present study validates the antioxidant, antityrosinase and antigenotoxicity potential of *Amaranthus viridis* extracts and their phytochemical properties were also determined. Herein, *A. viridis* showed the promising pharmacological properties in several in vitro model systems by total reduction capability, DPPH and ABTS free radical scavenging activity, and metal chelating activity. In addition, NO radical scavenging activity, as well as LPO inhibition, was determined by TBARS assay. In addition, the results of the present study revealed the presence of strong phenolic antioxidant components mainly gulonic acid, kaempferol, and chlorogenic acid in *A. viridis* extract as evidenced from UPLC-MS/MS. The current study reveals that the plant extracts have excellent pharmacological properties. Further studies are needed to identify and isolate the bioactive compounds present in the evaluated plant species; further, experiment on *in vivo* animal model will be highlighted for the treatment of oxidative stress-related disease. Thus, these *A. viridis* leaf extracts might be utilized as natural agents in pharmaceutical and food industries.

**The significance of findings**

The present study validates that the *A. viridis* has excellent antioxidant activity as well as inhibition of LPO ability. Thus, *A. viridis* might be a promising ingredient for food with potential health and nutritional benefits.

**Acknowledgment**

We are thankful to the Department of Science and Technology, New Delhi, for providing financial assistance to carry out this research work.

**Financial support and sponsorship**

We are thankful to the Department of Science and Technology, New Delhi, for providing financial assistance to carry out this research work.

**Conflicts of interest**

There are no conflicts of interest.
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