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Original Article

Exploring Polyphenol Based Bioactive Antioxidants of Underutilized Herb *Amaranthus Spinosus* L. for Medicinal Purposes

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Abstract

Background and objectives: Though *Amaranthus spinosus* L. or 'spiny amaranth' belonging to the family Amaranthaceae is widely used in folklore and for ethnomedicinal purposes, little is known about the ecotype-based bioavailability of bioactive polyphenolic compounds that could cause health benefits. Hence, this was the objective of the present study.

Methods: Reversed-phase high-performance liquid chromatography coupled with a photodiode assay was used to investigate pharmacologically significant bioactive flavonoids and phenolic acids from hydroethanolic leaf extract of two different ecotypes (the Rarh region and coastal plain of West Bengal, India) of *Amaranthus spinosus* L. Furthermore, the antioxidant capacity of the leaf tissue extract of both the ecotypes of this promising crop was evaluated in terms of the metal chelating property, total antioxidant capacity (2,2-diphenyl-1-picryl-hydrazyl-hydrate assay), anti-lipid peroxidation property, and the total pool of flavonoids and phenolics for validating their health-promoting anti-degenerative chemical properties.

Results: The results exhibited a rich source of pharmacologically important bioactive flavonoids and phenolic acids derived from the chalcone synthase and cinnamate-derived pathways for both the ecotypes, but when comparing the ecotype of the Rarh region, it proved to be superior to the ecotype of the coastal region.

Conclusions: Overall, the study suggests a region-specific ecotype effect on the accumulation of dietary flavonoids, phenolic acids, and antioxidant traits of *Amaranthus spinosus* L., thus substantiating their utility in the prevention of degenerative diseases. The study also highlighted the significance of plant-environment interaction in a secondary metabolic pathway, which may be explored in the future for improving the medicinal and functional food properties of underutilized crops for the prevention of degenerative diseases.

Keywords: *Amaranthus spinosus*; Bioactive polyphenols; Ecotypes; Antioxidant property; Phytonutrients.

Abbreviations: DPPH assay, 2, 2-diphenyl-1-picrylhydrazyl assay; ROS, reactive oxygen species; RP-HPLC, reverse phase-high performance liquid chromatography; *rpm*, revolutions per minute; RT, retention time.

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Introduction

In recent times, research on the effects of dietary polyphenols derived from plants on human health has developed considerably.[1–3] Polyphenols generally exert protective action against degenerative diseases, particularly cancers, as well as neurodegenerative and cardiovascular diseases.[4–6] Current studies have also exposed that several of these diseases are associated to oxidative stress triggered by reactive oxygen and nitrogen species.[7,8] However, plant secondary metabolites, especially polyphenols, have been found to be

one of the most potent classes of compounds responsible for conferring antioxidant properties in plants. Additionally, polyphenols significantly reduce oxidative stress by hindering the formation of or neutralizing the reactive oxygen species and precursors of oxyfree radicals. In several cases, they have acted as direct radical scavengers of the intermediates of the chain reaction of lipid peroxidation, thereby acting as chain breakers.[8–10] In fact, chain breakers donate an electron to the oxyfree radicals, deactivating the radicals, and themselves becoming stable (less reactive) radicals, thus stopping the chain reactions of lipid peroxidation.[7,8] Furthermore, apart from the direct scavenging of reactive oxygen species (ROS), polyphenols act as transition metal chelators necessary for the Fenton reaction. Chelation of transition metals, such as Fe^{2+} can directly reduce the rate of the Fenton reaction, thus preventing the formation of highly reactive hydroxyl radicals.[11,12] Moreover, several previous studies indicated the pharmacological role of flavonoids as anti-inflammatory, gastro and hepato-protective, antimicrobial, antifungal, antiviral, antimalarial, antineoplastic, and antidiabetic agents, while corroborating their strong antioxidant functions.[13–15] Recently, serious focus has been paid in discovering the dietary sources of natural antioxidants of plants to prevent such degenerative diseases in the backdrop of significant negative effects of synthetic antioxidants.

Amaranthus spinosus L. or 'spiny amaranth', commonly consumed as a leafy vegetable, is cultivated widely in tropical countries and warm temperate regions of Asia, including India, Bangladesh, and Sri Lanka. This underutilized medicinal herb, belonging to the family Amaranthaceae, has frequently been used in traditional systems of medicine.[16] In addition, the plant has been explored for diverse purposes as a diuretic, laxative, antipyretic, and febrifuge, as revealed from the Ayurveda and other Indian traditional systems of medicine. There are also records of utilization of this medicinal herb to treat diverse medical conditions like blood diseases, bronchitis, leprosy, piles, leucorrhea, anorexia, flatulence, nausea, *etc*.[17–21] Previous works based on phytochemical investigations revealed that spiny amaranth is a good reservoir of various medicinally important secondary metabolites like glycosides, phenolic compounds, steroids, terpenoids, betalain, stigmasterol, saponin, linoleic acid, carotenoids, tannins, *etc*. that strongly assure their medicinal properties as well as antioxidant potential.[22–25]

Most of the bioactive substances or secondary metabolites of plant origin are produced as a result of plant-environment interaction during their evolutionary history. The genesis of these compounds associated with the phytonutrient promise was therefore found to have a significant correlation with the plant-environmental interaction.[26,27] For example, the contents of phenolic compounds in crops were found to be affected by different agroclimatic environments, consequently causing an alteration of their phytonutrient properties.[27,28] Till now, several studies have considered *Amaranthus spinosus* L. for its ethnomedicinal uses and nutritional attributes, but there is a dearth of literature which considers the ecotype effect on the bioaccumulation of polyphenol-based antioxidants with phytonutrient promise. Therefore, identification of the right ecotype of medicinal plants like *Amaranthus spinosus* deserves special attention in view of realizing their phytonutrient promise. Moreover, the role of the phenotypic plasticity and genotypic diversity necessary for adaptation in diverse ecological conditions, while regulating the secondary metabolic pathway for improving the medicinal and functional food properties has been seldom studied; hence, this is the primary objective of the present study. In the current study, an effort was made to compare the ecotype specific variation of the accumulation of bioactive polyphenolic compounds (derived from the phenylpropanoid pathway) and associated antioxidant properties necessary for characterizing a low-cost supplement of natural antioxidants for the mitigation of degenerative diseases.

Materials and methods

Amaranthus spinosus L. plant was collected from the local harvest of two different phytogeographical regions of West Bengal, India [the Rarh region (comprising of the phytogeographical region of three districts: Burdwan, Bankura, Birbhum, with alluvial soil and average rainfall of 135 cm) and the coastal plain (the phytogeographical region of Diamond Harbor with clay loamy soil and average rainfall of 200 cm)]. Then, the plant materials were identified from the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India [Voucher Specimen no.-CNH/Tech./II/2020/5b, reference no.-BU/SB/AK-004 (Rarh region) and BU/SB/AK-006 (Diamond Harbor)]. The leaves were separately harvested and dried at 45°C for 48 h in a hot air oven. Then, the samples were ground to a fine powder and were kept in an opaque bottle for further experimental work.

Sample preparation for the HPLC study

In the Soxhlet apparatus, 15 g of oven-dried and powdered leaves from each experimental sample were extracted with 150 ml 95% of ethanol for three to four cycles. The extract was taken and filtered. The filtrate was concentrated and dried in a rotary vacuum evaporator (Eyela) at 50 ± 2°C and subsequently filtered using membrane filter paper (Millipore) before being subjected to high-performance liquid chromatography (HPLC).

RP-HPLC study for the estimation of the phenolic acids and flavonoids

Reversed-phase high-performance liquid chromatography (RP-HPLC) analyses were conducted by a Dionex Ultimate 3000 liquid chromatography system using a diode array detector (DAD) with a 5 cm flow cell with a Chromeleon system manager as the data processor. A reversed-phase Acclaim C18 column (5 µ particle size; 250 × 4.6 mm) was used to separate the phenolic compounds. 20 µl of the sample was loaded into the HPLC column. The mobile phase was made up of solvent A (methanol) and solvent B (0.05% of aq. acetic acid) and the column was thermostatically regulated at 25°C. In the mobile phase (methanol and acetic acid), the dried crude extract was dissolved. Subsequently, a gradient elution was done by varying the amount of the solvent A to solvent B. Per sample, the total analysis time was 105 m. We observed the HPLC chromatograms at three different wavelengths (272, 280, and 310 nm, respectively) using a photodiode array UV detector. We identified each phenolic compound by its RT value and by enriching it with the known standards used in this experiment under the same conditions. The quantitative estimation of the 21 experimental polyphenolic compounds (gallic acid, protocatechuic acid, ferulic acid, caffeic acid, sinapic acid, p-hydroxyl-benzoic acid, chlorogenic acid, ellagic acid, syringic acid, p-coumaric acid, salicylic acid, gentisic acid, vanillic acid, naringin, apigenin, catechin, myricetin, quercetin, naringenin, kaempferol, and rutin) presented in the methanolic leaf extract was conducted by measuring the integrated peak area against the peak areas of the corresponding standard samples. For the preparation of the standard solutions of the 21 polyphenolic compounds

(Sigma Chemicals), a stock solution of each in methanol (HPLC-grade) with a concentration of 10 µg/ml was prepared. The HPLC grade membrane filters (0.45 mm; Millipore) were used to filter all the standard solutions.[29]

Sample preparation and extraction for the quantification of the pool of polyphenolic compounds

50 ml of 95% of methanol was added to 5 g of each powder plant material for extraction for 48 h. In order to analyse the phytochemical content, we centrifuged the extracts at 10,000 *rpm* for 15 m at room temperature, and then stored the supernatants in the refrigerator.

Total phenolic content

The total phenol content in the extracts was determined using the spectrophotometric Folin-Ciocalteu method of Djeridane *et al*.[30] 2 mL of the Folin-Ciocatleu reagent and 2 ml of saturated $NaHCO_3$ were added to the extract solutions. After incubating the reaction mixture at 45°C at room temperature, the absorbance was read at 765 nm. Based on a standard curve of gallic acid, the amount of the total phenolics in the leaf samples of the two ecotypes was calculated as gallic acid equivalents per gram of dry weight.

Total flavonoid content

For the quantification of the flavonoids present in the leaf extracts, the process of Chang *et al*. was used.[31] An aliquot of 0.1 ml of methanolic leaf extract solution was mixed with 0.1 ml of $AlCl_3$ solution and 0.1 ml of CH_3COOK solution with a volume made up to 3 ml with distilled water. The absorbance was performed at 415 nm, and the concentration was calculated using the standard curve for the quercetin equivalents per gram of dry mass.

DPPH radical scavenging assay

For the determination of the total antioxidant capacity of the leaf extracts, a 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay was conducted according to the process of Shyu and Hwang.[32] In the DPPH radical scavenging assay, the dark violet colored stable DPPH radical was made to react with the plant extract, where depending on the reducing capacity of the experimental plant extracted the dark violet color to become pale yellow or colorless. To determine the scavenging activity, 1 ml of plant extract was mixed with 3 ml of the DPPH solution. Then, the solution was incubated for 30 m at 35°C and the absorbance was measured. The absorbance of the DPPH without the plant sample (control) was also measured. Radical scavenging by DPPH was calculated by using the formula:

$$\%\ \text{of inhibition} = \frac{Ac - As}{Ac} \times 100$$

where Ac was the absorbance of the control, and As was the absorbance with the plant extract.

Finally, the IC_{50} of the antioxidant capacity of each plant extract was determined, as the inhibitor concentration (estimated on the basis of the dried leaf extract) required inhibiting 50% of the radical scavenging activity in terms of the DPPH assay.

Metal chelating property

Based on the process described by Lin *et al*., the metal chelating property of the experimental plant sample was estimated.[33] Briefly, 1 ml of the plant extract was added with 0.02 ml of ferrous chloride (20 mM) and 0.04 ml of ferrozine (5 mM). At 562 nm, the absorbance was measured after shaking the mixture for 10 m at room temperature. The metal chelating ability was calculated by:

$$\text{Activity}\ (\%) = \frac{Ac - As}{Ac} \times 100,$$

where Ac was the absorbance of the control, and As was the absorbance with the plant extract.

Anti-lipid peroxidation assay

The inhibition of the lipid peroxidation of the experimental foliar sample was evaluated according to the process of Amabye by measuring the oxidation of the linoleic acid.[34] The plant extracts were added to 0.13 ml of linoleic acid solution, 10 ml of 99.8% ethanol, 10 ml of 0.2 M sodium phosphate buffer, and the volume was made up to 25 ml with distilled water. Briefly, 0.2 ml of ammonium thiocyanate solution (30%), 10 ml of ethanol, and 0.2 ml of ferrous chloride solution (20 mM in 3.5% HCl) were added to the mixture and after 3 m of stirring, the absorption was measured at 500 nm. The negative control contained all the reagents except the plant extracts. The maximum peroxidation was observed at 360 h (15 days). Oxidation was evaluated by the following equation:

$$\frac{1 - \text{change in absorbance of treated sample}}{\text{change in absorbance of the control sample}} \times 100.$$

Statistical analysis

To ascertain the differences in the content of bioactive polyphenols and antioxidant capacity between the two ecotypes of Amaranthus spinosus L., we collected leaf samples from each ecotype in triplicate, with each sample analyzed in duplicate to ensure reproducibility and reliability of the results. The experimental procedures were independently replicated on two separate occasions to validate the consistency of the findings. For the statistical treatment of data, we employed the unpaired two-sample t-test, which facilitated the comparison of mean values for the two sets of unpaired data. The t-tests were executed using GraphPad Prism 8.0, which provided a robust platform for data analysis. Data are expressed as means ± standard errors. $p < 0.05$ was considered statistically different.

Results

In the present investigation, we targeted 21 polyphenolic compounds of the phenylpropanoid pathway with the established antioxidant promise for the comparative RP-HPLC analysis of the hydro-ethanolic leaf extracts of two experimental ecotypes. This was because the complete qualitative and quantitative profiling of the entire individual polyphenolic compounds was not possible because of their huge structural and functional diversity. For this, we explored pharmaceutically important phenolic acids and flavonoids, which were derived from the phenylpropanoid pathway involving chalcone and cinnamic acid (salicylic acid, chlorogenic acid, protocatechuic acid, p-hydroxyl benzoic acid, gentisic acid, p-coumaric acid, syringic acid, caffeic acid, sinapic acid, ellagic acid, ferulic acid, vanillic

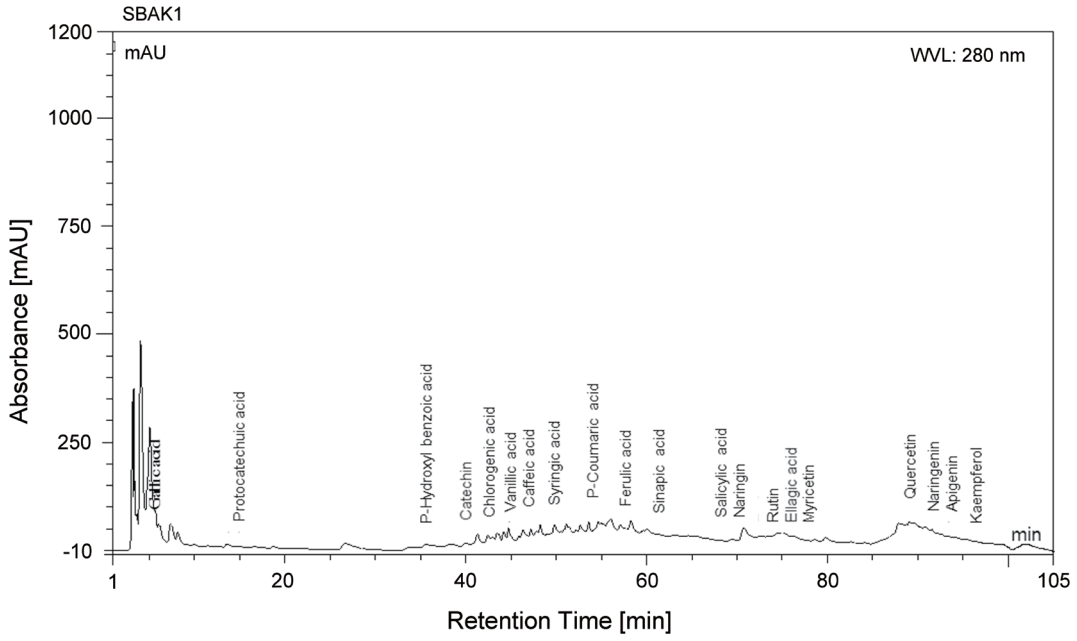


Fig. 1. RP-HPLC derived chromatogram of pharmacologically important phenolic acids and flavonoids of the leaf tissue of *Amaranthus spinosus* L. collected from the Rarh region (Burdwan) of West Bengal. RP-HPLC, reverse phase-high performance liquid chromatography; WVL, wavelength.

acid, and gallic acid, apigenin, rutin, naringin, naringenin, myricetin, catechin, quercetin, and kaempferol) and correlated their bioavailability with important biomarkers of the antioxidant properties. The leaf extract of the Rarh region exhibited greater abundance of some phenolic acids like gallic acid, chlorogenic acid, p-hydroxyl benzoic acid, p-coumaric acid, caffeic acid, salicylic acid, and syringic acid (Fig. 1). On the contrary, the leaf extract of the coastal plain revealed greater abundance of ellagic acid, protocatechuic acid, ferulic acid, and syringic acid (Fig. 2). When we compared the bioavailability of eight bioactive flavonoids derived from the chalcone synthase dependent phenylpropanoid pathway, we found the maximum bioavailability of naringin, rutin, quercetin, apigenin, and catechin in the leaf tissue extract of the Rarh region, whereas the leaf extract of the coastal plain exhibited greater abundance of myricetin and naringenin (Table 1). Overall, the leaf extract of the Rarh region exhibited a presence of 20 polyphenolic compounds tested as compared to

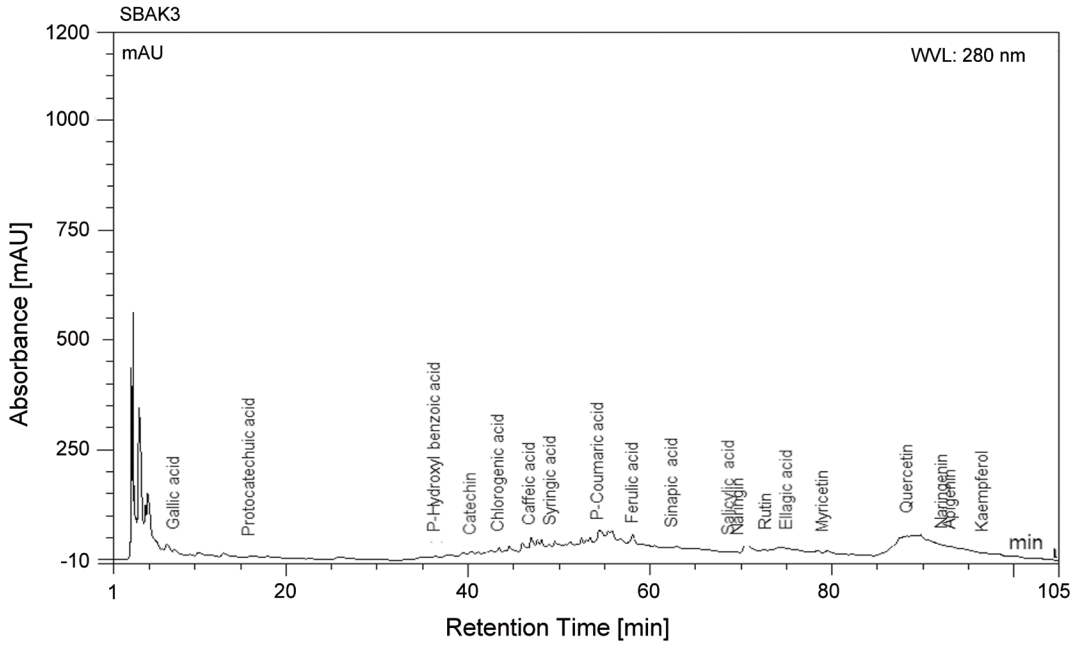
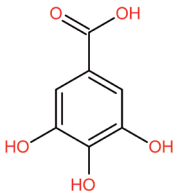
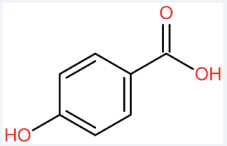
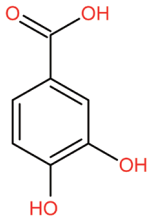
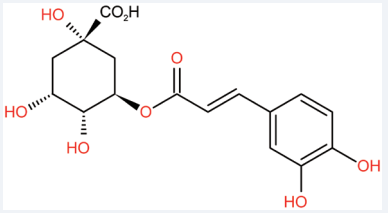
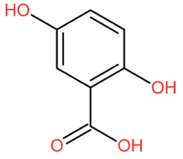


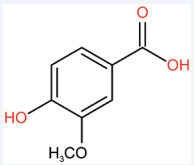
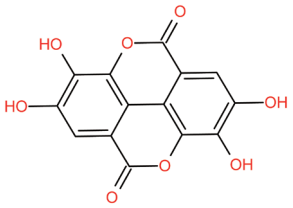
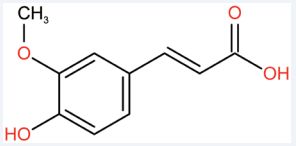
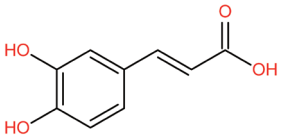
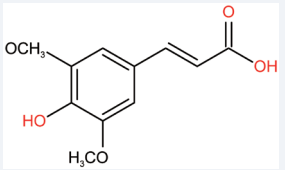
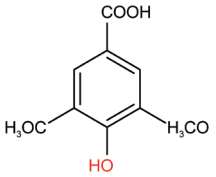
Fig. 2. RP-HPLC derived chromatogram of the pharmacologically important phenolic acids and flavonoids of the leaf tissue of *Amaranthus spinosus* L. collected from the coastal region (Diamond Harbor) of West Bengal. RP-HPLC, reverse phase-high performance liquid chromatography; WVL, wavelength.

Table 1. Comparative account of the availability of pharmacologically important bioactive polyphenolic compounds and their pharmacological activities of the two different ecotypes of *Amaranthus spinosus* L.

Name of the polyphenolic compound	Structure	Pharmacological activities	Amount (µg-^{100g}dm) in *Amaranthus spinosus* ecotypes	
			Ecotype of Rarh region (Burdwan)	Ecotype of the coastal plain (Diamond Harbor)
Gallic acid		Antioxidant, Anti-inflammatory, Antineoplastic, Anticancer	602.92	232.75
P-hydroxyl benzoic acid		Antimutagenic, Antiestrogenic, Anti-inflammatory	60.44	20.5
Protocatechuic acid		Antioxidant, Anticancer, Antiulcer, Antidiabetic, Antiaging, Anti-inflammatory, Antiartherosclerotic, Hepatoprotective, Cardioprotective	35.8	113
Chlorogenic acid		Antioxidant, Anti-inflammatory, Hepatoprotective, Cardioprotective, Neuroprotective	98	36.73
Gentisic acid		Antioxidant, Hepatoprotective, Anti-inflammatory, Neuroprotective	–	–

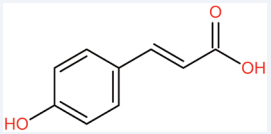
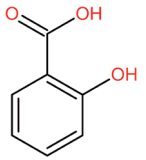
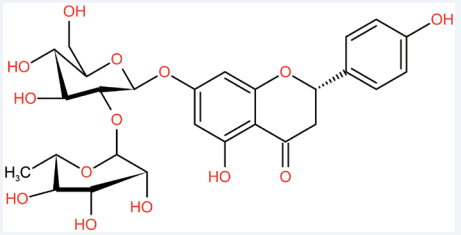
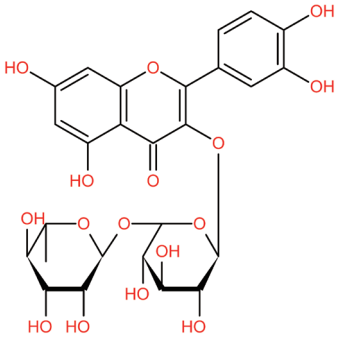
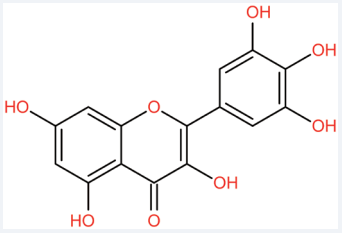
(Continued)

Table 1. *(Continued)*

Name of the polyphenolic compound	Structure	Pharmacological activities	Amount (µg-^{100g}dm) in *Amaranthus spinosus* ecotypes	
			Ecotype of Rarh region (Burdwan)	Ecotype of the coastal plain (Diamond Harbor)
Vanillic acid		Anti-inflammatory, Neuroprotective	29.4	–
Ellagic acid		Anticancer, Anti atherogenic, Neuroprotective, Anti-inflammatory	160.76	169.55
Ferulic acid		Anticancer, Antioxidant, Cardioprotective, Neuroprotective, Anti-inflammatory, Antidiabetic	100.9	119.75
Caffeic acid		Anticarcinogenic, Antioxidant, Anti-inflammatory	194.8	99.47
Sinapic acid		Antioxidant, Anti-inflammatory, Neuroprotective, Antimutagenic, Anticancer	2.1	14.45
Syringic acid		Cardioprotective, Anticancer, Hepatoprotective, Neuroprotective, Antioxidant, Anti-inflammatory	117.42	66.92

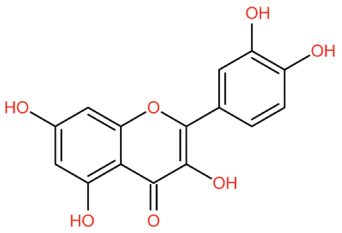
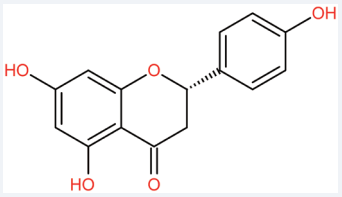
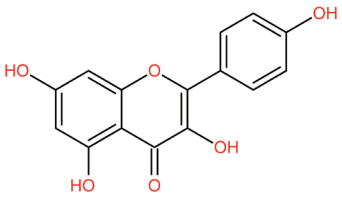
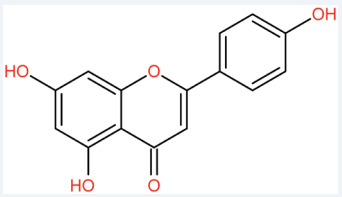
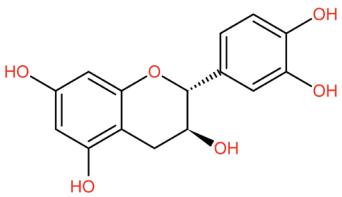
(Continued)

Table 1. *(Continued)*

Name of the polyphenolic compound	Structure	Pharmacological activities	Amount (µg-^{100g}dm) in *Amaranthus spinosus* ecotypes	
			Ecotype of Rarh region (Burdwan)	Ecotype of the coastal plain (Diamond Harbor)
P-coumaric acid		Anti-inflammatory, Antioxidant, Antineoplastic	168.4	87.6
Salicylic acid		Anti-inflammatory	161.6	23.95
Naringin		Anticancer, Anti-inflammatory, Hepatoprotective, Antiapoptotic, Antimutagenic	106.54	79.6
Rutin		Anti-inflammatory, Antidiabetic, Cytoprotective, Vasoprotective, Anticarcinogenic, Neuroprotective, Cardioprotective, Antiarthritic, Antiulcer, Neuroprotective	20.68	20.25
Myricetin		Anti-inflammatory, Antidiabetic, Anticancer	28.7	123.95

(Continued)

Table 1. *(Continued)*

Name of the polyphenolic compound	Structure	Pharmacological activities	Amount (µg-^{100g}dm) in *Amaranthus spinosus* ecotypes	
			Ecotype of Rarh region (Burdwan)	Ecotype of the coastal plain (Diamond Harbor)
Quercetin		Anti-inflammatory, Anticancer, Antitumor	326.6	186.97
Naringenin		Antidiabetic, Antiatherogenic, Antitumor, Antiinflammatory	8.94	24.57
Kaempferol		Anticancer, Antiapoptotic, Anti-inflammatory, Antidiabetic, Cardioprotective	13.38	2.32
Apigenin		Anti-inflammatory, antitoxicant, anticancer	5.7	.327
Catechin		Anticancer, Cardioprotective, Protect against Neodegenerative disease, Anti-inflammatory, Antiallergic	141.32	124.5

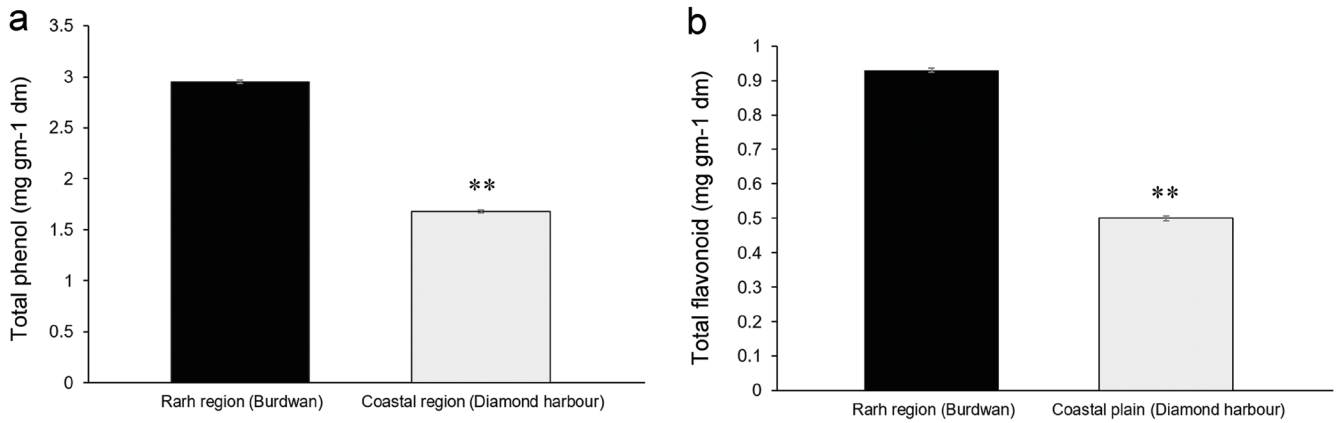


Fig. 3. Comparative assessment of non-enzymatic antioxidant content in *Amaranthus spinosus* L. ecotypes. The differential accumulation of total phenolic and flavonoid content in leaf extracts from two ecotypes of *Amaranthus spinosus* L. (a) The total phenolic content and (b) showcases the total flavonoid content, both measured in the Rarh region and coastal plain ecotypes. The data suggest ecotype-specific variations in the concentration of these critical antioxidant compounds. Data are expressed as means ± standard errors. $^{**}p < 0.01$.

19 compounds from the leaf tissue extract of the coastal plain, thus making the ecotype of the Rarh region to be superior based on the diversity of the phenylpropanoid pathway dependent polyphenolic compound available.

The estimation of the total pool of foliar flavonoids and phenols of the two different experimental ecotypes substantiated the data of the abundance of polyphenolic compounds tested through RP-HPLC (Figs. 3a, b). For the comparative estimation of the antioxidant properties and associated phytonutrient attributes, we standardized the sensitive biomarkers of the antioxidant potential like the total antioxidant capacity (radical scavenging property), metal chelating property, and anti-inflammatory property (anti-lipid peroxidation ability). The leaf extract of the Rarh region showed a significantly better metal chelating property compared to the leaf extract of the coastal region (Fig. 4a). The maximum DPPH radical scavenging ability was observed in the leaf extract of the Rarh region followed by the coastal

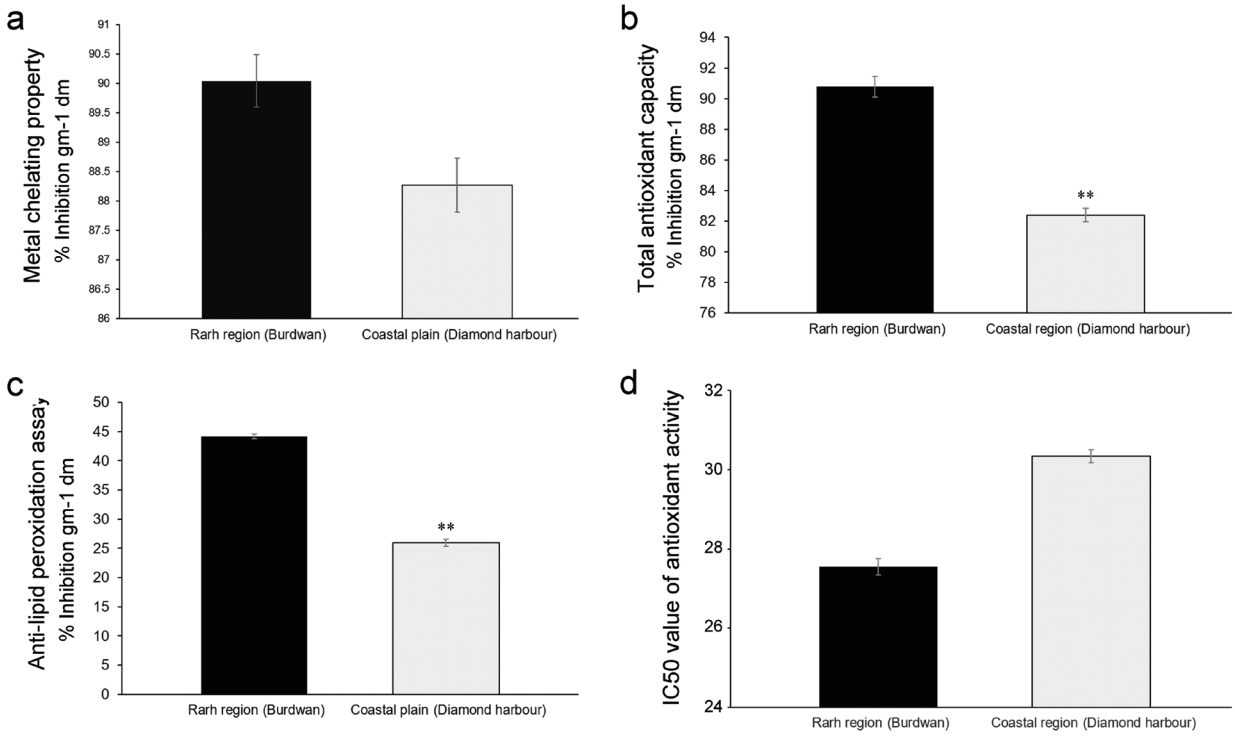


Fig. 4. Comparative analysis of antioxidant activity in *Amaranthus spinosus* L. ecotypes. A comparative evaluation of the antioxidant capabilities of leaf extracts from two distinct ecotypes of *Amaranthus spinosus* L. (a) demonstrates the metal chelating properties, (b) details the DPPH radical scavenging activity, and (c) shows the anti-lipid peroxidation potential of the extracts. The IC50 value, representing the concentration required to inhibit 50% of radical scavenging activity, is depicted in (d). The results underscore the superior antioxidant activity of the Rarh region ecotype compared to the coastal plain ecotype across all tested parameters. Data are expressed as means ± standard errors $^{**}p < 0.01$. DPPH assay, 2, 2-diphenyl-1-picrylhydrazyl assay.

Table 2. The correlation coefficient between the total antioxidant capacity (DPPH assay) and phenolic compounds of the leaf extract of the two ecotypes of *Amaranthus spinosus* L.

	DPPH assay	Total phenol	Total flavonoid	Gallic acid	Ellagic acid	p-hydroxyl benzoic acid	Chlorogenic acid	Caffeic acid	Myricetin	Naringin	Quercetin	Apigenin
DPPH assay	1											
Total phenol	1	1										
Total flavonoid	1	1	1									
Gallic acid	1	1	1	1								
Ellagic acid	−1	−1	−1	−1	1							
p-hydroxyl benzoic acid	1	1	1	1	−1	1						
Chlorogenic acid	1	1	1	1	−1	1	1					
Caffeic acid	1	1	1	1	−1	1	1	1				
Myricetin	−1	−1	−1	−1	1	−1	−1	−1	1			
Naringin	1	1	1	1	−1	1	1	1	−1	1		
Quercetin	1	1	1	1	−1	1	1	1	−1	1	1	
Apigenin	1	1	1	1	−1	1	1	1	−1	1	1	1

DPPH assay, 2, 2-diphenyl-1-picrylhydrazyl assay.

plain (Fig. 4b). The inhibition of the oxidation of linoleic acid, explored as the basis of the anti-lipid peroxidation assay, showed the same result with the foliar extract of the Rarh region exhibiting a significantly higher inhibition of peroxidation of the lipid followed by the leaf extract of the coastal region (Fig. 4c). The comparative estimation of the total pool of the flavonoids and phenolic acids from the methanolic foliar extracts of the experimental ecotypes strongly substantiated the data of all the important parameters or biomarkers of the antioxidant potential investigated (Figs. 3a, b).

The IC_{50} value was determined to estimate the sample concentration needed to inhibit 50% of the radical. The higher the antioxidant activity of the samples, the lower the IC_{50} value. The observed IC_{50} value showed that the leaf extract of the Rarh region exhibited the highest antioxidant capacity, as the IC_{50} value (27.54 ± 0.20) was lower than the IC_{50} value (30.34 ± 0.16) of the coastal plain (Fig. 4d).

Table 2 represented the correlation co-efficient between the total antioxidant capacity in terms of the DPPH assay, total phenol, total flavonoid, and polyphenolic compounds estimated through the RP-HPLC. A positive correlation was found between the total antioxidant capacity in terms of the DPPH assay, total phenol, total flavonoid, and polyphenolic compounds except with ellagic acid and myricetin.

Therefore, taken as a whole, the data of the different standardized biomarkers of the antioxidant potential and accumulation of the pool of the polyphenolic compounds not only exhibited significant antioxidant-based phytonutrients promise for both ecotypes, but also showed the ecotypes impact on the accumulation of the same.

Discussion

In our present study, we assessed and compared the foliar polyphenol-based antioxidant promise of two contrasting ecotypes of *Amaranthus spinosus* grown in different phytogeographical locations of West Bengal, India based on the bioavailability of the pharmacologically important bioactive polyphenolic compounds that in general showed significant potential in the treatment of several degenerative infectious diseases through the mitigation of oxidative stress. Plant-derived polyphenolic compounds showed a significant ROS quenching property utilizing their structural chemistry, thus showing their antioxidant activity.[35–38] Though there were several mechanistic aspects that supported the antioxidant properties of the phenolic acids (due to the presence of the strong reaction of the phenol moieties), the radical scavenging ability through the donation of a hydrogen atom was found to be the primary mechanism involved. Dietary flavonoids also exhibited antioxidant effects by preventing the generation of ROS and scavenging them. The aim of the current work was to assess the phytogeographical region-specific variation in the accumulation of pharmacologically important bioactive polyphenolic compounds derived from the phenylpropanoid pathway exploring cinnamic acid and chalcone synthase along with their promising antioxidant promise.

Because of the extremely complex chemistry of natural antioxidants showing different structure-activity relationships, it would not be advisable to depend on an individual method to assess and evaluate antioxidant properties. Hence, in the present investigation, we explored several important biomarkers of antioxidant properties like metal chelating property, DPPH-radical scavenging property, anti-lipid peroxidation property, and the total pool of the phenolic compounds of the leaf extracts from the experimental plants. The RP-HPLC data of the polyphenolic compounds of the hydro-methanolic leaf extracts of both the experimental ecotypes of *Amaranthus spinosus* L. were derived by computing the R_t values against their corresponding standards. In general, this exhibited significant accumulation of several polyphenolic compounds tested like protocatechuic acid, chlorogenic acid, gentisic acid, p-hydroxyl benzoic acid, gallic acid, catechin, syringic acid, p-coumaric acid, chlorogenic acid, salicylic acid, vanillic acid, caffeic acid, ferulic acid, sinapic acid, naringin, rutin, quercetin, naringenin, apigenin, and kaempferol myricetin, (Table 1). These bioactive polyphenols were recognized as powerful natural antioxidants with antibacterial, anti-inflammatory, anti-allergic, food additive, antiviral, hepatoprotective, signaling molecules, antithrombotic, and other biological and pharmacological activities.[39–40]

Early investigations also reported that the accumulation of polyphenolic compounds varied according to the ecotype and climatic conditions.[26,28] The differences in the accumulation of the bioactive compounds might be due to the differences in the genetic make-up of the ecotypes and their interaction with the environmental conditions. It was also reported that the phenolic contents increased more with more exposure to sunlight.[39] Hence, the availability of those bioactive polyphenolic compounds and the related antioxidant attributes were largely affected by the interaction of the plant and environment. In our experiments, the foliar extract of the Rarh region exhibited greater abundance of bioactive polyphenolic compounds compared to the coastal plain, thus supporting the earlier findings.[26,27]

Some important parameters, such as the DPPH-radical scavenging property, metal chelation property, and anti-lipid peroxidation property in the linoleic acid system and total phenols and flavonoids were assessed and compared between the two ecotypes of West Bengal. The foliar tissue extracts of the Rarh region showed significantly greater antioxidant property in terms of all the important biochemical techniques assessed. In general, we observed a significant association between the availability of the phenolic acids and flavonoids quantified through the RP-HPLC and biomarkers of the antioxidant properties. Clearly, the results of the current investigations showed that ecotype variation influenced the antioxidant properties of *Amaranthus spinosus*. The germplasms specific alteration in the bioavailability of the bioactive flavonoids and phenolic acids and associated antioxidant based phytonutrients promise among the two ecotypes of *Amaranthus spinosus* of West Bengal might be due to the different genetic ability and competence to mitigate the environmental odds or differences in a genotype-environment interaction.[41,42] Previous studies by Siracusa and Ruberto[41] as well as Aditya[43] noticed a strong correlation between the accumulation of individual bioactive polyphenolic antioxidants as well as the pool of flavonoids and other phenolic compounds with their corresponding antioxidant properties. The central role of the genotype-environmental interaction in the germplasms-specific accumulation of the polyphenolic compounds had significant physiological relevance in the redox regulation caused by the environmental fluctuations of the habitats of the ecotypes.[42,43] The present work in this aspect strongly corroborated the *Amaranthus spinosus* ecotype-specific variation in the bioavailability of the tested individual bioactive phenolic acids and flavonoids, as well as associated antioxidant-based traits. In most of the cases, the ecotypes of the Rarh region and coastal plain of West Bengal exhibited a significant extent of variation at the individual level of the polyphenolic compounds, as well as total pool of flavonoids and phenolic compounds. Thus, this substantiated the genotype influence of the chalcone synthase and cinnamate dependent pathways of polyphenol production in *Amaranthus spinosus*.[44,45] Previous research by Kalinova and Dadakova,[46] Schröter *et al*.,[47] and Sen and Bhattacharjee[11] exhibited species-specific variation of the polyphenolic compounds, hydrocinnamic acid, and other flavonoid glycosides. Nevertheless, it was pertinent to advocate that the better genetic traits associated with the cultivation of the experimental amaranth genotype would be required to evaluate the analysis of the impact of the genotype on the bioaccumulation of the bioactive flavonoids and phenolic acids.

Hence, in this present work, the RP-HPLC based comparative evaluation of the pharmacologically significant polyphenolic compounds along with the standardized biomarkers of the antioxidant properties (*in vitro* metal chelating property, DPPH assay, and anti-lipid peroxidation property) strongly corroborated the ecotype impact on the antioxidant-based phytonutrient promise of spiny amaranth belonging to two different phytogeographical regions (the coastal region and Rarh region, West Bengal, India). The methods employed in the present communication showed a significant ecotype impact on the bioavailability of the representative polyphenolic compounds derived from the phenylpropanoid pathway and antioxidant based functional food property associated with the medicinal attributes, thus confirming the significance of the phenotypic plasticity and genotypic diversity of the crops necessary for plant-environment interaction and the enhancement of the phytonutrients promise.[11,48–50]

Future directions

The findings of the present work supporting the ecotype impact on the bioavailability of polyphenolic compounds and associated phytonutrient promise would be beneficial for the selection of germplasms for cultivation and improvement of functional food properties of spiny amaranth.

Conclusions

Overall, the current work characterized the ecotype impact on the bioaccumulation of pharmaceutically important polyphenolic compounds derived from the phenylpropanoid pathway that assured their rich *in vitro* antioxidant properties. The work also highlighted the significance of phenotypic plasticity and genotypic diversity necessary for adaptation in diverse environmental conditions, while regulating the secondary metabolic pathway, which may be explored in the future for improving the medicinal and functional food properties of spiny amaranth.

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Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

Contributed to the study concept and design (SB), acquisition of the data (AK and SB), assay performance and data analysis (AK and SB), drafting of the manuscript (SB), critical revision of the manuscript (AK and SB), and supervision (SB).

Data sharing statement

No additional data are available.

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