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Evaluation of Free Radical Scavenging Activity and Vitamin-C (Ascorbic Acid) Contents in Selected *Chenopodium* Species

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A B S T R A C T

In the contemporary study, a Double Beam UV-Vis spectrophotometer was used to measure in vitro radical scavenging activity percentage and ascorbic acid contents in some selected *Chenopodium* species. Ascorbic acid content of some selected *Chenopodium* species was determined by the 2,4-Dinnitrophenylhydrazine method (DNPH). The DPPH methodology was utilized to determine the percentage of radical scavenging activity in the extract of *Chenopodium* species. The obtained results were denoted as mean ± standard deviation values. The maximum vitamin C amount in *Chenopodium giganteum* leaves was found to be 161.20 ± 0.008 mg/0.4g FW and the minimum was 51.07 ± 0.011 mg/0.4g FW in *Chenopodium giganteum* root. The leaves of *Chenopodium* species have had the highest percentage of radical scavenging activity. The obtained results were indicating that *Chenopodium* species are potential sources of natural antioxidants and vitamin C.

***Key words*:** Ascorbic acid, Antioxidant activity, *Chenopodium* species, Vitamin C, Spectrophotometer

White crystallized vitamin C is a tiny molecule. It has a faint acid flavor and a little sourness [1]. Ascorbic acid is acting as a key regulator in plant growth and development [2]. Vitamins are found in abundance in citrus fruit and vegetables [3]. L-ascorbic acid acts as an antioxidant [4]. Vitamin C is concerned with scurvy's cause, treatment, and prevention [5]. Numerous studies have shown that plant-derived antioxidant nutraceuticals scavenge free radicals [6-7]. Vitamin C is used to improve the human immune system [8]. Earlier studies suggest that antioxidant-rich food reduces the risk of heart disease and cancer [9]. Antioxidants have the capability to protect the human body against injury [10]. Several species of *Chenopodium* were conveyed to have many homeopathic properties. The contemporary medicinal investigation has similarly confirmed these plants' potent bactericidal, anti-itching, antifungal agent, and antitumor activities [11-15].

Chenopodium album, *Chenopodium giganteum* and *Chenopodium murale* of the family *Chenopodiaceae* belong to the genus *Chenopodium* (Linn). The genus *Chenopodium* Linn. (Family-*Chenopodiaceae*) is a native plant of westerns Asia [16]. *Chenopodium* species, mostly take possession of herbaceous annuals inhabiting large areas in the Americas, Asia, and Europe [17]. *Chenopodium album* has a high amount of vitamin C as the main active component [18]. *Chenopodiaceae* is a ridiculous source of protein, inorganic elements, and vitamin C. So, many *Chenopodium* species are cultivated for humans [19]. The primary objective of the research was to determine the amount of ascorbic acid and the radical scavenging activity percentage from selected *Chenopodium* species.

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MATERIALS AND METHODS

Collection of plant materials

Fresh plants of *C. album*, *C. giganteum*, *and C. murale* were collected from Jaipur, Rajasthan in the month of April 2022 and certified by Dr. Praveen Mohil Assistant Professor, Department of Botany, University of Rajasthan, Jaipur, India. In the herbarium at the Department of Botany, University of Rajasthan, Jaipur, India, specimens of selected *Chenopodium* species were preserved. (RUBL No. *C. album* 211805, *C. giganteum* 21273, and *C. murale* 21241). The leaves, stems, and roots were taken from the plants. The leaves, stem, and root were washed with distilled water to eliminate adherences, dirt, and other surface impurities, shade dried, and powdered with the mechanical grinder. The powder was stored in sealed boxes until further use.

Chemicals, reagents, and instruments

Instruments used in the study were a Double Beam UV-Vis's spectrophotometer, a weighing machine, a Volumetric flask, Pipettes, a Conical flask, Test tubes, Beakers, a Measuring cylinder, Whatman No.1 filter paper, a Glass marker, a Tissue roll, Cuvettes, etc. Chemicals and reagents used in the study were ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), metaphosphoric acid, 2,4-

dinitrophenylhydrazine reagent (DNPH), sulphuric acid, thiourea, copper sulfate, trichloroacetic acid (TCA), Dimethyl Sulphoxide (DMSO), etc.

Preparation of extract for determination of ascorbic acid

The extraction was performed according to Roe and Kuenthar [20] 1943 method. About 0.4g of each fresh plant material was soaked with 10 ml acetate buffer at 24 h (pH= 4.8), centrifuged (1200 rpm, 20 m), and Collected supernatants were diluted with 100 ml metaphosphoric acid acetic acid solution and stored in a refrigerator further use.

Preparation of an extract for determining the percentage of radical scavenging activity

1g of pulverized plant materials was soaked in ethanol (10 ml of 98%) for 48 h. The soaked material was mixed using a mechanical shaker for 24 h and left for 24 h. Centrifuged 5 min with 10000 rpm. The Whatman filter paper was used to filter the final extracts. The collected filtrates were concentrated at room temperature and kept for further use in a refrigerator. The stock solution of extracts (10mg/ml) was prepared by dissolving in dry extract in 98% ethanol. The working stock solutions (0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml) were prepared using appropriate dilutions of the extracts.

Determination of ascorbic acid

Some selected *Chenopodium* species' ascorbic acid content was determined by the 2,4-Dinnitrophenylhydrazine method (DNPH) [21] (Riemenschneider *et al*., 1976). Take 1.5 ml extract in test tubes separately and add 5 ml of 5% TCA, and 1 ml of Dinitrophenylhydrazine- thiourea – copper sulfate reagent to each tube and mix well. Incubate all tubes for 1 h at 60^0C in a water bath. After 1 hour removes the tubes and cools them instantly in ice for 15 minutes. Add 5 ml of 9N sulphuric acid to each test tube with careful shaking. 3 ml TCA used a black without extract. All the test tubes from the water bath and cool stand at room temperature for 20 min after which they can be read at 540 nm. The ascorbic acid concentration was expressed as mg/10ml ascorbic acid equivalent (AA/gFW) using a standard curve equation: $y = 0.0593\times + 0.0084$, R^2= 0.9999. The test was conducted thrice. A UV spectrophotometer scanning at a wavelength of 540 nm can be measured the amount of ascorbic acid present from the yellowish-orange coloration that results. Optical density vs. concentration was utilized to create the standard curve.

Determination of antioxidant activity

The DPPH test was used by Chang *et al.* [22] (2001) to measure the extract's radical scavenging activity percentage. A DPPH solution (4 mg/100 ml) was made in ethanol. With the addition of 1 ml of DPPH (0.1nM) solution, various volumes (0.02-0.1 ml) of plant extracts were produced up to 0.1 ml using DMSO. The experiment was conducted in a room with no light. For 30 min, all test tubes were incubated in the dark. After 30 min, the mixture's absorbance (which ranged from orange to yellow) was spectrophotometrically measured at 517 nm compared to a blank. As a reference, ascorbic acid (10 mg/ml DMSO) was employed. Using the following equation, the percentage of radical scavenging activity was determined.

$$\text{Percent of RSA} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RSA= Radical Scavenging Activity
Abs control= Absorbance of DPPH radical + ethanol
Abs sample= Absorbance DPPH radical + plant extract

In the process of creating antiradical curves for computing the EC_{50} values, several sample concentrations were utilized. The experiment was done three times.

Statistical analysis

The whole tests were in triplicates. The Statistics were dispensed as mean ± SD. GraphPad Prism-9 and AAT Bioquest Online Software were used. Microsoft 2019, and Microsoft Excel 2019 were used for statical and graphical assessments.

RESULTS AND DISCUSSION

Quantification of concentration of vitamin C

Results are represented as mean and standard deviation values. *C. album* root, stem, and leaves show 60.07 ± 0.046, 76.87 ± 0.029, and 79.13 ± 0.0 59 mg/0.4gFW amount of vitamin C. *C. murale* root, stem, and leaves show 76.87 ± 0.040, 115.13 ± 0.049, and 152.33 ± 0.005 mg/0.4gFW amount of vitamin C. *C. giganteum* root, stem, and leaves show 51.07 ± 0.011, 152.20 ± 0.015, and 161.20 ± 0.008 mg/0.4gFW amount of vitamin C. The highest ascorbic acid content was found in the leaves of all examined *Chenopodium* species. Ascorbic acid of extracts was found to increase in the order root < stem < leaves (Table 1, Fig 1).

DPPH radical scavenging activity

The radical scavenging activity percentage of an ethanol extract of the root, stem, and leaves of *C. album* and ascorbic acid is displayed in (Table 2, Fig 2-3). *C. album* leaves showed the highest percentage of radical scavenging activity of all the extractives. At 0.1 mg/ml concentration, of *C. album* root, stem, and leaves scavenging activity percentages were found to be 52.95 ± 0.224%, 65.54 ± 0.045% and 77.0 ± 0.030%. Standard ascorbic acid was 84.73 ± 0.008% at this concentration. Increased order in the root<stem<leaves indicated that *C. album* extracts had a higher percentage of radical scavenging activity.

Table 1 Ascorbic acid concentration in selected species of *Chenopodium*

Chenopodium species	Conc. of ascorbic acid mg/0.4gFW (Mean ± SD)		
	Root	Stem	Leaves
Chenopodium album	60.07 ± 0.046	76.87 ± 0.029	79.13 ± 0.059
Chenopodium murale	76.87 ± 0.040	115.13 ± 0.049	153.33 ± 0.005
Chenopodium giganteum	51.07 ± 0.011	152.20 ± 0.015	161.20 ± 0.008

Mean and standard deviation used to represent the data

The ethanol extract of *C. murale*'s root, stem, and leaves, as well as standard ascorbic acid, were shown a higher percentage of radical scavenging activity in (Tables 3, Fig 4-5). The highest percentage of radical scavenging activity was observed in *C. murale* leaves among the extractives. At 0.1 mg/ml concentration of radical scavenging activity, the

percentage of *Chenopodium murale* root, stem, and leaves were found at 47.92 ± 0.108%, 56.95 ± 0.181% & 81.35 ± 0.079%. At this concentration, the standard ascorbic acid was 84.73 ± 0.008%. The radical scavenging activity percentage of *Chenopodium murale* extract was found in increased order root < stem < leaves.

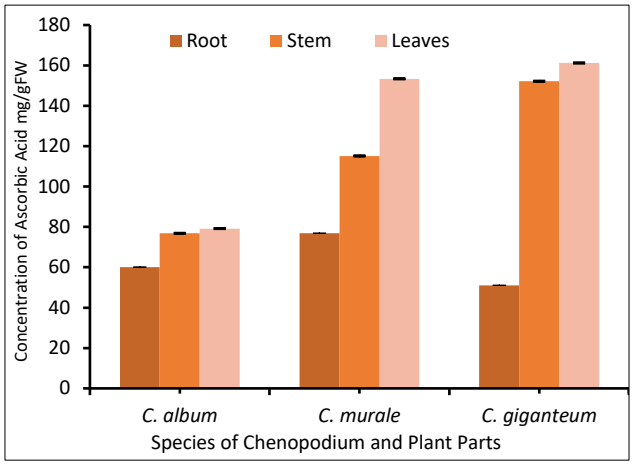

Fig 1 Ascorbic acid concentration in selected *Chenopodium* species (mg/0.4gFW, Mean ± SD). *Chenopodium album*, *Chenopodium murale* and *Chenopodium giganteum*

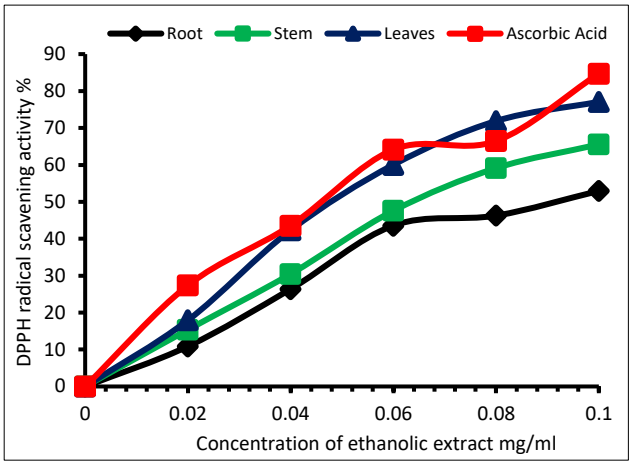

Fig 2 The percentage of DPPH radical scavenging activity of various *C. album* plant parts and ascorbic acid measured in mg/ml ethanolic extracts

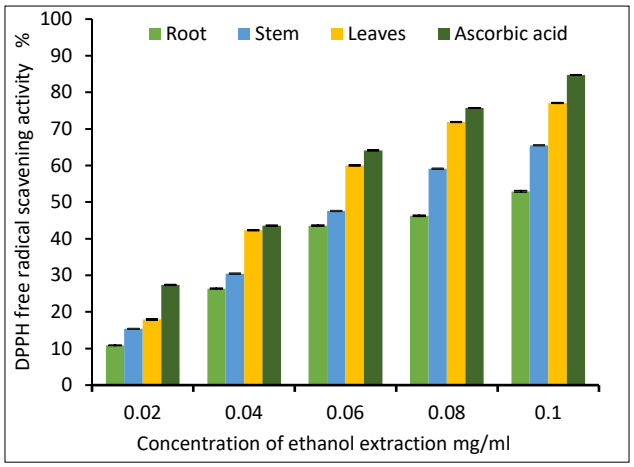

Fig 3 The percentage of DPPH radical scavenging activity of various *C. album* plant parts and ascorbic acid measured in mg/ml ethanolic extracts

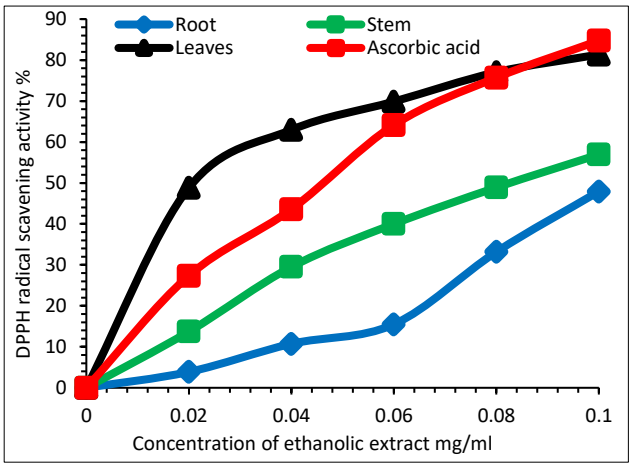

Fig 4 The percentage of DPPH radical scavenging activity of various *C. murale* plant parts and ascorbic acid measured in mg/ml ethanolic extracts

Table 2 The DPPH radical scavenging activity of *Chenopodium album*

Concentration (mg/ml)	Root	Stem	Leaves	AA
0.02	10.87 ± 0.085	15.37 ± 0.037	17.93 ± 0.028	27.37 ± 0.051
0.04	26.37 ± 0.143	30.46 ± 0.046	42.32 ± 0.011	43.56 ± 0.034
0.06	43.57 ± 0.180	47.58 ± 0.047	60.03 ± 0.049	64.15 ± 0.005
0.08	46.26 ± 0.186	59.12 ± 0.056	71.88 ± 0.032	75.71 ± 0.009
0.1	52.95 ± 0.224	65.54 ± 0.045	77.00 ± 0.030	84.73 ± 0.008

Mean and standard deviation used to represent the data

Table 3 The DPPH radical scavenging activity of *Chenopodium murale*

Concentration (mg/ml)	Root	Stem	Leaves	AA
0.02	3.87 ± 0.023	13.71 ± 0.084	48.83 ± 0.075	27.37 ± 0.051
0.04	10.74 ± 0.057	29.50 ± 0.149	62.96 ± 0.072	43.56 ± 0.034
0.06	15.49 ± 0.082	39.98 ± 0.144	69.87 ± 0.073	64.15 ± 0.005
0.08	33.18 ± 0.128	48.90 ± 0.184	77.03 ± 0.082	75.71 ± 0.009
0.1	47.92 ± 0.108	56.95 ± 0.181	81.35 ± 0.079	84.73 ± 0.008

Mean and standard deviation used to represent the data

The percentage of *Chenopodium giganteum* root, stem, and leaves ethanol extract that scavenges radicals in comparison to standard ascorbic acid is displayed in (Table 4, Fig 6-7). *Chenopodium giganteum* leaves showed the highest percentage of radical scavenging activity of all the extractives. The root, stem, and leaves of *Chenopodium giganteum* had scavenging activity percentages of 23.12 ± 0.138%, 19.61 ± 0.014%, and 79.36 ± 0.020% at 0.1 mg/ml, accordingly. Standard ascorbic acid was 84.73 ± 0.008 percent at this concentration. The percentage of radical scavenging activity of *Chenopodium giganteum* extracts was found in increased order root < stem < leaves.

Table 4 The radical scavenging activity of *Chenopodium giganteum*

Concentration (mg/ml)	Root	Stem	Leaves	AA
0.02	4.38 ± 0.082	3.11 ± 0.021	50.91 ± 0.039	27.37 ± 0.051
0.04	9.87 ± 0.111	7.00 ± 0.021	67.96 ± 0.07	43.56 ± 0.034
0.06	13.98 ± 0.113	9.10 ± 0.013	73.25 ± 0.029	64.15 ± 0.005
0.08	19.59 ± 0.115	16.95 ± 0.027	76.31 ± 0.033	75.71 ± 0.009
0.1	23.12 ± 0.138	19.61 ± 0.014	79.36 ± 0.020	84.73 ± 0.008

Mean and standard deviation used to represent the data

Extracts of *Chenopodium* species showed different levels of concentration EC_{50} over the range of 0.003 to 0.203 mg/ml. The EC_{50} of ethanolic extracts of *C. album* root, stem, and leaves was 0.044±0.005, 0.054±0.001 and 0.045 ±0.001 mg/ml, *Chenopodium murale* root, stem, and leaves were 0.108±0.014, 0.083±0.014 and 0.014±0.001 mg/ml and *Chenopodium giganteum* root, stem and leaves were 0.203±0.032, 0.080±0.008 and 0.003±0.003 mg/ml, respectively. The EC_{50} Ascorbic acid (standard) was 0.047±0.003 mg/ml respectively. *Chenopodium giganteum* leaves showed strong antioxidant activity, with EC_{50} of 0.003±0.003 mg/ml. The result indicated that the antioxidant potential of the *Chenopodium giganteum* leaf extract was greater than that of ascorbic acid (Table 5, Fig 8).

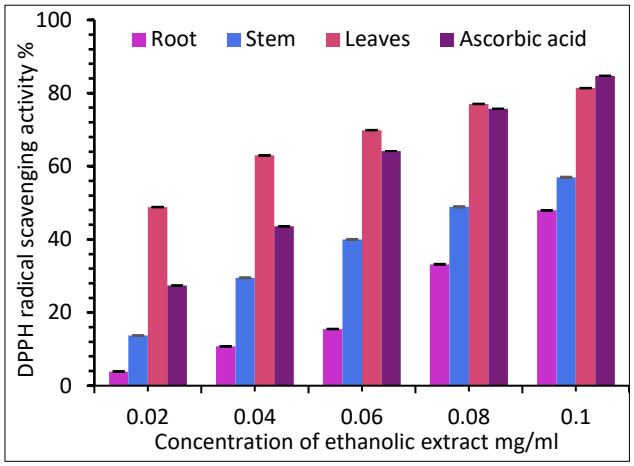

Fig 5 The percentage of DPPH radical scavenging activity of various *C. murale* plant parts and ascorbic acid measured in mg/ml ethanolic extracts

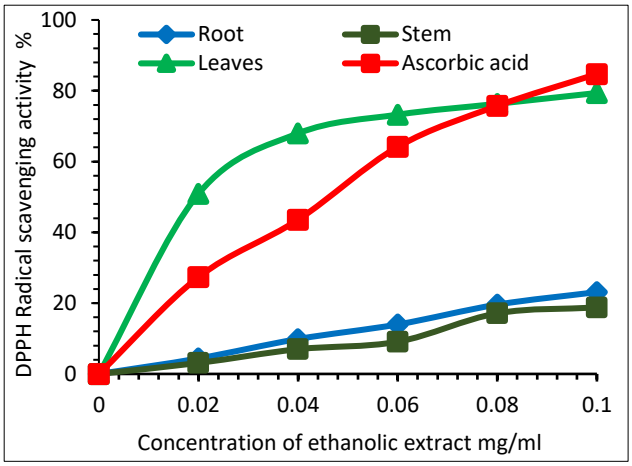

Fig 6 The percentage of DPPH radical scavenging activity of various *C. giganteum* plant parts and ascorbic acid measured in mg/ml ethanolic extracts

Table 5 EC_{50} of *Chenopodium* species

Ethanolic extracts mg/ml DW	EC $_{50}$ (mg/ml) (Mean ± SD)
CA-R	0.044 ± 0.005
CA-S	0.054 ± 0.001
CA-L	0.045 ± 0.001
CM-R	0.108 ± 0.014
CM-S	0.083 ± 0.014
CM-L	0.014 ± 0.001
CG-R	0.203 ± 0.032
CG-S	0.080 ± 0.008
CG-L	0.003 ± 0.003
AA	0.047 ± 0.003

The highest concentration of vitamin C (161.2±0.008 mg/DW) was observed in the leaves of *C. giganteum*, among all samples analyzed. *Chenopodium* species' leaves are a plentiful source of this vitamin. This figure is almost the same as that reported by Kuhnlein [20]. Aqueous and ethanol extracts of *C. album* were found to have antioxidant activity at 64.5% and 60.5%, correspondingly [21]. The effect of antioxidant and radical scavengers was assessed [22]. A previous study reported that the extracts of *C. album, C. giganteum, and C. murale* leaves have potential antioxidant activity with the IC_{50} values of 14.25 µg/ml, 17.31 µg/ml and 13.86 µg/ml respectively, where the value of standard ascorbic acid 9.56µg/ml. The highest DPPH inhibition (79.45% inhibition) was observed in the *C. murale* leaves while the lowest inhibition (68.24% inhibition) was observed in *C. giganteum* [23]. The highest DPPH inhibition (79.45% inhibition) was observed in the *C. murale* leaves while the lowest inhibition (68.24% inhibition) was observed in *C. giganteum* [24]. Some leafy vegetables in Bangladesh were reported to have antioxidant activity ranging from 55.15% to 86.65% [25]. In *Chenopodium* leaves, radical scavenging activity ranged from 9.28% to 24.90%, according to kulcu *et al.* [26]. The DPPH activity of the methanolic extract of *C. album* was reported to be 45 to 73 percent by Lone *et al.* [27].

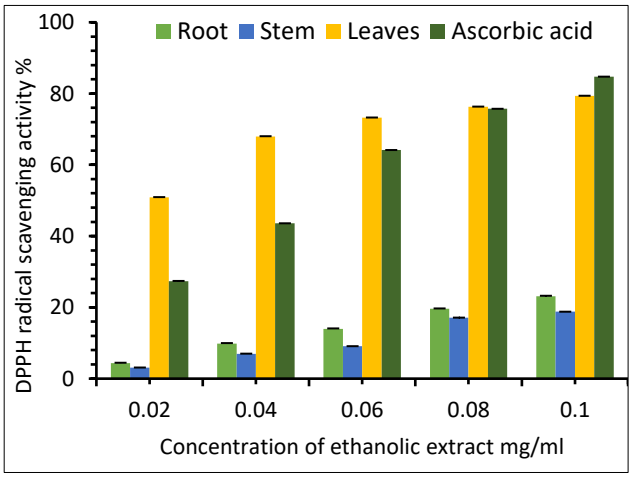

Fig 7 The percentage of DPPH radical scavenging activity of various *C. giganteum* plant parts and ascorbic acid measured in mg/ml ethanolic extracts

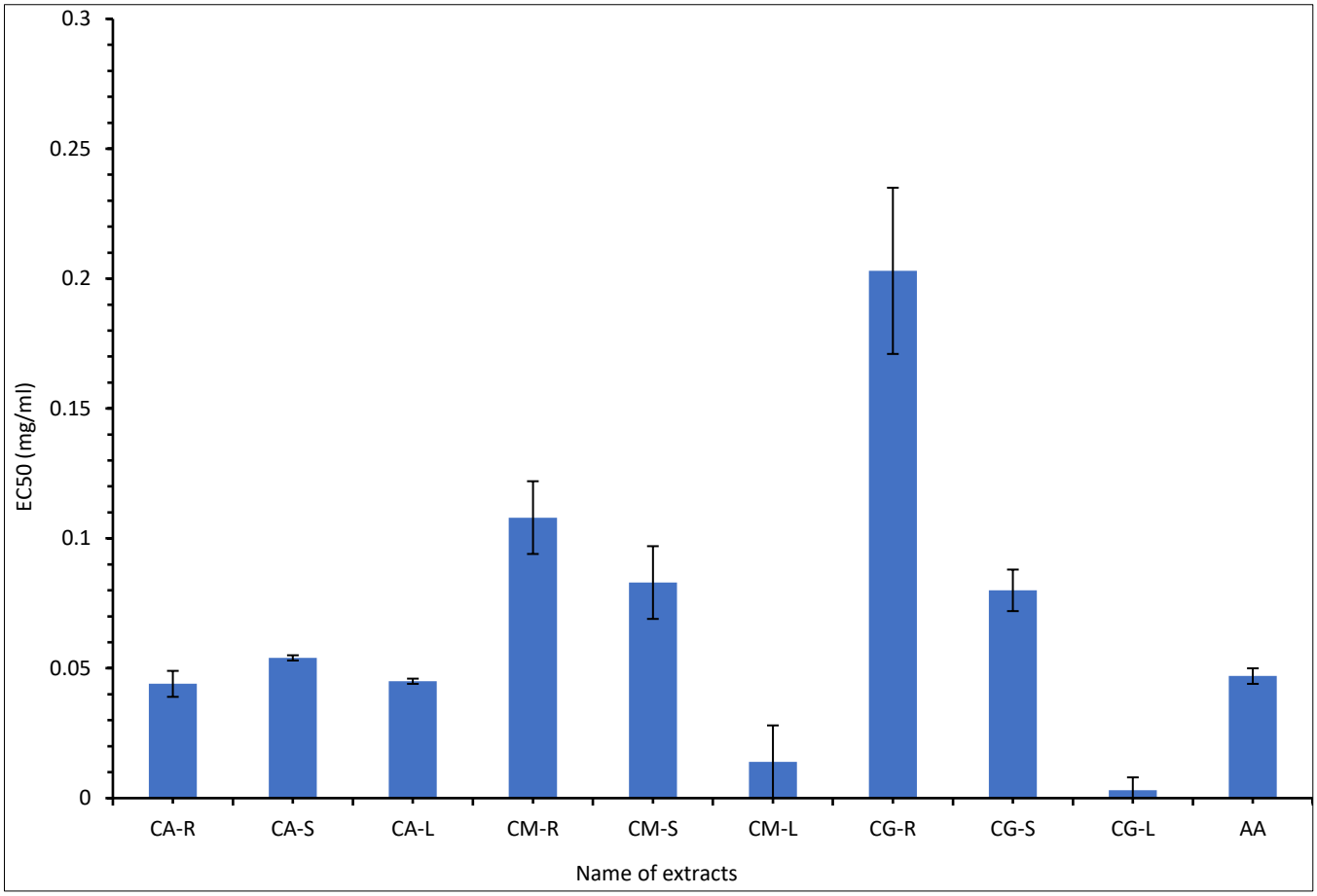

Fig 8 EC_{50} of ethanolic extract (Mean ± SD)

CONCLUSION

According to the present research, *Chenopodium album*, *murale*, and *giganteum* species exhibit potent antioxidant activity, good ascorbic acid levels, and low $EC_{50,}$ relatively good ARP values, which accounts for their efficiency. The conclusions of this study are that the *Chenopodium* species of root, stem, and leaf extract might be used as an easily available source of natural antioxidants. *Chenopodium* leaves, stem and root can be used for food supplement manufacture and in the medicinal industry.

Abbreviations
C. album– *Chenopodium album*
C. murale- *Chenopodium murale*
C. giganteum- *Chenopodium giganteum*
CA-R- *Chenopodium album* root
CAS-S- *Chenopodium album* stem
CA-L- *Chenopodium album* leaves
CM-R- *Chenopodium murale* root
CM-S- *Chenopodium murale* stem
CM-L- *Chenopodium murale* leaves
CG-R- *Chenopodium giganteum* root
CG-S- *Chenopodium giganteum* stem
CG-L- *Chenopodium giganteum* leaves
AA- Ascorbic Acid
ARP- Antiradical power

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