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Original Article

Comparative examination of phenolic content, flavonoid content, and antioxidant efficacy of *Chenopodium album* L. and *Chenopodium pumilio* R. Br.

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ABSTRACT

Background and Aims: Traditional indigenous system of medicine employed many *Chenopodium* species to treat a wide range of ailments. Therefore, the current study aims to evaluate the phenolic content, flavonoid content, and antioxidant capacity of the species *Chenopodium album* and *Chenopodium pumilio.*

Methods: Phenolics and flavonoid content were quantified using spectrophotometric techniques. The antioxidant activity of *Chenopodium* extracts was evaluated by assaying ferric reducing antioxidant power, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, phosphomolybdenum reducing power, ferrous ion chelating activity, hydrogen peroxide radical scavenging activity, hydroxyl radical scavenging activity, deoxyribose degradation activity, and β-carotene bleaching activity.

Results: In *C. album* and *C. pumilio*, total phenolic concentration was 17.5–23.8 and 13.3–25.3 mg tannic acid equivalent/g, respectively, and flavonoid content was 0.21–0.85 mg and 0.10–0.89 mg quercetin equivalent/g, respectively. Thus, *C. pumilio* had a higher content of phenolics and flavonoids than *C. album*. However, comparison of extracts in different solvents showed that ethanolic and aqueous extracts exhibited higher phenolics and flavonoid content than other solvent systems. The antioxidant capability of the species, determined by antioxidant assays, varied with the species evaluated and the solvents used for extraction. Remarkably, the correlation coefficient of different antioxidant assays with the phenolics and flavonoid content in both species showed discrepancy.

Conclusion: Ethanolic and aqueous extracts of the *Chenopodium* species studied had higher phenolics, flavonoids, and free radical scavenging activity than methanolic and acetonic extracts. The ethanolic and aqueous extracts of the studied species provide a potential source of antioxidants for drug formulation.

Keywords: *Chenopodium*; phenolics; flavonoids; free radicals; reducing power; correlation

INTRODUCTION

The genus *Chenopodium* includes numerous species of annual or perennial herbaceous plants that have worldwide distribution. *Chenopodium* comprises 250 species, of which only 21 are confined to India, which can be grown as aromatic and nonaromatic herbs (Fuentes-Bazan, Mansion, & Borsch, 2012). Several species of the genus *Chenopodium* have high medicinal value, with antibacterial, antifungal, antidiaphoretic, and antiasthmatic properties. Therefore, it has been documented as folk medicine in indigenous systems of medicine. Since ancient times, people in South America have prepared infusions from the leaves and seeds of *C. ambrosioides* (Mexican tea, Indian worm seed), considering it to be the best remedy for intestinal parasites. Similar infusions have been used in various countries. In Europe, infusions are used against pectoralis and nervous affections. In Brazil, infusions are effective in relieving flatulence, excessive sweating, anemia, cough, and chronic obstructive pulmonary diseases. In New Mexico, the seeds of *C. ambrosioides* are used as an abortifacient with low toxicity (Conway & Slocumb, 1979). In Madagascar and La Reunion, the juice of the plant is consumed undiluted or as a decoction in milk or water as an effective vermifuge (Yadav, Vasudeva, Singh, & Sharma, 2007). *Chenopodium album* L. improves appetite, fights intestinal worms, helps in bowel movement, acts as a diuretic, etc. It is useful in nausea, vomiting, abdominal pain, and eye diseases. A fine dusting powder from the leaves of *C.*

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album is applied over the external genitals of children to avoid skin irritation. Traditional ayurvedic medicine has indicated its use in various skin diseases, burns, wrinkles, etc. (Karwani & Sisodia, 2015). The therapeutic qualities of *Chenopodium* species have increased interest in research related to its phytochemistry, ethnopharmacology, and pharmacology. In this study, we examined the phenolic content, flavonoid content, and antioxidant potential of *C. album* and *C. pumilio*.

MATERIALS AND METHODS

Specimen collection

Samples of *C. album* and *C. pumilio* were collected from the premises of Karnataka State Akkamahadevi Women's University, Vijayapura. Dr. Sidanand V. Kambhar verified the authenticity of the plants. The herbaria voucher specimens and the herbaria for the species' (*C. album*: KSAWUV-159; *C. pumilio*: KSAWUV-160) were prepared and deposited in the Department of Botany, Karnataka State Akkamahadevi Women's University, Vijayapura (Karnataka).

Preparation of extracts

The whole plant (aerial part and root) was used for extraction. Deionized water, methanol, ethanol, and acetone were used as solvents to prepare the extracts on a dry weight basis. A mortar and pestle was used to homogenize 5 g of dried material in 25 mL of deionized water. The resultant extract was mixed well, shaken for 5–10 min, left on a rotary shaker for 24 h, and centrifuged for 14 min at 10,000 rpm. The supernatant was collected in a test tube, and the residue was centrifuged using 25 mL of solvent to extract remaining material. The supernatants were pooled and mixed with deionized water to a final volume of 50 mL. The same procedure was used to prepare extracts in different solvents. All the extracts were stored at 4°C, and 1% (v/v) extracts were used to quantify phenolics and flavonoids and evaluate antioxidant activity.

Total phenolic content

Total phenolic content in the plant samples was estimated by using the method of Gutierrez and Navarro (2010). The assay mixture was prepared by mixing 0.125 mL of sample extract with 0.125 mL of Folin–Ciocalteu reagent and 1.25 mL of saturated Na_2CO_3 solution. This was followed by incubation for 90 min at room temperature (25±2 °C), and optical density was measured at 760 nm. All the samples were analyzed in triplicate, and mean absorbance was measured. Based on the absorbance of standard tannic acid, a calibration curve was prepared (10–100 µg/mL, R^2 = 0.99). The results were expressed as mg tannic acid equivalent (TAE) per gram dry weight.

Total flavonoid content

Total flavonoid content of the plant samples was analyzed according to the spectrophotometric method (Basniwal et al., 2009). The assay mixture was prepared by incubating plant extract (1.5 mL) with 2 % methanolic $AlCl_3$ (1.5 mL) for 10 min at room temperature. Optical density was recorded at 420 nm. All the samples were analyzed in triplicate. Mean absorbance was recorded, and a calibration curve was plotted using standard quercetin (10–100 µg/mL, R^2 = 0.99). The final results were expressed as mg quercetin equivalent (QE) per gram dry weight.

Ferric reducing antioxidant power assay

The reducing power of the plant extracts was estimated using an advanced assay developed by Pulido et al. (2000). The assay mixture was prepared by mixing 100 µL of plant extract with 3 mL of ferric reducing antioxidant power (FRAP) assay reagent. The FRAP reagent contains one volume of 10 mM 2,4,6-Tripyridyl-S-triazine (TPTZ) solution, one volume of 20 mM $FeCl_3.6H_2O$, and ten volumes of 300 mM sodium acetate buffer (pH 3.6). The reaction mixture was incubated for 15 min at 37°C, and absorbance was recorded at 595 nm. A standard curve was obtained, utilizing an aqueous solution of ascorbic acid (10–100 µg/mL, R^2 = 0.98). The reducing ability of the extract was estimated as mg ascorbic acid equivalent (AAE) per gram dry weight.

DPPH free radical scavenging assay

The scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical in the plant extract was evaluated following the assay devised by Aquino et al. (2001). To prepare the assay mixture, plant extract (25 µL) was mixed with 25 mM DPPH solution (3 mL). The reaction mixture was incubated in the dark for 20 min at room temperature, and optical density was recorded at 515 nm against a blank. The following equation was used to calculate radical scavenging activity, and the results were measured as percent inhibition of DPPH radical.

$$\text{DPPH free radical scavenging activity (\%)} = A0 - A1/A0 \times 100$$

Where, A0 is the absorbance of the DPPH radical without sample and A1 is the absorbance of the DPPH radical with sample.

Phosphomolybdenum reducing power assay

The reducing power of the extracts was evaluated using the phosphomolybdenum reduction assay, according to Prieto et al. (1999). The reaction mixture was prepared by mixing 0.3 mL of sample with 3 mL of reagent. The reagent contained 28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6

M sulfuric acid. The tubes containing the reaction mixture were sealed with aluminum foil, incubated for 90 min at 95°C, and cooled. Absorbance was measured at 965 nm against a blank. A standard curve was plotted using ascorbic acid (10–100 µg/mL, R^2 = 0.98). On the basis of absorbance values, the reducing ability of the extracts was measured and represented as AAE per gram dry weight.

Ferrous ion chelating activity

The ability of the sample extracts to chelate ferrous ions was analyzed by using the assay developed by Chew, Goh, & Lim, (2009). An aliquot was incubated with 2 mM $FeCl_2$ (100 µL) and 5 mM ferrozine (300 µL) for 10 min. Optical density was recorded at 562 nm. Percent chelating activity of the sample extract was measured by using the formula of inhibition percentage as applied for DPPH free radical scavenging activity.

Hydrogen peroxide radical scavenging activity

The titration method was adopted to analyze hydrogen peroxide scavenging activity (Zhao et al., 2006). The assay mixture was prepared by mixing 200 µL of 40 mM H_2O_2, 200 µL of the test compound, 200 µL of 3 % ammonium molybdate, 200 µL of 2 M H_2SO_4, and 1.4 mL of 1.8 M KI. The reaction mixture was titrated against 5.09 mM NaS_2O_3 until color changed from yellow to colorless. The following formula was used to assess ability to scavenge hydrogen peroxide.

Hydrogen peroxide scavenging activity (%)
= [(Vc - Vs)/Vc] × 100

Where, Vc and Vs are control and samples volumes, respectively.

Hydroxyl radical scavenging activity

To measure hydroxyl radical scavenging activity, 1.5 mL of extract was mixed with 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 0.06 mL of $FeCl_3$ (1.0 mM), and 0.09 mL of 1,10- phenanthroline (1 mM) (Fadda, Barberis, & Sanna, 2018). Oxidation was initiated by adding 0.15 mL of hydrogen peroxide (0.17 M). The reaction mixture was incubated at room temperature for 5 min, and absorbance was recorded at 560 nm with a spectrophotometer. Hydroxyl radical scavenging activity was measured using the formula applied for DPPH free radical scavenging activity.

Deoxyribose degradation assay

Deoxyribose degradation ability of the extracts was determined by the assay devised by Cheng & Chang, (2003). The reaction mixtures were prepared by mixing 50 µL of deoxyribose (50 mM), 300 µL of phosphate buffer (0.2 M, pH 7.4), 50 µL of Na2EDTA (1 mM), 50 µL of FeCl3 (3.2 mM), and 50 µL of H_2O_2 (50 mM). Then 50 µL of ascorbic acid (1.8 mM) was added to the reaction mixture, and final volume was made up by adding 800 µL buffer. This was followed by incubation at 50°C for 20 min. The reaction was completed by adding 250 µL of trichloroacetic acid (10 %, w/w). Then, the reaction mixture was incubated with 150 µL of thiobarbituric acid (5 %) in 1.25 % aqueous NaOH at 105 °C in an oven for 15 min, which led to color change, and cooled. Absorbance was recorded at 530 nm against a blank (buffer). Inhibition of deoxyribose degradation was calculated in percentage using the same formula applied for DPPH free radical scavenging activity.

β-carotene bleaching assay

The β-carotene bleaching assay was conducted to determine antioxidant activity in the sample (Prieto, Rodríguez-Amado, Vázquez, & Murado, 2012). The assay mixture was prepared by adding 1 mL of β carotene solution (0.2 mg/mL chloroform) in a round bottom flask (50 mL) with 20 µL of linoleic acid and 200 µL of Tween 20 (100 %). The mixture was placed on a rotary evaporator for 10 min at 40°C to evaporate excess chloroform. Distilled water was added gradually with a burette to agitate the mixture into forming an emulsion. Then 3 mL of emulsion was mixed thoroughly with 200 µL of sample in a test tube, placed in a water bath for 2 h at 50°C, and cooled. Absorbance was recorded at 470 nm at two different times—immediately after preparation (t = 0 min) and after 2 h against a blank. All the aliquots were analyzed in triplicate. Total antioxidant activity was measured by using the following equation:

$$BBA\% = 1 - \frac{A1_{(t=0)} - A1_{(t=120)}}{A0_{(t=0)} - A0_{(t=120)}} \times 100$$

where:
BBA % is percent β-carotene bleaching activity
A1 (t = 0) is absorbance of test sample at time 0
A1 (t = 120) is absorbance of test sample after 2 h
A0 (t = 0) is absorbance of the control at time 0
A0 (t = 120) is absorbance of the control after 2 h

Statistical analysis

All experiments were conducted three times, and the results were analyzed and displayed as mean ± standard deviation. Statistical analysis was performed using MS Excel and GraphPad InStat 3 software. *P* value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phenolics and flavonoid content

In flowering plants, phenolics and flavonoid are the most important antioxidants, because they adsorb and neutralize

free radicals (Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018). Therefore, the total content of phenolics and flavonoids in different extracts of two *Chenopodium* species (*C. album* and *C. pumilio*) was assessed. The quantity of total phenolics is expressed as mg TAE/g of sample and the quantity of total flavonoids as mg QE/g of sample (Table 1). The results revealed that the amount of phenolics greatly varied among the two taxa of the genus Chenopodium, i.e., *C. album* and *C. pumilio*. *C. album* showed the maximum amount of total phenolics in the ethanolic extract (23.8 mg TAE/g) and the lowest in the methanolic extract (14.9 mg TAE/g). Similarly, *C. pumilio* exhibited the highest phenolic content in the aqueous extract (25.3 mg TAE/g) and the lowest in the acetonic extract (13.3 mg TAE/g). Significantly high results were found for phenolic content in this order: ethanol > aqueous > acetone > methanol for *C. album* and aqueous > ethanol > methanol > acetone for *C. pumilio*. Water, ethanol, methanol, acetone, and their aqueous mixes are the most commonly used solvents for phenolic extraction (Michiels, Kevers, Pincemail, Defraigne, & Dommes, 2012). According to Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer (2002), the antioxidant properties of plants are primarily attributed to phenolic chemicals, which are categorized as hydrophilic antioxidants. This could explain our findings that water and ethanol were the most effective extraction solvents. This might have resulted from interactions (hydrogen bonds) between the solvent and polar sites of the antioxidants, which improved the solvation of the chemicals found in Chenopodium species.

C. album and *C. pumilio* showed significant variation in flavonoid content with the solvents. Among the solvents, ethanolic and aqueous extracts showed the highest amounts of total flavonoids in *C. album* (0.89 mg QE/g) and *C. pumilio* (0.85 mg QE /g), respectively (Table 1). The content in different solvents was in this order: ethanol > methanol > aqueous > acetone for *C. album* and aqueous > ethanol > methanol > acetone for *C. pumilio*. Comparatively, *C. pumilio* exhibited a greater amount of TFC than *C. album*. When the phenolic and flavonoid content of different species was compared, *C. pumilio* had higher total phenolic and flavonoid content than *C. album*. According to Repo-Carrasco-Valencia et al. (2010), the polyphenol content of the Chenopodiaceae is high, with phenolics and flavonoids derivatives acting as radical scavengers. However, our findings contradict those of Repo-Carrasco-Valencia. There are reports that are consistent with our findings, according to which phenolic derivatives in plants have higher antioxidant potencies, because they neutralize lipid free radicals or prevent the breakdown of H_2O_2 (Cai, Luo, Sun, & Corke, 2004; Pitchaon, Suttajit, & Pongsawatmani, 2007). Thus, although the concentration of phenolics and flavonoids varies among species, they are both potent antioxidants that can scavenge free radicals (Muflihah, Gollavelli, & Ling, 2021).There is a direct correlation between the antioxidant activity of plants and their phenolic or flavonoid content (Joshi, Deepa, & Sharma., 2022).

FRAP assay

The reduction potential of *Chenopodium* species assessed by the FRAP assay revealed that the reducing power of both plants is significantly high, and highest activity is exhibited by the ethanolic extract of C. album (37.5 mg AAE/g) and aqueous extract of *C. pumilio* (37.9 mg AAE/g). *C. pumilio* had higher ferric reducing activity than *C. album* (Table 2). The reduction potential of the studied *Chenopodium* species is supported by data from earlier studies on other medicinal plants. Antioxidants found in plants were responsible for ferric reducing antioxidant properties (Rajurkar and Hande, 2011, Fernandes et al., 2016, Al-Laith, Alkhuzai, & Freije, 2019). The reason for this is the extract's ability to donate hydrogen atoms through cleaving (Marimuthu, Balakrishnan, & Nair, 2013). The plant primarily contains phenolic composites with redox properties, which enable them to function as singlet oxygen quenchers, reducing agents, and hydrogen donors. The determination of antioxidant potential was significantly influenced by this reduction potential. The phenolics and flavonoid compounds of the investigated Chenopodium species may be responsible for their reduction potential.

DPPH free radical scavenging activity

The DPPH free radical scavenging ability of the extracts was established in the following order: ethanol > methanol > aqueous > acetone for *C. album* and aqueous > ethanol > methanol > acetone for *C. pumilio*. *C. album* showed highest DPPH scavenging activity in the ethanolic extract (96.7 %), and *C. pumilio* exhibited the highest DPPH scavenging activity in the aqueous extract (90.6 %). The study indicated that all the extracts acted as potent hydrogen donors; they might serve as free radical scavengers and primary antioxidants. The DPPH radicals show a biphasic response to many plant-derived antioxidants. Numerous phytochemicals are present in plant extracts, and plant extracts with different phytochemicals may also show different biological activity (Molole, Gure, & Abdissa, 2022). Because of the combination of antioxidants present, plant extracts may be more reactive against DPPH radicals. Based on the findings, it is perceived that both species exhibited stable DPPH free radical scavenging activity and that the antioxidant radicals present in sample extracts may have contributed to this capacity. Although the DPPH free radical scavenging activity of *C. album* and other Chenopodium species has been investigated (Nowak, Szewczyk, Gawlik-Dziki, Rzymowska, & Komsta, 2016), the results of various studies can be difficult to compare because different experimental conditions are used in each study (Hirose et al., 2010). However, when comparing scavenging activity of *C. album* reported by Adedapo, Jimoh, & Afolayan (2011), the methanolic and acetonic extracts demonstrated the highest

Table 1. Total phenolics and flavonoid content of *Chenopodium album* and *Chenopodium pumilio*.

Species	Solvents	Total phenolic content (mg TAE/g dw)	Total flavonoid content (mg QE/g dw)
C. album	Aqueous	19.8±0.04	0.31±0.09
	Ethanol	23.8±0.06	0.85±0.06
	Methanol	14.9±0.10	0.37±0.07
	Acetone	17.5±0.12	0.21±0.01
C. pumilio	Aqueous	25.3±0.10	0.89±0.07
	Ethanol	15.6±0.09	0.31±0.06
	Methanol	15.1±0.10	0.15±0.01
	Acetone	13.3±0.07	0.10±0.03

Values are expressed as mean ± SD of triplicate measurements.
mg TAE/g **dw**: milligram tannic acid equivalent per gram **dry weight**.
mg QE/g **dw**: milligram quercetin equivalent per gram **dry weight**.

Table 2. Ferric reducing antioxidant power and DPPH free radical scavenging activity of *Chenopodium album* and *Chenopodium pumilio.*

Species	Solvents	Ferric reducing antioxidant power (mg AAE/g dw)	DPPH free radical scavenging activity (%)
C. album	Aqueous	20.6±0.15	79.6±0.07
	Ethanol	37.5±0.11	96.7±0.11
	Methanol	18.5±0.19	91.5±0.09
	Acetone	17.1±0.13	74.3±0.11
C. pumilio	Aqueous	37.9±0.08	90.6±0.11
	Ethanol	23.2±0.40	86.1±0.09
	Methanol	20.5±0.08	75.5±0.19
	Acetone	15.2±0.30	53.4±0.11

Values are expressed as mean ± SD of triplicate measurements.
mg AAE /g dw : milli gram ascorbic acid equivalent per gram dry weight.
% - percentage inhibition per gram dry weight.

activity, whereas the aqueous extract demonstrated the lowest scavenging activity.

Phosphomolybdenum reducing power

The reducing ability of the extracts could be considered a significant indicator of their potential as antioxidants (Eshwarappa, Iyer, Subbaramaiah, Richard, & Dhananjaya, 2014). The increasing amount of total antioxidant activity is directly proportional to the reducing power of the extracts. The phosphomolybdenum reducing power of the studied *Chenopodium* species varied with respect to different solvents and was found to be in the order ethanol > aqueous > methanol > acetone for *C. album* and aqueous > methanol > ethanol > acetone for *C. pumilio* (Table 3). Reducing power was lowest for acetone extracts in both *Chenopodium* species. *C. pumilio* (43.3 mg AAE/g) exhibited the highest reductive ability compared with *C. album* (34.7 mg AAE/g). Numerous studies have demonstrated a correlation between antioxidant activity and the ability of bioactive compounds to donate electrons (Gupta, Maurya, Agarwal, Kushwaha, & Kumar, 2016; Kolar, Kambhar, Chavana, Kadam, & Nadaf, 2021). The reducing power of *Chenopodium* species' chemical components reported may allow them to function as primary and secondary antioxidants by acting as electron donors and reducing the oxidized intermediates of lipid peroxidation.

Ferrous ion chelating activity

The Fe^{2+} chelating activities of the extracts were in the following order: ethanol > aqueous > methanol > acetone in *C. album* and aqueous > ethanol > methanol > acetone in C. pumilio (Table 3). According to the results, metal chelating activity was highest in the ethanolic extract of *C. album* (97.0 %) and aqueous extract of C. pumilio (65.4 %). Therefore, it was suggested that the Fe^{2+} chelating effects of *C. album* and *C. pumilio* could help in protecting against free radical damage. The oxidative degradation of lipids can be controlled by reducing metal

Table 3. Phosphomolybdenum reducing power and ferrous ion chelating activity of *Chenopodium album* and *Chenopodium pumilio.*

Species	Solvents	Phosphomolybdenum reducing power (mg AAE/g dw)	Ferrous ion chelating activity (%)
C. album	Aqueous	33.8±0.80	92.6±1.12
	Ethanol	34.7±0.12	97.0±0.68
	Methanol	27.5±0.09	57.9±0.93
	Acetone	22.5±0.64	44.6±1.36
C. pumilio	Aqueous	43.3±0.39	65.4±1.79
	Ethanol	34.4±0.17	61.4±0.89
	Methanol	36.5±0.32	59.1±0.89
	Acetone	20.6±0.10	56.9±1.61

Values are expressed as mean ± SD of triplicate measurements.
mg AAE /g dw: milli gram ascorbic acid equivalent per gram dry weight.
% - percentage inhibition per gram dry weight.

concentration through the metal chelating capacity of the compound (Gulcin, & Alwasel, 2022). The chelators have more affinity for metal; therefore, the chelating agents form covalent bonds with metal ions. Therefore, chelating agents are considered secondary antioxidants. They can reduce and stabilize metal ion oxidation (Sahat, Ibrahim, & Alsaid, 2015). Our data revealed that the phytocompounds in the extracts demonstrated strong ability toward iron binding, and the antioxidant action may be owed to its iron-binding ability.

Hydrogen peroxide radical scavenging activity

Both *Chenopodium* species showed effective scavenging activity for hydrogen peroxide. The activity for *C. album* follows the order ethanol > aqueous > methanol > acetone, and that for *C. pumilio* follows the order aqueous > methanol > ethanol > acetone. Highest activity was recorded for the aqueous extract of *C. pumilio* (66.6 %) and the ethanolic extract of *C. album* (53.3 %). Several dietetic polyphenols, such as catechin, quercetin, caffeic acid, and gallic acid, are main components of plant-based foods, especially fruits and vegetables. High amounts of hydrogen peroxide in plant and animal cells may cause oxidative stress, and extremely high concentrations may lead to cell death. Dietary polyphenols establish a protective shield against cell damage in mammalian and bacterial cells (Saffoon et al., 2014). On the basis of these results, phenolic and flavonoid compounds of *Chenopodium* species are involved in quenching H_2O_2, and hence, show significant hydrogen peroxide radical scavenging activity.

Hydroxyl radical scavenging activity

Antioxidant activity is comparable to hydroxyl radical scavenging activity. In our study, ethanolic and aqueous extracts exhibited the highest hydroxyl radical scavenging activity in *C. album* (74.4 %) and *C. pumilio* (78.5 %), respectively (Table 4). Hydroxyl radicals are highly reactive and have a short half-life; hence, they can damage all three important classes of biological molecules, including nucleic acids, proteins, and lipids (Das & Roychoudhury, 2014). The initiation of lipid peroxidation is carried out by radicals. This process is completed by taking hydrogen atoms from unsaturated fatty acids. As a result, one of a living body's most efficient defense against numerous diseases is the elimination of hydroxyl radicals. The *Chenopodium* species studied exhibited effective hydroxyl radical scavenging activity. Hence, it can act against various diseases by removing hydroxyl radicals.

Deoxyribose degradation activity

The deoxyribose degradation test showed that the *Chenopodium* species were effective in neutralizing OH radicals, where percentage inhibition followed the order ethanol > aqueous > methanol > acetone for *C. album* and aqueous > methanol > ethanol > acetone for *C. pumilio* (Table 5). The ethanolic and aqueous extracts exhibited the highest degradation activity in *C. album* (89.6 %) and *C. pumilio* (57.0 %), respectively. *C. album* is more potent at degrading deoxyribose than *C. pumilio*. The low intensity of red color indicated that antioxidant activity is comparable to hydroxyl radical scavenging activity (Gulcin, Berashvili, & Gepdiremen, 2005). The result showed that degradation of deoxyribose was prevented due to the addition of fractions of *Chenopodium* in the reaction mixture. Moreover, it helped initiate scavenging of hydroxyl radicals.

β-carotene bleaching activity

The ability of *Chenopodium* species to bleach β-carotene differed depending on the species and solvent type. The ethanolic and aqueous extracts of *C. album* (91.7 %) and *C. pumilio* (93.1%) had the highest β-carotene bleaching activity, respectively (Table 5). The order of activity for *C. album* is ethanol > aqueous > acetone > methanol, whereas the sequence for *C.*

Table 4. Hydrogen peroxide radical scavenging activity and hydroxyl radical scavenging activity of *Chenopodium album* and *Chenopodium pumilio.*

Species	Solvents	Hydrogen peroxide radical scavenging activity (%)	Hydroxyl radical scavenging activity (%)
C. album	Aqueous	48.5±1.05	67.5±1.04
	Ethanol	53.3±1.34	74.4±1.38
	Methanol	47.8±1.05	62.3±1.11
	Acetone	40.5±1.46	55.5±1.36
C. pumilio	Aqueous	66.6±1.15	78.5±0.98
	Ethanol	57.3±0.99	71.8±0.68
	Methanol	61.9±1.55	58.8±1.06
	Acetone	57.1±1.40	60.3±1.36

Values are expressed as mean ± SD of triplicate measurements.
% - percentage inhibition per gram dry weight.

Table 5. Deoxyribose degradation and β- Carotene bleaching activity of *Chenopodium album* and *Chenopodium pumilio.*

Species	Solvents	Deoxyribose degradation activity (%)	β- Carotene bleaching activity (%)
C. album	Aqueous	80.4±0.67	89.3±1.05
	Ethanol	89.6±1.10	91.7±0.94
	Methanol	74.9±0.97	82.8±0.77
	Acetone	57.8±0.67	85.4±0.62
C. pumilio	Aqueous	57.0±0.91	93.1±0.60
	Ethanol	44.5±0.59	82.5±1.03
	Methanol	53.2±1.08	87.7±1.25
	Acetone	37.4±0.64	78.4±1.16

Values are expressed as mean ± SD of triplicate measurements.
% - percentage inhibition per gram dry weight.

Table 6. Regression coefficients (r^2 value) for antioxidant activity assessed by different methods and the relative influence of antioxidant components.

Species		FRAP	DPPH	PMR	FC	HPS	HRS	DD	β- CB
C. album	**TPC**	0.783	0.125	0.522	0.648	0.406	0.637	0.443	0.951
	TFC	0.957	0.728	0.449	0.445	0.717	0.735	0.652	0.464
C. pumilio	**TPC**	0.968	0.296	0.620	0.877	0.793	0.704	0.584	0.794
	TFC	0.967	0.531	0.570	0.912	0.666	0.821	0.474	0.691

TPC = Total phenolic content; TFC = Total flavonoid content; FRAP = Ferric reducing antioxidant power; DPPH = DPPH free radical scavenging activity; PMR= Phosphomolybdenum reduction; FC=Ferrous ion chelation; HPS: Hydrogen Peroxide Scavenging; HRS: Hydroxyl Radical Scavenging; DD=Dexyribose degradation; β- CB= β- Carotene bleaching activity.

Table 7. ANOVA for antioxidant activity among different extracts by different antioxidant assays of two *Chenopodium* species.

Antioxidant assays	df	MS	*P*
FRAP	1	1.110	0.916
DPPH	1	5.661	0.628
PMR	1	33.17	0.491
FC	1	304.0	0.379
HPS	1	348.6	0.008
HRS	1	11.66	0.709
DD	1	11.90	0.013
β- CB	1	7.566	0.622

FRAP = Ferric reducing antioxidant power; DPPH = DPPH Radical scavenging; PMR= Phosphomolybdenum reduction; FC=Ferrous ion chelation; HPS: Hydrogen Peroxide Scavenging; HRS: Hydroxyl Radical Scavenging; DD=Dexyribose degradation; β- CB= β- Carotene bleaching activity.

pumilio is aqueous > methanol > ethanol > acetone. Compared to methanolic and acetone extracts, the β carotene bleaching inhibitory activity was relatively higher in ethanolic and aqueous extracts. The amount of bleaching is reduced by antioxidants in plants, which neutralize the linoleic hydroperoxyl free radicals produced (Kulisic et al., 2004). As a result, the antioxidant activity of the sample extracts influences the rate at which β-carotene degrades. The findings indicate that the extracts of *Chenopodium* species studied could prevent β-carotene from bleaching, making them effective antioxidants.

Correlation between phytochemical constituents and antioxidant activity

The relationship of the phytocompounds with the antioxidant ability of the plants examined was determined (Table 6). Phenolic content displayed a significant correlation with most of the antioxidant tests, such as ferric reducing antioxidant power ($r = 0.783$) and β-carotene bleaching activity ($r = 0.951$) in C. album, and with ferric reducing antioxidant power ($r = 0.968$), ferrous ion chelation ($r = 0.877$) and β-carotene bleaching activity ($r = 0.794$) in *C. pumilio*. Similarly, the flavonoid content of C. album indicated a good correlation with antioxidant assays, such as ferric reducing antioxidant power ($r = 0.957$), DPPH free radical scavenging activity ($r = 0.728$), hydrogen peroxide scavenging activity ($r = 0.728$), and hydroxyl radical scavenging activity ($r = 0.735$). The flavonoid content of *C. pumilio* revealed a significant correlation with ferric reducing antioxidant power ($r = 0.967$), ferrous ion chelation ($r = 0.912$) and hydroxyl radical scavenging activity ($r = 0.821$). The remaining assays exhibited average correlation in both species. The antioxidant capacity of different extracts shows great variation in results, probably due to factors such as genetics, agro-technical practices, and environmental circumstances, which have great impact on the presence of phenolics and flavonoid compounds. Several reports have indicated that antioxidant activity and phenols correlate directly (Aryal et al., 2009; Muflihah, Gollavelli, & Ling, 2021; Alnsour, Issa, Awwad, Albals, & Al-Momani, 2022). The inconsistencies observed in the correlation in this study explained that different plants are rich in different specialized metabolites; therefore, it is difficult to consider which is predominant. The literature reviews showed a discrepancy in the correlation of phytochemical constituents with antioxidant activity. This inconsistency is due to variations in the representation and elucidation of results, methodology used, and evaluation of interfering substances, such as ascorbic acid, saccharides, and carotenoids. Moreover, the antioxidant activity of an extract can fluctuate due to many factors, such as the method of mixture preparation, antioxidant solubility, oxidation state, pH, and type of oxidation-prone substrate (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010).

ANOVA was used to evaluate significantly mean differences amid the different solvent extracts of *Chenopodium* species, and a significant difference was confirmed at the $P < 0.05$ level in different assays (Table 7). The examined plant extracts had promising antioxidant and free radical scavenging activity.

This study has some limitations. Instead of analyzing pure chemicals, the studies were performed on extracts and fractions. From the perspective of single molecule-based medicine, they are inconclusive, although they offer highly valuable insights into the use of plant-based medicine in chronic conditions. The results of the *in vitro* tests performed in this work must be validated *in vivo*.

CONCLUSION

The results of this study demonstrated the presence of flavonoids, phenolics, and antioxidant activity in the two *Chenopodium* species. Based on the results, in various test procedures, all the solvent extracts of both the species showed varying levels of antioxidant activity. A sufficient quantity of flavonoids and phenolics was present, which accounts for the observed antioxidant efficiency. When compared with other solvent extracts, aqueous and ethanolic extracts showed the highest concentrations of phenolics and flavonoids and the strongest antioxidant activity. Therefore, aqueous and ethanolic extracts of *C. album* and *C. pumilio* can be utilized as readily available sources of natural antioxidants. Further studies on the role of these antioxidants are warranted. The purification, separation, and identification of active compounds, as well as their function in disease prevention, must be evaluated.

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