



COMPARATIVE ANTIOXIDANT POTENTIAL AND PHYTOCHEMICAL COMPOSITION OF LEAF AND STEM EXTRACTS OF NASTURTIIUM OFFICINALE L

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Abstract

Nasturtium officinale L. (Watercress) is a Brassicaceae vegetable rich in redox-active phytochemicals, yet antioxidant recovery may vary with solvent and plant organ. This study compared acetone, methanolic, ethanolic and aqueous extracts of leaves and stems using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, reducing-power and ferrous-ion-chelating assays, together with total phenolic and total flavonoid determinations. Extracts were evaluated at four reported quantities (25, 50, 75 and 100 µg), with three observations per treatment; results were retained exactly as supplied and expressed as mean $\pm$ standard error of the mean where available. Antioxidant responses increased with concentration in every solvent-organ series. Methanolic leaf extract produced the highest DPPH inhibition ($87.63 \pm 0.22\%$), reducing activity ($87.93 \pm 0.47\%$) and ferrous-ion chelation (81.93% ; standard error not reported) at 100 µg. Corresponding methanolic stem values were 73.78% (standard error not reported), $80.51 \pm 1.15\%$ and $77.41 \pm 2.12\%$, respectively. Methanolic leaves also contained more total phenolics (81.53 ± 1.25 mg/100 g) and flavonoids (33.21 ± 0.45 mg/100 g) than stems (69.88 ± 0.85 and 20.86 ± 0.39 mg/100 g, respectively). Ethanol generally ranked second, whereas aqueous fractions were commonly least active. The concordance between methanol-extractable phenolics/flavonoids and antioxidant performance supports their plausible contribution through electron donation, radical stabilization and metal coordination. However, incomplete error reporting, inconsistent concentration notation and absence of inferential statistics restrict definitive comparisons.

Keywords: Antioxidant activity; DPPH; Ferrous-ion chelation; Phenolic compounds; Flavonoids; Watercress

Introduction

Reactive oxygen species (ROS), including superoxide, hydroxyl radicals and hydrogen peroxide, arise during normal metabolism and in response to environmental stress. At controlled concentrations, ROS participate in signaling and host defense, but excessive formation or inadequate antioxidant protection creates oxidative stress that can damage lipids, proteins and nucleic acids (Halliwell & Gutteridge, 2015; Lobo *et al.*, 2010). Oxidative processes are therefore relevant to food deterioration and to the pathophysiology of several chronic disorders. Antioxidants can limit such reactions by donating electrons or hydrogen atoms, stabilizing radical intermediates, interrupting oxidation chains or binding transition metals that catalyze radical formation (Apak *et al.*, 2016; Huang *et al.*, 2005). Because a single assay captures only part of this mechanistic diversity, antioxidant evaluation is strengthened when radical-scavenging, reducing and metal-chelating measurements are interpreted together (Prior *et al.*, 2005).

Plant phenolics are prominent contributors to non-enzymatic antioxidant defense. Their hydroxylated aromatic structures can transfer an electron or hydrogen atom to a reactive species, while the resulting phenoxyl radical may be stabilized by resonance (Rice-Evans *et al.*, 1996; Shahidi & Ambigaipalan, 2015). Flavonoids, a major polyphenol subclass, exhibit activities that depend on hydroxylation pattern, conjugation and carbonyl functionality; selected structures can also coordinate iron or copper and thereby reduce metal-catalyzed radical production (Pietta, 2000; Procházková *et al.*, 2011). Total phenolic content (TPC) and total flavonoid content (TFC) are consequently useful, although nonspecific, indices for relating extract composition to antioxidant behavior. The Folin–Ciocalteu reaction measures overall reducing capacity rather than phenolics exclusively, and colorimetric flavonoid assays respond differently to distinct flavonoid subclasses; both therefore require cautious interpretation (Everette *et al.*, 2010; Singleton *et al.*, 1999).

Nasturtium officinale L., commonly known as watercress, is a perennial aquatic member of the Brassicaceae consumed as a leafy vegetable and used traditionally as a medicinal plant. Its nutritional and phytochemical profile includes vitamin C, carotenoids, chlorophylls, phenolic acids, flavonoids, glucosinolates and glucosinolate-derived isothiocyanates (Klimek-Szczykutowicz *et al.*, 2018; Manchali *et al.*, 2012). Comparative surveys of leafy vegetables have identified watercress as a rich source of vitamin C and antioxidant activity (Martínez-Sánchez *et al.*, 2008). Watercress extracts have demonstrated radical-scavenging, reducing and lipid-protective effects in vitro and in experimental models (Bahramikia & Yazdanparast, 2010; Ozen, 2009; Yazdanparast *et al.*, 2008). Phenolic profiling has further revealed organ-dependent distributions of coumaric-acid derivatives, caftaric acid, sinapic acid and quercetin derivatives (Zeb, 2015). These findings make watercress an attractive candidate for natural-antioxidant, nutraceutical and functional-food research.

Measured activity nevertheless depends strongly on sample history and analytical design. Genotype, developmental stage, light, mineral nutrition, postharvest storage and drying can alter antioxidant metabolite abundance (Klimek-Szczykutowicz *et al.*, 2019; Payne *et al.*, 2015; Pinela *et al.*, 2018). Extraction is equally decisive because solvent polarity, hydrogen-bonding capacity, water content, temperature and time determine which constituents enter the analytical fraction (Dai & Mumper, 2010; Do *et al.*, 2014; Naczek & Shahidi, 2004). Methanol and ethanol often recover a broader spectrum of moderately polar phenolics than water alone, while acetone may favor other phenolic or pigment fractions. Higher activity of a methanolic extract should therefore be interpreted as superior recovery

under the tested conditions, not as proof that methanol itself enhances bioactivity or that a single identified compound is responsible.

The DPPH assay estimates the capacity of an extract to reduce a stable nitrogen-centered radical, producing a concentration-dependent decline in absorbance at 517 nm (Brand-Williams *et al.*, 1995). The ferricyanide reducing-power assay evaluates electron transfer from extract constituents to ferric species, with greater color development at 700 nm indicating stronger reduction (Oyaizu, 1986). In the ferrozine assay, compounds compete with ferrozine for ferrous ions; diminished formation of the magenta Fe^{2+} –ferrozine complex indicates chelation (Decker & Welch, 1990). Together, these assays distinguish direct radical quenching, general reducing ability and preventive metal sequestration.

The present study compared acetone, methanolic, ethanolic and aqueous extracts of watercress leaves and stems across DPPH radical scavenging, reported reducing activity and ferrous-ion chelation, and related these responses to methanolic TPC and TFC. The objectives were to identify solvent-, concentration- and plant-part-dependent trends without inventing missing statistics; to preserve the experimental values exactly as supplied; and to assess whether the phytochemical pattern offers a plausible explanation for the observed antioxidant hierarchy. This integrated comparison addresses the practical question of which plant organ and extraction solvent most effectively recover antioxidant constituents for subsequent purification and validation.

Materials and Methods

Plant material and preparation

Whole plants were collected during the 2023–2024 flowering season from Bajjachak, Karanjiya and sites in Raipur, Chhattisgarh, India, including the Botanical Garden of Indira Gandhi Krishi Vishwavidyalaya and local gardens. Identification was confirmed by the specialists named in the supplied document, and voucher specimens were deposited in the institutional herbarium; voucher number was not reported. Plant material was shade-dried in open air for 5–10 days and pulverized. Leaves and stems were evaluated separately.

Extraction

Finely powdered material (20 g) was extracted separately with acetone, methanol, ethanol or distilled water. The source method states that each mixture was maintained in darkness at room temperature for 72 h and gently agitated every 24 h. Extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at approximately 30°C. Solvent volume, solid-to-solvent ratio, extraction yield, final stock concentration, storage temperature and duration were not reported. Although a Fig. caption in the source refers to Soxhlet extraction, the written protocol describes 72-h room-temperature maceration; the latter was used here, and the inconsistency is acknowledged.

DPPH radical-scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay followed Liyana-Pathirana and Shahidi (2005) with modifications. Fresh 0.135 mM DPPH in methanol (1 mL) was mixed with 1 mL extract, vortexed and incubated for 30 min in darkness at room temperature. Absorbance was measured at 517 nm. A DPPH reagent mixture without extract served as the control; rutin and butylated hydroxytoluene were identified as reference antioxidants, but their results were not reported. Radical-scavenging activity was calculated as $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. The results tables report extract quantities of 25, 50, 75 and 100 μg .

Reducing-power assay

Reducing power was assessed by the ferricyanide method (Oyaizu, 1986). Extract in 1 mL distilled water was combined with 2.5 mL 0.2 M phosphate buffer (pH 7.6) and 2.5 mL 1% potassium ferricyanide, then incubated at 50°C for 30 min. After adding 2.5 mL 10% trichloroacetic acid, mixtures were centrifuged at 3000 rpm for 10 min. Supernatant (2.5 mL) was mixed with 2.5 mL water and 0.5 mL 0.1% ferric chloride; absorbance was read at 700 nm. α -Tocopherol, butylated hydroxyanisole and butylated hydroxytoluene were stated as comparators, but comparator results were not reported. The source tables express the outcome as percentage “reducing power” at 25, 50, 75 and 100 μ g, although the method describes absorbance and a 20–140 μ g range; a formula converting absorbance to percentage was not reported.

Ferrous-ion-chelating assay

Chelation was determined by the ferrozine method (Decker & Welch, 1990). Extract (1 mL) was mixed with 3.7 mL deionized water and 0.1 mL 2 mM ferrous chloride. The source describes preliminary incubation intervals of 5, 10, 30 and 60 min, after which 0.2 mL 5 mM ferrozine was added and color developed for 10 min at room temperature. Absorbance was measured at 562 nm. A reaction without extract was the negative control, and ethylene di amine tetra-acetic acid (0.037 mg/mL) was the reference chelator; its results were not reported. Chelation was interpreted using $[1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$. Results were reported at 25, 50, 75 and 100 μ g, which differs from the method’s 0.25–1.00 mg/mL notation; the basis for conversion was not reported.

Total phenolic and flavonoid contents

TPC was measured with the Folin–Ciocalteu method. Diluted filtrate (5 μ L) was mixed with 60 μ L 7.5% sodium carbonate, 15 μ L Folin–Ciocalteu reagent and 200 μ L water, held for 5 min at 60°C, cooled and read at 700 nm. Gallic acid standards (200–3000 mg/L; reported $r^2 = .9987$) were used. TFC was determined by mixing 30 μ L diluted extract with 90 μ L methanol, 6 μ L 10% aluminum chloride, 6 μ L 1 mol/L potassium acetate and 170 μ L water; absorbance was read at 415 nm after 30 min. Quercetin standards (25–200 mg/L; reported $r^2 = .9994$) were used. Only methanolic leaf and stem extracts were reported. Although the methods specify gallic-acid equivalents per gram dry weight and quercetin equivalents per gram dry weight, result tables state only mg/100 g; values and displayed units were retained exactly, and the missing equivalence/dry-weight basis is marked as not reported.

Replicates and statistical treatment

The supplied tables state that data were obtained from three observations and were expressed as mean $\pm$ standard error of the mean (SEM). SEM values were absent for all stem DPPH observations and for leaf chelation observations above 25 μ g; these are marked “NR.” Raw replicate values, variance assumptions, randomization, analytical batch structure, normality testing, analysis of variance, post hoc comparisons, exact p values and software were not reported. Accordingly, the present paper provides descriptive comparisons only and makes no claim of statistical significance.

Results

DPPH radical scavenging

Every extract showed increasing DPPH inhibition across the reported concentration sequence (Table 1; Fig.1). Methanol was the leading solvent for both organs. At 100 μ g, leaf methanol reached $87.63 \pm 0.22\%$, exceeding stem methanol ($73.78 \pm 0.27\%$). Leaf ethanol ($70.76 \pm 0.07\%$) and acetone ($69.82 \pm 1.23\%$) were similar descriptively at the highest quantity, whereas aqueous leaf extract reached $55.85 \pm 0.54\%$. Corresponding stem values were 69.76% for ethanol, 68.82% for acetone and 58.85% for

aqueous extract, with SEM not reported. Leaves exceeded stems at 100 μg for methanol, ethanol and acetone, whereas stems exceeded leaves for aqueous extract. No inferential comparison was possible.

Table 1. DPPH radical-scavenging activity of leaf and stem extracts of *Nasturtium officinale*

Plant Part (N. officinale)	Quantity (μg)	Acetone	Methanol	Ethanol	Aqueous
Leaves	25	23.15 ± 0.28	51.20 ± 0.08	33.11 ± 0.40	26.20 ± 0.22
	50	47.84 ± 0.09	68.35 ± 0.26	52.56 ± 0.29	39.41 ± 0.26
	75	59.49 ± 0.15	76.57 ± 0.31	64.54 ± 0.35	47.67 ± 0.37
	100	69.82 ± 1.23	87.63 ± 0.22	70.76 ± 0.07	55.85 ± 0.54
Stems	25	30.23 ± 1.12	54.42 ± 0.15	39.38 ± 0.52	40.11 ± 0.07
	50	49.44 ± 0.07	69.45 ± 0.45	49.57 ± 0.09	44.81 ± 0.06
	75	56.45 ± 0.08	70.77 ± 0.05	60.64 ± 0.32	50.87 ± 0.08
	100	68.82 ± 0.25	73.78 ± 0.27	69.76 ± 0.17	58.85 ± 0.82

Data are multiple of three observation

Values (%) $\pm$ SEM

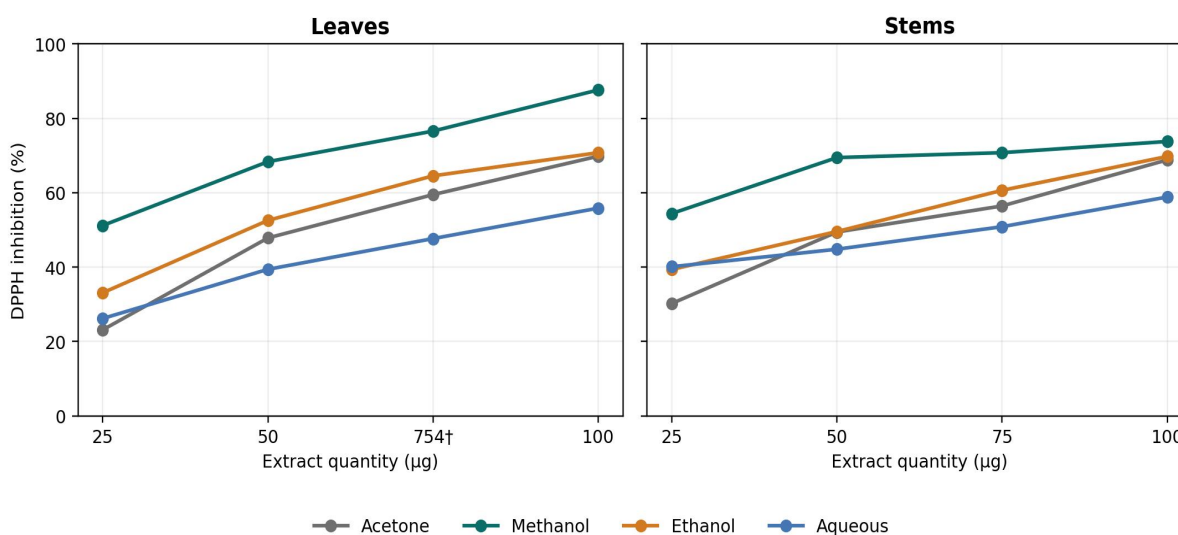


Fig. 1. Concentration-dependent DPPH radical-scavenging activity of leaf and stem extracts

(Error bars are omitted to maintain legibility; comprehensive SEM values appear in Table 1.)

Reducing power

Reported reducing activity rose monotonically from 25 to 100 μg in all eight solvent–organ series (Table 2; Fig. 2). Methanolic leaves gave the maximum value, increasing from $43.11 \pm 0.25\%$ to $87.93 \pm 0.47\%$; methanolic stems increased from $50.33 \pm 0.65\%$ to $80.51 \pm 1.15\%$. At 100 μg , the common solvent order in both organs was methanol > ethanol > acetone > aqueous. Leaves exceeded stems for

methanol ($87.93 \pm 0.47\%$ vs. $80.51 \pm 1.15\%$) and ethanol ($73.42 \pm 1.02\%$ vs. $73.34 \pm 0.25\%$), whereas stem acetone ($63.14 \pm 0.95\%$) was slightly below leaf acetone ($64.44 \pm 0.60\%$) and stem aqueous extract ($59.85 \pm 1.11\%$) exceeded leaf aqueous extract ($54.53 \pm 0.22\%$). These are descriptive differences only.

Table 2. Reducing-power activity of leaf and stem extracts of *Nasturtium officinale*

Plant Part (N. officinale)	Quantity (μg)	Acetone	Methanol	Ethanol	Aqueous
Leaves	25	29.57 ± 0.94	43.11 ± 0.25	35.30 ± 0.12	25.11 ± 0.62
	50	33.64 ± 0.87	59.71 ± 0.42	41.64 ± 0.55	29.61 ± 0.24
	75	53.72 ± 1.00	75.83 ± 0.89	62.73 ± 0.75	35.43 ± 0.69
	100	64.44 ± 0.60	87.93 ± 0.47	73.42 ± 1.02	54.53 ± 0.22
Stems	25	32.11 ± 1.02	50.33 ± 0.65	40.15 ± 0.68	29.18 ± 0.48
	50	41.66 ± 1.11	61.66 ± 0.27	48.85 ± 0.09	32.65 ± 0.92
	75	55.14 ± 0.9	70.61 ± 1.02	64.66 ± 0.88	47.67 ± 1.2
	100	63.14 ± 0.95	80.51 ± 1.15	73.34 ± 0.25	59.85 ± 1.11

Data are multiple of three observation

Values $\pm$ SEM

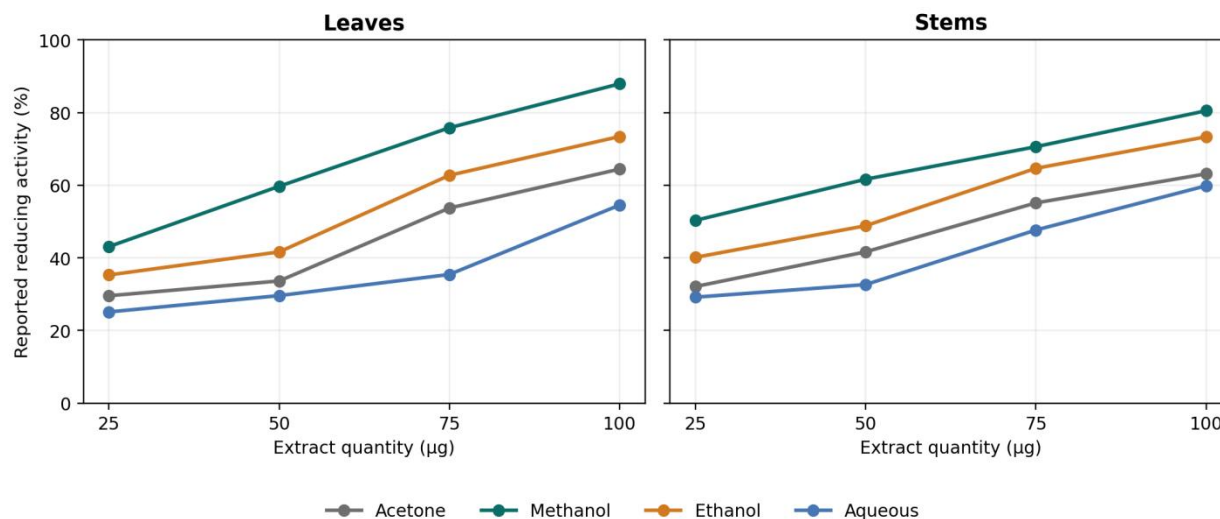


Fig. 2. Concentration-dependent reducing-power comparison of leaf and stem extracts.

(Error bars are omitted to maintain legibility; comprehensive SEM values appear in Table 2.)

Ferrous-ion chelation

Chelating activity increased with concentration for every solvent and organ (Table 3; Fig. 3). At 100 μg , methanol was highest for leaves (81.93% ; SEM not reported) and stems ($77.41 \pm 2.12\%$), followed by ethanol (74.42% , SEM not reported; and $70.47 \pm 0.26\%$), acetone (63.44% , SEM not reported; and

61.57 ± 1.42%) and aqueous extract (50.53%, SEM not reported; and 53.89 ± 0.92%). Leaves exceeded stems for methanol, ethanol and acetone at 100 µg, but the stem aqueous fraction was higher. At 25 µg, methanolic leaves also had the strongest activity (48.83 ± 0.15%), while aqueous stems had the weakest (20.16 ± 0.49%).

Table 3. Ferrous-ion-chelating activity of leaf and stem extracts *Nasturtium officinale*

Plant Part (<i>N. officinale</i>)	Quantity (µg)	Acetone	Methanol	Ethanol	Aqueous
Leaves	25	18.62 ± 1.32	48.83 ± 0.15	28.36 ± 0.26	22.10 ± 0.91
	50	31.64 ± 1.02	55.11 ± 0.09	42.69 ± 0.15	29.61 ± 0.34
	75	50.72 ± 0.07	70.73 ± 0.11	60.73 ± 1.15	35.43 ± 1.27
	100	63.44 ± 0.25	81.93 ± 2.31	74.42 ± 0.09	50.53 ± 0.28
Stems	25	25.23 ± 1.19	40.15 ± 0.99	32.22 ± 1.24	20.16 ± 0.49
	50	31.26 ± 1.20	52.90 ± 1.20	40.85 ± 0.89	27.18 ± 0.42
	75	47.64 ± 1.00	66.81 ± 0.15	60.42 ± 0.56	42.52 ± 0.85
	100	61.57 ± 1.42	77.41 ± 2.12	70.47 ± 0.26	53.89 ± 0.92

Data are multiple of three observation

Values ± SEM

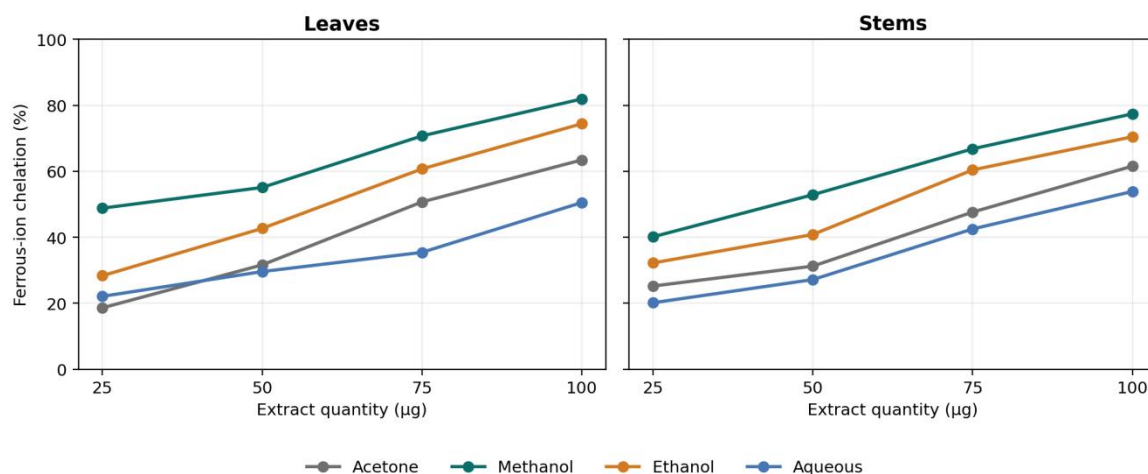


Fig. 3. Concentration-dependent ferrous-ion-chelating activity *Nasturtium officinale*

(Error bars are omitted to maintain legibility; comprehensive SEM values appear in Table 3.)

Phenolic and flavonoid contents

Methanolic leaves contained 81.53 ± 1.25 mg/100 g total phenolics and 33.21 ± 0.45 mg/100 g total flavonoids, whereas methanolic stems contained 69.88 ± 0.85 and 20.86 ± 0.39 mg/100 g, respectively (Table 4; Fig. 4). Thus, leaves had descriptively greater levels of both indices. The source narrative incorrectly states that the leaf–stem flavonoid difference was 4.74 mg/100 g; subtraction of the displayed table means yields 12.35 mg/100 g. The table values were treated as authoritative, and no inferential significance was assigned.

Table 4. Total phenolic and total flavonoid contents of methanolic leaf and stem extracts.

Plant part	Extract	Total phenolics (mg/100 g)	Total flavonoids (mg/100 g)
Leaves	Methanol	81.53 ± 1.25	33.21 ± 0.45
Stem	Methanol	69.88 ± 0.85	20.86 ± 0.39

Data are multiple of three observation

Values $\pm$ SEM

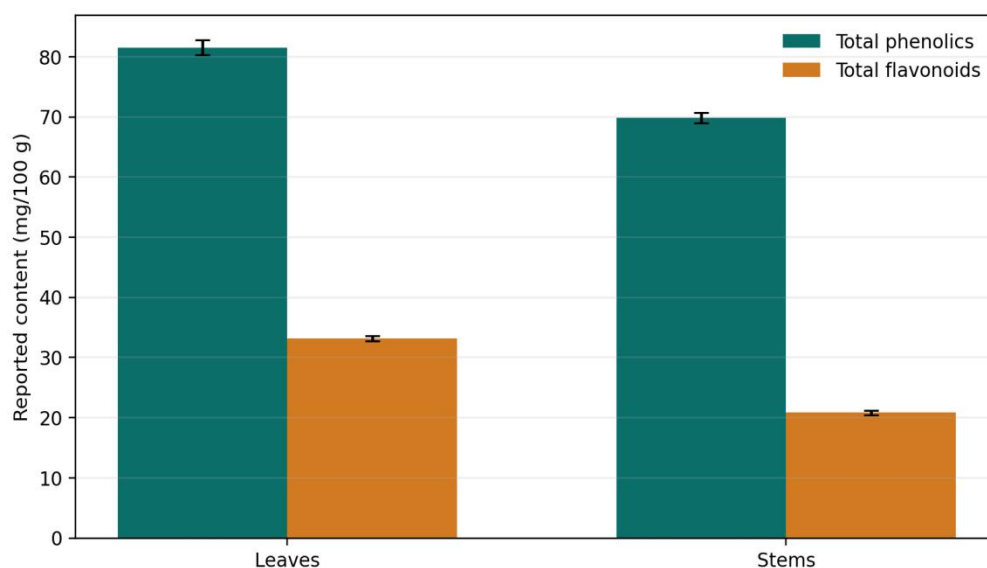


Fig. 4. Total phenolic and flavonoid contents of methanolic leaf and stem extracts

Discussion

The combined assays produced a coherent solvent-dependent pattern: methanol generally recovered the fraction with the greatest radical-scavenging, reducing and iron-chelating responses, ethanol usually ranked second, and water was commonly least effective. This hierarchy agrees with reports that methanolic watercress fractions can contain more phenolics and show stronger radical-scavenging activity than corresponding aqueous fractions (Zeb, 2015), as well as with broader evidence that extraction solvent governs phenolic yield and measured antioxidant capacity (Dai & Mumper, 2010; Do *et al.*, 2014). Bahramikia and Yazdanparast (2010) likewise documented multiple antioxidant functions in watercress extract, including DPPH scavenging, reducing capacity and Fe^{2+} chelation, although differences in extraction, concentration, standards and expression units prevent direct numerical comparison.

Methanol is a polar protic solvent with strong hydrogen-bonding capacity and can solubilize many phenolic acids, flavonoid glycosides and other moderately polar constituents while penetrating dehydrated tissues efficiently. Water may extract highly polar compounds but can be less effective for less-polar phenolics and can coextract macromolecules that dilute measured activity. Acetone and ethanol occupy different polarity and selectivity ranges; their substantial activities show that the antioxidant pool is chemically heterogeneous. These explanations remain extraction-based hypotheses because the present study did not identify individual compounds or quantify solvent yields. It would therefore be inappropriate to attribute activity to named phenolics, glucosinolates or isothiocyanates solely from colorimetric tests.

The concentration-dependent responses are compatible with a larger number of antioxidant molecules becoming available to interact with DPPH radicals, ferricyanide or ferrous ions as extract quantity increases. DPPH reduction reflects electron- and hydrogen-transfer capacity (Brand-Williams *et al.*, 1995), whereas reducing power reflects the ability to convert ferric species to ferrous forms (Oyaizu, 1986). Phenolic hydroxyl groups can donate an electron or hydrogen atom; delocalization of the resulting unpaired electron across the aromatic system stabilizes the phenoxyl radical and can terminate oxidative chains (Rice-Evans *et al.*, 1996; Shahidi & Ambigaipalan, 2015). Flavonoid behavior depends on structural features such as catechol substitution, a 2,3-double bond conjugated with a 4-oxo group, and hydroxyl positioning (Pietta, 2000; Procházková *et al.*, 2011). Because structural profiling was not reported, these mechanisms explain plausible class-level contributions rather than demonstrated compound-specific action.

Ferrous-ion chelation represents a complementary preventive mechanism. By coordinating Fe^{2+} through hydroxyl, carbonyl or carboxyl donor groups, some phenolics and flavonoids can reduce the pool of metal available for Fenton-type chemistry and thereby limit hydroxyl-radical formation (Halliwell & Gutteridge, 2015). The close ranking of methanol > ethanol > acetone > water in the chelation and reducing assays suggests that the more active fractions contained both electron-donating and metal-binding constituents. However, the Folin–Ciocalteu assay responds to any sufficiently reducing substance, and the aluminum-chloride method does not capture all flavonoids equally (Everette *et al.*, 2010). Association among TPC, TFC and antioxidant outcomes therefore cannot establish causality without correlation based on replicate-level data and chromatographic confirmation.

Plant-part differences were most evident for methanol. Leaves had higher TPC and TFC than stems and also produced higher 100- μg methanolic responses in all three assays. This direction is consistent with Zeb (2015), who reported organ-dependent phenolic profiles and stronger antioxidant performance in methanolic watercress extracts, and with studies showing that watercress phytochemistry varies with tissue, environment and developmental conditions (Klimek-Szczykutowicz *et al.*, 2019; Payne *et al.*, 2015). Leaves experience direct light exposure and require photoprotective and defense metabolites, offering a biologically plausible reason for greater polyphenol accumulation. Nevertheless, stems were not uniformly inferior: aqueous stem fractions exceeded aqueous leaf fractions at 100 μg in each antioxidant dataset, indicating that organ differences depend on the chemical selectivity of the solvent. Ozen (2009) found ethanolic watercress extract particularly active in several antioxidant assays, while the current dataset favored methanol. This is not necessarily contradictory: cultivar, locality, organ composition, drying, solvent strength, solid-to-liquid ratio and assay conditions can change extraction selectivity and outcome. Modern watercress research also demonstrates substantial effects of storage,

drying, mineral nutrition and cultivation conditions on phenolics and antioxidant capacity (Klimek-Szczykutowicz *et al.*, 2019; Kijkuokool *et al.*, 2024; Pinela *et al.*, 2018). The present results should thus be viewed as specific to the supplied material and protocol, while still supporting methanolic leaves as the most promising fraction for bioassay-guided chemical characterization.

Conclusion

Across the supplied dataset, antioxidant activity was concentration dependent and strongly influenced by solvent and plant part. Methanol produced the highest DPPH scavenging, reducing activity and ferrous-ion chelation in both leaves and stems, with methanolic leaves reaching the overall maxima and containing more total phenolics and flavonoids than methanolic stems. Ethanol generally represented the next most effective solvent, whereas aqueous fractions were commonly weaker. The convergent assay pattern is compatible with the contribution of methanol-extractable phenolics and flavonoids through electron donation, resonance stabilization of radical products and transition-metal chelation. These findings identify the methanolic leaf fraction as the most suitable candidate for further fractionation and validation.

Conflict of Interest: The authors declare no conflict of interest.

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Data availability: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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