



Metal Chelation and Antioxidant Capacities of *Helianthus annuus* Extracts: A Promising Approach for Green Chelators

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Abstract: This study systematically investigated the iron, zinc and copper chelating capacities, along with the antioxidant potential, of hexane (HE), chloroform (CE), ethyl acetate (EAE), and methanolic (ME) extracts obtained from the leaves, stems, and roots of *Helianthus annuus*. Metal-chelating activities were evaluated *in vitro* using the ferrozine assay for iron and the murexide method for zinc and copper, with EDTA serving as the reference chelator, while antioxidant activity was assessed using the DPPH radical-scavenging assay. Among the extracts tested, the hexane stem extract (HSE) exhibited the strongest iron-chelating activity (91%), approaching that of EDTA (97%). Zinc chelation was highest in the chloroform leaf extract (CLE), reaching 55%, which notably exceeded the activity of EDTA (37%). For copper, the chloroform stem extract (CSE) demonstrated substantial chelating efficiency (78%), comparable to EDTA (81%). All extracts showed pronounced DPPH radical-scavenging activity, with the methanolic leaf extract (MLE) achieving a maximum inhibition of approximately 91.3%. Corresponding IC₅₀ values further confirmed the superior antioxidant capacity of methanolic extracts relative to the standard antioxidant, ascorbic acid. Overall, these results indicate that *H. annuus* extracts possess significant metal-chelating and antioxidant properties, supporting their potential use as natural, plant-based green chelators and antioxidants in food, environmental, and agricultural applications as sustainable alternatives to synthetic agents.

Key words: Chelation, *Helianthus annuus*, DPPH, Heavy metal, Ferrozine method, Eco-friendly detoxification.

Sunflower (*Helianthus annuus*) is one of the most important oilseed crops cultivated worldwide. Its cultivation areas are frequently exposed to multiple biotic and abiotic stresses, including drought, pathogen infection, and temperature fluctuations, which often occur simultaneously and result

in significant reductions in crop productivity (Vemanna *et al.*, 2016). Heavy metals, also referred to as trace metallic elements (ETM), represent a large group of chemical elements that play a dual role in modern societies—being both economically valuable and environmentally hazardous. Due to their toxic, persistent, and non-biodegradable nature, ETM pose serious risks to ecosystems and human health (Niencheski *et al.*, 2000; Bencheikh *et al.*, 2025).

Globally, a large number of plant species exhibit remarkable biological properties, with applications ranging from pharmacology to environmental remediation. Among these, *Helianthus annuus* has attracted considerable attention for its rapid growth, high biomass production, and ability to tolerate and accumulate metals from contaminated environments. This species has been effectively used for the phytoextraction and reclamation of soils polluted with ETM such as Pb, Cd, Zn, Cu, and Fe, making it a promising candidate for phytoremediation in industrial areas, including plastic, paper, dye, and textile sectors (Chen *et al.*, 2011). In addition to its phytoremediation potential, *Helianthus annuus* is also known for its antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and antihypertensive activities, which are attributed to its diverse phytochemical constituents (Guo *et al.*, 2017; Muhtadi, 2021). Native to North America and domesticated in regions of Mexico, this species has become globally distributed due to its agronomic and therapeutic importance (Lentz *et al.*, 2015).

Considering these diverse biological and ecological potentials, the present study aims to evaluate the *in vitro* metal-chelating (Fe^{2+} , Zn^{2+} , and Cu^{2+}) and antioxidant activities of *Helianthus annuus* extracts obtained from leaves, stems, and roots using different solvents (hexane, chloroform, ethyl acetate, and methanol). Statistical analysis was performed using the *t*-Student test to assess the significance of differences among the extracts. This investigation seeks to highlight the potential of *H. annuus* as a natural, eco-friendly source of chelating and antioxidant agents for possible environmental and biotechnological applications.

Unlike most previous studies on *Helianthus annuus*, which have primarily focused on seeds

or single plant organs and mainly evaluated antioxidant activity, the present work provides a systematic and integrative assessment of both antioxidant capacity and multi-metal chelating activity. Specifically, this study compares Fe^{2+} , Zn^{2+} and Cu^{2+} chelation across three plant organs (leaves, stems, and roots) extracted using solvents of different polarity. This comprehensive organ- and solvent-dependent approach allows a deeper understanding of the metal-binding and antioxidant potential of sunflower tissues and highlights their possible application as natural, eco-friendly chelating agents, extending the current knowledge beyond conventional antioxidant screening.

To date, there is a lack of integrated profiling of Fe-Zn-Cu chelation capacities in different organs of sunflower using multiple solvent systems. By combining metal-chelating and antioxidant assay, our results fill this gap and identify organ and solvent-specific extracts with strong chelation potential, supporting their possible use as eco-friendly chelators in environmental remediation and food and nutraceutical applications.

Materials and Methods

Plant materials: At the flowering stage, leaves, stems, and roots of *Helianthus annuus* were collected in May 2025 from the Bougaa region, located north of Setif (eastern Algeria). The collected plant materials were rinsed thoroughly with running tap water to remove adhering impurities and then were air-dried in the shade for four weeks. After complete drying, the samples were ground using a Moulinex mill until a fine, homogeneous powder was obtained. The powder was prepared immediately prior to extraction.

Twenty grams of the air-dried leaf, stem, and root powders were separately subjected to Soxhlet extraction using 200 ml of solvents of increasing polarity hexane, chloroform, ethyl acetate and methanol for six hours each. The obtained extracts were then concentrated to dryness at 45°C using a rotary vacuum evaporator (Büchi). Finally, the dried extracts were stored in sterile containers at 4°C until further use (Sellal *et al.*, 2018).

Ferrous in chelating activity: The chelation of iron (II) ions by the *H. annuus* extracts was determined according to the method of

Gulzar *et al.* (2013); Sellal *et al.* (2016), with slight modifications. It involved mixing 500 μL of each extract solution (hexane, chloroform, ethyl acetate and methanol; 1 mg ml^{-1} in methanol) or EDTA solution (1 mg ml^{-1} in distilled water) with 50 μL of FeSO_4 (2 mM in distilled water) and 450 μL of methanol. After 5 minutes of incubation, 50 μL of ferrozine solution (5 mM in distilled water) was added to the reaction mixture. The control samples contained all reagents except the plant extract. After a 10-minute equilibration period, the absorbance of each solution was measured spectrophotometrically at 562 nm.

The percentage of inhibition of the ferrozine- Fe^{2+} complex formation was calculated using the following equation:

$$\% \text{ inhibition} = [(AC - AS) / AC] \times 100 \quad \dots(1)$$

where: AC was the absorbance of the control and AS was the absorbance in the presence of the sample of *H. annuus* extracts or standard.

Zinc and copper chelating assay: The chelating activity of zinc (Zn^{2+}) or copper (Cu^{2+}) ions using solutions of *H. annuus* plant extracts (1 mg ml^{-1} in methanol) was measured according to the murexide method described by Watak and Patil (2012), with slight modifications. Thereafter, 0.5 mL of methanol containing various volumes (25, 50, 100, 150, 200, 250, 300, 400, and 500 μL) of hexane (HE), chloroform (CE), ethyl acetate (EAE), or methanol (ME) extracts from leaves, stems and roots or EDTA (1 mg ml^{-1} in deionized water, used as a standard), was mixed with 1.6 mL of deionized water and 0.1 mL of ZnCl_2 or CuSO_4 (8 mM in 0.1 M HCl buffer prepared with 0.1 M KCl, pH 5). After 30 seconds of incubation, 0.1 mL of murexide solution (5 mM in the same buffer) was added. Murexide reacts with divalent zinc or copper ions to form stable magenta-colored complexes that are highly soluble in water. The mixture was then kept in the dark at room temperature for 10 minutes, and the absorbance was measured at 562 nm (Belattar and Sellal, 2020). The control contained deionized water without plant extract. A lower absorbance of the reaction mixture indicated higher Zn^{2+} or Cu^{2+} chelating activity. The experiment was performed in triplicate, and the percentage inhibition of murexide- Zn^{2+} or - Cu^{2+} complex formation was calculated using the following equation:

$$\% \text{ inhibition} = [(AC - AS) / AC] \times 100 \quad \dots(2)$$

where: AC was the absorbance of the control and AS was the absorbance in the presence of the sample of plant extracts and standard.

DPPH radical-scavenging assay: The free radical scavenging activity of the hexane (HLE), chloroform (CLE), ethyl acetate (EAE), and methanol (MLE) extracts obtained from the leaves, stems, and roots of sunflower (*Helianthus annuus*) was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, according to the method of Huang *et al.* (2011) with slight modifications. In the DPPH assay, antioxidants reduce the stable purple DPPH radical to the yellow-colored diphenylpicrylhydrazine. This method is based on the reduction of an alcoholic DPPH solution in the presence of hydrogen-donating antioxidants, resulting in the formation of the non-radical DPPH-H form (Huang *et al.*, 2011).

A 0.1 mM DPPH solution in methanol was freshly prepared, and 1.95 mL of this solution was mixed with 50 μL of distilled water containing different volumes of extract solutions (10 mg/mL in methanol) or ascorbic acid solution (0.5 mg/mL in methanol) as a reference antioxidant. The mixture was then incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm (Sellal *et al.* 2019a; Sellal *et al.* 2019b).

The control sample contained distilled water without plant extract. A lower absorbance value indicated stronger DPPH radical scavenging activity. All experiments were performed in triplicate.

Statistical analysis: The results were expressed as mean $\pm$ standard deviation (SD) of three replicates. Statistical analyses were performed using Microsoft Excel and IBM SPSS Statistics (version 25). Student's *t*-test, conducted with Microsoft Excel, was used to compare each extract and standard with the control. One-way ANOVA followed by Duncan's or least significant difference (LSD) post hoc test was carried out using IBM SPSS Statistics to compare mean values among groups for the *in vitro* assays. Differences were considered statistically significant at $p \leq 0.05$ with a 95% confidence interval.

IC_{50} values were determined by linear interpolation of the dose-response data. For

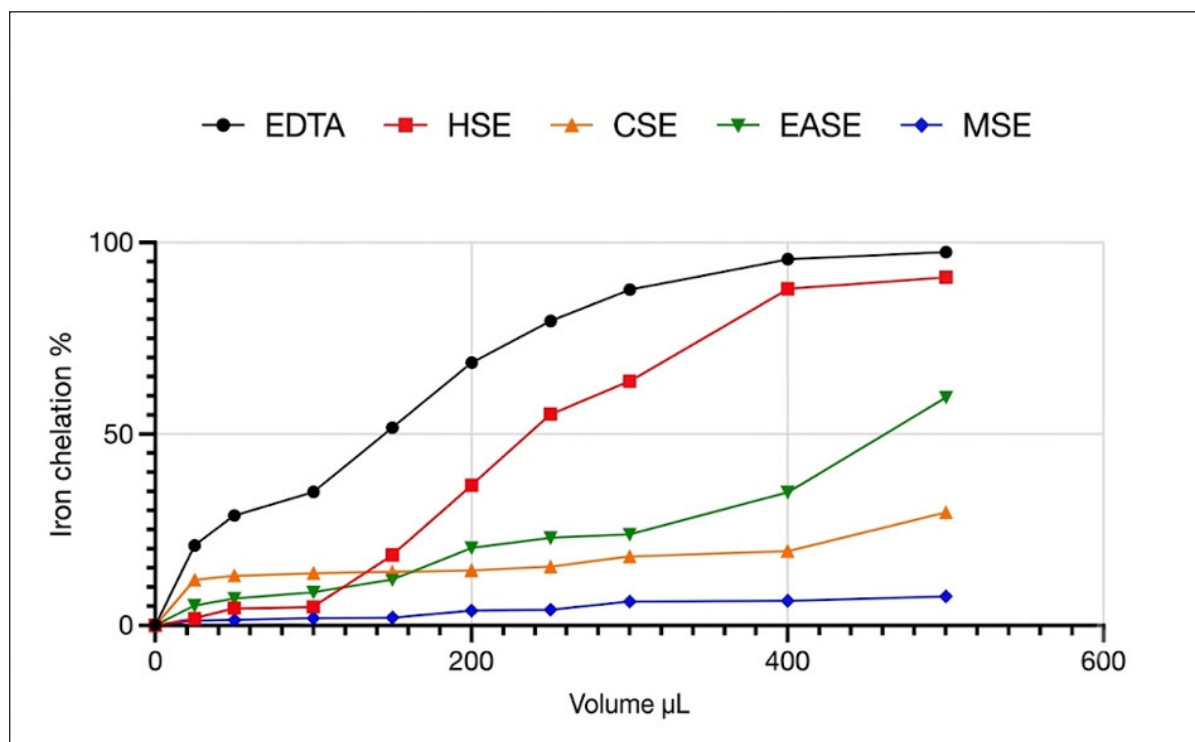


Fig. 1. Iron chelation activity by increasing volumes (25 to 500 μL) of hexane stem extract (HSE), chloroform stem extract (CSE), ethyl acetate stem extract (EASE), methanol stem extract (MSE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

each sample, the two experimental volumes surrounding 50% chelating activity were identified, and the volume corresponding to 50% chelation (V_{50}) was calculated using the equation: $V_{50} = V_1 + [(50 - A_1) \times (V_2 - V_1)] / (A_2 - A_1)$, where V_1 and V_2 are the two volumes surrounding 50% activity and A_1 and A_2 are their corresponding chelating activities (%). The V_{50} was then converted to IC_{50} (mg/mL) based on the concentration of the tested solution (1 mg/mL). When 50% chelation was not achieved within the tested concentration range, the IC_{50} was reported as "not reached"

Results and Discussion

Ferrous ion chelating activity: The iron-chelating activity was determined based on the absorbance measurement of the ferrous ion (Fe^{2+})-ferrozine complex, which produces a red chromophore exhibiting maximum absorbance at 562 nm. Chelating agents are capable of sequestering ferrous ions before they interact with ferrozine.

As shown in Fig. 1, increasing volumes of the hexane stem extract and EDTA (used as a standard chelator) resulted in a significant ($p \leq 0.001$), volume-dependent decrease in

absorbance at 562 nm compared with the control. At 500 μL , the hexane stem extract and EDTA produced 91.1% and 97.8% inhibition of murexide- Zn^{2+} complex formation, respectively, with absorbance values of 0.048 ± 0.001 and 0.012 ± 0.003 , compared with 0.54 ± 0.0028 for the control. These results indicate strong Zn^{2+} -chelating activity of the hexane stem extract, although its activity was lower than that of EDTA.

In contrast, the addition of the same volumes of the other stem extracts (chloroform, ethyl acetate and methanol) resulted an absorbance values ranging between 0.21 ± 0.007 and 0.39 ± 0.002 , which are markedly lower than those obtained with HSE and EDTA. This indicates that these extracts exhibited a weak iron-chelating effect compared with the negative control, showing markedly lower activity than HSE and EDTA, with inhibition percentages of 29% for chloroform, 59% for ethyl acetate and 7% for methanol.

Analysis of variance (ANOVA) among the tested samples (extracts and EDTA) revealed a highly significant difference, following the order: EDTA > HSE > EASE > CSE > MSE.

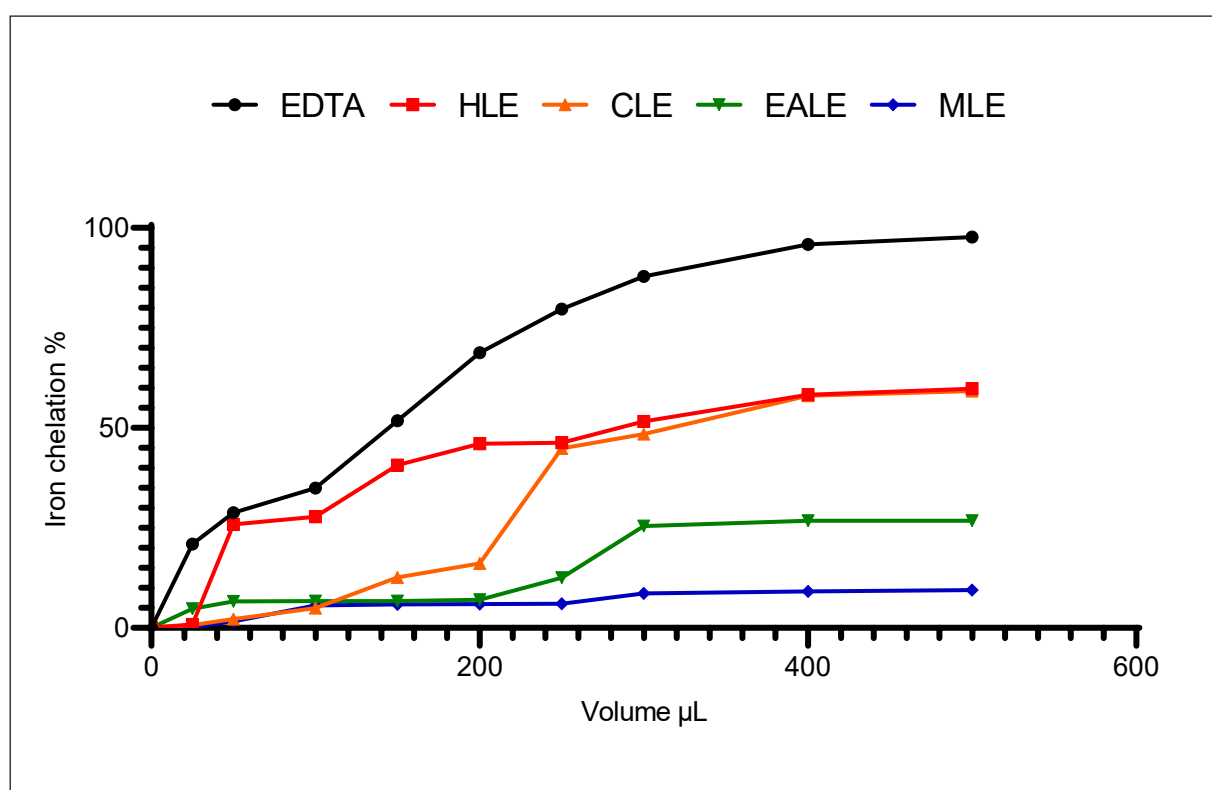


Fig. 2. Iron chelation activity by increasing volumes (25 to 500 μL) of hexane leaf extract (HLE), chloroform leaf extract (CLE), ethyl acetate leaf extract (EALE), methanol leaf extract (MLE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

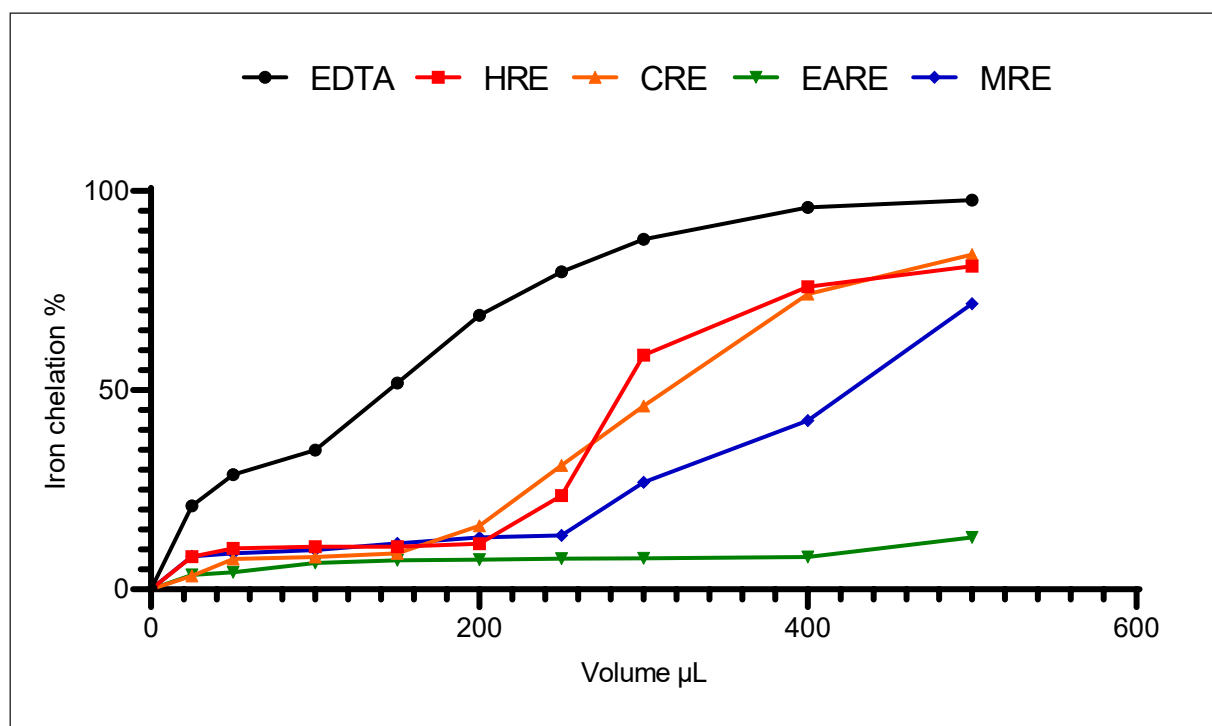


Fig. 3. Iron chelation activity by increasing volumes (25 to 500 μL) of hexane root extract (HRE), chloroform root extract (CRE), ethyl acetate root extract (EARE), methanol root extract (MRE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

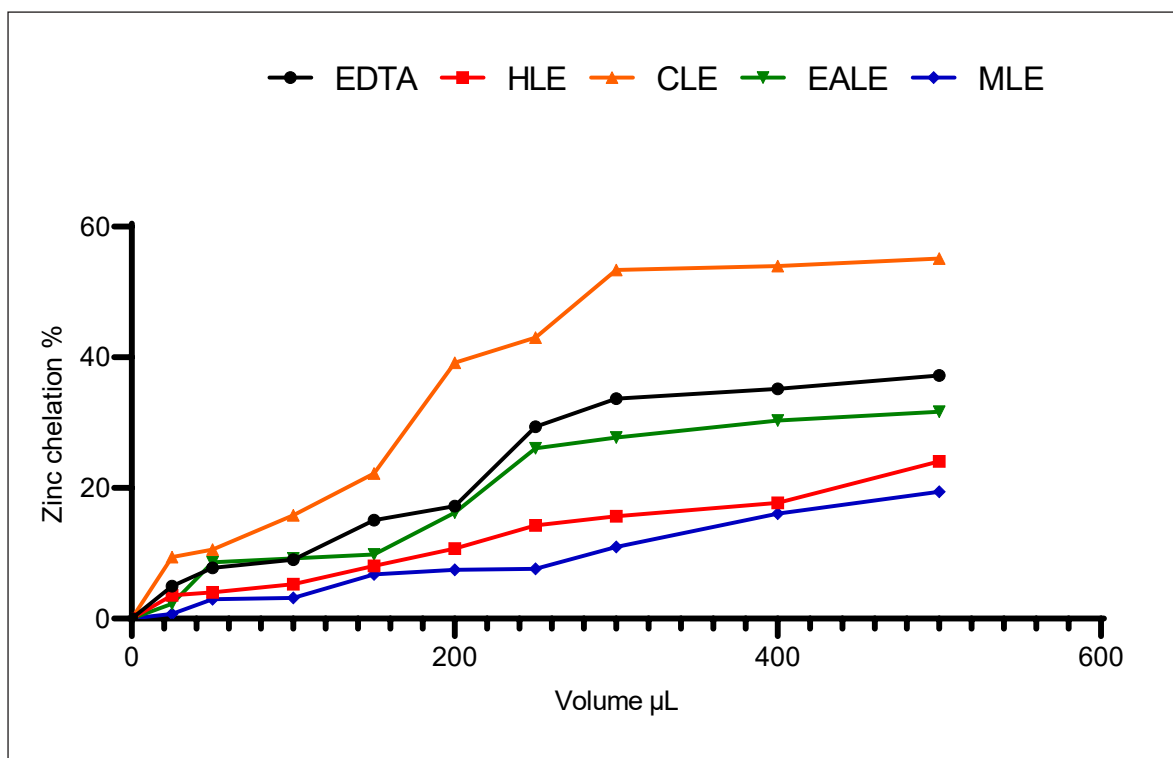


Fig. 4. Zinc chelation activity by increasing volumes (25 to 500 μ L) of hexane leaf extract (HLE), chloroform leaf extract (CLE), ethyl acetate leaf extract (EALE), methanol leaf extract (MLE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

Furthermore, the analysis showed that EDTA and HSE formed two distinct groups according to their iron-chelating activity. At 500 μ L, EDTA exhibited nearly complete iron chelation (97%), confirming its strong affinity for Fe^{2+} . Hexane and chloroform leaf extracts plateaued at 59%, indicating notable chelating potential at higher concentrations, whereas ethyl acetate (26%) and methanol (9%) remained weak. Statistical analysis showed that both extract type and volume, as well as their interaction (volume $\times$ extract), were highly significant ($p = 0.001$). All volumes differed significantly except 400 and 500 μ L, which belonged to the same range. The inhibition hierarchy followed EDTA > HSE > CSE > EASE > MSE. In roots, chelation markedly increased at 500 μ L: hexane (84%) and chloroform (83%) showed strong activity, methanol rose sharply to 71%, while ethyl acetate remained low (13%). The results for the leaf and root extracts are presented in Figures 2 and 3, respectively.

Zinc chelating assay: The Zn^{2+} -murexide complex exhibits an absorption maximum at 562 nm, where lower absorbance values indicate higher chelating activity. For zinc, the addition

of increasing volumes (25, 50, 100, 150, 200, 250, 300, 400, and 500 μ L) of HLE, CLE, EALE, MLE or EDTA (used as a standard chelator) revealed that CLE and EALE displayed the most significant ($p \leq 0.001$) dose-dependent chelating capacity, exceeding that of EDTA in comparison with the control. These extracts showed at 500 μ L the decreased absorbance values of 0.12 ± 0.003 , 0.15 ± 0.005 and 0.19 ± 0.004 respectively, as compared to the negative control of absorbance 0.3 ± 0.01 (Fig. 4). These values corresponding to inhibition percentages of 55%, 31% and 37% respectively.

For the stem extracts, CSE exhibited comparable inhibition rates with the EDTA (37%), while EASE demonstrated a high significant ($p \leq 0.001$) dose-dependent activity, with an absorbance of 0.092 ± 0.01 and inhibition percentage of 35% at 500 μ L compared to the negative control (Fig. 5) which represents an inhibition of 35%.

Regarding root extracts, both EARE and EDTA showed the most significant ($p \leq 0.001$) dose-dependent chelating capacities, with absorbance values of 0.15 ± 0.005 and 0.19 ± 0.004 relative to the negative control (Fig. 6)

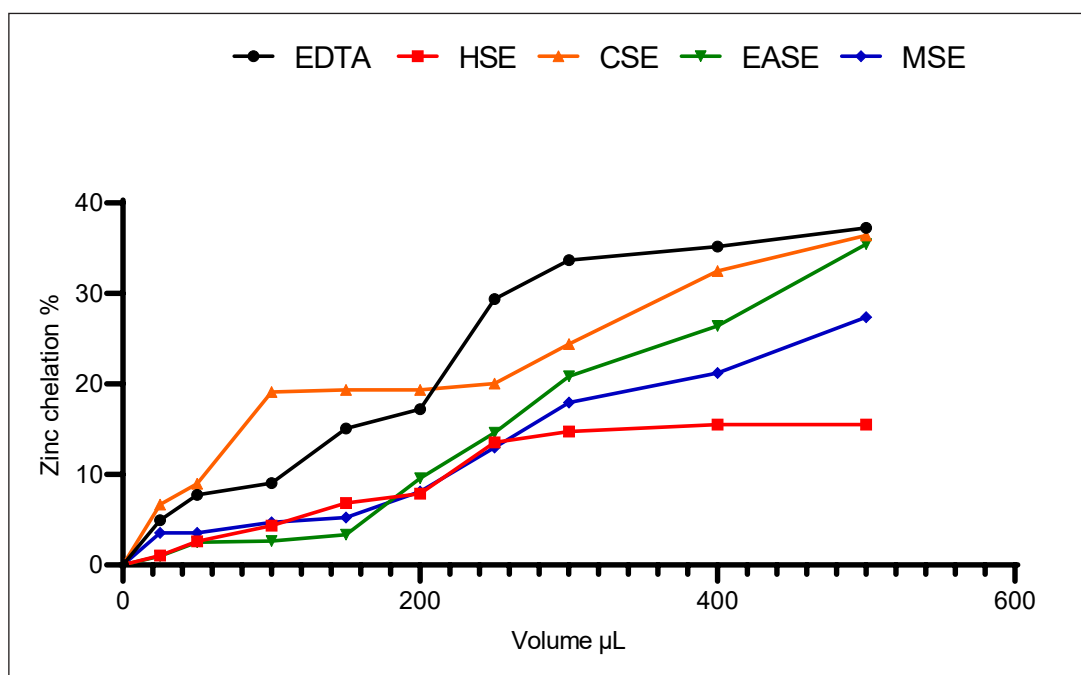


Fig. 5. Zinc chelation activity by increasing volumes (25 to 500 μ L) of hexane stem extract (HSE), chloroform stem extract (CSE), ethyl acetate stem extract (EASE), methanol stem extract (MSE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

and corresponding inhibition percentages of 52% and 37% at 500 μ L, respectively.

Copper chelating assay

The results showed that none of the extracts exhibited a statistically significant ($p > 0.05$) dose-dependent chelating capacity compared

with EDTA. Nevertheless, MLE, CSE, and HRE displayed slight decreases in absorbance values (0.013 ± 0.003 , 0.002 ± 0.004 , and 0.01 ± 0.01 , respectively) relative to the negative control (Figures 7-9). At 500 μ L, the highest inhibition percentages were observed for MLE (70%; Fig.

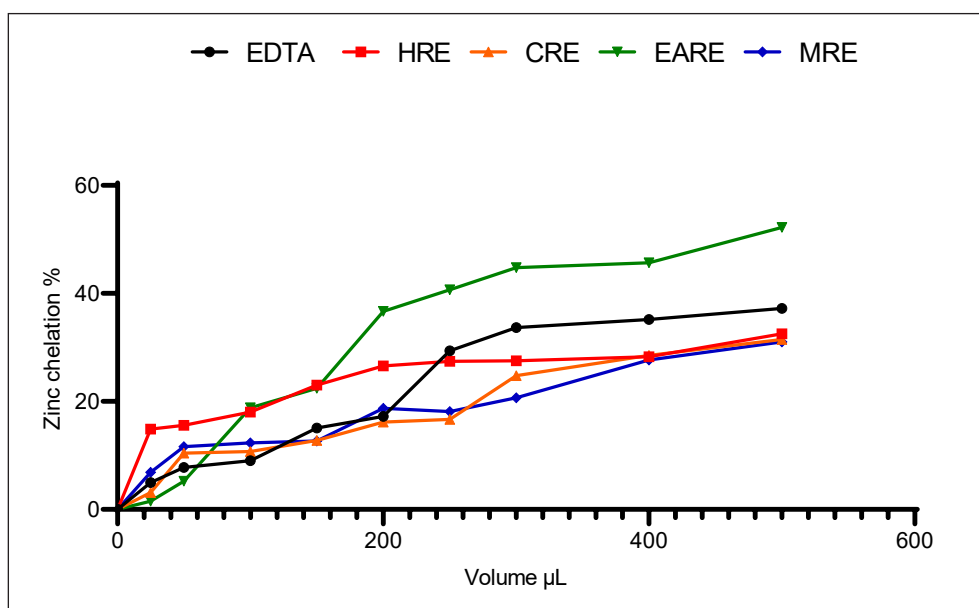


Fig. 6. Zinc chelation activity by increasing volumes (25 to 500 μ L) of hexane root extract (HRE), chloroform root extract (CRE), ethyl acetate root extract (EARE), methanol root extract (MRE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

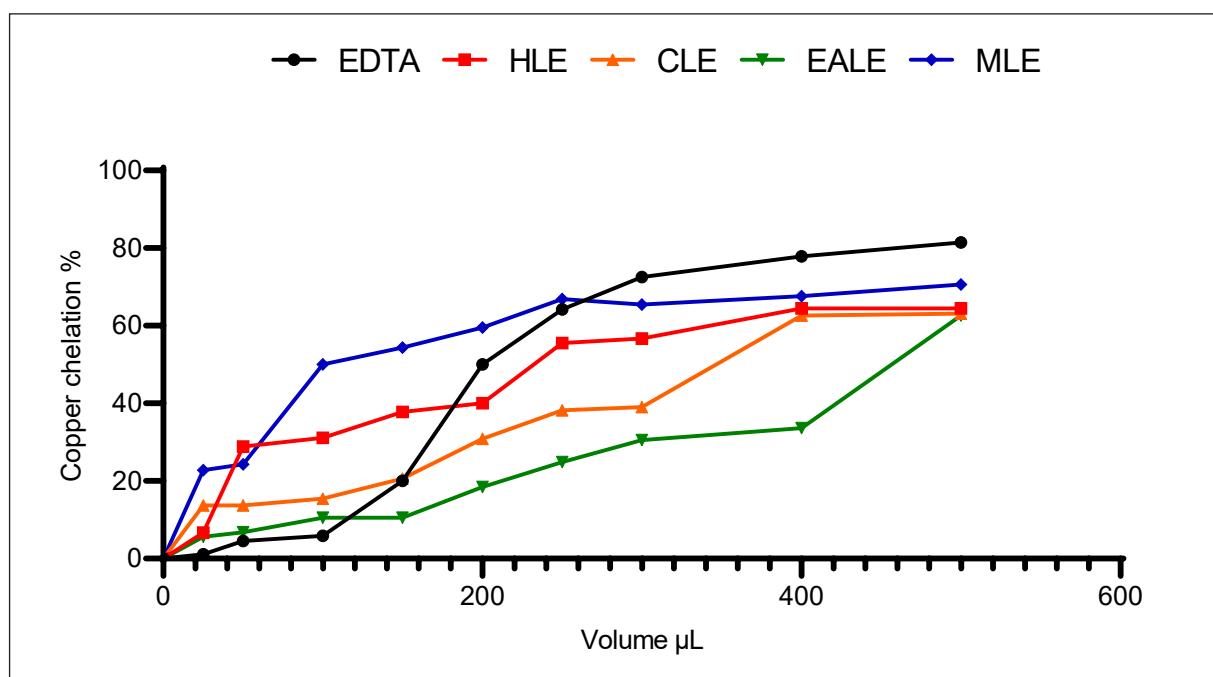


Fig. 7. Copper chelation activity by increasing volumes (25 to 500 μL) of hexane leaf extract (HLE), chloroform leaf extract (CLE), ethyl acetate leaf extract (EALE), methanol leaf extract (MLE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

7), CSE (81%; Fig. 8) and HRE (74%; Fig. 9), respectively.

To facilitate comparison of the metal chelating efficiency among the different

extracts, the maximum inhibition percentages obtained for each metal ion were summarized according to plant organ and extraction solvent. As shown in Table 1, distinct extracts exhibited preferential chelation toward specific metals,

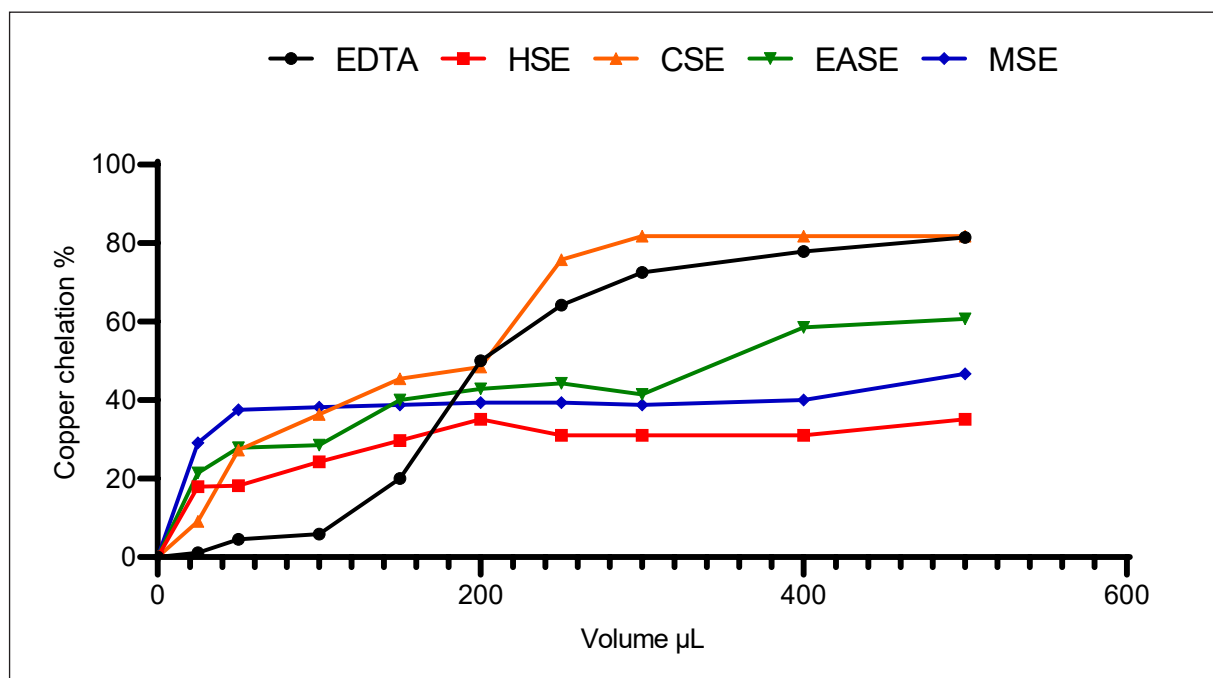


Fig. 8. Copper chelation activity by increasing volumes (25 to 500 μL) of hexane stem extract (HSE), chloroform stem extract (CSE), ethyl acetate stem extract (EASE), methanol stem extract (MSE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

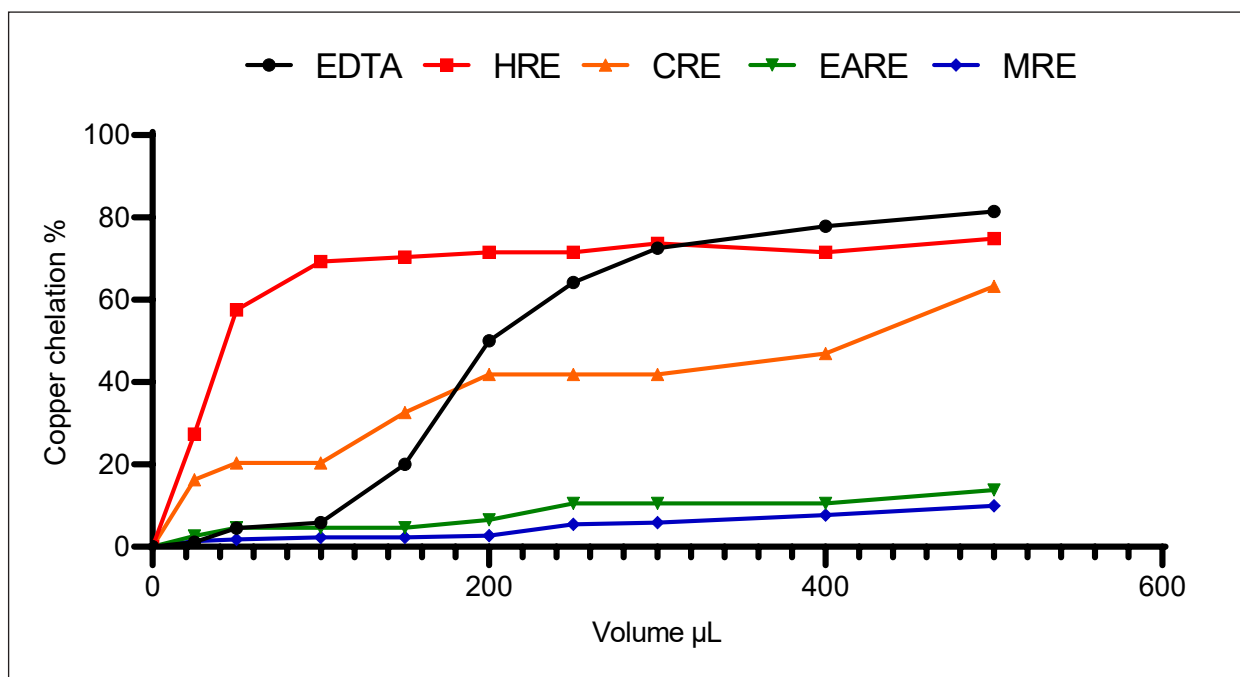


Fig. 9. Copper chelation activity by increasing volumes (25 to 500 μL) of hexane root extract (HRE), chloroform root extract (CRE), ethyl acetate root extract (EARE), methanol root extract (MRE) of *H. annuus* and EDTA (as a standard) at 562 nm. The results present mean $\pm$ SD ($n = 3$).

Table 1. Maximum metal chelation activity (% inhibition) of *Helianthus annuus* extracts according to organ and solvent

Metal ion	Extract (organ-solvent)	Maximum inhibition (%)
Cu^{2+} (Copper)	CSE	78
Zn^{2+} (Zinc)	CLE	55
Fe^{2+} (Iron)	HSE	91

highlighting the influence of both tissue type and solvent polarity on metal-binding capacity.

The copper-chelating activity of the extracts was quantified using IC_{50} values (Table 2). Among all extracts, the root hexane extract (HRE) exhibited the strongest chelating capacity ($\text{IC}_{50} = 0.087 \text{ mg ml}^{-1}$), surpassing even the reference chelator EDTA ($\text{IC}_{50} = 0.40 \text{ mg ml}^{-1}$). Methanolic leaf extract (MLE) also showed strong activity ($\text{IC}_{50} = 0.20 \text{ mg ml}^{-1}$). In contrast, several extracts did not reach 50% chelation within the tested concentration range, and their IC_{50} values could not be determined.

To facilitate comparison with reference chelators and among different plant organs and solvents, IC_{50} values (mg ml^{-1}) were calculated for copper, zinc, and iron chelation assays and are summarized in Tables 2-4. These values were derived from the dose-response curves

based on the inhibition percentages presented above. When 50% chelation was not achieved within the tested concentration range, the IC_{50} was reported as "not reached". The tables are provided solely for comparative purposes.

The IC_{50} values highlight marked differences in metal-chelating efficiency depending on plant organ, solvent polarity, and metal type. For copper chelation, several extracts showed strong activity, particularly the methanolic leaf

Table 2. IC_{50} values of copper-chelating activity of *Helianthus annuus* extracts

Organ	Extract	$\text{IC}_{50} \text{ mg/mL}$	Remark
–	EDTA	0.4	Positive control
Leaf	HLE	0.464	–
	CLE	0.69	–
	EAL	0.91	–
	MLE	0.2	Strong activity
Stem	HSE	–	IC_{50} not reached
	CSE	0.41	–
	EASE	0.7	–
	MSE	–	IC_{50} not reached
Root	HRE	0.087	Very strong activity
	CRE	0.84	–
	EARE	–	IC_{50} not reached
	MRE	–	IC_{50} not reached

Table 3. IC₅₀ values of zinc-chelating activity of *Helianthus annuus* extracts

Organ	Extract	IC ₅₀ mg/mL	Remark
Leaf	EDTA	-	IC ₅₀ not reached
	HLE	-	IC ₅₀ not reached
	CLE	0.567	-
	EALE	-	IC ₅₀ not reached
	MLE	-	IC ₅₀ not reached
Stem	HSE	-	IC ₅₀ not reached
	CSE	-	IC ₅₀ not reached
	EASE	-	IC ₅₀ not reached
	MSE	-	IC ₅₀ not reached
	HRE	-	IC ₅₀ not reached
Root	CRE	-	IC ₅₀ not reached
	EARE	0.93	IC ₅₀ not reached
	MRE	-	IC ₅₀ not reached

Table 4. IC₅₀ values of iron-chelating activity of *Helianthus annuus* extracts

Organ	Extract	IC ₅₀ mg/mL	Remark
Leaf	EDTA	0.29	Positive control
	HLE	0.55	-
	CLE	0.63	-
	EALE	-	IC ₅₀ not reached
	MLE	-	IC ₅₀ not reached
Stem	HSE	0.47	-
	CSE	-	IC ₅₀ not reached
	EASE	0.92	-
	MSE	-	IC ₅₀ not reached
	HRE	0.575	-
Root	CRE	0.63	IC ₅₀ not reached
	EARE	-	-
	MRE	0.85	-

extract (MLE) with IC₅₀ = 0.2mg/mL and hexane root extract (HRE) with IC₅₀ = 0.087 mg/mL, which exhibited IC₅₀ values comparable to or lower than EDTA, indicating a high affinity for Cu²⁺. In contrast, many stem extracts did not reach 50% inhibition, suggesting a lower abundance or accessibility of copper-binding compounds in this organ. Zinc chelation was generally weaker, with IC₅₀ values reached only for a limited number of extracts, and

EDTA itself not reaching IC₅₀ under the tested conditions, reflecting the higher stability of Zn²⁺ complexes. For iron chelation, moderate activity was observed mainly in leaf and root extracts, with some hexane and chloroform fractions approaching the activity of EDTA (IC₅₀=0.29). Overall, these results indicate a clear metal-specific and organ-dependent chelation behavior, likely linked to differences in the

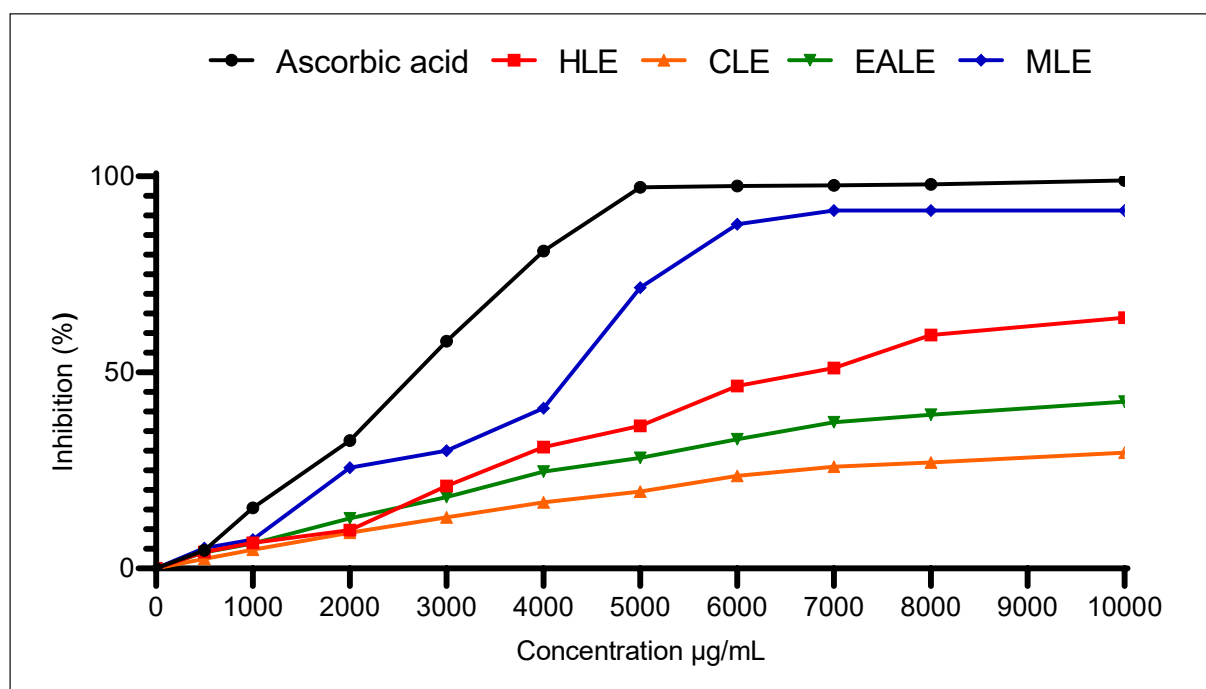


Fig. 10. DPPH leaf extract inhibition percentage DPPH radical scavenging activity by increasing concentration (0 to 10000 µg/mL) of HLE, CLE, EALE and MLE extracts and ascorbic acid as a standard at 517 nm. The results present mean ± SD (n = 3). The results were compared to the control by the Student's t-test (compared to the positive control).

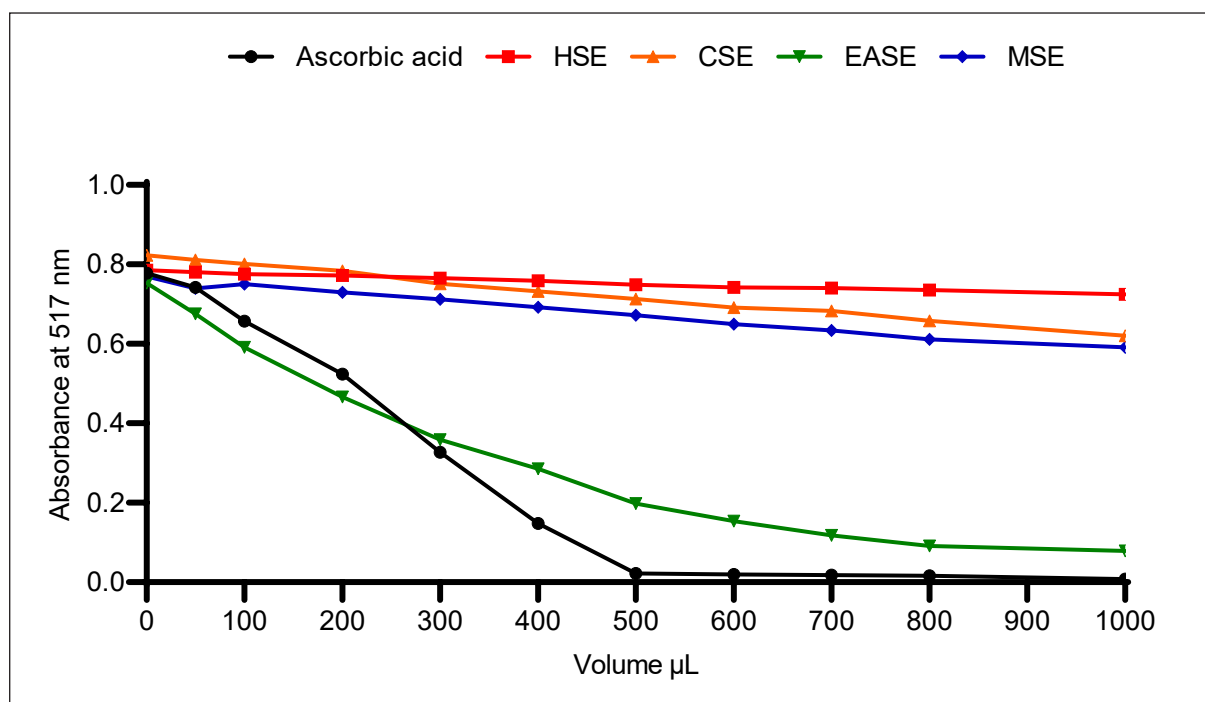


Fig. 11. DPPH radical scavenging activity by increasing volumes (25 to 1000 μL) of HSE, CSE, EASE and MSE extracts and ascorbic acid as a standard at 517 nm.

distribution and chemical nature of bioactive constituents.

DPPH assay: Figure 10 illustrates the percentage of inhibition obtained with *Helianthus annuus* leaf extracts. The differences between ascorbic acid (used as a standard) and the extracts (HLE, CLE, EALE, and MLE) were highly significant ($p \leq 0.001$). Methanol leaf extract (MLE) showed the highest inhibition rate (91%) at 500 μL , compared with 98% for ascorbic acid. HLE, EALE, and CLE exhibited inhibition rates of 63%, 42%, and 29%, respectively.

As shown in Fig. 11, the DPPH scavenging activity of stem extracts revealed that ethyl acetate stem extract (EASE) exhibited the highest inhibition (89%) with an absorbance of 0.079 ± 0.004 at 500 μL , compared to ascorbic acid (0.008 ± 0.0005 for the same volume). The differences between ascorbic acid and HSE, CSE and MSE were statistically significant ($p = 0.001$), whereas the difference with EASE was not significant ($p = 0.139$). The inhibitory capacity followed the order: EASE > MSE > CSE > HSE.

Figure 12 shows that the ethyl acetate root extract (EARE) exhibited the highest inhibition (70%), with an absorbance of 0.2 ± 0.01 at 500 μL , compared to ascorbic acid (0.008 ± 0.0005).

The differences between ascorbic acid and all root extracts were statistically significant ($p = 0.001$). The scavenging efficiency decreased in the following order: ascorbic acid > EARE > CRE > HRE > MRE.

In the DPPH radical-scavenging assay, ascorbic acid, used as a positive control, exhibited strong antioxidant activity ($\text{IC}_{50} = 0.13 \text{ mg/ml}$). All *Helianthus annuus* extracts showed lower scavenging efficiency, with IC_{50} values strongly influenced by both plant organ and extraction solvent.

Among leaf extracts (Fig. 13), the methanolic extract (MLE) displayed the highest antioxidant activity ($\text{IC}_{50} = 3.83 \text{ mg ml}^{-1}$), followed by the hexane (HLE, 6.72 mg ml^{-1}), ethyl acetate (EALE, 9.59 mg ml^{-1}), and chloroform extracts (CLE, 13.34 mg ml^{-1}). For stem extracts (Fig. 14), the ethyl acetate stem extract (EASE) was the most active ($\text{IC}_{50} = 3.11 \text{ mg ml}^{-1}$), whereas chloroform (CSE) and methanolic (MSE) extracts showed comparable but weaker activity ($\text{IC}_{50} \approx 20 \text{ mg ml}^{-1}$). The hexane stem extract (HSE) exhibited the lowest activity ($\text{IC}_{50} = 65.13 \text{ mg ml}^{-1}$). In root extracts (Fig. 15), the ethyl acetate extract (EARE) showed the strongest activity ($\text{IC}_{50} = 5.67 \text{ mg ml}^{-1}$), followed by chloroform (CRE) and methanolic extracts (MRE) with similar IC_{50}

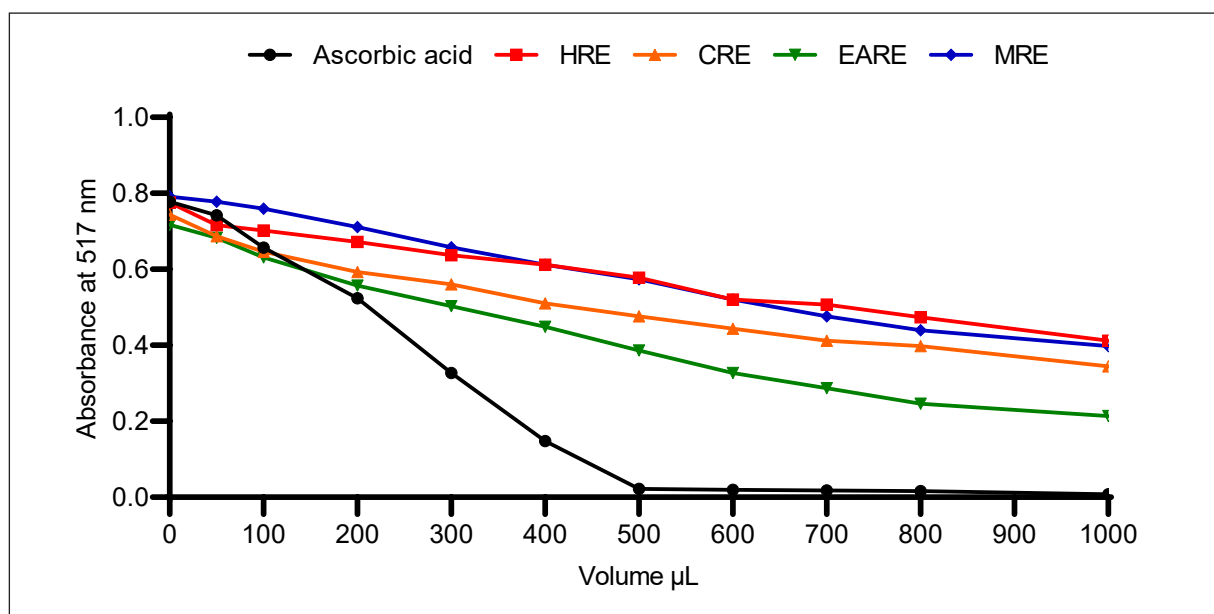


Fig. 12. DPPH radical scavenging activity by increasing volumes (25 to 1000 μL) of HRE, CRE, EARE and MRE extracts and ascorbic acid as a standard at 517 nm.

values ($\approx 8 \text{ mg ml}^{-1}$), while the hexane extract (HRE) was the least active ($\text{IC}_{50} = 10.4 \text{ mg ml}^{-1}$).

Statistical analysis (SPSS) revealed highly significant effects of plant organ, extraction solvent, and their interaction (organ $\times$ solvent) on IC_{50} values ($p < 0.001$). Post hoc tests confirmed significant differences between ascorbic acid and all plant extracts. Homogeneous subset analysis grouped ascorbic acid separately, with roots and leaves forming a second subset and stems a third, indicating significantly lower antioxidant activity. Regarding solvents, all

differences were significant ($p < 0.005$), with antioxidant effectiveness decreasing in the order: ethyl acetate $>$ methanol $>$ chloroform $>$ hexane.

The antioxidant capacity of selected *H. annuus* extracts was further expressed as both percentage inhibition and IC_{50} values (Table 5), providing a quantitative comparison of their relative DPPH radical-scavenging efficiency.

The results obtained in this study revealed that the iron chelation activity of *Helianthus annuus* stem extracts is both solvent-dependent

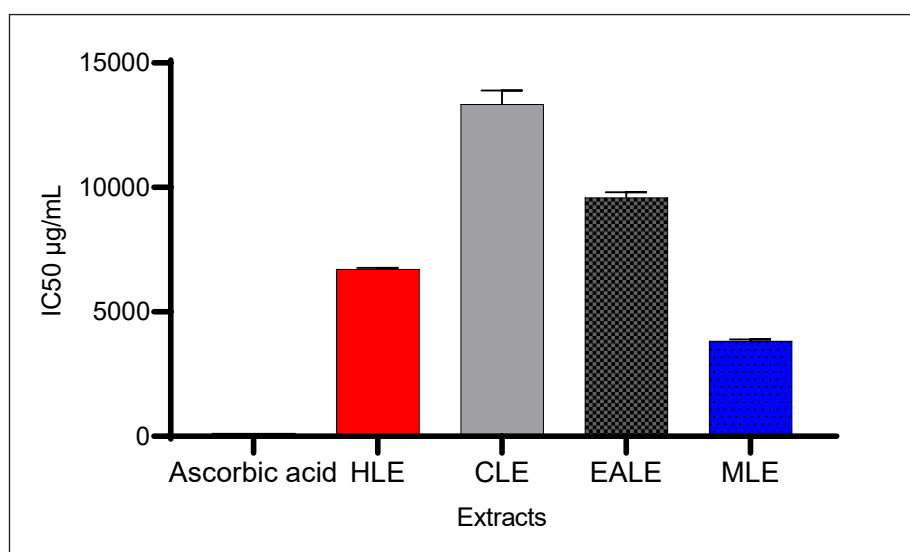


Fig. 13. IC_{50} values ($\mu\text{g/mL}$) of ascorbic acid and leaf extracts (HLE, EALE, CLE, and MLE) determined by the DPPH assay. The results present mean $\pm$ SD ($n = 3$).

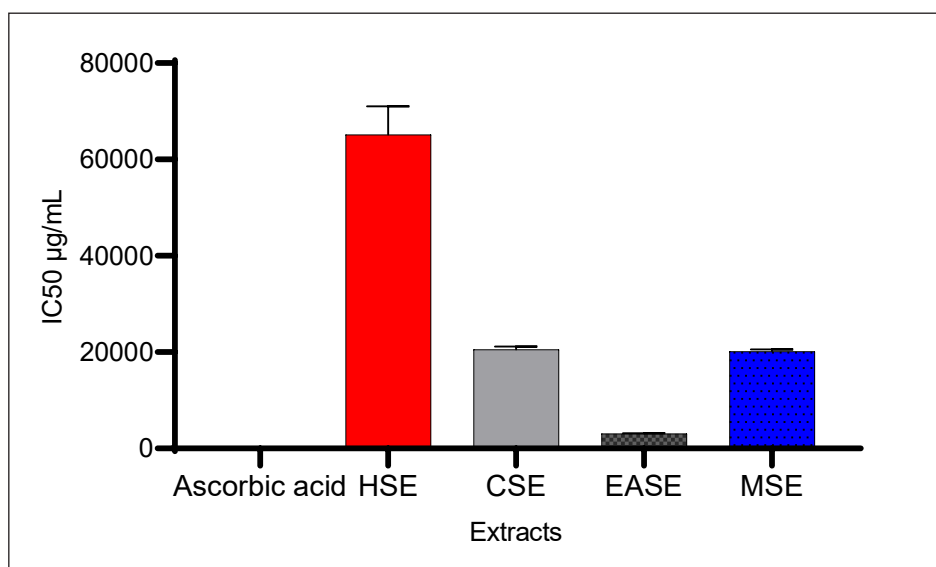


Fig. 14. IC₅₀ values (µg mL⁻¹) of ascorbic acid and stem extracts (HSE, EASE, CSE, and MSE) determined by the DPPH assay. The results present mean ± SD (n = 3).

and concentration-dependent. Among the tested fractions, the hexane extract exhibited the highest chelating activity, particularly at higher volumes, whereas the methanolic extract showed the lowest capacity to bind Fe²⁺ ions. These observations suggest that the bioactive compounds responsible for metal chelation are mainly lipophilic molecules that are efficiently extracted with non-polar solvents.

This finding is consistent with previous reports demonstrating that *Helianthus annuus* contains a wide spectrum of secondary

Table 5. DPPH radical scavenging activity and IC₅₀ values of selected *Helianthus annuus* extracts

Extract	Organ	DPPH inhibition (%)	IC ₅₀ mg mL ⁻¹
MLE	Leaf	91	3.83
EASE	Stem	89	3.11
EARE	Root	70	5.67

metabolites, including phenolic acids, flavonoids, coumarins, tocopherols, and sesquiterpene lactones (Al-Snafi, 2018; Gai *et al.*, 2020). Among these, tocopherols (vitamin E) and certain sesquiterpenes possess strong metal-binding capacities due to their phenolic

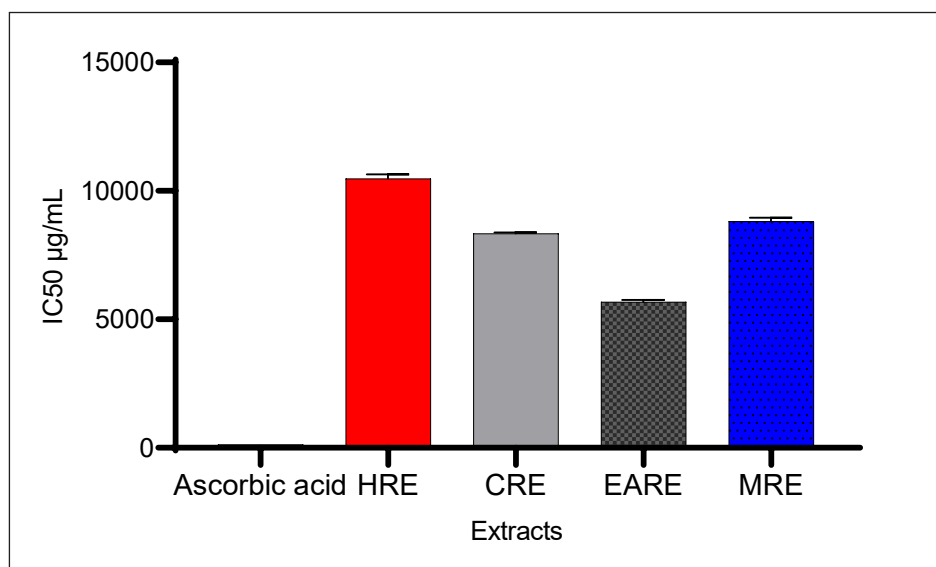


Fig. 15. IC₅₀ values (µg mL⁻¹) of ascorbic acid and root extracts (HRE, EARE, CRE, and MRE) determined by the DPPH assay. The results present mean ± SD (n = 3).

hydroxyl groups, which can form stable complexes with transition metals (Al-Snafi, 2018). Their lipophilic nature could explain the superior performance of the hexane extract observed in the present work, as non-polar solvents such as hexane are particularly effective in extracting fat-soluble bioactive compounds including tocopherols (vitamin E), sterols, terpenoids and sesquiterpene lactones (Sellal and Belattar, 2023; Sellal and Belattar, 2024). These molecules possess hydrophobic structures with functional groups (such as hydroxyl or carbonyl moieties) capable of forming coordination bonds with transition metal ions like Fe^{2+} . The non-polar environment provided by hexane not only favors the solubilization of these compounds but also preserves their native conformations and redox properties, which are often diminished in polar media. Consequently, the enrichment of the hexane fraction with these lipophilic chelators enhances its overall capacity to sequester metal ions, supporting the hypothesis that the iron-binding activity of *Helianthus annuus* stems is primarily attributed to such non-polar metabolites (Al-Snafi, 2018; Pokorný and Schmidt, 2001; Gai *et al.*, 2020).

In contrast, polar extracts such as those obtained with methanol or ethyl acetate are rich in phenolic acids (chlorogenic, caffeic, ferulic, and p-coumaric acids) and flavonoids (quercetin, luteolin, apigenin, and kaempferol) (Gai *et al.*, 2020; Heim *et al.*, 2002). Although these compounds are recognized for their antioxidant and radical-scavenging properties, their chelating efficiency toward Fe^{2+} may vary depending on structural factors such as the number and position of hydroxyl and carboxyl groups (Sroka and Cisowski, 2003). The relatively low chelation observed for the methanolic extract suggests that, under the experimental conditions, these hydrophilic phenolics exhibit limited affinity for iron ions, possibly due to competitive solvation or steric hindrance.

Moreover, the dose-dependent trend observed in the hexane extract indicates that the chelating compounds act in a concentration-proportional manner, with enhanced activity at higher volumes. This behavior has been similarly described for other lipophilic plant extracts rich in tocopherols or terpenoids, which increase in metal-binding potential with concentration (Rice-Evans *et al.*, 1996).

Overall, these findings highlight the complementary roles of lipophilic and hydrophilic antioxidants in *Helianthus annuus*. While phenolic and flavonoid compounds contribute mainly to radical scavenging, the lipophilic constituents, particularly tocopherols and sesquiterpenes, appear to dominate the iron-chelating activity of sunflower stem extracts. This duality underlines the complex phytochemical balance governing the antioxidant and metal-binding potential of sunflower-derived fractions.

Copper ions are known to form stable complexes with ligands containing nitrogen and oxygen donor atoms, such as hydroxyl, carbonyl, and amine groups (Sarkar *et al.*, 2015). The activity observed in the methanolic and chloroformic fractions could thus be attributed to the presence of phenolic and flavonoid compounds, which are recognized for their ability to bind Cu^{2+} through catechol or carbonyl-hydroxyl systems (Sroka and Cisowski, 2003). The performance of the hexane extract may be attributed to the presence of lipophilic chelators, such as tocopherols or terpenoids, whose hydrophobic matrices stabilize metal-ligand interactions in non-polar environments (Al-Snafi, 2018; Pokorný and Schmidt, 2001).

Overall, the results indicate that *Helianthus annuus* possesses a diverse array of metal-binding metabolites capable of complexing transition metals like Fe^{2+} , Cu^{2+} , and Zn^{2+} . The zinc-chelating activity seems to favor semi-polar extracts (chloroform and ethyl acetate), whereas copper and iron chelation activities are enhanced in non-polar fractions, particularly the hexane extract, underscoring the complementary roles of the plant's phytochemical classes. Such versatility reflects the coexistence of both hydrophilic and lipophilic antioxidants, providing *Helianthus annuus* with a broad-spectrum metal-detoxifying potential relevant for biomedical and environmental applications (Gai *et al.*, 2020).

The higher DPPH radical scavenging activity observed in this study reflects the presence of various antioxidant compounds distributed across the leaf, stem, and root extracts of *Helianthus annuus*. In the DPPH assay, antioxidants were able to reduce the stable purple DPPH radical to the yellow-colored diphenylpicrylhydrazine, a reaction

based on hydrogen donation leading to the formation of the non-radical DPPH-H form (Huang *et al.*, 2011).

Among the tested extracts, the methanolic leaf extract (MLE) exhibited the highest inhibition rate (91%) at the maximum tested volume, compared to 98% for the standard antioxidant, ascorbic acid. The hexane, ethyl acetate, and chloroform leaf extracts (HLE, EALE, and CLE) displayed inhibition percentages of 63%, 42%, and 29%, respectively, all showing statistically significant differences compared to the control ($p \leq 0.001$). This result highlights the superior efficiency of the methanolic extract, likely due to its richness in polar antioxidant compounds such as phenolics and flavonoids, which are known for their high hydrogen-donating ability and radical-scavenging capacity.

The antioxidant activity of *Helianthus annuus* leaves observed in the present study is in agreement with previous reports indicating that leaves represent the most potent antioxidant organ of the plant. Muhtadi (2021) reported that a single ethanolic extract of sunflower leaves exhibited the highest DPPH scavenging activity among the tested plant parts, with an IC_{50} value of 48.841 $\mu\text{g/mL}$. In contrast, the present work employed a sequential Soxhlet extraction of the same leaf material using solvents of increasing polarity (hexane, chloroform, ethyl acetate and methanol), resulting in distinct antioxidant capacities across fractions. Among these, the methanolic leaf extract showed the strongest activity ($IC_{50} = 3.83 \text{ mg mL}^{-1}$), followed by hexane, ethyl acetate, and chloroform extracts. The higher IC_{50} values observed here are likely attributable to differences in extraction strategy, solvent polarity, and fractionation of antioxidant compounds, rather than inconsistencies in antioxidant potential. Sequential extraction allows the redistribution of phenolic and flavonoid compounds among fractions, whereas single-solvent ethanol extraction may concentrate these compounds in a single extract. Similarly, Gai *et al.*, (2020) confirmed that *Helianthus annuus* contains diverse classes of bioactive molecules-including flavonoids, alkaloids, tannins, saponins, steroids, and carbohydrates-which contribute to its antioxidant potential.

Therefore, the strong radical scavenging performance observed in the methanolic

fraction can be attributed to the high solubility of phenolic and flavonoid compounds in polar solvents. These compounds act as potent hydrogen or electron donors, capable of neutralizing free radicals by converting them into more stable, non-reactive species. The results thus confirm that the antioxidant potential of *Helianthus annuus* is largely dependent on the solvent polarity and the presence of phenolic-rich fractions, particularly in leaf tissues.

The DPPH assay showed that ascorbic acid had the strongest antioxidant activity, while all plant extracts exhibited higher IC_{50} values, reflecting lower radical scavenging efficiency typical of crude extracts. Ethyl acetate and methanol extracts consistently displayed greater antioxidant activity than chloroform and hexane extracts, highlighting the key role of solvent polarity in extracting phenolic and flavonoid compounds. In addition, leaves and roots showed significantly stronger antioxidant activity than stems, likely due to their higher accumulation of antioxidant metabolites involved in plant defense. These findings are consistent with previous reports on the influence of plant organ and extraction solvent on DPPH radical scavenging capacity.

Mechanistically, the metal-chelating and radical-scavenging activities can be attributed to specific structural motifs present in plant-derived phenolic compounds. Phenolic hydroxyl groups, particularly in catechol-type structures, are capable of coordinating Fe^{2+} and Cu^{2+} ions through bidentate chelation, thereby reducing metal-catalyzed redox reactions and oxidative stress. This metal-binding capacity is closely linked to antioxidant behavior, as phenolic moieties can also donate hydrogen atoms or electrons to neutralize free radicals such as DPPH. Consequently, a strong relationship between metal chelation and radical scavenging can be established, as previously described for polyphenolic compounds (Perron and Brumaghim, 2009)

The metal-chelating and antioxidant properties of *Helianthus annuus* extracts suggest their potential as eco-friendly, plant-based chelators for practical applications. Such extracts or purified fractions could be used to remove residual metals from food systems, treat contaminated wastewater or soils, and

act as co-formulants in agriculture to mitigate metal toxicity and enhance plant health. Similar strategies using plant-derived chelators have been reported; for example, polyphenol-rich extracts can bind heavy metals in aqueous systems (Sharma and Dubey, 2005; Ali *et al.*, 2013), and combined antioxidant-chelator formulations have been proposed to prevent metal-induced oxidative damage in food and environmental matrices (Khan *et al.*, 2020). These findings support the dual functionality of sunflower extracts as natural antioxidants and metal chelators, offering sustainable alternatives to synthetic agents such as EDTA (Yin *et al.*, 2024).

Plant-derived chelators and antioxidant-chelating systems have been increasingly proposed as sustainable alternatives to synthetic agents such as EDTA for the removal of residual metals from food systems, wastewater, and contaminated soils (Yin *et al.*, 2024; Khare *et al.*, 2024). This dual chelating and antioxidant behavior is mainly attributed to phenolic hydroxyl, catechol, and carbonyl functional groups, which enable both transition metal coordination and free radical scavenging. In this context, the strong metal-chelating and antioxidant activities observed in the present study suggest that *Helianthus annuus* extracts or purified fractions could be effectively exploited as green chelators for environmental remediation and agro-food applications, as well as co-formulants in agriculture to mitigate metal-induced stress and improve micronutrient management.

Conclusions

This study provides a comprehensive evaluation of the chelating and antioxidant potential of *Helianthus annuus* extracts across leaves, stems, and roots using solvents of varying polarity (hexane, chloroform, ethyl acetate, and methanol). Unlike previous studies that largely focused on sunflower seeds or individual plant organs and primarily assessed antioxidant activity, our work systematically examined multi-metal chelation (Fe^{2+} , Zn^{2+} , Cu^{2+}) across all major organs and solvent fractions. The results revealed organ- and solvent-specific differences in metal-binding capacity, with hexane extracts showing the strongest iron chelation, chloroform extracts excelling for zinc, and root extracts, particularly with

non-polar and mid-polar solvents, exhibiting the highest overall chelating activity. These findings suggest the presence of lipophilic and mid-polar metabolites-such as tocopherols, terpenoids, and sesquiterpene lactones-capable of forming stable complexes with divalent ions. The study highlights the dual functionality of *H. annuus* extracts, where polar fractions provide potent antioxidant activity and non-polar fractions deliver efficient multi-metal chelation, underscoring their novel potential as natural, eco-friendly chelators for environmental, food, and biomedical applications.

Conflict of Interest Statement

The authors declare that the research was conducted without any commercial or financial relationships that could potentially create a conflict of interest.

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