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Investigation of Secondary Metabolite Content and Antioxidant and Inhibitory Activities of Extracts from Parts of *Salsola richteri* L. Plant

Biki Abdelmalek[ab],Ghemam Amara Djilani[ac], Laiche Ammar Touhami[cd], Mohammed Messaoudi[ac] , Abdelbasset Kaddour[e],Bentahar Assia[f], Alia zeid[ga], Ahmed Elkhalifa Chemsa[cd],Nour Elhouda Mekhadmi[cd],Nezar cherrada[c], Yassin bouras[ab], Samir aydi[h]

[a] Laboratory of Biology, Environment and Health, El Oued University, El Oued, Algeria.

[b] Department of Cellular and Molecular Biology, Faculty of Natural Science and Life, El Oued University, El Oued, Algeria.

[C] Department of Biology, SNV Faculty, University of Chahid Hamma Lakhdar, El-Oued, Algeria.

[d] Laboratory of Biodiversity and Applications of Biotechnology in the Agriculture Field, University of ChahidHamma. Lakhdar, El-Oued, Algeria.

[e] Higher School of Agriculture Saharian, El-Oued, Algeria.

[F] Laboratory of Phytotherapy Applied to Chronic Diseases SNV Faculty, University of Setif 1, Setif, Algeria.

[G] Department of Agronomic Faculty of Natural Science and Life, El Oued University, El Oued, Algeria.

[H]bLaboratory of biodiversity and valorisation of bioresources in arid zones (LR18ES36). Faculty of Sciences at the University of Gabes, Zrig 6072 Gabes, Tunisia.

***Corresponding Author**: Ghemam Amara Djilani, Laboratory of Biology, Environment and Health, El Oued University, El Oued, Algeria Email: djilani-ghemamamara@univ-eloued.dz ; ghemamamaradjilani@gmail.com.

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Abstract

The *Salsola richteri* plant is traditionally used for the treatment of many diseases. The objective of this study was to investigate the content of active compounds and antioxidants in three different plant parts (leaves, flowers, and roots) of *Salsola richteri*. Total polyphenol content (TPC) was assessed usingthe Folin-Ciocalteu reagent, flavonoid content (TFC) was evaluated using quercetin as a reference compound, and tannin content (TTC) was measured using tannic acid. To evaluate antioxidant properties, the assays used included 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS++ radical cation scavenging activity, iron-chelating activity, and Hydrogen Peroxide Radical Scavenging (activitywere activity).

Among the extracts studied, the root extract had the highest tannin content (25.75 ± 2.08 mg AT/g DR) and the highest extraction yield (34%). The leaf extract showed the highest total polyphenol and flavonoid contents (173.98 ± 1.63 mg GAE/g DM, 135.93 ± 1.44 mg QE/g DM, respectively). The root extract also exhibited the strongest antioxidant activity, as measured by DPPH, ABTS, and FRAP assays, with lower IC_{50} and EC_{50} values indicating higher efficacy. The results indicated that all extracts demonstrated anti- hemolytic activity, with the leaf extract showing the highest inhibition rate, estimated at 93.14 ± 0.39%. Therefore, *Salsola richteri* extracts contain various bioactive compounds, validating their use in traditionaltreatments.

Keywords: *Salsola richteri*; Antioxidants; Anti-hemolysis; Secondary metabolites.

Introduction:

The aggregation of wild botanicals and herbs represents a pivotal reservoir for forage sustenance and serves as an innate antioxidant, antiparasitic, and antimicrobialagent in both classical and contemporary pharmacology ((D'Ambola et al. 2019; Mennai et al. 2021). Presently, the quest for antioxidants and their pharmaceutical derivatives is focused on scrutinizing essential oils and raw plant extracts ((ElNaggar et al. 2022; Tasdemir et al. 2020).The taxonomic entity Salsola, previously classified under Chenopodiaceae, belongs to the Amaranthaceae family and is one of the largest and most prevalent genera globally, encompassing approximately 150 species((Borger et al. 2008). These taxa predominantly inhabit fixed and semi-fixed sandy terrains worldwide, characterized by saline conditions, notably in regions such as North Africa, Central Asia, and Europe. Given their therapeutic and fodder significance, these flora are deemed crucial desert taxa, with Salsola species playing a pivotal role in the reclamation of saline pastures. Enduring adversities such as drought and salinity, these species constitute approximately 45% of desert ecosystems. Moreover, they manifest facile seed dispersal mechanisms and are recognized for their commendable fodder (ElNaggar et al. 2022).

Scholarly evidence suggests that a substantial proportion of Salsola species are palatable, with *Salsola soda* being particularly prominent, known as agretti, in Italy (Centofanti and Bañuelos 2015). Species within the genus Salsola, characterized by elevated protein content, digestibility of dry matter, and metabolizable energy, along with low plant fiber, confer manifold advantages. Notable among these is *Salsola tragus*, esteemed as livestock forage during arid spells, owing to its abundance in bioactive compounds such as steroids, phenols, flavonoids, saponins, and nitrogenous constituents (Murshid et al. 2022). These botanicals are also employed in the management of hypertension, neurological disorders, and headaches, and possess immunomodulatory, antioxidant, diuretic, lipotropic, and antidiabetic properties (Colás et al. 2006; ElNaggar et al. 2022). Furthermore, they are useful for treating rheumatic ailments, autoimmune conditions, dermatological afflictions, contraception, and dental maladies. Active compounds isolated from the *Salsola tubercultiformis* shrub exhibit anti-inflammatory properties akin to the adverse effects of dexamethasone, in addition to displaying efficacy against microbial pathogens and cytotoxicity (Ahmad et al. 2008). These botanicals are also harnessed in the production of soda, which is utilized in soap and glass formulations (ElNaggar et al. 2022).

In Algeria, the genus Salsola comprises 17 species that are extensively distributed across arid expanses and predominantly inhabit saline terrains. These plants exhibit xerophytic attributes, typified by pilosity, abbreviated cylindrical foliage, leafless stems, and parenchymatous storage structures (Centofanti and Bañuelos 2019; Hameed et al. 2010; Toderich 2008). It is

Salsola richteri L is renowned for its efficacy in combating desertification and stabilizing sand dunes owing to its vigorous growth, prolific seed production, and resilience to aridity and salinity (Shomurodov et al. 2013) . It serves as a primary repository of alkaloids, which are beneficial for the management of hypertension. Characterized by its low toxicity, this species thrives in restricted sandy locales in Algeria. Research endeavors aim to unravel its biological potential by assessing its secondary metabolite repertoire and the efficacy of its phytochemical and pharmacological extracts.

Materials and methods

- plants samples

Samples of *Salsola richteri* plant parts were collected from the desert regions of El Oued City in Algeria. The plant samples were identified by Professor Youssef Helis from the Physical and Chemical Analysis Unit in Touggourt, which is affiliated with the Scientific and Technical Research Center for Arid Regions in Biskra, Algeria. The samples were then stored in the herbarium of the Department of Biology and Environment Health (Faculty of Natural and Life Sciences, University of El Oued, Algeria) under reference number LBEH-03/2020. The plant samples were thoroughly washed with distilled water to remove surface dust contamination. Subsequently, they were air-dried outdoors, in the shade, away from sunlight and moisture, for a period of 4 weeks until a constant weight was attained. Subsequently, the samples were ground into a fine powder and stored in sterilized glass vials under conditions protected from light and moisture.

Preparation and yeild of Extracts

The plants were extracted using distilled water, yielding ten grams of a fine powder. The solvent was then removed using a rotary vacuum evaporator at 45 °C. The resulting dry extracts were placed in glass tubes and stored at -20 °C for further analysis. The yield was calculated using the following equation:

$$Y\ (\%) = (m/M) \times 100$$

Where *m:* dry residue mass (gdr), *and M:* plant material mass (g).

Total phenolic content (TPC)

The total phenolic content of *Salsola richteri* L. extracts was estimated spectrophotometrically according to the Folin-Ciocalteu method, with minor modifications (Aydi et al. 2023). 75 µl of each plant extract (1 mg/ml), 275 µl of distilled water, and 150 µl of Folin-Ciocalteu reagent (0.1

N) were mixed. The mixture was incubated for 3 min in a laboratory hood and 250 µL of sodium carbonate (7.5 percent) was added. Subsequently, it was incubated again for half an hour in the laboratory in the dark, and the absorbance of the product was estimated at a wavelength of 760 nm using a spectrophotometer. A calibration curve was prepared using gallic acid (0–250 mg/L) as the positive control. The results are expressed as mg Gallic Acid equivalents (GAE)/g dry matter.

Total flavonoid content (TFC)

Total flavonoid content was determined according to the method described by Bahorun et al., (1996). This method involved the use of an aluminum chloride solution by mixing 1 ml of the extract with 1 ml of 2% AlCl3. The absorbance at 430 nm was read after 20 min. Quercetin solution was used as the standard. The results are expressed as quercetin equivalents per gram of extract (mg QE/g).

Total Tannin content (TTC)

Tannin content was determined by the Folin-Ciocalteu-based method. A homogeneous mixture of 0.1 ml of sample extract with 7.5 ml of distilled water, 0.5 ml of Folin Ciocalteu phenol reagent, and 1 ml of 35% sodium carbonate solution was prepared in a flask-to-flask solution. Then dilute the mixture to 10 ml. The mixture was shaken well and incubated at laboratory temperature for half an hour, while a series of similarly treated reference concentrations of tannic acid were prepared. The absorbance of all solutions is read at a wavelength of 700 nm with a UV-Vis spectrophotometer. Tannins are expressed as mg tannic acid equivalent/g WD

DPPH Antioxidant Assay

Antioxidant activity The antioxidant activity of aqueous extracts of Salsola richteri L. was tested using the DPPH method as described by (AMARA 2020), with some modifications. A mixture of 180 µl of the extract (1 mg/ml) was prepared with 1500 µl of DPPH solution (0.2 mM) to obtain a concentration of 50 µg/ml in the well. It was left under laboratory conditions in the dark for 30 min, and then the absorbance was determined at a wavelength of 570 nm in a spectrophotometer, using ascorbic acid as a reference. The percentage inhibition (DPPH) was calculated using the following equation: Inhibition ratio = 100 × (blank– sample)/ blank.

The effective concentration required to inhibit 50% of the free radicals (IC50) was calculated from the inhibition percentage curve (I) with the concentrations of the extracts, and the lowest IC50 value represented the highest chelating effect of the tested extract.

Iron Chelation Assay

Ferrozine is the most commonly used chelating agent for assessing the chelating capacity of extracts. Ferrozine forms a complex with the free iron present in the medium, forming Ferrozine-Fe^{2+} with an intense purple color. The clearer the color of the solution containing the tested extract, the stronger is the chelating power of the extract (Khadhri et al., 2013).

Procedure: The chelating activity of iron by the plant extract was measured by inhibiting the formation of the complex - Fe^2+-ferrozine according to the method described by Le et al. (2007). To 250 µL of various concentrations of the plant extract, 50 µL of 24 FeCl^3 and 450 µL of methanol were added after mixing the mixture and leaving it for 10 min at room temperature. Then, 50 µL of ferrozine was added, and after 10 min of reaction, the absorbance was read at a wavelength of 562 nm compared to the control, which contained all reactants except the plant extract. The results obtained were compared with the EDTA control prepared using the same method for preparing the extract samples.

The ABTS Assay:

The ABTS assay was developed as a simple method to measure total antioxidant capacity, which measures its ability to neutralize ABTS radicals (Re et al., 1999). This involves the use of the stable cationic radical 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), which exhibits a stable blue-green color when mixed with any substance that can oxidize it. Upon reduction, the solution returns to its original colorless form (Munteanu, 2003; Erel et al., 2021).

Procedure: The effect of displacement on the ABTS radical was estimated according to the method described by Re et al. (1999). First, ABTS radicals were prepared by mixing the ABTS solution (7 mM) and potassium peroxodisulfate solution (2.45 mM). The prepared solution was then incubated in the dark at room temperature for 16 h. This solution was then diluted with methanol to obtain an absorbance of 0.7 at a wavelength of 734 nm. Next, 1 ml of the methanolic ABTS solution was added to 50 µL of various concentrations of the plant extract. BHT was used as the standard for comparison. The mixture was then incubated at room temperature in the dark for 30 min. Absorbance was measured and compared to the control, which contained all reaction components except the plant extract. The inhibition percentage (I) was calculated usingthe same method as that for the

Hydrogen Peroxide Radical Scavenging Activity

Among the other tests used to assess the potential of scavenging free radicals is the H2O2 assay. The principle of this experiment revolves around studying the ability to scavenge H2O2, which

is not considered a free radical, but causes DNA damage, disrupts the plasma membrane, and leads to the release of calcium ions inside the cell, resulting in the activation of calcium-dependent enzymes (Saikat et al., 2010).

Procedure: The ability of the extract to scavenge hydrogen peroxide radicals was measured according to the fold-dilution method (Mukhopadhyay et al., 2016). A total of 380 µL of the plant extract at varying concentrations was collected, and 62.5 microliters of ammonium solution was added. After thorough mixing, 17.5 microliters of a hydrogen peroxide solution was added. After thorough mixing, the solutions were incubated for 5 min. After the incubation period, 380 Ml ferroinwas added and the mixture was incubated for an additional 10 min. The absorbance was measured at a wavelength of (230 nm). The obtained results were compared with those of the control, vitamin C, prepared using the same method as described above.

The percentage of hydrogen peroxide radical scavenging was calculated as follows:

1% [(AC-AE)/AC] ×100

Statistical Analysis

The content of secondary metabolites and the capacity of various antioxidants were estimated in triplicate, with results reported as the average of three measurements ± standard deviation (SD). The Excel program was used to conduct ANOVA tests to assess statistical differences, with a P-value equal to or less than 0.05 indicating significance.

Results and discussion

yield of Extracts

Table 1 illustrates the crude extract content of *Salsola richteria*, where the roots showed the highest extraction yield (34%), whereas the leaves had the lowest extraction yield at 13%. This is because of the positioning of the roots, which makes them more efficient in extracting organic compounds, as they are the organs that store and produce the most biologically active compounds, facilitating their extraction from the plant. This indicates that plant organs play a role in the quantity and quality of secondary metabolites and their extraction methods. Other studies have found differences between the young and mature leaves of plants(Anwar et al. 2017). Another report stated that the extraction yield is influenced by several factors, including the age of the plant, the type of plant organ, and agricultural practices(García-Mier et al. 2013).

Table 1: yield of Extracts at different plant parts

	S-racines	S-feuilles	S-fleures
yield of Extracts%	34	**13**	22

TPC, TFC and TTC in plant parts

Table 2 illustrates the content of polyphenols, flavonoids, and tannins in various parts of the *Salsola richteri* plant, with polyphenols represented using the standard curve of ascorbic acid (AGE: mg/g). Flavonoids were represented using quercetin (mg/g), whereas tannins were measured with tannic acid (mg/g) based on a linear curve. The results indicated that extracts from the *Salsola richteri* contain high levels of polyphenols, ranging from 92.44 to 173.98 mg/g. Flavonoid values range from 13.5 to 20 mg/g in the methanolic extract, as reported by (Abbas et al. 2021). The results revealed that the highest phenolic content was found in leaf extracts, followed by flower and root extracts, indicating that the aerial parts of the plant are more exposed to environmental stresses, such as heat, humidity, sunlight, wind, and attacks by microorganisms. This suggests that the leaves are the center of secondary metabolite formation before these compounds migrate to the roots.

Table 2: polyphenols, flavonoids, and tannins content at different plant parts

	TPC	TFC	TC
S-racines	$92.43^{c} \pm 0.55$	**$30.81^{c} \pm 0.75$**	$25.75^{a} \pm 2.08$
S-feuilles	$173.79^{a} \pm 1.63$	**$135.93^{a} \pm 1.44$**	$14.67^{c} \pm 0.46$
S-fleures	$136.02^{b} \pm 2.41$	**$90.62^{b} \pm 0.54$**	$19.19^{b} \pm 0.87$
LSD	3.33	1.92	2.59

The results also showed that *Salsola richteri* extracts contain a significant amount of tannins, ranging from 14.69 to 25.75 mg/g compared to the findings by Maryana et al.(2019) in the leaves of *Hibiscus cannabinus*. with the highest level in root extracts (25.75 mg/g), followed by flower and leaf extracts. This variation in content was explained by (Maryana, Rahman, and Liew 2019) and (Igual et al. 2011), who noted that it is due to the specific function of each plant organ, with roots being the site of tannin accumulation and storage, whereas the aerial parts serve as the center for the production and migration of compounds to other plant parts (Boulaaba et al. 2019).

This aligns with other findings that indicate that the plant contains compounds with carboxyl functional groups, responsible for antioxidant and biological activity, while also acting as insecticides and helping to reduce some human health issues. Plants contribute to nutrient

absorption by aiding in mineralization and breaking down soil minerals into absorbable forms (Chen et al. 2020; Um, Han, and Lee 2018).

Salsola richteri is rich in secondary compounds compared to *Salsola Kali*. (Boulaaba et al. 2019) Boulaba 2019 reported that its content was the highest among five regions in Tunisia in terms of polyphenols, flavonoids, and tannins, with levels of 17.23, 15.282, and 2.03 mg/g, respectively. Nizar 2023, on the other hand, recorded levels of 46.55, 13.17, and 11.23% for *Salsola Tetragonia,* consistent with (Boulaaba et al. 2019) and (Lisiewska, Kmiecik, and Korus 2006) for *Salsola Kali*. Polyphenols play a crucial role in protecting photosynthetic organs by reducing reactive oxygen species generated during photosynthesis. (Naczk and Shahidi 2004) showed that phenolic compounds accumulate in plants living under harsh conditions, whereas (Sokolowska-Krzaczek, Skalicka-Wozniak, and Czubkowska 2009) confirmed that Salsola species grow easily in dry soil and can withstand pH fluctuations and harsh climates. Furthermore, (Dixon and Paiva 1995) noted that the presence of secondary metabolites in roots helps in the absorption of iron, nitrates, and phosphorus.

Antioxidant activity:

Radical scavenging activity DPPH

In this study, the antioxidant activity of Salsola plant extracts (roots, leaves, and flowers) was evaluated using the DPPH assay. The antioxidant activity of the leaf, flower, and root extracts from *Salsola richteri* was expressed in terms of IC50 values (mg/ml), along with the standard compound BHT(Cherrada et al. 2023; Oluwole et al. 2022). The inhibition results in Figure 1 show that the extracts exhibited higher antioxidant activity than BHT. The results also indicate that there were no statistically significant differences between the samples. The antioxidant activity was estimated to (30., 336, 368)ug/ ml for roots, leaves, and flowers, respectively, while the activity for BHT was estimated to be 26 ug/ml . This is consistent with previous studies on plants of the Salsola genus (Beyaoui et al. 2012; Elwekeel et al. 2023; Golovchenko et al. 2022; Oueslati, Bouajila, and Jannet 2017). Despite the variation in secondary metabolite content, root extracts showed a higher inhibition rate, which contradicts what (Boulaaba et al. 2019) for *Salsola Kali* plant extracts. This variability is linked to the total phenolic and flavonoid content, both qualitatively and quantitatively. This is due to the presence of antioxidant molecules such as ascorbic acid, quercetin, and chlorogenic acid.

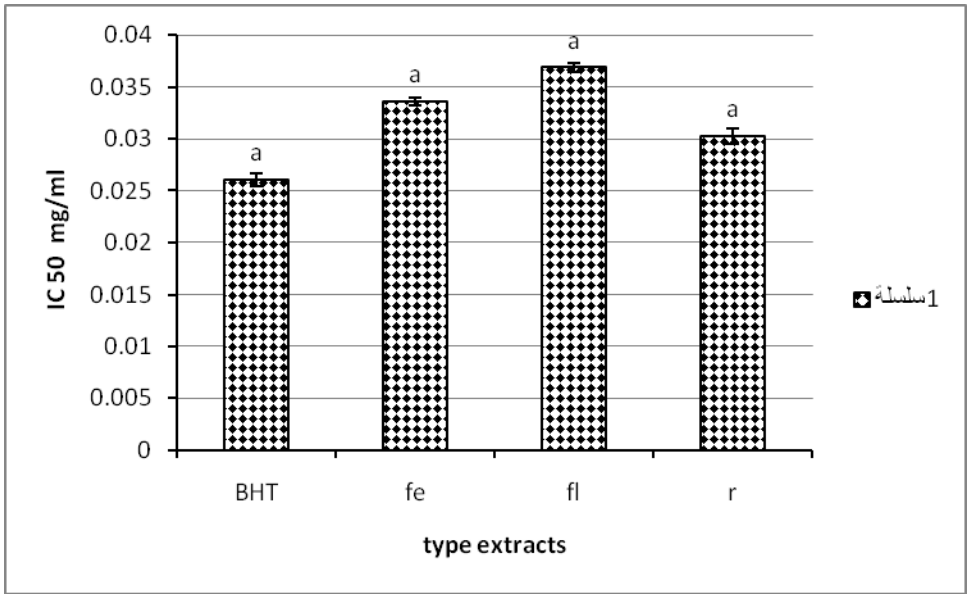

Figure 1: **Dpph of three parts at plant *Salsola richteria***

(Akopian et al. 2020) noted that *Salsola richteri* contains alkaloids salsolin and salsolodin, which represent 50% of the plant's total active content, and these compounds contribute significantly to antioxidant activity. Moreover, alkaloid content decreased in the flowers and increased in the roots. The results showed that this plant's extracts have a very high scavenging capacity, exceeding what (Cherrada et al. 2023) found in *Salsola Tetragona* (478.3 ± 0.66), (Mohammed et al. 2021) found in the methanolic extract of *Salsola Cyclophylla* (reaching 1350 ± 0.16), and (Amin et al. 2022) found in the methanolic extract of *Salsola Villasa*(1050 µg/ml). (El-Bassossy, Abdelgawad, and Elazab 2023) reported that the methanolic extracts of *Salsola Kali* had IC_{50} values of 8064 ± 4.53.

The overall higher antioxidant capacity of the root extracts, with greater reducing power and more substantial radical-scavenging activity compared to leaves and flowers, can be attributed to the presence of potent antioxidant compounds, such as phenolic and flavonoid substances (Liazid et al. 2007; Magalhães et al. 2009) . The number of phenolic hydroxyl groups and their positions strongly influence the antioxidant activity (Rodríguez-Bonilla et al. 2017) , as these compounds can donate a hydrogen atom to the DPPH radical, forming a non-radical molecule (Ak and Gülçin 2008).

The ABTS Assay

The results infigure2 show that extracts from *Salsola richteri a*exhibit high ABTS free radical scavenging activity, with varying capabilities. Radical proton scavenging is an important antioxidant characteristic. This activity indicates that the use of this plant might be beneficial in

treating the pathological damage associated with free radicals (Roy et al. 2011; Valko et al. 2007). The root extract demonstrated a significant antioxidant effect at a value of 6.25 mg/ml, which is considerably better than that of the reference compound BHT, whose activity was estimated at 15.2 mg/ml. This result is similar to that (Daniels et al. 2011) for the *Gethyllis villosa* plant, whereas it was found that the leaf extract was more effective in the *Gethyllis multifolia* plant. This is confirmed by the results obtained by (Lachowicz-Wiśniewska et al. 2022) that the root extract is highly effective against ABTS roots compared to the leaf and flower extracts from the *Stachys Palastris* plant. In contrast, (Rao et al. 2022) found that the activity of the flowers was higher than that of the root extract but lower than that of BHT.

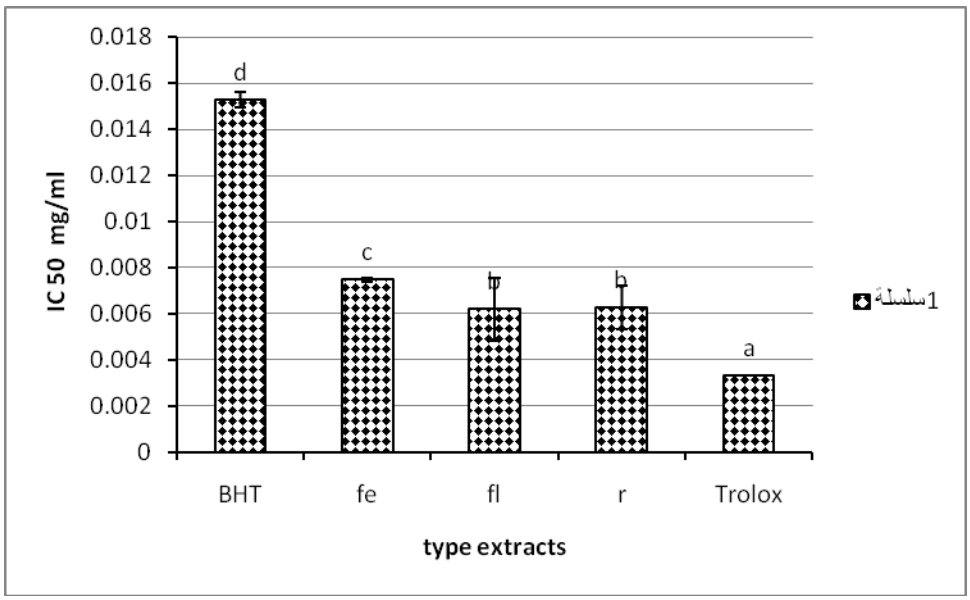

Figure 2: **ABTS of three parts at plant *Salsola richteria***

Iron Chelation Assay

The results outlined in figure 3 show that the ferric reducing power of the plant root extracts was higher than that found in the leaf and flower extracts of the *Salsola richteri* series, but lower than that of the reference compound BHT. These findings align with the results from (Boulaaba et al. 2019) on the *Salsola Kali* plant and (Maisuthisakul, Suttajit, and Pongsawatmanit 2007) , which revealed significant variation in radical scavenging responses in seeds and fruits of the *Nephellium Lappaceum* plant. (Falleh et al. 2013), in halophytic plants, found that antioxidant activities varied based on the plant organs, with root extracts showing the highest ferric reducing power. On the other hand, (Lachowicz-Wiśniewska et al. 2022) reported that flower extracts had significantly higher iron-reducing power than root, stem, leaf, and fruit extracts. (Rao et al. 2022) found similar results with two genetic varieties (Gianni and Cascade) of *Humulus lupulus*. Furthermore, the researcher (Chiancone et al. 2023) demonstrated that root extract was more

effective than other extracts in iron-recovering activity, while (Boulaaba et al. 2019) noted that leaf and root extracts were more efficient in chelating iron ions, with an estimated EC50 of approximately 5.85 - 6.15 mg/ml.

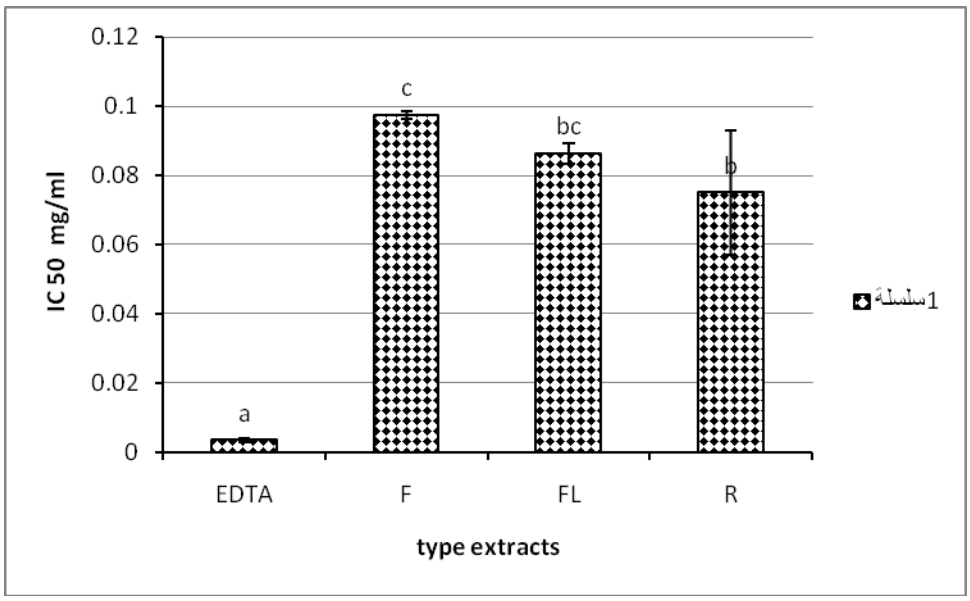

Figure 3: **Iron Chelation Activity of three parts at plant *Salsola richteria***

Hydrogen Peroxide Radical Scavenging Activity

The effectiveness of the extracts against red blood cell hemolysis is shown in Figure 4, where all extracts showed satisfactory results in protecting red blood cells from hemolysis at low concentrations. The leaf extract exhibited a hemolysis inhibition rate of 93.14%, with maximum anti-hemolytic activity at a concentration of 0.39 mg/ml. In contrast, the root and flower extracts had hemolysis inhibition rates of 60.79% ± 0.37 and 57.76% ± 0.27, respectively, at concentrations of 9.04 mg/ml and 1.36 mg/ml. This is reflected in the IC50 values, where a lower IC50 value indicates higher activity. The IC50 values for the plant organ extracts showed significant differences: the leaf extract had an IC50 of 0.083 mg/ml ± 0.004, indicating that it was the most efficient among the extracts, with the exception of vitamin C, which had an IC50 of 0.017 mg/ml. The root extract had an IC50 of 1.66 mg/ml, whereas the flower extract had an IC50 of 0.509 mg/ml. These values suggested that the leaf extract was the most potent in inhibiting red blood cell hemolysis.

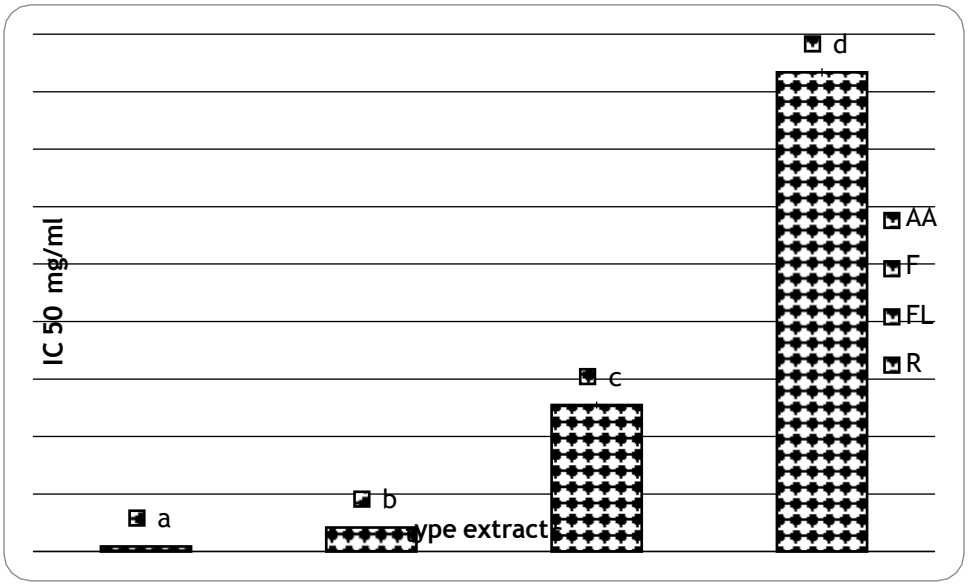

Figure 4:**Hydrogen Peroxide Radical Scavenging Activity of three parts at plant *Salsola richteria***

The observed variation in the protective effect against red blood cell hemolysis can be attributed to the high content of secondary metabolites, particularly phenolic compounds. The difference in antioxidant content can be influenced by the type of plant organ, the choice of solvents, even within the same organ, and the harvesting stage. Notably, red blood cell hemolysis can occur for various reasons, such as oxidative damage to membrane lipids, hemoglobin abnormalities, oxidizing drugs, increased levels of certain minerals, or radiation exposure (Afsar et al. 2016; Ebrahimzadeh, Nabavi, and Nabavi 2009). According to a previous study (Naim et al. 1976), hemolysis is exacerbated by the presence of toxic substances, such as oxidized water. As noted by (Chomchan et al. 2018), concentrations above 90 mg/ml are considered nontoxic (Elizondo-Luévano et al. 2023).

In the results obtained, all extracts demonstrated low hemolytic activity, with IC50 values above 100 mg/ml, indicating that these extracts have very low toxicity toward red blood cells.

Conclusion

The study demonstrated that *Salsola richteria*is a plant abundant in secondary metabolites with antioxidant propertiesin all its parts, despite their differences. Furthermore, it exhibited significant efficacy against free radicals such as DPPH and ABTS, in addition to iron radicals and hydrogen peroxide. These findings suggest its potential as a valuable resource for pharmaceutical purposes and as an efficient alternative medicinal ingredient.

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