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ANTIOXIDANT ACTIVITIES OF SOME LAMIACEAE HERBS FROM IRAQI AND TURKISH ORIGIN: A COMPARATIVE

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ABSTRACT

This work aims to compare and evaluate the in-vitro antioxidant properties of Lamiaceae herbs, rosemary salvia (*Rosmarinus officinalis L.*), sage (*Salvia officinalis L.*) and marjoram (*Origanum majorana L.*) from Iraq and Turkey. Oxidative stress is implicated in many chronic and degenerative disorders processes and consequently, antioxidants have received considerable scientific attention in recent years as potential contributors to the inhibition of free radical propagation and the limitation of oxidative damage. Plant materials, especially medicinal and aromatic herbs are often sources of various phytochemicals with antioxidant properties such as phenolics acids, flavonoid compounds, terpenoids, and other bioactive compounds. That can act as metal-chelating, free radical-scavenging or reducing agents. **Methodology:** Aerial parts were dried and extracted with ethanol. For analysis of total phenolic content (TPC), total flavonoids content (TFC), DPPH radical-scavenging ability, reducing power and Fe- chelating activity. **Results:** Turkish *S. rosmarinus* presented higher values for TPC (2.36 0.06 mg GAE/g dry extract) with up to 100.0 3.8% inhibition of DPPH at 500 µg/mL. Turkish *O. majorana* had the highest TFC (0.0720 ± 0.0028mg QE/g dry extract) and the best reducing power amongst extracts 1.99 ± 0.10. The absorbance at 700 nm at 500 µg/mL). The strongest Fe chelating activity was exhibited by S. Officinalis from Iraq at 300 µg/mL (97.0 3.2%) followed by *O. majorana* from Iraq which presented higher values at 500 µg/mL (98.3 9.2%). Our data strongly indicate that there are differential and complementary antioxidants-related chemicals responsible for the tested activities in the selected Lamiaceae species from Iraq and Turkey depending on plant species, origin, concentration and assay system. These findings provide a valuable basis for advanced biochemical, phytochemical, and pharmaceutical investigations of these herbs.

KEYWORDS: Lamiaceae plant; antioxidant; *Rosmarinus officinalis*; *Salvia officinalis*; *Origanum majorana.*

INTRODUCTION

Oxidative stress is caused by the over-production of reactive oxygen species, and other radical species, which is greater than the rate at which can be neutralize by the endogenous defense system. Prolonged oxidative imbalance has been related to lipid peroxidation, protein and nucleic-acid damage and membrane alteration and seems to play role in number of chronic and degenerative disease.[1,2] The antioxidants have been found to reduce oxidative damage by mechanisms including: the scavenging of radical, donation of electron or hydrogen atoms, interference of the chain reactions and chelation of transition metals which participates in initiation radical.[1-3] Phenolic acids, flavonoids, tannins, terpenoids and a range of other specialized secondary plant compounds are available from medicinal plants and aromatic herbs for antioxidant and metal-chelating functions in vitro. Even as the total amount of phenolic compounds or total flavonoid content are widely used for an overall comparative ranking of the samples for their antioxidant potential, a high value of either does not ensure better activity in all performed tests.[3-5] In general, plant family Lamiaceae (also known as the mint or dead-nettle family) is a recognized source of herbs with ethnopharmacological potential, many of which also show interesting antioxidant activities due to their phytochemistry, like rosemary, sage or marjoram have been shown to provide a variety of phenolic and terpenoid compounds.[6-8] *Salvia rosmarinus* Spenn is a popular aromatic herb that contains significant quantities of rosmarinic acid, carnosic acid and carnosol.[6,7] These are known as compounds of contribution toward antioxidant activity. The latter is a typical Lamiaceae with literature that suggests its phenolic character and biological activity in extracts. The antioxidant character of the herb has similarly been stated by *Origanum majorana* L., reported to have phenolic acids, flavonoids and volatile compounds, the amount and nature of which depends on the plant source and extract.[8, 9] Rosemary, sage and marjoram are popular medicinal and nutraceutical herbs,[10,11] however limited comparative information comparison were available on Iraqi and Turkish materials under the same condition of extraction and analyses. In present work the ethanolic extracts of *Salvia rosmarinus*, *Salvia officinalis*, and *Origanum majorana* sold by well-established herbal marketers in Iraq and Turkey were studied and compared their profile for their total phenolic content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability (DPPH) and reducing power activity (RPA) as well as their Fe chelating ability (FCA).

MATERIALS AND METHODS

Plant materials and extraction

The herbs were purchased from licensed sources in Iraq and Turkey. Extraction of dried plant samples was completed using ethanol as a solvent. Following extraction, filtration was performed using Whatman filter paper, and the filtrate was collected to remove the solvent. The resulting solids were weighed, dissolved in ethanol at the desired concentrations, and a stock solution was prepared.

Determination of total phenolic contents

The total phenolic content of each extract was determined using the Folin- Ciocalteu method; gallic acid was used as the calibration standard.[12] Total phenolic content was expressed as mg of gallic acid equivalent per gram of dry extract (mg GAE/g dry extract).

Determination of total flavonoid content

The total flavonoid content was estimated using the aluminum chloride colorimetric method employing quercetin as the calibration standard.[13] The results were reported as milligrams of quercetin equivalents per gram of dry extract (mg QE/g dry extract).

DPPH radical-scavenging activity

The DPPH radical activity of plants and standard substances was determined according to the method used by Brandt-Williams et al. A DPPH solution was prepared in ethanol. Various concentrations of plant extracts (in the range of 100, 200, 300, 400, and 500 µg/mL) were added to this solution and left to stand for 30 minutes at room temperature. Then, absorbance values were measured at a wavelength of 517 nm using a spectrophotometer. Trolox was used as standards. The experiments were repeated three times. The decreasing absorbance value indicates the free radical scavenging activity, which is a measure of the DPPH solution. The capacity of the DPPH free radical was calculated using the following equation.[14,15]

DPPH scavenging activity (% inhibition) = [(A0-A1) / A0] x 100

A0 = Control absorbance value

A1 = Absorption value of sample or standard

Reducing-power assay

Power reflects the electron donating capacity of antioxidant compounds. İt is determined by the reduction of potassium ferricyanide to potassium ferrocyanide. The reduced product then reacts with ferric chloride to form a colored complex and the absorbance of which is measured at 700 nanometers. İn this study reducing power was determined according to Oyaizu's method. The plant extracts at concentrations of (100, 200,300,400, and 500 µg/ mL). Were mixed with 0.2 M phosphate buffer pH 6.6 and 1% potassium ferricyanide solution. The prepared mixtures were incubated in a water bath at 50°C for 20 minutes. Thereafter 10% trichloroacetic acid was added and the mixtures were centrifuged in 3000 revolutions per minute for 10 minutes. The supernatant was then mixed with distilled water and 0.1% ferric chloride solution. L -ascorbic acid was used as the positive reference antioxidants and absorbance was measured at 700 nanometers using a spectrophotometer a higher absorbance value indicated greater reducing power and each experiment was performed in triplicate.[16]

Ferrous-ion-chelating activity

The Fe-chelating activity was evaluated with ferrozine method[17]. The extracts at concentrations of 100, 200, 300, 400 and 500 µg/mL and EDTA as a reference chelator were incubated at 37 °C for 1 hour in iron II solutions and then ferrozine was added. After 10 min of reaction at room temperature, absorbance at 562 nm was measured and percentagechelation was calculated by ($[A_{control}$ $A_{sample})/A_{control}]$ 100.

Statistical analysis

All experiments were conducted triplicates under same conditions. Data were represented asmean±standard deviation (SD).

RESULTS

1. Total phenolic content (TPC) and total flavonoid content (TFC)

In Table 1 and Fig. 1 there is the TPC and TFC of extracts obtained from Iraqi and Turkish plants. In our work, the sample Turkish *Salvia rosmarinus* recorded the higher level in TPC, 2.36 ± 0.06 mg GAE/g dry extract, while Iraqi *S. officinalis* has a TPC of 2.22±0.12 mg GAE/g dry extract and the sample with the lowest TPC in our experiments was Iraqi *S. rosmarinus* (1.07 ± 0.04 mg GAE/g dry extract). The highest amount of TFC was recorded in Turkish *Origanum majorana* (0.0720±0.0028 mg QE/g dry extract), while Iraqi *S. rosmarinus* resulted in having the lowest values (0.0202±0.0009 mg QE/g dry extract).

Table 1: ***Total phenolic and flavonoid contents of the selected Lamiaceae extracts.***

Species	Origin	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)
Salvia rosmarinus	Iraq	1.07 ± 0.04	0.0202 ± 0.0009
Salvia rosmarinus	Türkiye	2.36 ± 0.06	0.0320 ± 0.0019
Salvia officinalis	Iraq	2.22 ± 0.12	0.0348 ± 0.0017
Salvia officinalis	Türkiye	1.98 ± 0.08	0.0313 ± 0.0013
Origanum majorana	Iraq	1.86 ± 0.04	0.0353 ± 0.0011
Origanum majorana	Türkiye	1.99 ± 0.07	0.0720 ± 0.0028

Values are expressed as mean ± SD. GAE: gallic acid equivalents; QE: quercetin equivalents.

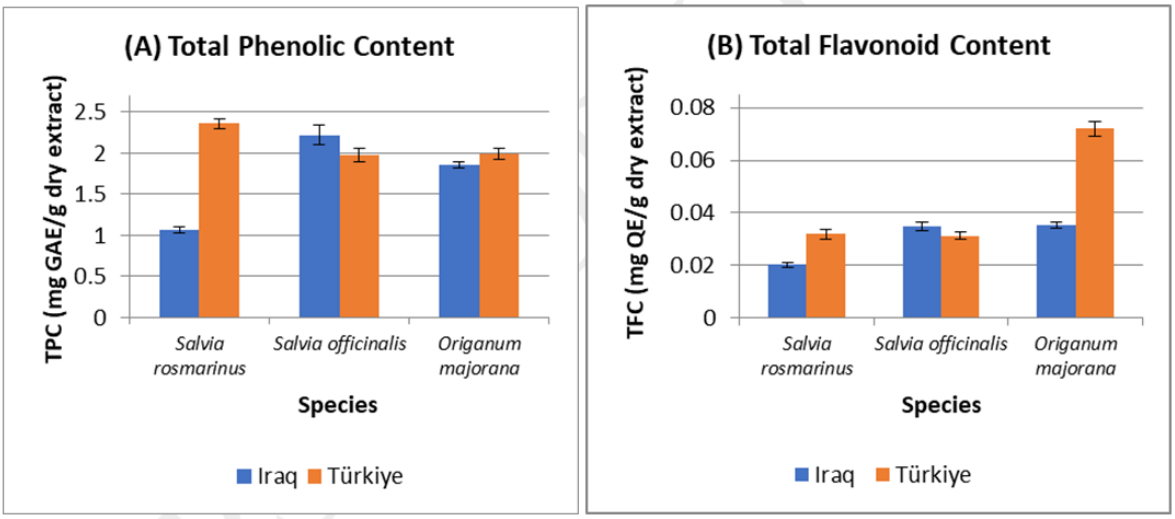


Figure 1: Total phenolic content (A) and total flavonoid content (B) of Iraqi and Turkish *Salvia rosmarinus*, *Salvia officinalis*, and *Origanum majorana*. Values are mean ± SD.

2. DPPH radical-scavenging activity

DPPH radical-scavenging activity showed concentration-dependent increases for all extracts (Table 2 and Fig. 2). In the plant extracts tested, Turkish *S. officinalis* gave best result for the higher activity (91.9 2.1% inhibition) when incubated at concentration 100 µg/mL. Turkish *S. rosmarinus* gave inhibition of 73.3 1.4% inhibition at 100 µg/mL whereas increased to 100.0 3.8% inhibition at concentration 500 µg/mL and for Iraqi *O. majorana* it gave 100.0 2.1% inhibition at 500 µg/mL concentration. Trolox showed 92.5 1.8 and 99.9 0.3% inhibition at 100 µg/mL and 500 µg/mL concentration respectively.

Table 2: ***DPPH radical-scavenging activity of the selected Lamiaceae extracts.***

Species	Origin	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Salvia rosmarinus	Iraq	19.0 ± 0.4	53.2 ± 0.9	87.4 ± 0.4	89.75 ± 1.4	92.1 ± 0.8
Salvia rosmarinus	Türkiye	73.3 ± 1.4	85.65 ± 0.7	98.0 ± 1.4	99.0 ± 1.0	100.0 ± 3.8
Salvia officinalis	Iraq	38.4 ± 0.9	63.2 ± 1.9	88.0 ± 3.1	90.0 ± 0.6	92.0 ± 2.9
Salvia officinalis	Türkiye	91.9 ± 2.1	94.3 ± 1.2	96.7 ± 1.6	97.5 ± 2.9	98.3 ± 2.0
Origanum majorana	Iraq	43.1 ± 1.3	65.3 ± 2.9	87.5 ± 1.2	93.75 ± 1.9	100.0 ± 2.1
Origanum majorana	Türkiye	71.2 ± 1.2	81.85 ± 1.6	92.5 ± 0.7	94.9 ± 2.4	97.3 ± 4.7
Trolox	Reference	92.5 ± 1.8	96.2 ± 1.4	98.7 ± 0.9	99.5 ± 0.6	99.9 ± 0.3

Values are expressed as percentage inhibition (mean ± SD). Trolox was used as the reference antioxidant.

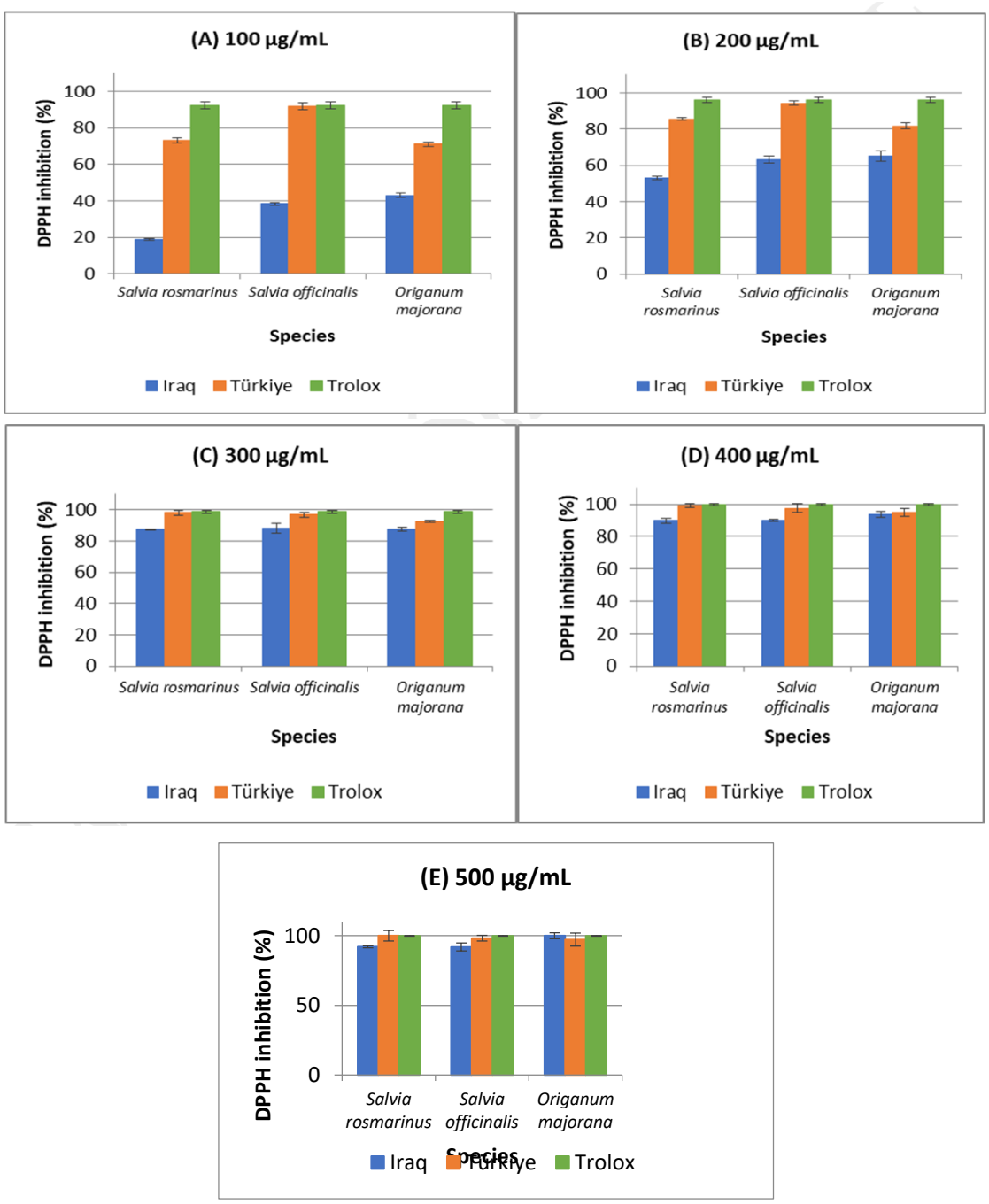


Figure 2: DPPH radical-scavenging activity of Iraqi and Turkish *Salvia rosmarinus, Salvia officinalis, and Origanum majorana*. Trolox was used as the reference antioxidant. Values are mean ± SD.

3. Reducing-power

The reduction power of all extracts was directly proportional to the concentration (Table 3 and Fig. 3). Turkish *O.majorana* made a better response for all concentrations with reducing-powers being highest of plant extracts, ranging from 0.66±0.02(100 µg/mL) to1.99±0.10(500 µg/mL).

For plant extracts of Turkish *S. rosmarinus* and Turkish *S. officinalis* the absorbance values at 500 µg/mL was found to be 1.77±0.05 and 1.76±0.12, respectively. On comparing it with Iraq extracts the values were less at all concentrations studied. L-ascorbic acid yielded the highest values.

Table 3: Evaluation of the Reducing Power of the Selected Lamiaceae Extracts.

Species	Origin	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Salvia rosmarinus	Iraq	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.09 ± 0.00
Salvia rosmarinus	Türkiye	0.80 ± 0.02	1.16 ± 0.02	1.53 ± 0.01	1.65 ± 0.03	1.77 ± 0.05
Salvia officinalis	Iraq	0.03 ± 0.00	0.06 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.00
Salvia officinalis	Türkiye	0.58 ± 0.01	0.92 ± 0.02	1.26 ± 0.04	1.51 ± 0.08	1.76 ± 0.12
Origanum majorana	Iraq	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
Origanum majorana	Türkiye	0.66 ± 0.02	1.16 ± 0.03	1.66 ± 0.04	1.82 ± 0.07	1.99 ± 0.10
L-ascorbic acid	Reference	0.88 ± 0.02	1.48 ± 0.02	2.05 ± 0.02	2.62 ± 0.03	3.05 ± 0.04

Values are given as absorbance at 700 nm (mean± SD). The reference antioxidant was L-ascorbic acid.

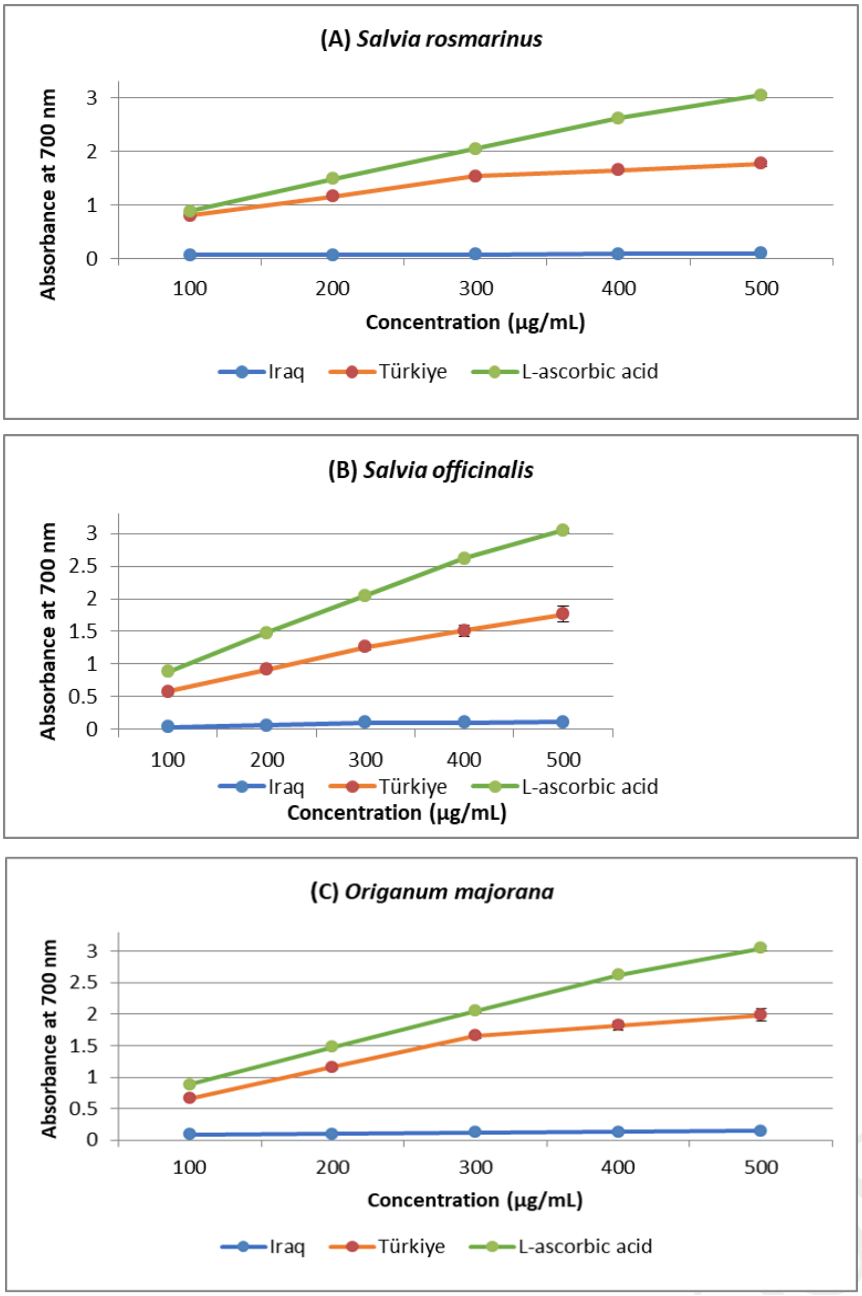


Figure 3: Concentration-dependent reducing power capability of Iraqi and Turkish extracts of *Salvia rosmarinus* (A), *Salvia officinalis* (B) *and Origanum majorana* (C) compared with L-ascorbic acid as the reference sample.

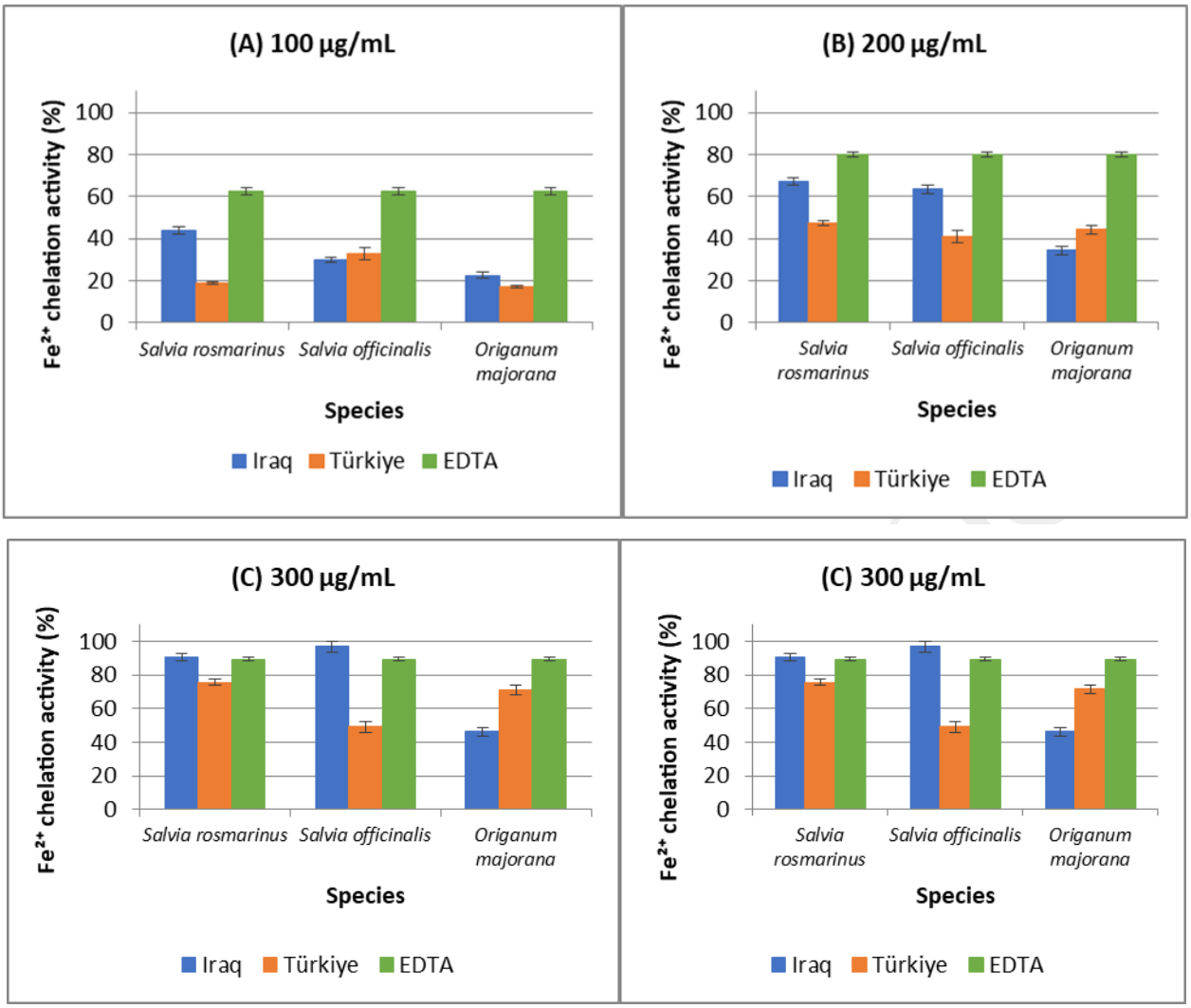
4. Ferrous ion chelation

The Fe(II)-chelating activity of all extracts increased with the concentration (Table 4 and Fig. 4). Iraqi *S. officinalis* attained a chelation of 97.0 3.2% at 300 µg/mL and achieved 97.4 4.1% and 97.8 5.1% chelating activity at 400 and 500 µg/mL, respectively. Among the extracts of these spices, Iraqi *O. majorana* resulted in maximum chelating activity of 98.3 9.2% at 500 µg/mL. The percent of chelation of Iraqi *S. rosmarinus* was higher than that of Turkish *S. rosmarinus* at all concentrations and reached 96.5 10.2% at 500 µg/mL. EDTA provided 99.7 0.2% at 500 µg/mL.

Table 4: Fe-chelation capacity of Selected Lamiaceae Extract.

Species	Origin	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Salvia rosmarinus	Iraq	43.8 ± 1.8	67.2 ± 1.9	90.7 ± 2.1	93.6 ± 5.1	96.5 ± 10.2
Salvia rosmarinus	Türkiye	18.8 ± 0.7	47.2 ± 1.3	75.8 ± 1.6	78.7 ± 1.9	81.5 ± 2.1
Salvia officinalis	Iraq	29.8 ± 1.1	63.4 ± 2.2	97.0 ± 3.2	97.4 ± 4.1	97.8 ± 5.1
Salvia officinalis	Türkiye	32.6 ± 3.0	40.9 ± 3.0	49.3 ± 3.2	72.0 ± 2.6	94.6 ± 2.1
Origanum majorana	Iraq	22.6 ± 1.5	34.4 ± 2.0	46.2 ± 2.6	72.3 ± 5.5	98.3 ± 9.2
Origanum majorana	Türkiye	17.0 ± 0.7	44.2 ± 1.8	71.5 ± 2.9	77.4 ± 1.5	83.2 ± 1.1
EDTA	Reference	62.5 ± 1.5	79.8 ± 1.2	89.6 ± 0.9	96.8 ± 0.6	99.7 ± 0.2

Values are expressed as Fe^{2+} chelation (%) (mean ± SD). EDTA was included as the reference chelator



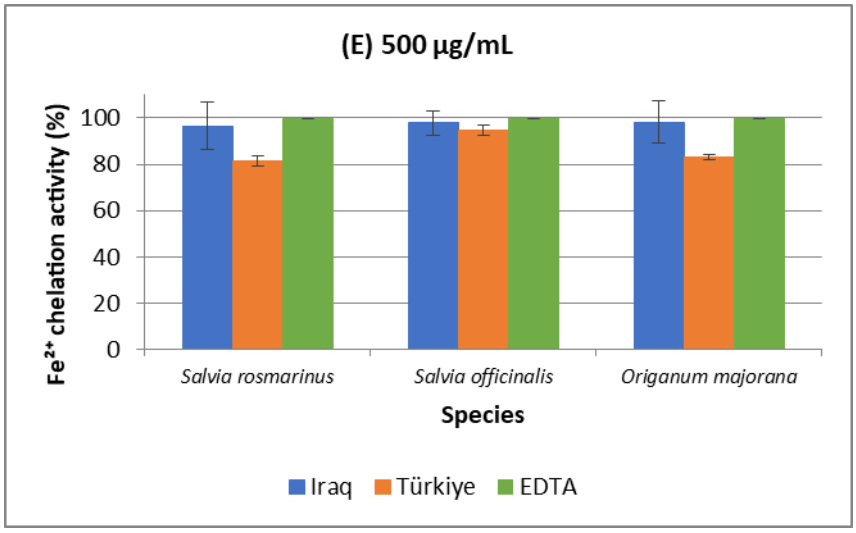


Figure 4: The iron- (Fe (II))-chelating activity of Iraqi and Turkish *Salvia rosmarinus, Salvia officinalis* and *Origanum majorana.* EDTA was taken as reference chelator. All the values are mean± SD.

5. DISCUSSION

Differences between TPC and TFC values would mean that Folin-Ciocalteu reactive reducing compounds and flavonoid responsive compounds did not change in the same way in extracts. The fairly high TPC value reported for Turkish *S. rosmarinus* is in good agreement with claims of rosemary as an herb in the category of phenolic-rich herbs. There is also evidence that phenolic content and antioxidant effectiveness of rosemary differ across cultivars and growing locations.[6,7] Similarly, S. Officinalis extracts, along with those of other Mediterranean salvia species, have been found to contain significant amounts of phenolic acids and flavonoid.[7,18] The high TFC of Turkish *O. majorana* is in line with reports that marjoram includes some phenolic and flavonoid elements, the profile which will, to a degree differ according to plant developmental phase and plant extract type.[19] Cautious numerical comparisons should be made with reference to previously published literature as the extraction solvent constituents, sample preparation Methods, drying before extraction, calibration factors and reporting units can be different. Differences found between Iraqi and Turkish materials may be attributed to resource location of plants, botanical factors and post-harvest care. Individual compounds are unable to be identified since chromatographic analysis of extracts has not been undertaken.[3-5] In DPPH antioxidant activity measurement, the DPPH radical inhibiting percentage increase versus the increasing the concentration of DPPH showed that more compounds were more effective to scavenging radical when used in higher concentration within the reaction mixture. The high DPPH antioxidant activity of *S.rosmarinus* and *S.officinalis* of Turkey presented are relatively similar with the antioxidant activities of rosemary and sage extracts as previously reported[6,7,18,20,21] although the conclusion agreement should be made on quality basis. This fact is Due to difference on the DPPH measurement which affected by solvent system of extraction, the concentration, the conditions of the reaction, standards and expression of results.

Because DPPH radical is a single chemical assay, in vitro, which do not fully reflect a biological antioxidant activity, or because the DPPH radical is sensitive to color of extract and the solvent system used to dilute extracts.[1,3] It is appropriate to interpreted in association with results of reducing-power and Fe(II)-chelating measurements. It can be said that the pronounced reducing power of Turkish *O. majorana* shows that the compounds in the assay condition have a significant donating ability for electron. Reductive activities were previously showed for Salvia and *Origanum*

extracts, as comparing the Mediterranean species of Salvia it was shown that reducing power is related to phenolic-acids, to the content of rosmarinic acid.[18] It was demonstrated the antioxidant activity in DPPH, metal chelating, and reducing power assay for *O. majorana* too.[19]

A high absorbance at 700 nm has not been stated as indication of highest quantity of antioxidant, but high reducing power of compounds present under the assay condition. Difference among the Turkish and Iraqi extracts could perhaps stand at difference in the proportion of reducible agents that are extracted,[3] but more directed chemical analysis along with standard way of sampling of plant materials should be addressed to find them.[1] The results obtained on Fe(II)-chelation were not correlated to the rank-order in the order of: TPC, TFC, DPPH inhibition, and reducing power. This may be explained by the fact that metal chelating depends on the amount and the arrangement in space of metal binding functional groups and not simply on the quantity of total phenolics and flavonoids.[4,5,17] Moreover, according to previous reports concerning Mediterranean Salvia species, radical scavenging activity, reducing power and metal binding activity of extract could show variation of individual activity in relation to one another.[18,21] Thus, the high chelating activity exhibited by Iraqi Salvia officinalis and Iraqi *Origanum majorana* can indicate a high metal binding affinity under ferrozine assay and not its activity under in-vivo situation. In conclusion, variation in species and origin influenced differently activities (radical scavenging, reducing activity, metal chelating behavior), which may be interpreted as one of the reasons to support a multi-assay approach for evaluated plant extracts for their antioxidant activities.[3,5,17]

6. CONCLUSIONS

The presented study makes a comparative in-vitro screening on the antioxidant activities and antioxidant-related properties of selected Lamiaceae herbs collected from Iraq and Turkey. The primary conclusions drawn are:

1. Turkish *S. rosmarinus* presented the highest total phenolic content and high radical scavenging ability against DPPH radicals, indicating a high proportion of Folin-Ciocalteu-reactive reducing substances under the tested experimental circumstances.
2. Turkish *O.majorana* extract had the highest total flavonoid content and the best ability to reduce the ferric iron (in comparison to others). It exhibits strong electron-donating capacity in terms of reducing ferricyanide ions.
3. Iraqi *S. officinalis* and Iraqi *O. majorana* had high Fe(II) chelating activity at determined concentrations, implying their ability to chelate iron ions under iron binding conditions (ferrozine assay).
4. The varied rankings in the different antioxidant related essays (TPC, TFC, DPPH inhibition, Reducing Power, Fe(II) Chelating) indicate that these assays are all determining somehow related but different antioxidant properties, which highlights the need for a multi-assay approach for plant extracts evaluation of antioxidant power.
5. In comparing the studies performed up to now focusing on single herbs of rosemary, sage or marjoram; this work comparing several such herbal preparations that used commercial products obtained from Iraq and Turkey and tested under standard conditions provided Comparative information of how plant origin and species affects the antioxidant properties.
6. The current study useful as comparative references for further biological, phytochemical and pharmaceutical studies related to this herbal medicine. Nonetheless, further studies with additional botanicals and comprehensive biological and safety studies should precede to any therapeutic or nutraceutical claim to the uses of these herbs.

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