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Total Phenolic, Flavonoids and Vitamin C Contents with Antioxidant Activity of *Urtica dioica* L. Leaves Growing in Zakho, Kurdistan Region-Iraq

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Abstract

The genus *Urtica dioica* L. (Urticaceae) is frequently grown in the Kurdistan region of Iraq and is used as a folk remedy by the locals to heal a variety of illnesses. The purpose of this study is to evaluate the total phenolic, flavonoid and vitamin C contents using spectrophotometric method with the determination of antioxidant activities for different solvents such as (aqueous, ethanol, ethyl acetate, chloroform and n-hexane) using different antioxidant methods namely (1,1-diphenyl-2-picrylhydrazyl, reducing power assay, total antioxidant capacity, nitric oxide scavenging, hydroxyl radical, β -carotene-Linoleic acid and iron chelating assays) and ascorbic acid as standard reference. Our results showed that polar solvent extracts exhibited a significant high phenolic and flavonoid contents while ethyl acetate extract had a high vitamin C content. In addition, the findings showed that the extracts had remarkable antioxidant effects compared with standard ascorbic acid. The ethanol extract of *Urtica dioica* L. leaves had stronger scavenging activities than other solvent extracts for 1,1-diphenyl-2-picrylhydrazyl and hydroxyl radicals. Water extract exhibited higher antioxidant effect than other extracts for reducing power and nitric oxide scavenging assays while lower antioxidant activity for β -carotene/Linoleic acid and total antioxidant capacity tests. In contrast, non-polar hexane had the highest antioxidant activity for the iron chelating assay. The present study shows that *Urtica dioica* L. leaf extracts are a viable natural source of antioxidants and may be used in food products as well as nutraceutical applications.

Keywords: Antioxidant activity, Total phenolic contents, Total flavonoid contents, Total vitamin C contents, Utica dioica L. (Stinging nettle).

Introduction

Reactive oxygen species (ROS) are generated as a result of oxygen consumption that occurs naturally during cell growth. They are created by the body's regular oxygen usage, including respiration and some cellular immunity functions. Active oxygen can occur in the form of free radicals such as Hydroxyl radical ($OH^{\bullet}$), superoxide anion (O_2^-) and non-free radicals such as singlet oxygen($^1O_2$) and hydrogen peroxide(H_2O_2), a product of normal metabolism that attacks biological molecules,

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causing cell or tissue damage[1,2]. When the system of antioxidant defence is disrupted by exogenic and endogenic factors enhanced polymorphonuclear leukocytes , macrophages and peroxisomes, resulting diseases such as cancer, cardiovascular disease, inflammation, and accelerated ageing process [3,4].

However, antioxidant substances or antioxidant-rich foods may be utilized to assist the reducing oxidative harm caused by free-radicals as well as active oxygen in human body. Natural antioxidants with their site-specific action mechanisms may be more efficient but even less hazardous than the naturally occurring and synthetic antioxidants currently employed in the food processing industry[5]. Hence, it is necessary to identify alternative safe and natural sources of food-antioxidants, the search for natural antioxidants, particularly those of plant origin, has increased significantly in recent years [6].

Phenolic and polyphenolic components are a most notable groups of secondary plant metabolites that have antioxidant activity primarily because of their chemical reactivity that can be interacting directly with ROS, hydrogen donors, and chelating metal ions [6,7].

Urtica dioica L. is a member of the family *Urticaceae* (Fig.1). It is a perennial herbaceous species often known as nettle [8,9] and it is utilized as a medicine in many countries all over the world[10]. Small trichomes may be seen on the stem and leaves of nettles as they contain formic acid and histamines, the major causes of skin allergies when interacting with this plant. This plant grows in nitrogen-rich soil [11]. In traditional medicine, the entire plant is employed to treat kidney stones allergies, burns, diabetes, leukopenia, internal bleeding, rashes and other ailments[12]. In our society (Kurds), Kurdistan Region, Iraq, *U. dioica* L., known as Kazink, is considered as herb and used as a tea choice.

Moreover, the leaves of nettle have a significant amount of biologically active substances, including terpenoids, flavonoids, carotenoids, polyphenolic compounds, chlorophyll, amino acids, vitamins, tannins, carbohydrates and minerals[10,12,13]. Additionally, *U. dioica* L. extracts have anti-inflammatory, antihyperglycemic, antimicrobial and antiaging properties due to the presence of phenolic and flavonoids compounds in nettle [14,15].

The initial goal of this study, the first to be done on this plant, is to assess the antioxidant activity by employing different methods for each extract of nettle leaves grown in Kurdistan region, Iraq. Additionally, the present research evaluates the total phenolic, flavonoid and vitamin C contents for different solvent extracts of *U. dioica* L. leaves.

Figure 1. **Stinging nettle (*Urtica dioica* L.).**

Materials and Methods

Plant collection and reagents

Urtica dioica L. leaves were harvested from Zakho City, Kurdistan region of Iraq (April-May 2021). Identified by taxonomist from College of Agriculture/ Duhok University. The leaves of *U. dioica* were washed with tap water, then distilled water and left to dry in darkness for 15 days, ground to powder and stored in dark containers in freezer until the day use.

All applied reagents were of the highest purity available and purchased from the Sigma Aldrich Chemical Company.

Instrument:

(Lambda-25 Perkin-Elmer UV-VIS spectrophotometer).

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Methods:

Preparation of *Urtica dioica* L. leaves extract:

Five different solvents were prepared from leaves *U. dioica* using Soxhlet apparatus and procedure described by this method [16].

Total phenolic content: Total phenolic content of all extracts have been estimated by Folin-Ciocalteu method with modifications [17] . A standard curve was prepared using same method with serial concentrations of standard tannic acid solution (50-500 mg/ ml) for determine the concentration of TPC in each extract.

Total flavonoid content: The aluminum chloride method was used to determine the total flavonoid content (TFC) with slight modifications[18] and Quercetin was used as a standard. To prepare the quercetin standard curve, a stock solution was prepared by dissolving 20 mg of quercetin in 100 ml methanol (200 µg/ml). From this stock solution, a series of different concentrations 10 - 180 µg/ml were prepared. To estimate TFC, a standard curve of quercetin was obtained by plotting quercetin concentration versus absorbance. Quercetin equivalents were calculated using the regression equation of this curve and the result was demonstrated as a percentage w/w (mean S.E.).

Total ascorbic acid content: The total ascorbic acid contents for different solvent extracts of *Urtica dioica L*. leaves were determined by [19] method. Ascorbic acid was used as standard as ten milligrams of ascorbic acid were dissolved in 100 ml of distilled water (100 µg/ml). For preparing standard calibration curve of ascorbic acid, pipettes various volume from the stock solution aliquots of 0.05, 0.1, 0.25, 0.5, 1.0 and 1.5 ml and transfer the volume into each volumetric flack and complete the volume to 10 ml by distilled water. For the preparation of extracts, weighted 10 mg and mixed with 10 ml of suitable solvents (1mg/gm). The concentration of ascorbic acid in the extracts was determined by extrapolation from the standard curve and calculated as ascorbic acid equivalents per gram of the dry plant material (mg.AAE.g-1)

Antioxidant activity

To prepare different solvent extracts of *U. dioica* L. leaves and standard ascorbic acid: 50 mg of ascorbic acid and each extract were dissolved in 50 ml of methanol (1000 µg /ml). From the stock solution prepare the series of dilution 6.25 - 100 µg/ml.

DPPH free radical-scavenging activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used to assess the extracts' ability to scavenge free radicals was evaluated by [20] with modifications. The percent inhibition of DPPH scavenging of free radicals was determined by using following Eq. 1:

$$\% \text{ Inhibition of scavenging free radical} = [(A_C - A_S) / A_C] \times 100 \quad \ldots. 1$$

Where: A_C = absorbance of freshly prepared DPPH and A_S = absorbance of extracts and standard.

Reducing power activity: The activity of reducing power for different solvent extracts of *Urtica dioica L*. leaves was measured by Oyaizu assay [21].

Total antioxidant capacity (Phosphomolybdate assay): The phosphomolybdenum method was used to determine the antioxidant capacity of *U. dioica* L. leaves extracts and ascorbic acid as standard [22].

Nitric oxide scavenging activity assay: Nitric oxide (NO^-) produced by sodium nitroprusside (SNP) was evaluated the Marcocci et al method [23]. The amount of nitric oxide scavenging was calculated by this Eq. 2:

$$\% \text{ Nitric oxide scavenging} = [(A_C - A_S) / A_C] \times 100 \ldots. 2$$

Where: A_C = absorbance of control and A_S = absorbance of sample extract and standard (ascorbic acid)

Hydroxyl radical (OH^-) scavenging activity: Fenton's reaction was carried out to evaluate the scavenging of hydroxyl radicals capacity for different solvent extracts of *U.dioica* L. leaves using Smirnoff and Cumbes method [24], with slight modifications. The percent scavenging of hydroxyl radical was calculated the following Eq. 3:

$$\% \text{ Scavenging of hydroxyl radical } OH^- = [(A_C - A_S) / A_C] \times 100 \quad \ldots\ldots 3$$

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Where: A_C = absorbance of control and A_S = absorbance of sample extract and standard (ascorbic acid)

Effect of ß-carotene/linoleic bleaching assay: A method described by [25] was used to assess the antioxidant activity of *U. dioica* leaves extracts. The following Eq. 4 was used to calculate the antioxidant activity (AA) in terms of bleaching β-carotene:

AA% = [Absorbance after 2 hours incubation / Absorbance at zero time] × 100 …… 4

Iron- cheating activity: The iron chelating ability for different solvent extracts was determined by 1,10 phenanthroline method [26] with few modifications. The % inhibition of iron chelating was calculated by the following Eq. 5:

% Inhibition of Fe radical = $[(A_C - A_S) / A_C] \times 100$ 5

Where: A_C= absorbance of control and A_S= absorbance of sample extract and standard (ascorbic acid)

Statistical analysis:
Data were displayed mean ±SEM (standard error of mean) of triplicates and were statistically evaluated by one way analysis of variance (ANOVA). The values were separated by Duncan multiple tests using (SPSS software version 26). Differences with values were considered significant at $p<0.05$. Linear regression analysis and intercept data was used to calculate the IC_{50} values.

Results and Discussion

Total Phenolic, Flavonoid Contents and vitamin C contents of *Urtica dioica* L.

The total phenolic content of *U. dioica* L. leaves for each extract are shown in Table 1. The total phenol content of each extract was significantly increased $p < 0.05$ showing the water extract had the highest TPC while the chloroform had the lowest value according to the following order: water extract > ethanol extract > n- hexane > ethyl acetate > chloroform.

On the other hand, the total flavonoid content of *U. dioica* L. leaves for each extract are shown in Table 1. The total flavonoid content of each extract was remarkably elevated followed the order: ethanol extract > n-hexane > ethyl acetate > chloroform > water extract.

The vitamin C contents for each extract of leaves *U. dioica* is demonstrated in the same table. Our results demonstrated that total vitamin C content followed the order: ethyl acetate > ethanol > water > hexane > and chloroform extract.

Table 1. Total phenol and flavonoid contents and Vitamin C from *Urtica dioica* L. leaves extract.

Extraction type	Total phenolic content (mg TAE /gm)	Total flavonoid content (mg QE/gm)	vitamin C (mg AA/gm)
Water	680.826667 ± 13.91374^{d}	30.49 ± 2.62439^{a}	9.7957 ± 0.00345 ^{c}
Ethanol	359.71 ± 9.41433^{c}	215.176667 ± 2.03415^{e}	14.9430 ± 0.30142^{d}
Ethyl acetate	63.415 ± 1.50043^{a}	83.4533333 ± 3.47413^{b}	50.1203 ± 0.81297^{e}
Chloroform	57.4666667 ± 8.07056^{a}	53.3466667 ± 2.44270^{c}	1.7470 ± 0.00416 ^{a}
n-hexane	136.633333 ± 16.86876^{b}	135.89 ± 0.16258^{d}	7.2953 ± 0.08127^{b}
p-value	< 0.0001	< 0.0008	< 0.0006

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- Note: Values are means of three replicates M ± S. Error. Numbers in the same column followed by the same letter are not significantly different $p < 0.05$.

Antioxidant activity of *Urtica dioica L.* leaves: Different antioxidant methods were used to determine the antioxidant activity of *U. dioica* leaves extracts.

DPPH free-radicals scavenging activity*:* Our findings showed that all extracts of *U. dioica* L. leaves and ascorbic acid had a moderate level of 1,1-diphenyl-2-picrylhydrazyl scavenging activity as presented in Table 2. The scavenging activity of standard ascorbic acid was significant increase $p < 0.05$ more than extracts while, ethanol extract had the highest scavenging activity among other extracts. At 50 µg/ml of the ethanol and aqueous extracts had a slightly stronger DPPH scavenging activity than standard of ascorbic acid.

Additionally, the IC_{50} values of all extracts and ascorbic acid of *U. dioica* L. leaves followed the order: ethyl acetate > chloroform > hexane > Aqueous > ascorbic acid > ethanol extract.

Table 2. DPPH scavenging activity of *Urtica dioica* L. leaves for different solvent extracts and ascorbic acid.

Urtica dioica L. **Conc. (µg/ml)**	**DPPH scavenging activity (%)**						**p-value**
	Aqueous	**Ethanol**	**Ethyl acetate**	**chloroform**	**n- Hexane**	**Ascorbic acid (standard)**	
6.25	40.7333^{r}	44.4200^{u}	13.2238^{b}	11.9867^{a}	16.1167^{c}	39.8067^{p}	
	± 0.02028	± 0.03055	± 0.02340	± 0.01202	± 0.01764	± 0.02404	
12.5	41.5200^{s}	48.2400^{x}	30.7433^{h}	13.2433^{b}	16.3500^{d}	40.0500^{q}	
	± 0.01732	± 0.03055	± 0.01764	± 0.01764	± 0.02887	± 0.01528	
25	44.1200^{t}	54.5133^{AA}	33.4533^{i}	19.0433^{f}	18.2167^{e}	40.7433^{r}	P<0.0008
	± 0.02082	± 0.01856	± 2.34334	± 0.03383	± 0.01764	± 0.01202	
50	47.1900^{w}	68.0967^{AB}	34.6733^{j}	19.2000^{g}	37.2567^{k}	46.4367^{v}	
	± 0.02082	± 0.02963	± 0.31339	± 0.01155	± 0.03712	± 0.02028	
75	50.8200^{y}	81.2000^{AC}	38.2625^{m}	38.5700^{n}	37.7667^{l}	85.5633^{AD}	
	± 0.01155	± 0.01155	± 0.08683	± 0.03000	± 0.02404	± 0.11200	
100	52.4700^{z}	91.3567^{AE}	38.6233^{n}	39.0600^{o}	38.2467^{m}	97.0600^{AF}	
	± 0.02082	± 0.01453	± 0.08950	± 0.02887	± 0.02728	± 0.02309	
IC_{50}	74.6058	15.9219	142.801	129.6698	125.652	32.2294	

-Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly. $P < 0.05$

Reducing power assay (RPA): The reducing power of various extracts increased with the increasing concentration of extracts which is considered to be statically significant in all extract $P < 0.05$ as shown

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in Table 3. The *U. dioica* extracts demonstrated slightly lower reducing power than ascorbic acid at all concentrations tested, besides water extract has a greater reducing power than ascorbic acid at concentration 6.25 µg/ml. As a result, the reducing power was discovered to be in the following order: ascorbic acid > water > ethanolic > chloroform > ethyl acetate > and hexane extract.

Table 3. Effect on reducing power of *Urtica dioica* L. extracts and ascorbic acid.

Urtica dioica L. **Conc. (µg/ml)**	**Reducing power assay (O.D 700 nm)**						**p-value**
	Aqueous	**Ethanol**	**Ethyl acetate**	**chloroform**	**n- Hexane**	**Ascorbic acid (standard)**	
6.25	2.3042^{hij} ± 0.00400	2.2788^{bcde} ± 0.00137	2.2582^{a} ± 0.00263	2.2652^{ab} ± 0.00160	2.2658^{abc} ± 0.00377	2.2941^{fgh} ± 0.00703	
12.5	2.3110^{ijk} ± 0.00157	2.2881^{efg} ± 0.00653	2.2767^{bcde} ± 0.00027	2.27007^{abc} ± 0.00280	2.2718^{abcd} ± 0.00015	2.3127^{ijk} ± 0.00225	
25	2.3278^{lmno} ± 0.00207	2.3121^{ijk} ± 0.00177	2.2809^{cdef} ± 0.00233	2.2871^{defg} ± 0.00356	2.2723^{abcd} ± 0.00015	2.3238^{klmn} ± 0.00603	P< 0.0003
50	2.3326^{no} ± 0.00256	2.3286^{lmno} ± 0.00237	2.2980^{ghi} ± 0.00404	2.3049^{hij} ± 0.00393	2.2726^{abcd} ± 0.00260	2.3321^{no} ± 0.00333	
75	2.3381^{nop} ± 0.00087	2.3317^{mno} ± 0.00739	2.3155^{jkl} ± 0.00226	2.3148^{jklm} ± 0.00110	2.2802^{bcdef} ± 0.00207	2.3517^{p} ± 0.00733	
100	2.3407^{op} ± 0.00285	2.3410^{op} ± 0.00208	2.3253^{klmno} ± 0.00410	2.3290^{mno} ± 0.00200	2.2850^{defg} ± 0.00200	2.3738^{q} ± 0.01894	

-Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly. P < 0.05

Total antioxidant capacity: Our results displayed that antioxidant capacity of all extracts and ascorbic acid as standard increased with increasing concentration. As a result, it was found that the order of increasing total antioxidant activity as; ascorbic acid > ethyl acetate > ethanol > chloroform > n-hexane > water. The total antioxidant capacity of all extracts showed a statistically significant differences $p < 0.05$ using ANOVA as illustrated in Table 4.

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Table 2. Total antioxidant capacity of different extracts of *U. dioica* L. leaves and against ascorbic acid.

Urtica dioica L. Conc. (µg/ml)	Total antioxidant capacity (O.D 695 nm)						p-value
	Aqueous	Ethanol	Ethyl acetate	chloroform	n- Hexane	Ascorbic acid (standard)	
6.25	0.0660^{a} ± 0.00065	0.0770^{bc} ± 0.00043	0.0685^{ab} ± 0.00529	0.0681^{ab} ± 0.00260	0.1085^{ef} ± 0.00127	0.0753^{abc} ± 0.00033	
12.5	0.0681^{ab} ± 0.00003	0.1169^{fg} ± 0.00052	0.0769^{bc} ± 0.00470	0.0929^{d} ± 0.00253	0.1150^{fg} ± 0.00137	0.1510^{jk} ± 0.00003	P< 0.0009
25	0.0684^{ab} ± 0.00010	0.1335^{h} ± 0.00030	0.0815^{c} ± 0.00613	0.1200^{g} ± 0.00096	0.1203^{g} ± 0.00405	0.2611^{o} ± 0.00027	
50	0.0756^{abc} ± 0.00003	0.1448^{ij} ± 0.00018	0.1396^{hi} ± 0.00115	0.1323^{h} ± 0.00098	0.1315^{h} ± 0.00204	0.3467^{p} ± 0.00009	
75	0.0906^{d} ± 0.00003	0.1948^{m} ± 0.00012	0.2036^{m} ± 0.00044	0.1593^{k} ± 0.00757	0.1460^{ij} ± 0.00056	0.4890^{q} ± 0.00018	
100	0.1036e ± 0.00072	0.2025^{m} ± 0.00054	0.2173^{n} ± 0.00642	0.1961^{m} ± 0.01063	0.1800^{l} ± 0.00428	0.5445^{r} ± 0.00029	

-Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly. P < 0.05.

Effect of nitric oxide scavenging assay: The % inhibition of nitric oxide increasing with the increase concentration of the plant extracts and ascorbic acid which is found to be statically significant p < 0.05 as shown in Table 5. Besides that, it was found that the rate of scavenging NO for ethyl acetate extract at concentration 12.5 µg/ml was higher than 25 and 50 µg/ml respectively. Moreover, aqueous extract of *U. dioica* L. leaves were found to be the highest NO scavenging activity than other solvents and ascorbic acid as well. The all extracts were observed to be included in the sequence: aqueous > chloroform > hexane > ethyl acetate > ethanol. The value of IC_{50} for all extracts was found to be as follows: ethanol > ethyl acetate > hexane > chloroform > and water. The IC50 value of standard ascorbic acid was 47.6099 µg/ml.

Table 3. The effect of nitric oxide NO assay for different extract of leaves *U. dioica* L. against ascorbic acid.

Urtica dioica L. Conc. (µg/ml)	Scavenging of hydroxyl radical (%)						p-value
	Aqueous	Ethanol	Ethyl acetate	chloroform	n- Hexane	Ascorbic acid (standard)	
12.5	8.6407^{hij} ± 0.01613	1.6889^{a} ± 0.05880	5.3185^{e} ± 0.00979	8.4777^{hi} ± 0.04205	6.0185^{f} ± 0.02063	2.2851^{b} ± 0.03229	
25	11.8148^{m} ± 0.17514	2.9555^{c} ± 0.01923	6.8148^{g} ± 0.03291	8.8926ij ± 0.03866	6.7111^{g} ± 0.05133	4.7888^{d} ± 0.01285	
50	20.7555^{q} ± 0.34145	5.5407^{e} ± 0.08520	8.2777^{h} ± 0.01285	10.3555^{l} ± 0.19159	9.0074^{jk} ± 0.04730	5.2073^{e} ± 0.03647	
75	21.5000^{r} ± 0.05130	24.6481^{s} ± 0.01334	8.4074^{h} ± 0.02593	11.5363^{m} ± 0.16293	12.4592^{n} ± 0.00370	9.3417^{k} ± 0.62794	P < 0.0001
100	25.4407^{t} ± 0.04900	31.1926^{u} ± 0.01961	13.4925^{o} ± 0.02063	15.8555^{p} ± 0.07564	15.9074^{p} ± 0.07155	33.9926^{v} ± 0.06553	
IC_{50}	223.5930	120.3848	573.0551	547.7306	400.7308	144.6722	

-Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly. P < 0.05.

Hydroxyl radical (OH^-) scavenging assay: The scavenging of hydroxyl radicals OH^- activity of plant extracts and standard ascorbic acid increased with increasing concentration as shown in Table 6. The

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results demonstrated that the *U. dioica* L. extracts and ascorbic acid are statistically significant p<0.05. In general, maximum hydroxyl radical scavenging ability was observed in ethanol extract among other solvent extracts. The increasing of scavenging activity of plant extracts and ascorbic acid was found to be: ascorbic acid > ethanol> water> hexane > chloroform > ethyl acetate. In addition, the greatest IC50 value was showed in ethyl acetate followed by chloroform > hexane > water > ethanol > and ascorbic acid respectively.

Table 6. The effect of hydroxyl radical scavenging for different extract of leaves *U. dioica* L. against ascorbic acid.

Urtica dioica L. **Conc. (µg/ml)**	**Scavenging of hydroxyl radical (%)**						**p-value**
	Aqueous	**Ethanol**	**Ethyl acetate**	**chloroform**	**n- Hexane**	**Ascorbic acid (standard)**	
12.5	8.6407^{hij} ± 0.01613	1.6889^{a} ± 0.05880	5.3185^{e} ± 0.00979	8.4777^{hi} ± 0.04205	6.0185^{f} ± 0.02063	2.2851^{b} ± 0.03229	P<0.0001
25	11.8148^{m} ± 0.17514	2.9555^{c} ± 0.01923	6.8148^{g} ± 0.03291	8.8926ij ± 0.03866	6.7111^{g} ± 0.05133	4.7888^{d} ± 0.01285	
50	20.7555^{q} ± 0.34145	5.5407^{e} ± 0.08520	8.2777^{h} ± 0.01285	10.3555^{l} ± 0.19159	9.0074^{jk} ± 0.04730	5.2073^{e} ± 0.03647	
75	21.5000^{r} ± 0.05130	24.6481^{s} ± 0.01334	8.4074^{h} ± 0.02593	11.5363^{m} ± 0.16293	12.4592^{n} ± 0.00370	9.3417^{k} ± 0.62794	
100	25.4407^{t} ± 0.04900	31.1926^{u} ± 0.01961	13.4925^{o} ± 0.02063	15.8555^{p} ± 0.07564	15.9074^{p} ± 0.07155	33.9926^{v} ± 0.06553	
IC_{50}	223.5930	120.3848	573.0551	547.7306	400.7308	144.6722	

-Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly. P < 0.05.

***β*-carotene- Linoleic acid bleaching assay**: Table 7 illustrates the inhibition of oxidative damage in all extract for *U. dioica* L. leaves with different concentrations compared with ascorbic acid. All of the extracts and ascorbic acid displayed significant activity ($p < 0.05$) ranged from 19.57 to 90.77%. The absorbance increase rates among the extracts were shown as: water > hexane > ethyl acetate > ethanol > chloroform. Furthermore, the order of the strength of the IC_{50} of all extracts and ascorbic acid was followed the order; chloroform > ethanol > ethyl acetate > hexane > water > ascorbic acid.

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Table 7. Antioxidant capacity of *Urtica dioica* L. leaves extracts and ascorbic acid was evaluated using theβ -carotene / linoleic acid assay.

Urtica dioica L.	**β -carotene bleaching assay (%)**						**p-value**
Conc. (µg/ml)	**Aqueous**	**Ethanol**	**Ethyl acetate**	**chloroform**	**n- Hexane**	**Ascorbic acid(standard)**	
6.25	65.3311^{kl} ± 0.87105	19.5785^{a} ± 1.98511	45.0663^{ef} ± 0.76847	23.5433^{ab} ± 1.72262	35.5522^{cd} ± 1.96215	69.9847^{lmn} ± 0.95114	P<0.0005
12.5	69.5452^{lmn} ± 1.29121	30.4448^{bc} ± 5.95962	48.3575^{fgh} ± 2.55502	40.1008^{de} ± 1.76050	49.9522^{fgh} ± 0.45137	73.6216^{mno} ± 1.23452	
25	70.6776^{lmn} ± 0.83582	62.6163^{jkl} ± 1.60964	54.8751^{ghi} ± 1.17906	49.0719^{fgh} ± 0.57820	51.7773^{fghi} ± 0.61021	75.6989^{no} ± 0.14737	
50	84.0421^{pqr} ± 0.31100	65.4606^{klm} ± 9.83730	55.8352^{hig} ± 0.55930	44.3976^{ef} ± 0.06188	66.8552^{klm} ± 2.15605	78.5136^{op} ± 1.36339	
75	86.2690^{qr} ± 0.95686	67.2313^{lm} ± 1.28215	59.1646^{igk} ± 3.08256	47.3086^{efg} ± 0.96465	67.5730^{lm} ± 1.31594	86.8560^{qr} ± 0.95490	
100	88.0031^{r} ± 4.48284	69.9204^{lmn} ± 2.56996	79.5693^{opq} ± 1.11247	51.4594^{fghi} ± 1.17772	86.5850^{qr} ± 1.37067	90.7710^{r} ± 2.07625	
IC_{50}	42.0698	54.513	54.300	74.0631	49.3096	41.6077	

-Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly. P < 0.05.

Iron chelating activity: Our data revealed that the high-level activity of scavenging ferrous chelating for extracts of leaves *U. dioica* L. and ascorbic acid ranged from 79.06 % to 93.24%. The % inhibition of metal chelating increasing with the increase concentration of the plant extracts and ascorbic acid which is discovered to be statically significant (p <0.05) as shown in Table 8. Moreover, it was observed that the rate of aqueous and ethanol extract's iron chelating initially fluctuated with concentration but later increased with increasing concentration. On the other hand, the percent of chelating ferrous activity of all extracts of leaves nettle was found to be higher than standard ascorbic acid which is found to be: hexane > water > chloroform > ethanol > ethyl acetate > ascorbic acid. Additionally, the IC_{50} values of *U. dioica* L. leaves extracts and standard was decreased with following order: ascorbic acid > water > ethyl acetate > ethanol > chloroform > hexane.

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Table 8. Chelating effect of different extracts of *Urtica dioica* L. leaves and ascorbic acid on Fe^{+2} ion.

Urtica dioica L. Conc. (µg/ml)	Iron chelating agent (%)						p-value
	Aqueous	Ethanol	Ethyl acetate	chloroform	n- Hexane	Ascorbic acid (standard)	
6.25	84.0841^{c} ± 0.00969	85.3251^{e} ± 0.05933	86.4912^{f} ± 0.01226	89.0209^{hi} ± 0.00736	88.1758^{g} ± 0.02200	79.0689^{a} ± 0.27110	P<0.0006
12.5	84.5437^{d} ± 0.01120	85.5137^{e} ± 0.02769	89.1838^{i} ± 0.62690	89.2792^{i} ± 0.01181	88.8133^{h} ± 0.00763	79.7360^{b} ± 0.03387	
25	84.5839^{d} ± 0.00850	92.2419^{lm} ± 0.01123	91.3547^{k} ± 0.00559	91.5242^{k} ± 0.03813	92.7419^{nop} ± 0.00423	90.0518^{j} ± 0.03376	
50	92.7502^{nop} ± 0.01676	92.6043^{mno} ± 0.00367	91.9584^{l} ± 0.16642	91.8948^{l} ± 0.08812	92.5557^{mno} ± 0.03940	92.0325^{l} ± 0.06794	
75	92.6107^{mno} ± 0.04851	92.5299^{mno} ± 0.03601	92.4645^{mn} ± 0.01100	92.7081^{nop} ± 0.00210	92.8881^{opqr} ± 0.01481	92.5620^{mno} ± 0.06981	
100	93.1655pqr ± 0.00764	92.8902^{opqr} ± 0.03363	92.7886^{nopq} ± 0.15253	93.0914^{pqr} ± 0.02149	93.2482^{or} ± 0.02357	92.8224^{nopq} ± 0.00213	
IC_{50}	38.5119	38.2467	38.2731	38.1679	38.0430	38.5564	

-Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly. P < 0.05.

Discussion

Phenolic compounds in nettle leaves have received a significant amount of attention due to their antioxidant effects provided by their OH- groups [8] as many research groups demonstrated that these leaves are rich in phenolic compounds than other wild plants[27], and the most abundant one is Rutin [28]. Zeković and his co-workers used different extraction techniques for determining TPC and TFC as they found that total phenolic contents from subcritical water extraction gave the highest value and the lowest total flavonoid [29].

Fattahi et al. [30] reported that total phenolic content from water extract was two-fold lower than our findings while the total flavonoid contents were ~ 4.5-fold higher than our findings, this might be attributed to various standards used for quantifying flavonoids. In addition, Ghaima et. al.[28] found that TPC in ethyl acetate extract was lower than our result using same solvent. Külcü study revealed that both TPC and TFC of ethanol, chloroform and n-hexane extracts were much lower than our results [31]. Moreover, Vitamins are a unique class of organic substances that has important biochemical and biological functions in living cells [32- 34].

Common nettle (*Urtica dioica* L.) is a beneficial plant source of vitamins and is used both traditionally and medicinally as homeostatic, vitamin stimulants[35] and as infusions,[36]. According to Ioana and his group, who collected nettles from March to July, the maximum concentration of vitamin C was found in a 50% ethanol extract of U. dioica leaves in March [37]. Moreover, the amount of vitamin C for boiled water as (a tea) of leave Urtica dioica L. from two different location in Serbia were 32 to 35 mg/g [38]. Similarly, ethanol extract of nettle leaves was discovered to be most effective for isolating vitamin C and pigments[39]. There are no research findings about vitamin C content for ethyl acetate, chloroform and n-hexane extracts of nettle.

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Over free-radicals production beyond the body's capability to defend itself through antioxidant defense system results in oxidative stress, which is the basis of biological chronic disease [40]. Evaluating the scavenging abilities of stable DPPH free radicals for the estimation of antioxidant effect in vitro is a prevalent and quick method when comparing to the other methods. Therefore, DPPH is typically utilized as a substrate to assess the antioxidant capacity of antioxidants[41].

Therefore, the results of this study demonstrated *Urtica dioica* L. leaves for each extract to be a moderate free- radical scavenger, due to the presence of hydroxyl group of phenolic and flavonoid contents in *U. dioica* plant. Depending on the previous research, Flórez et al evaluated DPPH radical assay using different solvents and techniques, their results revelated water extract had the high antioxidant activity than ethanol extract utilized stirring extraction technique [42].Similarly, Külcü and co-workers found that the *Urtica dioica* L. have moderate antioxidant activity in DPPH radical method, in our result showed the ethanol extract had a highest inhibition activity than chloroform and hexane extract [31]. On the other hand, another groups demonstrated that fresh leaves of ethanol extract in stinging nettle has a minimal amount of antioxidant capacity in DPPH scavenging assay [43].In addition, the IC50 values of *Urtica dioica* L. from Nepalese using DPPH method showed that hexane extract had the highest value while methanol had the lowest [44]. Other authors claim that, plants harvested under different conditions illustrate notably different antioxidant activity. According to Paulauskien et al., [13] the antioxidant activity of *Urtica dioica* L. leaves varied considerably (p 0.05) from 52.9 to 95.1% from April to September, with May of harvesting the leaves with the highest antioxidant activity.

Substances with reducing power that are electron donors can minimize the oxidized substituents of lipid per-oxidation systems, allowing them to act as primary and secondary antioxidants [45]. The findings of our investigation on leaves *Urtica dioica L*. for all extract revealed that have strong reducing agents.

According to the Zeković et al [29] reported about *U. dioica* L of different extracting solvents using three non-conventional extraction techniques such as microwave assisted, ultrasonic-assisted and sub-critical water extraction. That results revelated the water extract using ultrasonic assisted extraction had the highest antioxidant activity in reducing power assay among other solvents. Similarly, Fattahi et al, [30] considered that the reducing power of aqueous extract *Urtica dioica L.* had the highest value while, the study of Singh & Sengar confirm that the 70% ethanol extract of *U. dioica* L. had a higher reducing power than ascorbic acid as a standard [46]. Chloroform extract of *U. dioica* L. leaves is done using this assay for the first time.

The phosphomolybdate method is commonly used to assess the total antioxidant capacity of stinging nettle extracts. The antioxidant activities of the plant extract are strongly related to the solvent used, due to different antioxidant property of compounds with varying polarities. Moreover, phytochemical screenings indicated the existence of numerous different bioactive phytochemicals, may be contributed antioxidant capacity of *Urtica dioica L*. leaves. Our findings are in agreement with the results of Joshi et al. [47] reporting flavonoids such as rutin and quercetin, phenolic acids such as chlorogenic acid and caffeic acid, and carotenoids (β-carotene and hydroxyl- β-carotene) and also fatty acids, essential oils and other constituents such as vitamins and minerals in leaves U. dioica L. extracts were accountable for the potent antioxidant activity.

Considering the earlier research Sharma et al results disagree with our findings, they estimated that the methanol extract of nettle leaves had the highest antioxidant activity than hexane and ethyl acetate extracts[48]. On the other hand , other authors demonstrated that the Turkish *U. dioica* L leaves for ethanol extract had a highest antioxidant activity than chloroform and hexane extracts [31]. Another groups revealed that the growing U .*dioica* L. leaves for 70% alcoholic extract under different intensity of light and wavelength obtained different quantity of phenolic, flavonoids and antioxidant activity levels [49].

Nitric oxide is one of the important antioxidant methods. Nitric oxide is a short-lived endogenous free radical and a significant chemical mediator

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produced by macrophages and endothelial cells, plays an essential role in pathogenies of different diseases associated with inflammation diabetes, cancer, epilepsy and cerebral ischemia[50]. Plants and plant products have the capability to prevent the effect of NO formation, which may be a great interest in avoiding the negative effects of excessive nitric oxide (NO^-) generation in human body. The Urtica dioica L. leaves extracts showed high nitric oxide scavenging. As the extract concentration increase, the percentage of inhibition increase as well.

Depending on the previous research, our results disagree with Semwa et al, findings, who revealed that the ethyl acetate had a higher NO^- scavenging effect than other solvent extracts.[51] . Similarly, Joshi et al.,[52] reported the effect of NO^- scavenging assay of *U. dioica* L. whole plant for the 80% of alcohol extract and it's fractioned by aqueous, butanol, ethyl acetate and petroleum ether and compared with ascorbic acid as standard. They demonstrated the ethyl acetate fraction had a significant nitric oxide scavenging potential comparing to hydroalcoholic extract as well and other fractions. Moreover, other authors revealed that the methanolic extract of nettle leaves had a significant of NO^- free-radical scavenging activity [53]. Chloroform and n-hexane extracts we measured for first time.

Hydroxyl radical (OH^-) is an active reactive oxygen component that has an effective oxidising ability. It can destroy red blood cells, cellular membranes, damaging DNA, macromolecules, and also lead to significant harm human health. Thus, removing hydroxyl radicals is considered one of the most efficient methods for preventing disease. Our results revealed that the *U. dioica* L. leaves for each extract have a moderate-low ability to scavenge the hydroxyl radicals.

Durović et al., [54] studied hydroxyl radical scavenging assay of *Urtica dioica* L. leaves for different location in Serbia by supercritical carbon dioxide. They revealed that the nettle had a high antioxidant activity scavenging of OH^- radical. Moreover, there is no report with the estimation of hydroxyl radical scavenging ability regarding different solvents increasing polarity (n-hexane, chloroform, ethyl acetate, ethanol and water) of leaves Urtica dioica L.

β -carotene/Linoleic acid bleaching assay is one of the widely methods used for determining antioxidant activity. Different extracts can reduce the amount of beta-carotene decolorization by neutralizing linoleate-free radical as well as other radicals established with in system [45]. Regarding the previous study by Mehdi [55] and groups agree with our results, they are estimated that the Iranian *Utica dioica* L. leaves for pre and post flowering steps; water had higher inhibition than ether extract. Similarly, Bashyigit et al.,[56] results showed the hydroalcoholic extract of nettle exhibited high value ability of antioxidants. Other groups assessed that the higher concentration of phenolic compounds in 80% ethanol extract was associated with the highest antioxidant activity in beta-carotene bleaching method [57]. Chloroform, ethyl acetate and hexane extracts of U. dioica L. leaves is done using this assay for the first time.

Finally, Iron is thought to be important cell functions including cellular respiration and oxygen transport and it serves as a co-factor by a number of Fe-metallic enzymes[58]. This method is based on the formation 1,10-Phenanthroline-Fe^{+2} complex and interruption in the appearance of chelating agents. The reduction will be utilized as an indicator of donating electrons action, which displaying an efficient mechanism of antioxidant property [59]. Metal chelating activity results for extracts of *Urtica dioica* L. Leaves showed that the highest value was found in n-hexane among other solvent extracts, while the lowest metal chelating ability was recorded in standard ascorbic acid. As a result, the iron-chelating ability for all extracts of *U. dioica* L. leaves would prevent transition metals from contributing to the beginning of oxidative stress.

Previous research reported for aqueous extract of *U. dioica* L. leaves revelated that the water extract had a lowest effect of metal chelating when compared to standard [60]. Similarly, Vajic et al [61] investigated that 54% methanol extract of nettle 18-times weaker than standard EDTA. In addition, Güder and co-workers [62] studied hydroalcoholic extract of different parts *U. dioica* L. in Turkey, they showed that the metal-chelating activity of leaves nettle was lower than another parts of plant, and standards of BHA, α-tocopherol and BHT. The antioxidant activity by

metal chelating assay for ethanol, ethyl acetate, chloroform and n-hexane extracts are no one detected.

Conclusion

The findings of this study demonstrated that *U. dioica* leaves are a good source of antioxidants (phenolic, flavonoid, and vitamin C contents). In comparison to other solvents, water extract had the highest total phenolic content, whereas ethanolic extract had the highest total flavonoid content. The extract with the greatest overall vitamin C levels was found to be ethyl acetate. Furthermore, in comparison to conventional ascorbic acid, our results showed high antioxidant activity (1,1-diphenyl-2-picrylhydrazyl scavenging, total anti-oxidant capability, reducing power, hydroxyl radical, NO scavenging, β -carotene bleaching scavenging, and iron chelating scavenging). Because of this, the examined Urtica dioica L species seem to be significant sources of natural antioxidants that could be exploited in agriculture and healthcare.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Zakho.

Authors' Contribution Statement

The manuscript was done by the cooperation by the two authors. Conceptualization: L. Y M.; Methodology: L. Y M.; Validation: L. Y. M.; Formal analysis: Gh. A. O.; Investigation: L. Y. M.; Resources: L. Y. M. and Gh. A. O.; Data curation: Gh. A. O.; writing—original draft preparation: Gh. A. O. Writing—review and editing: L. Y. M. Visualization: L. Y. M. and Gh. A. O.; Supervision: L. Y. M.

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إجمالي محتويات الفينول والفلافونويد وفيتامين ج مع نشاط مضاد للأكسدة لأوراق القراص التي تنمو في زاخو ، إقليم كوردستان ، العراق

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الخلاصة

يُزرع جنس Urtica dioica L. (Urticaceae) بشكل متكرر في إقليم كردستان العراق ويستخدم كعلاج شعبي من قبل السكان المحليين لعلاج مجموعة متنوعة من الأمراض. الغرض من هذه الدراسة هو تقييم إجمالي محتويات الفينول والفلافونويد وفيتامين ج باستخدام طريقة القياس الطيفي مع تحديد الأنشطة المضادة للأكسدة للمذيبات المختلفة مثل (المائي ، الإيثانول ، أسيتات الإيثيل ، الكلوروفورم و هكسان) المتدفقة التي تختلف باختلاف اختبارات مضادات الأكسدة مع تراكيز مختلفة وهي (1،1-ثنائي فينيل -2 بيكريل هيدرازيل ، اختبار القدرة المختزل ، القدرة الكلية لمضادات الأكسدة ، كسح أكسيد النيتريك ، جذور الهيدروكسيل ، β-carotene- حمض اللينوليك ومخلبات الحديد) وحمض الأسكوربيك كمرجع معياري. أوضحت نتائجنا أن تحديد إجمالي محتويات الفينول والفلافونويد باستخدام طرق فولين سيوكالتو وكلوريد الألومنيوم أظهر أن مستخلصات المذيبات القطبية أظهرت محتوى عاليًا من الفينول والفلافونويد بينما يحتوي مستخلص أسيتات الإيثيل على نسبة عالية من فيتامين ج. بالإضافة إلى ذلك، أظهرت النتائج أن المستخلصات لها تأثيرات مضادة للأكسدة ملحوظة مقارنة بمضادات الأكسدة القياسية مثل حمض الأسكوربيك. كان لمستخلص الإيثانول لأوراق U. dioica L أنشطة تنظيف أقوى من مستخلصات المذيبات الأخرى لجذور 1،1-ثنائي فينيل -2 بيكريل هيدرازيل و الهيدروكسيل. أظهر مستخلص الماء تأثيرًا مضادًا للأكسدة أعلى من المستخلصات الأخرى لتقليل مقاييسات القوة وأكسيد النيتريك مع انخفاض نشاط مضادات الأكسدة لـ β-carotene / حمض اللينوليك وإجمالي اختبارات القدرة المضادة للأكسدة. في المقابل ، كان للهكسان غير القطبي أعلى نشاط مضاد للأكسدة لمقايسة مخلب الحديد. تُظهر الدراسة الحالية أن مستخلصات أوراق U. dioica Lهي مصدر طبيعي حيوي لمضادات الأكسدة ويمكن استخدامها في المنتجات الغذائية بالإضافة إلى تطبيقات التغذية.

الكلمات المفتاحية: النشاط المضاد للأكسدة، إجمالي محتويات الفينول، إجمالي محتويات الفلافونويد ، إجمالي محتويات فيتامين سي ،نبات القراص.