Study of the antioxidant activity of crude extracts and phenolic compounds of leaves of *Populuseuphratica*against free radicals (DPPH)

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Summary

Diagnostics by HPLC proved that the *Populuseuphratica*plant growing on the banks of the Tigris River in the regions of Mosul and Tikrit contained a number of phenolic compounds, which included: hydrobenzoic acid, gallic acid, sacid, rutin, apigenin, ferulic acid, kaempferol, and sennapic.Andthe results showed that the phenolic compounds of the leaves of *Populuseuphratica* (Tikrit - Mosul) were identical in their presence and appearance in both plants, with a superiority in the total concentration of the phenolic compounds of *Populuseuphratica* growing in Tikrit over the same plant growing in the city of Mosul with a concentration of (3.35 and 2.367) mg. /gm and respectively.The results indicated that the use of phenolic compounds and crude petroleum ether and alcoholic extracts of the study plant as antioxidants at different concentrations, and comparing them with ascorbic acid as a standard sample, all of which led to sniping of the DPPH free radical; The highest sniping percentage of the phenolic compounds of the Euphrates/Tikrit and Mosul plants was (95.7% and 90.2%), respectively, at a concentration of 500 µg/ml, superior in proportion to all other products, as well as superior to the standard sample, whose sniping percentage of free radicals was (85.6%). And at the same concentration, and that the inhibition of free radicals increases with the escalation of the concentration of the natural active compounds of the plant.

Key words: *Populuseuphratica*, antioxidant, phenolic compounds.

Introduction

Medicinal plants have been used for centuries to treat ailments; Because they contain chemical components that have therapeutic value, and they are the result of secondary metabolism within plants (Ghourchian*et al*, 2016). They are known as natural products, and their products are known as active ingredients.These substances act as antioxidants and antibiotics, and they work to raise the efficiency of the immune system against viruses and many diseases that affect humans.Numerous studies have shown that plants contain many active ingredients that have an antimicrobial effect on microorganisms, and that the compounds isolated from plants are complex and important biomolecules used in treatment. Plants are rich in many biological compounds such as alkaloids, tannins, volatile oils, phenols, saponins, flavonoids, glycosides, and other compounds that have anti-microbial and antioxidant activity (Oran and Rais, 2000).Phenolic compounds are among the most prevalent secondary metabolite compounds in the plant kingdom, i.e. they are found in most plants, but they are scarce in algae, bacteria and fungi. Studies have indicated that 2% of the total carbon prepared during photosynthesis is converted into flavonoids. Phenolic acids are found in a variety of soluble forms and are associated with sugars in a glycosidic bond, or are associated with organic acids and are involved in the synthesis of complex compounds such as lignins and tannins (Lattanzio, 2013).

One of the medicinal plants used and which is under study is ***Populuseuphratica,***This plant is called locally by several names, including the Euphrates poplar, the gog, the Mercis, the multi-leaf poplar, and the ancient.It is a perennial plant that lives on the banks of rivers. It is a

medium-sized oily tree with a height of about 15 meters. The trunk is bent and forked, and the old stems have thick, rough bark, olive green in color, and the leaves are variable in shape (Dudonne, 2011).The flowers are borne in the form of pods. The male flowers are 25-50 mm long and the female flowers are 50-75 mm long. The fruits are oval-shaped capsules 7-12 mm long and contain small seeds encased in silky hairs (Ruisheng1 and Pei, 2005).It is a dioecious plant that is wind-pollinated and self-fertilizing. These plants grow in light sandy to medium heavy (clay) soils and prefer well-drained, acidic, neutral-basic soils. And it is one of the fast-growing species that loves light and heat and grows in conditions of drought and high salinity (Gonge, 2018).

Free radicals are atoms or molecules that result from the metabolic processes of normal cells, and they are unstable atoms because they have unpaired electrons in their outer shell, which exposes them to the loss of those electrons. Taking their electrons is also looking for electrons from other atoms and so on, which poses a great danger, as it is the cause of causing a lot of damage to the cells of the body (BAS, 2018).They are substances produced by the human body mostly against harmful substances, and when their production rate exceeds the necessary limit, oxidative stress occurs, and free radicals are produced as a result of the internal functions of the body, where free radicals are generated from internal sources by cells as a result of physiological and biochemical processes in the body such as activation of immune cells Inflammation, mental stress, phagocytic cells, and anemia(Rao *et al*, 2011), in addition to its production from external sources when the body is exposed to some polluting substances of air, water and toxic environmental pollutants, alcohol intake, heavy metals such as cadmium,

mercury, lead, iron, exposure to ultraviolet radiation and drug abuse (Kumar *et al*, 2011).DPPH is a common abbreviation for the organic chemical compound 2,2dipheny-1-picryl hydrazyl, which is a stable free radical and its chemical formula is $C_{18}H_{12}N_5O_6$ Figure 7 and its powder is greenish-black insoluble in water, and its methanolic solution is violet in color, it gets between it and the anti compounds The oxidation reacts directly and quickly, which gives it a proton, then the DPPH free radical turns into an inactive radical and loses its violet color, turning into a pale yellow color (Sharma and Tej, 2009).

The study aims to prepare crude extracts, separate phenolic compounds and characterize them using HPLC technology from the leaves of the *Populuseuphratica*plant, and study their inhibitory effect against free radicals (DPPH).

Materials Experiment

1- Collection and classification of *Populus*euphratica used in the study:

The leaves of *Populuseuphratica* were collected from the banks of the Tigris River in the cities of Mosul and Tikrit in September 2022. The plant was classified in the Directorate of the Project for the Development of Medicinal Plants in Mosul Dam of the Iraqi Ministry of Agriculture by Dr. TalalTaha.

After that, the leaves were cleaned from the dust and the like, and dried on leaves away from sunlight, taking into account turning them daily and monitoring them from rotting, then they were placed in paper bags and kept in conditions far from moisture until use.

2- The taxonomic position of the plant:

Kingdom : plantae

Order :Malpighiales

Family : Salicaceae

Genus :populus

Specie: *P.euphratica*

3- Preparation of Some Plant Extracts:

The plant extracts were prepared according to the method mentioned by Grand (1988) by placing 40 g of the study plant powder in a glass beaker and 500 ml of petroleum ether was added to it to separate the fixed oils from the study leaves and using a magnetic stirrer for a period of 72 hours, then ethanolic alcohol was added at a concentration of 70 % and in the same way as before. Then the extract was concentrated by a rotary vacuum evaporator (RVE) at a temperature of 40 °C, then the crude extract was placed in opaque glass bottles, tightly closed and placed in the refrigerator until use.

4- Separation and purification of phenols from plant leaves by acid hydrolysis:

5 ml of the crude ethanolic extract was taken and 25 ml of HCl (1N) acid was added to it. After that, thermal sublimation was carried out at a temperature of 100 °C for one hour, then the solution was cooled and placed in a separating funnel, and 50 ml of ethyl acetate was added to it twice with good shaking, after which two upper layers (the organic layer) of ethyl acetate and a lower layer were obtained. The upper layer was taken and 3 g of anhydrous magnesium sulfate MgSO¬4 was added to it. The samples were kept in sealed and darkened vials and placed in the refrigerator until they were diagnosed by HPLC and their antioxidant activity was studied (Hasan *et al*, 2019).

5- Identification of active phenolic compounds using HPLC:

The process of identifying phenolic compounds was carried out in the laboratories of the Ministry of Science and Technology / Department of

Environment and Water after conducting the acid hydrolysis process described in paragraph 3-4 by means of a high-performance liquid chromatography (HPLC) type SYKAM of German origin, with a flow rate of 1 ml / min. The mobile phase is A, B. .

A= (Methanol: D.W.: acetic acid) (85:13:2).

B= (Methanol: D.W.: acetic acid) (25:70:5).

Column 18-ODS with dimensions of 25 cm * 4.6 mm has been detected responses at a wavelength of 360 nm.

6- Study of the antioxidant efficacy of the separated compounds:

Weigh 7.9 mg of DPPH (Diphenyl pecril hydrazine) and dissolve it in 100 ml of methanol to obtain a 200mM. Concentrations of 200, 300, 400, 500 µg/ml were prepared, and ascorbic acid was used as a control sample, and then 1 ml of DPPH solution was added to each concentration in addition to the control sample. After that, the samples were incubated at room temperature for 30 minutes in the dark, and each sample was measured. At a wavelength of 517 nm and then the following equation was applied to find out the percentage of inhibition of free radicals (Sahu et al., 2013, Sumathy et al., 2013):

% = (AbB-AbS)/AbB * 100

AbB = absorbance of control sample

AbS = absorbance of the sample

Results and discussion

1- Identification of phenolic compounds by HPLC technology in the leaves of Euphrates plant:

Chromatographic analytical charts were obtained and the retention time of each phenolic compound was determined for the study and standard samples (Fig. 3-10), which included:

Hydrobenzoic with a retention time of 2.51 minutes, Galic acid with a retention time of 3.02 minutes, catchine with a time of 5.9 minutes, Rutin with a time of 6.52, Apigenine with a retention time of 7.01, Ferulic acid with a time of 8.74 minutes, Kaempferol with a time of 9.35 minutes and Sinapic with a retention time of 11.25 minutes.

The diagnosis showed the agreement of the phenolic compounds separated for a plant to study a number of standard phenolic compounds, Table (1) and Figure (1-2), which included:

1- 1- Hydrobenzoic The diagnosis confirmed the appearance of the hydrobenzoic phenolic compound in the leaves of *Populuseuphratica* (Tikrit Mosul) in the ethanolic extract with a retention time of (2.5-2.55) minutes and a concentration of (0.346-0.276) mg/g, respectively.
2- Galic acid The results of the chromatographic research showed the appearance of the compound Galic acid of the leaves of *Populuseuphratica* (Tikrit Mosul) in the ethanolic extract with a retention time of (5.89-5.84) minutes and a concentration of (0.412-0.350) mg / g, respectively.
3- Catchine The results indicated that the compound was present in the ethanolic extract with a retention time of (5.89-5.84) minutes and a concentration of (0.240-0.299) mg/g respectively.
4- Rutin The results showed the presence of the compound Rutin in the leaves of *Populuseuphratica* (Tikrit Mosul) in the ethanolic extract with a retention time of (6.58-6.55) minutes and a concentration of (0.273-0.191) mg/g, respectively.
5- Apgenine The results of the diagnosis confirmed the presence of the phenolic compound Apgenine in the ethanolic extract of the leaves of *Populuseuphratica* (Tikrit Mosul) with a retention time of (7.18-7.16) minutes and a concentration of (0.614-0.472) mg/g, respectively.
6- Ferulic acid The results of the research indicated the presence of the phenolic compound Ferulic acid in the extract of the leaves of *Populuseuphratica* (Tikrit - Mosul) with a retention time of (8.74 - 8.71) and a concentration of (0.197 - 0.144) mg / g, respectively.
7- Kaemeferol The results of separation and identification showed that the ethanolic extract of the leaves of *Populuseuphratica* (Tikrit - Mosul) contained the phenolic compound Kaemeferol with a retention

time of (9.35-9.34) minutes and a concentration of (0.434-0.033) mg/g, respectively.

8- Sinpic The results of the diagnosis showed that the ethanolic extract of the leaves of *Populuseuphratica* (Tikrit - Mosul) contained the presence of Sinpic compound with a retention time of (11.23-11.35) minutes and a concentration of (0.775-0.661) mg/g, respectively.

Table (1) showed the correspondence of the phenolic compounds of the leaves of *Populuseuphratica* (Tikrit - Mosul) in their presence and appearance in all of them in both plants, in addition to that, the results showed a slight superiority in the total concentration of phenolic compounds of *Populuseuphratica* growing in Tikrit over the same plant growing in the city of Mosul At a concentration of (3.35 and 2.367) mg/g, respectively. The reason may be due to the closeness of the environment in which the plant is grown, as well as environmental factors such as light, humidity, type of plant part, genetic factors, and the physiological state of the plant, which are close in producing phenolic compounds. As both of them grow on the outskirts of the Tigris River, therefore, it did not appear to change in the paths of secondary metabolism Metabolites Secandaires to produce phenolic compounds.

Phenolic compounds have an important role in the normal growth of the plant, as they work to protect it from external influences and defend it against infection with diseases, and they also play an important role in the biometabolism of the plant and regulating the action of auxins in it (Lattanzio, 2013).

Table (1): Phenolic compounds identified using HPLC technology for leaves of *Populuseuphratica*.

standard phenolic compounds	Standard retention time (min)	Phenolic compounds of *Populuseuphratica* (Tikrit)		Phenolic compounds of *Populuseuphratica* (Mosul)	
		retention time (min)	Concentration (mg/g)	retention time (min)	Concentration (mg/g)
Hydrobenzoic	**2.51**	**2.50**	**0.346**	**2.55**	**0.276**
Galic acid	**3.02**	**3.10**	**0.412**	**3.18**	**0.350**
Catchine	**5.90**	**5.89**	**0.299**	**5.84**	**0.240**
Rutin	**6.52**	**6.58**	**0.273**	**6.55**	**0.191**

Apigenine	**7.01**	**7.18**	**0.614**	**7.16**	**0.472**
Ferulic acid	**8.74**	**8.74**	**0.197**	**8.71**	**0.144**
Kaempferol	**9.35**	**9.35**	**0.434**	**9.34**	**0.033**
Sinapic	**11.25**	**11.23**	**0.775**	**11.35**	**0.661**
Total concentration			**3.35**		**2.367**

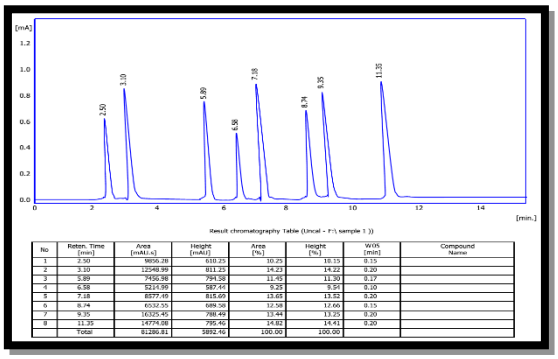

Figure 1: Isolated and HPLC-identified phenolic compounds from the leaves of Populuseuphratica (Tikrit)

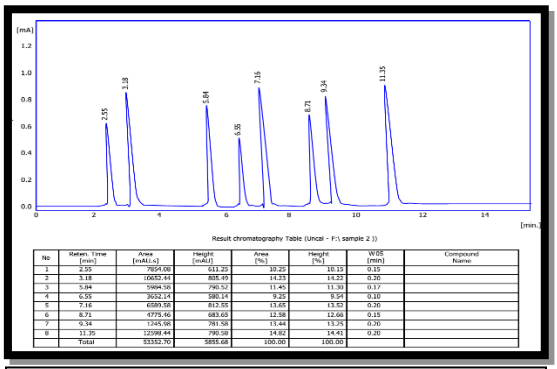

Figure 2: Isolated and HPLC-identified phenolic compounds from the leaves of Populuseuphratica (Mosul)

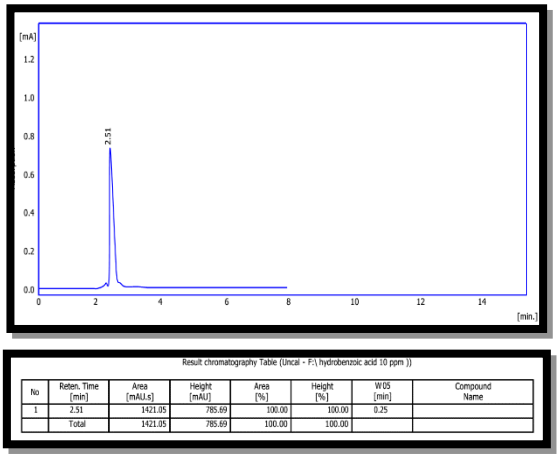

Figure 3: Standard curve of Hydrobenzoic Phenolic Compound by HPLC

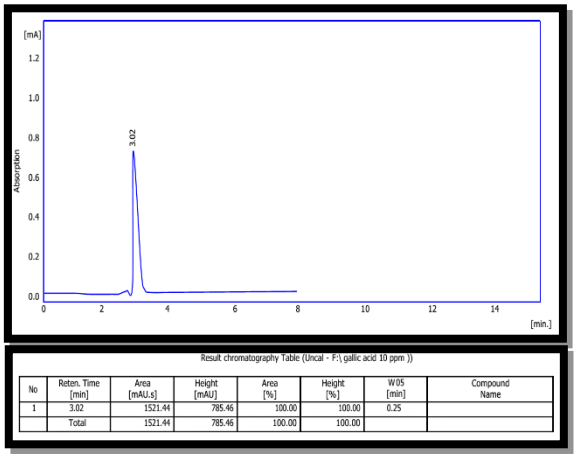

Figure (4): Standard curve of phenolic compound gallic acid by HPLC technique

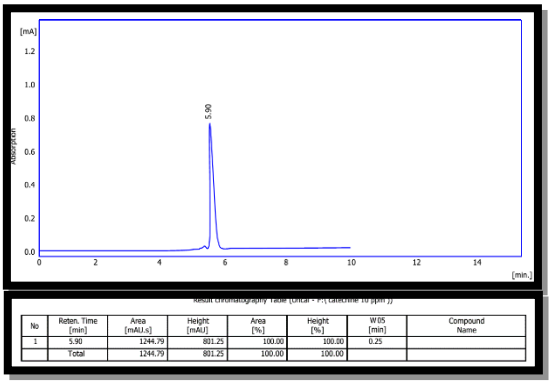

Figure (5): Standard curve of the phenolic compound Catechine by HPLC

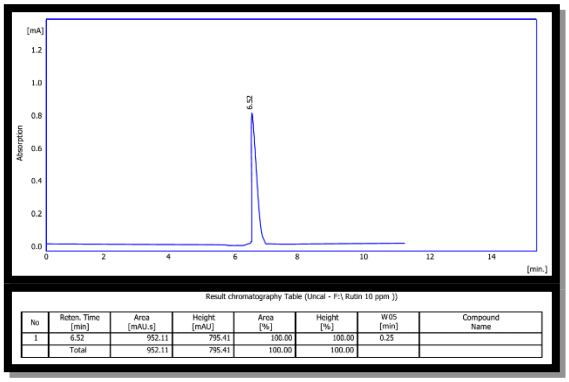

Figure (6): Standard curve of the phenolic compound Rutin by HPLC

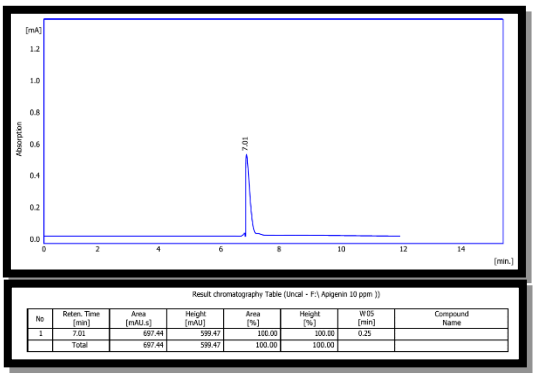

Figure 7: Standard curve of the phenolic compound apigenin by HPLC

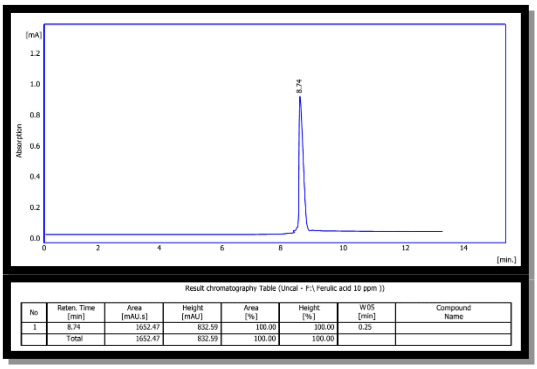

Figure 8: Standard curve of ferulic phenolic compound by HPLC

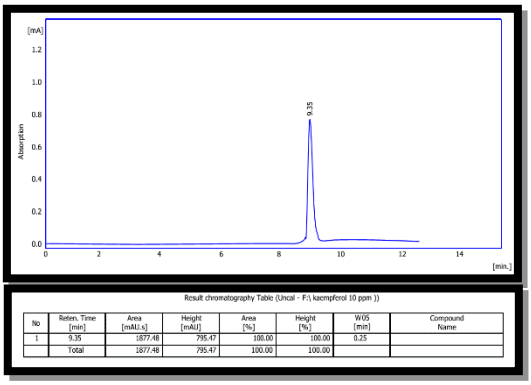

Figure (9): Standard curve of the phenolic compound Kaempferol by HPLC

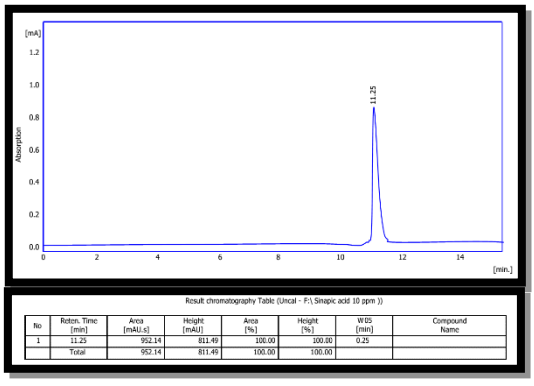

Figure (10): Standard curve of Sinapic phenolic compound by HPLC technique

2- A study of the antioxidant activity of the leaves of *Populuseuphratica*(Tikrit / Mosul):

The results of Table (4) indicated that the use of phenolic compounds and crude petroleum ether and alcoholic extracts of the study plant as antioxidants at different concentrations and compared with ascorbic acid as a standard sample all led to sniping of the free radical DPPH; The highest sniping percentage of the phenolic compounds of the Euphrates/Tikrit and Mosul plants was (95.7% and 90.2%), respectively, at a concentration of 500 µg/ml, superior in proportion to all other products, as well as superior to the standard sample, whose sniping percentage of free radicals was (85.6%). And at the same concentration.The table also indicates that there is a significant interaction between the natural antibiotics at different concentrations, but the lowest was the alcoholic extract / Tikrit and Mosul by (44.6% and 49.5%), respectively, at a concentration of 200 micrograms / ml, compared to the rest of the natural antibiotics and the standard sample, ascorbic, by (65.8%).) and at the same concentration. It is noted from the results that the increase in the inhibition process of free radicals increases exponentially with increasing concentration; This is because the higher the concentration, the greater the number of protons inhibiting the DPPH free radical.

It turns out that natural phenolic compounds are of great importance in capturing free radicals and reducing their danger. By giving a hydrogen atom to the free radical DPPH and stabilizing it, the reason for its strong effectiveness is due to the number of phenolic compounds in each plant and the content of each compound of hydroxyl groups, and this is in line with what was mentioned by Nagulendran et al. (2007) that the activity of phenolic antioxidants is mainly due It is essential to its redox properties, and its ability to donate hydrogen, thus inhibiting the reaction of free radical oxygen as well as stopping it from generating new free radicals, thus inhibiting the oxidation of fats, proteins and DNA, by inhibiting enzymes that contribute to the generation of free radicals (Blainski*et al*., 2013). These results are consistent with what Said (2002) indicated that the total content of phenols separated from the crude extracts of the leaves of Tamarixarticulata had a role in inhibiting the free radicals of DPPH. The

results of the study are also consistent with what was mentioned by Barnes (2003) that the Tunisian Tamarisk articulata extract has antioxidant activity according to the total phenolic content in the extract, and the results of the study are also consistent with what Upadhyay et al. (2010) found that antioxidant molecules such as polyphenols and flavonoids reduced the DPPH free radical capacity.

Moreover, the inhibition induced by the crude extracts is highly effective in preventing diseases caused by the overproduction of free radicals, and can also act as an important source of nutritional supplementation with the potential to maintain health protection (Mohammedi and Atik, 2011).

Table (2): the concentration and percentage of the standard sample, the compounds of fatty and phenolic acids, and the crude extracts of *Populuseuphratica* as antioxidants.

Concentration / Samples	200 µg/mL	300 µg/ml	400 µg/ml	500 µg/mL
Phenol Tikrit	78.5%c	83.5%b	88.3%ab	95.7%a
Phenol Mosul	73.3.%d	78.5%c	83%bc	90.2%ab
Tikrit Petroleum	55.1%h	58.7%g	63.4%f	67.5%e
Mosul etroleum	49.5%i	52.2%hi	62.1%f	66.8%e
Tikrit ethanol	44.6%ij	57.4%g	59.8%g	61.9%f
Mosul ethanol	46.4%ij	50.9%hi	57.1%g	60.9%fg
Standard sample (ascorbic acid)	65.8%ef	67.3%e	76%bc	85.6%ab

conclusions

The results showed a variation in the concentration of phenolic compounds of *Populuseuphratica* growing on the banks of the Tigris River and in two different regions, and that the highest concentration was of the plant growing in Tikrit.The results also indicated that phenolic compounds and crude extracts had an effect in inhibiting free radicals (DPPH) in different proportions, and that the higher the concentration, the greater the inhibition of free radicals.

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