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Study the Effect of Different Polar Extracts of Parsley Seeds (*Petroselinum Crispum*) and Its Phenolic Compounds as Antioxidants

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Abstract
In this study, four successive polar solvents (petroleum ether, acetone, ethanol 70% and distilled water) were used to obtain crude extracts from the seeds of the parsley plant (*Petroselinumcrispum*), as well as to separate the phenolic compounds of the plant. The HPLC diagnosis showed that the plant contains phenolic compounds (Campferol, Gallic acid, Quercetine, Rutin and Epigenine). The study proved that these plant extracts and phenolic compounds have an inhibitory effect on scavenging DPPH free radicals and that the best significant was for the ethanolic extract at a concentration of 500 and 400 µg at a concentration of 96.2% and 94.8%, respectively, compared to the control sample (ascorbic acid) at 85.9% and 85.4% At the same concentrations and respectively. As well as the superiority of the ethanolic extract over the rest of the plant extracts and phenolic compounds.And it appeared from the study that the higher the concentration of the antioxidant compounds, this led to an increase in the inhibition and stability of free radicals and preventing their damage.
Key words: *Petroselinumcrispum*, phenolic compounds, antioxidants

Introduction

Medicinal plants are considered to be of great importance in medicinal production against many diseases through their use in the form of raw extracts or the separation of their active components by manufacturing an antidrug that is more effective, safe and effective against diseases that arise from pathogens such as bacteria, fungi and viruses (Shrivastava and Leelavathi, 2010). According to the World Health Organization (WHO) World health organization, about 65-80% of the world's population, in developing countries rely mainly on traditional medicines as the primary means of health care, and the remaining 20-35% are mainly residents of industrializedrepublics also benefit circuitouslyof natural crops in health care (Ghourchian et al,2016).

Parsley (*Petroselinumcrispum*) is a biennial shrub belonging to the Apiaceae family It is native to the Mediterranean region and is now grown all over the world its height ranges between 60-30 cm, It has several stems that all grow from one root it is rounded, upright, and branched, and the leaves are compound, consisting of leaves of unequal size and lobed, and the tips of their blades are serrated and the flowers are compound, and the fruits are distinguished by their white color, and they are also tentative compounds (Mor. F *et al*., 2010). Parsley contains carbohydrates, fats, fiber, minerals and some vitamins, and its seeds contain high percentages of essential oils and also contains a number of fatty acids, including

omega-3, linolenic and palmitic acid, It also contains flavonoids, the most important of which are Apigenin, Lutolin, Kaempferol and Quercetin (Ozsoy *et al*., 2006). Parsley has antimicrobial effects (Ojala *et al*., 2000) and antioxidant effects and has a high ability to scavenge free radicals, among other biological effects (Potapovich and Kostyuk, 2003). Parsley is anti-inflammatory and has the potential to improve and enhance immunity, so it has been used in the treatment of urinary tract infection, inflammation of the kidneys, bladder and the prevention of kidney stones (Kareydiyyeh and Usta, 2002). The oil extracted from parsley is an antioxidant that helps stopinjury to cells produced by free extremists (Zhang *et al*., 2006). Research has proven that the ethanolic extract, as well as the aqueous extract of parsley, has a significant effect on stimulating antioxidant factors in the brain region of experimental mice (Maodaa *et al*., 2016).

The aim of the study was to use successive polar solvents to make various extracts of parsley seeds, separate its phenolic compounds and compare their effect on inhibiting free radicals of Dpph.

Materials and methods

Collecting and classifying plant seeds:

Parsley seeds (*Petroselinumcrispum*) were obtained from the local markets of the city of Mosul, and the seeds were classified in the Directorate of Medicinal Plants Development Project in Mosul Dam, which is affiliated to the Iraqi Ministry of Agriculture.

Plant classification:

Kingdom: Plantae.
Division: Angiosperms.
Class: Asterids.
Order: Apiales.
Family: Apiacea.
Genus: *Petroselinum.*
Species: *crispum.*
(Duke,2004).

Preparation of Plant Extracts Using Soxhlet Apparatus

Four solvents of various polarity were used within the extraction manner: petroleum ether, acetone, ethanol 70%, and distilled water.The removalprocedure was approved out according to the boiling point of all solvent. 25 gm of finely ground leaves and flowers powder were placed in the Batch in the Soxhlet device, with the addition of 500 ml of solvent. The removal continued at a rate of 7 hours per day until the solvent usage in the device became colorless, after which the second solvent was placed. Thus, the procedure was recurrent on the break of the thinners and on the same plant model, after which the extract was focused by means of a Rotary vacuum evaporator (RVE) at a temperature of 40C°.Then the bag was occupied and the plant physical was saturated in a beaker with 400 ml of purified water and located on a attractive stirrer to obtain the aqueous extract (Harborne,1984).

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Separation and purification of phenols from parsley seeds by acid hydrolysis:

mL of crude ethanol extract was taken and 25 mL of HCl (1N) acid was added to it. Then reflex was carried out at a temperature of 100 C° for one hour, then the solution was cooled and put in a separating funnel and 50 ml of Ethyl acetate was additional to it twice with good shaking, then, two upper layers (the organic layer) were obtained for ethyl acetate and a lower layer, the top layer was occupied and 3 gm of anhydrous magnesium sulfate MgSO¬4 was added to it. The sample was kept in an airtight, opaque vial and placed in the refrigerator until it was diagnosed by HPLC (Harborne,1998) and a study of its antioxidant activity.

Identification of active phenolic compounds using HPLC:

The process of diagnosing phenolic compounds was carried out in the laboratories of Al-Fadhel Foundation for Training and Scientific Services in the city of Mosul, using a Japanese HPLC device.

Study of the antioxidant activity of plant extracts and phenolic compounds:

Weigh 7.9 mg of DPPH (Diphenyl pecril hydrazine) and dissolve it in 100 mL of methanol to obtain 200 mM. Different concentrations of the study extracts were made 200, 300, 400 and 500 µg/ml, and ascorbic acid was usage as a control sample, and then 1 ml of DPPH solution was additional to each attentiveness as well as the control sample (Sahu *et al*.,2013 and Sumathy*et al*., 2013).

% = (AbB-AbS) /AbB * 100

AbB= Control sample absorbance.

AbS = Sample absorbance.

Results and discussion

Identification of phenolic compounds with high performance liquid chromatography (HPLC) for plants under study.

Analytical charts were obtained and theholding time of all sample was determined for the study sample compared to the sample time ofstandard,Campferol(3.052)min., Gallic acid(3.182)min., Quercetin(5.20)min., Rutin(5.62)min., Apigenin(7.02) min. table (1). The diagnosis showed the approval of phenolic compounds separated into the study plants for standard samples as shown in Figs.(1-6) table (1), which included:

Campferol: is a flavinoid compound, a cyclic structure containing four hydroxyl groups and its molecular formula $C_{15}H_{10}O_6$, which is a yellow crystalline solid, discovered in a spread of flora and plant-derived meals, acts as an antioxidant by means of lowering oxidative pressure and is effective against bacteria and inflammation. For its anti-cancer effectiveness (Calderon-Montano *et al*, 2011)In the parsley plant, the phenolic compound appeared with a retention time of (3.038) minutes. **Gallic acid:** (3,4,5-Trihydroxybenzoic acid) is a cyclic compound containing four hydroxyl groups, and its molecular formula $C_7H_6O_5$, has been found in a number of plants such as ammonia, hazelnuts, tea leaves, oak bark and other plants, a compound that possesses antioxidants (Kim. 2007).In the parsley plant, the phenolic compound appeared with a retention time of (3.299) minutes. **Quercetine** is a cyclic compound containing five groups of hydroxyl and its molecular formula $C_{15}H_{10}O_7$,

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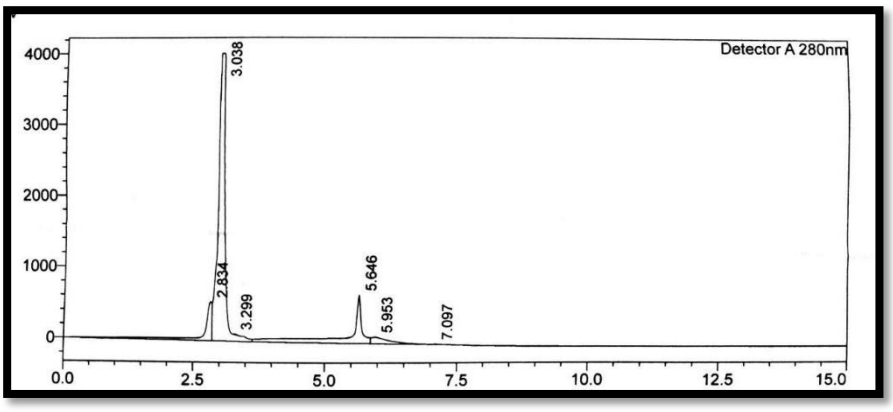
a plant color of the group of polyphenol and flavonoids contains four groups of hydroxyl, and enter into the manufacture of many drugs such as Fellodipine and Cisplatin and Digoxin and Estrogen, and is an antioxidant and catalyst for some vital tracks and inhibitor of Of enzymes.In the parsley plant, the phenolic compound appeared with a retention time of (5.646) minutes. **Rutin:** is one of the phenolic compounds and its molecular formula $C_{27}H_{30}O_{16}$. It is found in plant species such as violet, fruits and vegetables. It contributes to anti-bacterial, anti-oxidant, cancer, cardiovascular and neurotoxic properties, as well as lowering blood sugar level (Al-Dhabi *et al*, 2015).In the parsley plant, the phenolic compound appeared with a retention time of (5.953) minutes. **Epigenin**:is a form of crystalline solid and a hexagonal ring in which three groups of hydroxyl and its molecular formula $C_{15}H_{10}O_5$, a secondary metabolic product in the plant that is found in the leaves and which is a source of treatment for a number of diseases such as cardiovascular diseases and some cancers; Through its work as antioxidants (Cermak and Wolffra, 2006), as well as being an important vehicle in the treatment of Alzheimer's, also reduces the incidence of bacteria, viruses and fungi (Ross, 2002).In the parsley plant, the phenolic compound appeared with a retention time of (7.097 minutes.

Table (1) Phenolic compounds identified using HPLC techniquefrom*Petroselinum crispum* plant.

Standard phenolic compounds	Time of retention Standard (minutes)	Time of retentionSample (minutes)
Campferol	3.052	3.038
Gallic acid	3.182	3.299
Quercetine	5.20	5.646
Rutin	5.62	5.953
Epigenine	7.02	7.097



Standard curve for phenolic compounds of ***petrosselinum crispum***

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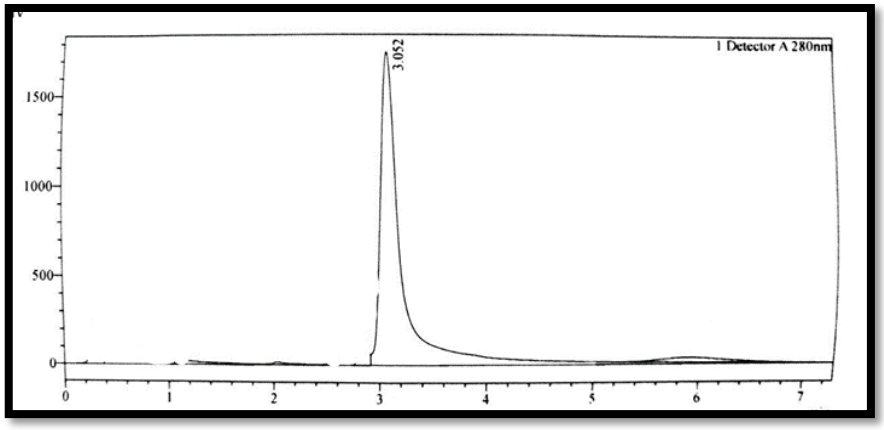
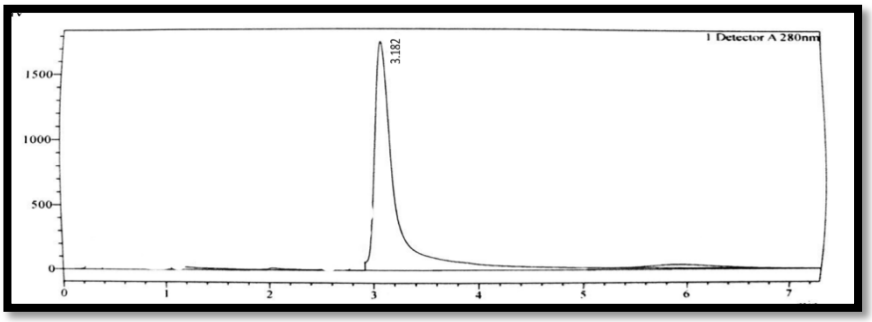
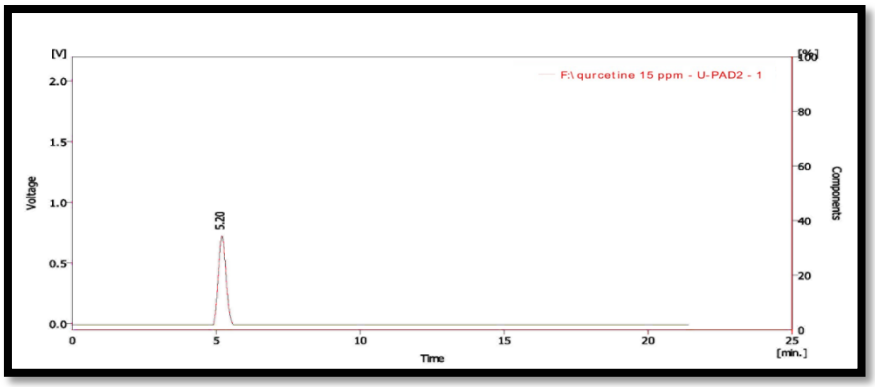


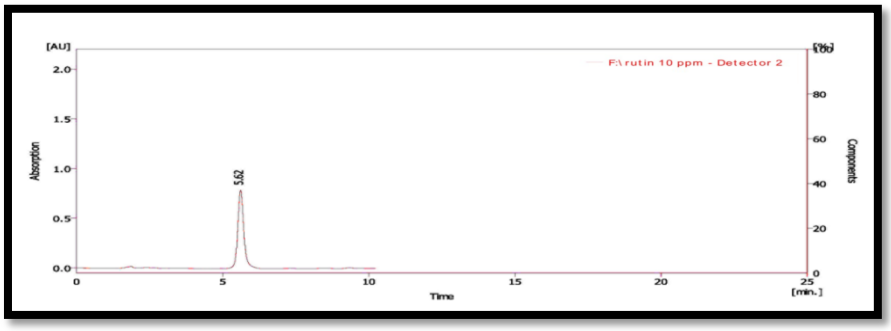
Standard curve for phenolic compound Camferol



Standard curve for phenolic compound Gallic acid



Standard curve for phenolic compound Quercetin



Standard curve for phenolic compound Rutin

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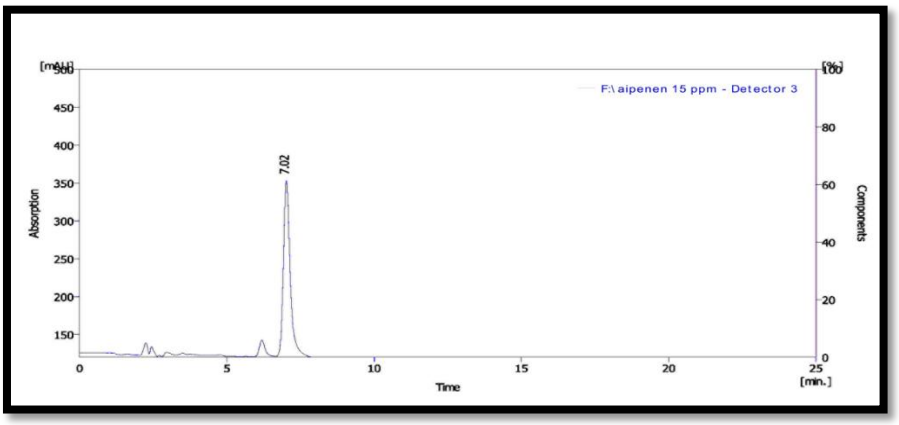

Standard curve for phenolic compound Epigenine

Study of the antioxidant efficacy of separated phenolic compounds and successive polar plant extracts:

The results of Table 2 designated that the use of phenolic mixesunglued from the seeds of the parsley plant and the successive polar crude extracts (petroleum ether, acetone, ethanol 70% and distilled water) as antioxidants at concentrations of 200, 300, 400 and 500 µg/ml and their comparison with ascorbic acid as a normal sample led to trigger DPPH free radical sniping, It was significantly higher in the inhibition of free radicals by the ethanolic extract at a attentiveness of 500 and 400 µg/ml, by 96.2% and 94.8%, correspondingly, likened to the switch sample ascorbic acid by 85.9% and 85.4%, respectively, in addition to the superiority of the ethanolic extract over the rest of the extracts at the same concentrations. . The results show a significant agreement in the inhibition of free radicals between the ethanol extract at a concentration of 300 µg/ml and the distilled water extract at a concentration of 500 µg/ml at a percentage of 91.6% and 90.3%, respectively.

The results confirm that there is a significant agreement in the inhibition of free radicals of phenolic compounds at a concentration of 400 µg/ml with acetone extract at a concentration of 300 µg/ml at 76.3% and 77.6%, respectively.

From the above, we find that the inhibition of free radicals increases with an increase in the concentration of phenolic compounds and plant extracts, and the reason for the superiority of raw plant extracts over phenolic compounds of the parsley plant is due to the abundance of the hydrogen proton ion in the crude extracts, which increases the inhibition of free radicals.

This is consistent with what Cosme *et al*. (2018) and Hasan *et al* (2019) reported that the activity of phenolic antioxidants is mainly due to their redox possessions and their aptitude to give hydrogen, thus constraining the allowedfundamental oxygen response as well as discontinuing the generation of novel free extremists, as well as inhibiting enzymes involved in the generation of free radicals (Blainski *et al*., 2013 and Gulcin, 2020).

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Table (2) Study of the antioxidant effect of crude extracts and phenolic compounds of *Petroselinum crispum*.

Concentration	Petroleum ether	Acetone	Ethanol 70%	distilled water	Phenolic compounds	Standard sample (ascorbic acid)
200µg/ml	77.3 jk	75.1 lm	89.5 cd	80.6 i	72.6 n	83.2 fgh
300µg/ml	81.8 hi	77.6 jk	91.6 b	83.6 fg	73.6 mn	84.8 ef
400µg/ml	82.4 gh	80.4 i	94.8 a	88.3 d	76.3 kl	85.4 e
500µg/ml	84.8 ef	83.3 fgh	96.2 a	90.3 bc	78.6 j	85.9 e

Conclusion:

The study proved the antioxidant activity of the successive polar plant extracts and their superiority over the control sample (ascorbic acid) and the phenolic compounds separated from the seeds of the parsley plant in inhibiting the free radicals of DPPH, and that the effect increases with the increase in the concentration of the extracts for the abundance of hydrogen ion that stabilizes the free radical and its association with the oxygen atom of the free radical.

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