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Polyphenol Profile, Antioxidant Potential, Vitamin C and Mineral Contents of Crude Parsley Leaves Extract (*Petroselinum crispum*) and Its Isolated Apigenin: A Comparative Study

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Abstract

Petroselium crispum or parsley is a typical culinary vegetable that is used as a flavouring and aromatic food additive. The aim of this research was to purify a major flavonoid present in parsley leaves, apigenin and evaluate the polyphenol, mineral and vitamin C contents in parsley leaves extract (PLE); in addition, the study compared antioxidant activities of ethanolic PLE with purified apigenin (AP), applying different non-enzymatic strategies using ascorbic acid (AA) as a reference. A complete screening for bioactive compounds in the ethanolic PLE was performed by HPLC analysis. The research outcomes indicated that purification of apigenin was successfully achieved by column chromatography with a high yield and purity; a single peak in the HPLC chromatogram confirmed this. The analysis revealed that contents of total phenolic and flavonoid in the ethanolic PLE were 75.91± 3.33 mg. g^{-1} TA Eq and 15.61 ± 0.18 mg. g^{-1} respectively; vitamin C is found to be 4.01 mg. g^{-1}. Our results indicated that among the trace minerals in parsley leaves, calcium and sodium documented the highest values; 14.13 mg. g^{-1} and 8.51 mg. g^{-1}, while copper and manganese had the lowest values; 0.02 mg. g^{-1} and 0.012 mg. g^{-1} respectively. Finally, our findings demonstrated that purified AP had superior potential than ethanolic PLE for reducing power, total antioxidant, nitric oxide and superoxide scavenging activities. In contrast, the ethanolic PLE exhibited greater antioxidant activity than AP towards suppressing the DPPH and hydroxyl radicals.

Keywords: Antioxidants, Apigenin, Chromatography, Parsley, Vitamin C.

Introduction

Medicinal plants with therapeutic and medicinal properties have a variety of pharmacological effects on human and animal health; therefore, it is regarded as essential for the existence of life[1, 2]. The World Health Organization (WHO) has assessed roughly 80% of individuals in developing nations relies on natural medication to address their healthcare needs. Most of plants have effective functional ingredients against diseases but some plants are too rich with these chemicals and are favored for the treatment of ailment[3]. Nowadays, pharmacological plants are acknowledged as an effective source of newly discovered compounds of therapeutic value. When compared to modern medicine, herbal medicines are still widely used, especially the former fail to be free from adverse effects and fail to cure some chronic conditions[4, 5]. *Petroselium crispum* (Mill.) or parsley, as a one of an important medicinal plant, belong to the family *Apiaceae* or *Umbelliferae*. It is produced in vegetable garden, which is frequently added to food for flavoring and aromatic additive[6]. Generally, parsley contains numerous natural bioactive

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components. For instance, flavonoids and polyacetylenes are main components of parsley[7]. Furthermore, it contains a number of crucial nutrients, including vitamins "C, A, B, K, tocopherols" and ergosterol. Minerals (iron, zinc, magnesium, calcium, sodium, potassium, phosphorus), furanocoumarins, carotenoids and fatty acids (linolenic and palmitic acid), are abundant in parsley[7–9]. Interestingly, *P. crispum* is being suggested for various therapeutic purposes in everyday medicine[10]. In this context, the effective diuretic and antispasmodic activities of parsley was investigated. Besides, the bioactive constituents in parsley are also associated with the variety of its beneficial effects, such as hepatoprotective, anti-diabetic, and analgesic characteristics. Remarkably, within numerous medical contexts, parsley leaves have demonstrated their therapeutic efficacy as medicinal herbs with anti-inflammatory, anti-anemic, anti-hyperlipidemic, anti-cancer, anti-hypertensive, and anticoagulant effects, which is mainly related to their component diversity[8]. Moreover, parsley leaves have been linked to a variety of formulations and bioactive compounds, including those for the treatment of immunological disorders, diabetes, antibacterial activities, as well as in the treatment of stroke, chronic bronchitis, dyspepsia, allergies[7, 11, 12]. Along with the beneficial roles of parsley, the kaempferol, quercetin, and other flavonols, as well as luteolin and apigenin (glycosylated flavones) are the two main flavonoids type compounds present in parsley. These classes of compound possess antioxidant properties and high ability for neutralization of free radicals. Thereby, exerts its sanogenous effects in the prevention of ailments linked with oxidative stress[13, 14].

Apigenin is a kind of secondary metabolites that is considered as one of main flavonoids found in large quantities in a set of natural provenances, for instance fruits and vegetables. Apigenin is identified as 4′, 5, 7-trihydroxy flavone, possessing a chemical composition of $C_{15}H_{10}O_5$[15]. Apigenin is particularly abundant in celery, parsley, chamomile, vine spinach, oregano and artichokes. The richest source of apigenin among these is dried parsley[16]. Interestingly, numerous biological effects including their anti-inflammatory, antioxidant, anticancer, antigenotoxic, anti-allergic, neuroprotective, cardioprotective and antimicrobial have been linked to flavones and its synthetic derivative, apigenin[17]. The present study basically aimed to: (i) purify the major flavonoid, apigenin from parsley leaves using column and thin-layer chromatography techniques; (ii) achieve a complete screening (qualitatively and quantitatively) for the main ingredients found in parsley leaves using HPLC chromatography; (iii) compare the antioxidant activity of crude parsley extract with its purified compound (apigenin) by applying different non-enzymatic methods; (iv) profile polyphenol (total phenolics and flavonoids), vitamin C and mineral contents in ethanolic PLE.

Materials and Methods

Plant Collection

Fresh parsley leaves were gathered from a local market in Dohuk city, Kurdistan region, Iraq. Parsley was first cleaned using tap water and followed by running demineralized water and then brought into the air in a dark room to dry. The dried parsley leaves were then crushed by a mill and stored in dark color containers at -20 °C until the day of use.

Methods

Preparation of Parsley Leaves Extracts (Crude extract)

About 20 g dried powder of parsley leaves were placed in a conical flask wrapped with aluminum foil containing 250 mL of 70% ethanol and soaked overnight, followed by stirring at room temperature for 24 hrs. using magnetic stirrer, after that the extract was filtrated by Buchner under vacuum utilizing filter paper Whatman No.1. After that, a rotary evaporator is utilized to concentrate purify solution at 45°C until it was completely dry. The parsley powder kept in a dark container and frozen until using for subsequent experiments.

Qualitative and Quantitative Analysis of Bioactive Compounds in Parsley Leaves Using HPLC Chromatography

In the next set of experiments, the HPLC analysis was carried out for quantification of individual phenolic compounds (crude extract and purified AP), in order to determine the bioactive constituents and purity of isolated AP respectively. The study was carried out using reversed phase HPLC and a Zorbax Eclipse Plus-C18-OSD (25cm/4.6mm) column with a SYKAMN HPLC chromatographic system outfitted with a UV detector. The column was heated at 30ºC in temperature and injected volume (100 µL) of samples and standards automatically took place employing autosampler. The spectrum was monitored at 280 nm[18]. The identity of each peak in

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the HPLC chromatography's of injected PLE and purified AP were compared with the peaks of authentic standard. Furthermore, the amount of each peak was also determined using the following formula:

$$\text{Conc. (ppm)} = \frac{\text{Area (sample)} * \text{standard conc.}}{\text{Area (Standard)}} * \frac{\text{NO.of dilution}}{\text{sample weight}}$$

Isolating Flavonoids from Parsley Leaves

The isolation of flavonoids from parsley leaves was conducted utilizing the methodology of[19], with certain changes. In this method, green thick solution of total flavonoids was obtained and used for subsequent apigenin separation.

Separation of Apigenin from Flavonoids by Chromatography Techniques

In order to separate apigenin from total flavonoids, two chromatography techniques were used. Column chromatography was applied firstly to isolate the bioactive compound, apigenin (AP) from flavonoid[20]. In this particular approach, a column measuring 60x2 cm was utilized, which was packed with slurry consisting of silica gel-H with a mesh size ranging from 60-120 μ (Hi media, Mumbai). After that, the fractions were eluted using a gradient solvent system consisting; n-butanol, water, and acetic acid (12: 2: 1 v/v/v). For detection AP in elutes, thin-layer chromatography technique (TLC) was performed for each fraction as primary detection of apigenin and compared the retention factor (R_f) of each spot with R_f value of standard apigenin[21].

Analysis of Polyphenol Profile and Vitamin C in Parsley Leaves Extract

Each test in this section was performed in triplicates as well as for standard solutions.

Estimation of Entire Phenolic Constituents

A total amount of phenolic constituents in PLE were measured via Folin-Ciocalteu method. The total phenolic concentration in this method was estimated depending on the tannic acid standard curve[22].

Estimation of Entire Flavonoid Constituents

The modified aluminum chloride method introduced by[23], was applied for quantification of total flavonoids in PLE. Different concentrations of quercetin were used also for preparation of quercetin standard curve, which was used to determine quercetin equivalents in sample.

Determination of Ascorbic Acid (Vitamin C) Contents

Ascorbic acid concentration in PLE assessed spectrophotometrically utilizing a method conducted by[24] with certain changes. In this approach, a standard curve was plotted using reference compound; AA to determine its concentration in the extract. The calculation the quantity of AA equivalents for each gram of extract (mg.AAE.g-1) was performed utilizing the formulation that reported by[25].

$V = C \times V/M$

V= AA content (mg.AAE.g^{-1}), C= Ascorbic acid Concentration extracted from standard curve (mg.ml^{-1}), V= Volume of PLE (ml) and M= PLE weight (g)

Trace Minerals Analysis

The concentrations of trace minerals (Ca, Na, Mg, Cu, Mn and Fe) in parsley leaves were determined using a wet acid digestion method and the analysis was carried out by Atomic Absorption Spectroscopy (AAS)[26].

Antioxidant Activity Assessments of Parsley Leaves Extract and Purified Apigenin

The antioxidant activity of PLE and its purified AP *in vitro* was carried out using different methods. A stock solution (1mg/ml) of each sample and standard was prepared and dissolved in distilled water (D.W.) and subsequently used for antioxidant analysis. Each test was done on three times as well as for standard solutions.

Reducing Power Assay

A reducing power of PLE and its purified AP has been estimated by the procedure described by[27], and ascorbic acid functioned as a basis for comparison in this test.

Comprehensive Antioxidant Activity Assay

In this test, a spectroscopic phosphomolybdenum procedure was done for determination of comprehensive antioxidant capacities of PLE and purified AP as described by[28]. Ascorbic acid functioned also as a basis for comparison in this assay.

Nitric Oxide Suppressing Assay

In order to evaluate the suppression of nitric oxide by PLE and purified AP, the approach introduced by[29]

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was used. In this assay, vitamin C was utilized as standard antioxidant for comparison. Further, control samples were also prepared by the same procedure without plant samples. The proportion (%) of nitric oxide radicals that are inhibited was established using the formula: % Inhibition of NO radical = [(Ao – A_1) /Ao]* 100
The absorptions of control and sample are referred as Ao and A1, respectively.

Hydroxyl Radical Scavenging Assay
The capability of PLE and purified AP to inhibit hydroxyl radicals was verified by the procedure done by[30]. The same context as previously mentioned for nitric oxide was applied for calculation of the % of inhibition.

Ferrous Ion Chelating Assay
The capacity of PLE and its bioactive component, AP to sequester the ferreous ions (Fe^{+2}) was estimated by the method of[31] , which is outlined by[32]. Vitamin C standard solution was used in this assay for comparison. Ferrous ion chelation capacity was calculated as follow: Bound Fe^{2+} (%) = [(Ac - As) /Ac] *100

Superoxide Anion Suppressing Analysis
In this experiment, the method of[33] was used for estimation the potential of PLE as well as AP in suppressing of superoxide anion radicals. In a similar fashion to the calculation of nitric oxide, the percentage of superoxide anion suppressing activity is determined.
Activity to scavenge superoxide anion radicals (%) = [(A_c-A_s)/A_c] × 100
Absorptions of control and sample are denoted by Ac and As, respectively.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Suppressing Assay
The activity of the specimens to suppress DPPH radicals was assessed as stated by[34]. In this assay, the blank and control solutions were also prepared and the percentage of suppressing activity (AA%) was calculated in accordance with[35].
AA%=100- [($O.D_{sample}$ – $O.D_{blank}$) /$O.D_{control}$] ×100
O.D = Optical density

Data Analysis
Results from each experiment were given as mean ± SE (standard error) values, which were carried out in triplicate. Duncan's multiple range analysis was used to separate the means from one another and an analysis of variance was used to assess the results ($P<0.05$). SPSS was used to analyze the outcomes[36].

Results and Discussion

Qualitative and Quantitative Analysis of PLE by HPLC
HPLC technique was applied in the present study for qualitative investigation of crude PLE as shown in HPLC chromatogram Fig. 1. The polyphenolic compounds found in crude alcoholic PLE berries compared with retention time of the standard phenolic compounds. The analysis revealed that the major bioactive components found in parsley leaves are apigenin, luteolin, p-Coumaric acid, epicatechin, kaempferol and quercetin. The main compounds and their corresponding concentration are presented in Table 1. As documented in Table 1, the highest polyphenol compound in PLE was epicatechin (45.99 ppm) followed by apigenin (41.56 ppm), while the other bioactive compounds were in lower concentrations. Due to the presence of a wide spectrum of active substances as confirmed by HPLC analysis, parsley therefore may be used as a medicinal plant with a diversity of antihypertensive and anticoagulant anti-inflammatory, anti-anemic, anti-hyperlipidemic and anticancer activities[8, 37]. Furthermore, the high number of flavonoids with antioxidant power and its capacity to suppress free radicals possibly the cause of parsley's sanogenous effects [13, 38].

Table 1. HPLC results for the qualitative and quantitative analysis (ppm) of crude PLE compared to standard phenolic and flavonoid compounds.

Standard compound (Rt/min.)	Alcoholic PLE (Rt/min.)	Concentration (ppm)
Apigenin, (7.22)	7.12	41.56
Luteolin, (7.72)	7.79	20.66
p-Coumaric acid, (8.02)	8.26	20.58
Epicatechin, (8.95)	8.89	45.99
Kaempferol, (9.09)	9.00	33.56
Quercetin, (13.92)	13.89	31.78

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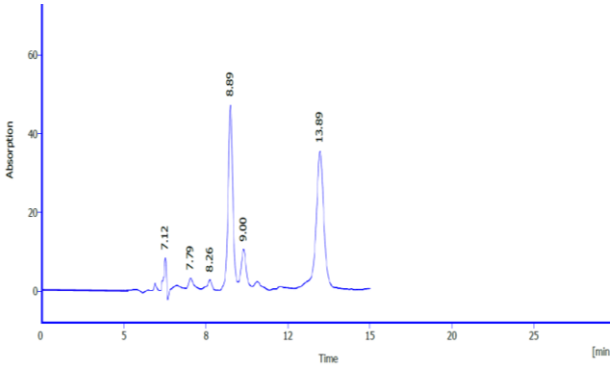


Figure 1. HPLC Chromatograms for main isolated bioactive compounds and their retention times accordingly (apigenin:7.12 min., Luteolin:7.79 min., p-Coumaric acid :8.26min., epicatechin:8.89min., kaempferol:9.00min and quercetin:13.89min).

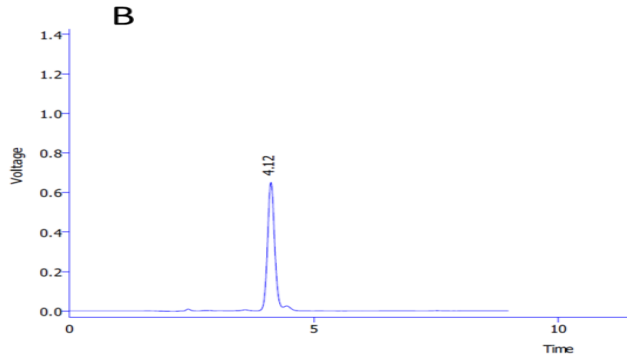
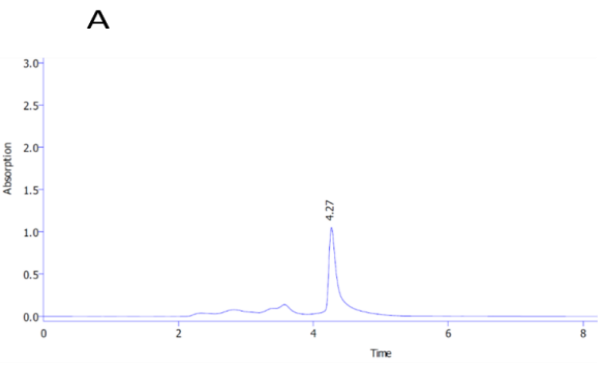


Figure 2. HPLC Chromatogram for A) Isolated apigenin from crude parsley leaves, B) HPLC chromatogram for the authentic apigenin injection.

Isolation of Flavonoids from Parsley Leaves

Due to its practicality, affordability, and availability in different stationary phases, both column chromatography and thin-layer chromatography (TLC) are still widely used[39]. However, the parsley leaf extract was used in the current work to isolate apigenin as mentioned in section 2.4 and 2.5. The results showed that the isolation of apigenin was successfully achieved by column chromatography with a high yield (1.5g) and purity. Furthermore, thin-layer chromatography was applied individually on all fractions obtained from the column with the same solvent system to evaluate the R_f value on TLC plate and compare it with standard apigenin. The R_f for the isolated AP was 0.86, which is close to the R_f of standard AP (0.84). As well, the HPLC analysis Figs. 2A and B for purified AP showed the characteristic single peak in HPLC chromatogram at 4.27 min. retention times (Rt) compared with standard apigenin compound (4.12 min). These data provided further verification of the purity of isolated aapigenin using chromatographic strategies.



Polyphenol Profile and Vitamin C Contents in Parsley Leaves Extract

Total Phenol and Flavonoids Contents

In view of the current study, the quantity of total phenolic components in ethanolic PLE was 75.91± 3.33 mg/g (TA Eq). In contrast, our data showed that the amount of total flavonoid in PLE was 15.61 ± 0.18 mg/g. Comparing our findings to a previous study of[40], the total phenolic contents were lower (21.63 ± 1.04 mg/g), while the total flavonoid was comparable to our result, with the study of[11] demonstrated that both contents were higher, possibly due to usage of different extraction conditions. Interestingly, the valuable amount of total phenolic and flavonoid constituents in parsley leaves may reflect their radical scavenging activity as well as potential chemo-preventive/ protective roles in maintaining human health and illness prevention[41]. Thereby, it possess a variety of biological activities, including organ protection, hypoglycemic, lipid-lowering and anti-inflammatory effects[42, 43].

Ascorbic Acid Contents

Vitamin C contents in ethanolic PLE were 4.01± 0.33 mg/g. According to several studies[44, 45], vitamin C is the most significant biological component of parsley leaves and one of excellent supply of this vitamin of the daily requirements[46, 47]. In this context, vitamin C is very essential for modulating the immune system and able to protect vital biomolecules from destruction resulting from oxidants, toxins, and pollutants by donating electrons because it is a potent antioxidant[48]. Furthermore, vitamin C also involved in the creation of amidated peptides including vasopressin and catecholamine hormones[49] and

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collagen synthesis[50]. Strikingly, vitamin C contents in parsley leaves may contribute to all of aforementioned health benefits for human.

Trace Minerals

The data shown in Table 2 indicate to the mineral analysis of parsley leaves. The analysis showed that among all minerals detected in PLE, calcium was highest (14.13 mg/g), followed by sodium (8.51 mg/g). A study by[51] revealed that, the nutritional value of parsley was1986 ±40 µg. g^{-1} of sodium. As shown in Table 2, magnesium was present in a reasonable amount (2.14 ± 0.01 mg/g). In contrast to[52], which indicated that Mg in parsley leaves was below the detection limit, possibly due to unsuccessful or un-sufficient digestion strategy. In our study, copper (Cu) and manganese (Mn) had low concentrations in parsley leaves, about 0.012 and 0.02 mg/g, respectively. Finally, the iron content of the parsley leaves under examination was 0.22 mg/gm. Extensive evidence exists about the usefulness of minerals for handling of common health problems. In this context, the mineral levels in blood and cellular fluids are of great importance for bones health and cardiac muscle[53, 54], nutrition[55], as enzyme cofactors[56], regulation of neuronal activity, immunological response, energy generation, and antioxidant defense[57, 58], and development of connective tissues[26]. Therefore, parsley may serve as source of trace minerals to support for normal bodily functions.

Table 2. Trace Elements Concentrations of PLE (mg/g).

Elements	Amount (mg/g) dry weight
Calcium	14.13 ± 0.05
Sodium	8.51 ± 0.01
Magnesium	2.14 ± 0.01
Copper	0.02 ± 0.01
Manganese	0.012 ± 0.01
Iron	0.22 ± 0.09

Antioxidant Activity of Parsley Leaves and Purified Apigenin

The parameters measuring antioxidant activity were presented as mean ± S.E of three replicates.

Reducing Power of PLE and Purified AP

In this study, the ability of PLE and AP to convert ferric ion to ferreous ion used as a measure for reductive power of both compounds compared to reference compound (AA), which is a well-known powerful reducer. As displayed in Table 3, both compounds showed the powerful of reducing properties. This finding may suggest that the reducing power of parsley leaves might be due to presence of the biologically active substances such as antioxidant polyphenols, minerals, essential oils, and pigments.

Our data showed also that with raising the concentrations of PLE and purified AP, the reducing power increased accordingly. This observation may be because of increasing the antioxidants' capacity to donate electrons. Furthermore, apigenin seems to have stronger reducing activity than PLE, and this difference was statistically significant. The reason can be attributed to the chemical nature of apigenin, which is trihydroxy phenols that have a greater number of hydroxyl groups are joined to ring, these group act as more powerful reducing agent and considered as good proton donors[59]. Research published by[13] showed that parsley roots have reducing activity as well.

Table 3. Dose-dependent Reducing Power of PLE and Purified AP.

concentrations (ppm)	PLE	AP	AA
23.53	0.02^{a} ± 0.01	0.07^{ab} ± 0.01	1.85^{c} ± 0.01
70.59	0.07^{ab} ± 0.01	0.09^{ab} ± 0.01	2.84^{d} ± 0.01
117.64	0.13^{c} ± 0.01	0.18^{b} ± 0.01	2.94^{d} ± 0.1

The numbers in the identical column and raw accompanied by similar alphabetical characters, mean no significant changes (P > 0.05).

Comprehensive Antioxidant Activity of PLE and Purified AP

Table 4 displays the total antioxidant activity of the PLE, AP in comparison to AA. The overall antioxidant capacity of PLE and AP are likely due to the flavonoids, which are widely distributed in parsley leaves. These substances may primarily act as antioxidants, stabilizing free radicals by resonance or through oxidation-reduction processes as reducing agents[60]. Hence, it provides a potent intracellular defense against "reactive oxygen species (ROS) and oxidative stress" related disease.

The findings revealed that purified AP has higher overall antioxidant properties than PLE. This may be explained by the high potential of AP for being a proton donor and hence potent antioxidant compound compared with PLE [61]. Our results

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concerning the overall antioxidant capacity of PLE and AP were dose-dependent. Compared with vitamin C, significant changes were noticed ($p<0.05$) in total antioxidant capacity when both, PLE and AP compared with AA, which was also dose dependent. Similar trend of total antioxidant activity of PLE was noticed from study done by[11], using different solvent system.

Table 4. Comprehensive Antioxidant Activity of PLE and Purified AP.

concentrations (ppm)	PLE	AP	AA
30.3	$0.44^{a} \pm 0.01$	$0.48^{ab} \pm 0.04$	$1.84^{e} \pm 0.07$
60.6	$0.54^{b} \pm 0.04$	$0.63^{C} \pm 0.02$	$2.2^{f} \pm 0.08$
90.9	$0.61^{c} \pm 0.01$	$0.72^{d} \pm 0.01$	$2.54^{g} \pm 0.07$

The numbers in the identical column and raw accompanied by similar alphabetical characters, mean no significant changes ($P > 0.05$).

Scavenging of Nitric Oxide (NO) by PLE and Purified AP

Based on the data given in Table 5, the *in vitro* NO scavenging assay suggests that both, PLE and purified AP have the capacity to scavenge NO radicals as displayed by high percentage (%) of inhibition at various concentrations compared to vitamin C. This scavenging activity against NO could be explained by the fact that the secondary metabolites in parsley are responsible for the antioxidant properties. As well, the results illustrated in Table 5 showed that AP exhibited a higher and significant degree of nitric oxide scavenging activity ($p<0.05$) in comparison to PLE. Indeed, the impact of phytochemicals on NO suppressing may be of great interest for their therapeutic applications. In this regards, multiple evidence showed that an activation of pro-inflammatory mediators linked to both acute and chronic inflammation could result in tissue damage and NO overproduction[62]. As a result, NO inhibitory medications may be useful for treating the inflammatory response[63].

Table 5. Nitric Oxide Suppressing Activity of PLE and Purified AP (% Inhibition).

concentrations (ppm)	PLE	AP	AA
66.67	$50.22^{abc} \pm 10.04$	$64.15^{de} \pm 4.3$	$59.46^{bcde} \pm 6.72$
200	$43.59^{a} \pm 8.4$	$49.93^{ab} \pm 3.8$	$52.73^{abcd} \pm 4.55$
333.33	$62.94^{de} \pm 7.35$	$63.86^{de} \pm 10.55$	$81.2^{f} \pm 7.25$

The numbers in the identical column and raw accompanied by similar alphabetical characters, mean no significant changes ($P > 0.05$).

Hydroxyl Radical Scavenging Activity of PLE and Purified AP

Table 6 demonstrates the impact of PLE and purified AP on the suppressing of OH radicals. The results of present investigation have substantiated the inhibitory influence of PLE and purified AP on generation of OH radicals that is relied on the dosage administered. This observation maybe related to secondary metabolites presence in PLE especially kaempferol, which is known to possess a higher ability to scavenge OH radicals[64]. On the other hand, the inhibitory effect of AP is likely due to the presence of high degree of hydroxylation on their aromatic rings, which may suggest its higher contribution in the inhibition of OH radicals' formation [61]. Moreover, the results in Table 6 showed also that PLE was more effective than AP in scavenging of OH radicals, and both possess lower scavenging activity compared to ascorbic acid ($p<0.05$). Similar findings have been established regarding the ability of numerous plant extracts and flavonoids, such as naringin and mangiferin in hydroxyl free radicals' neutralization[65, 66]. Along with the monitoring of the obtained data, plant extracts can interact with OH radical to reduce their harmful effects to cell and its components. Therefore, the assessment of OH radical suppressing activity of vegetable matrices holds significant importance from biological perspective, as it aids in the identification of efficacious extracts that are suitable for pharmacological purposes[67].

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Table 6. Hydroxyl Radicals Scavenging Activity of PLE and Purified AP (% Inhibition).

concentrations (ppm)	PLE	AP	AA
16.66	$28.69^{ab} \pm 2.01$	$21.56^{a} \pm 2.74$	$27.2^{ab} \pm 2.5$
25	$52.34^{def} \pm 4.47$	$36.98^{c} \pm 3.73$	$54.68^{ef} \pm 7.9$
33.33	$69.29^{hi} \pm 3.14$	$63.03^{gh} \pm 3.41$	$82.42^{j} \pm 1.52$

The numbers in the identical column and raw accompanied by similar alphabetical characters, mean no significant changes (P > 0.05).

Ferrous Ion Chelating Ability of PLE and Purified AP

Fe^{+2} binding ability (%) of the assays reviewed in Table 7. According to our findings, both, PLE and AP have iron chelation effect and so, preventing the reaction between the reagent and the ferrous complex. This effect may be consistent to the excessive antioxidant contents of parsley leaves. Where these antioxidants functioned as sequestering tools by decreasing the potential of oxidation-reduction reactions and stabilizing the metal ion's oxidized state[68, 69]. Besides, the studies introduced by[61] documented the efficacy of apigenin as a robust antioxidant and chelator of redox active metals both in vitro and in vivo. The data obtained demonstrated that activity of metal chelator of PLE and AP exhibited a gradual increase with increasing concentrations, thereby demonstrating the capacity of the plant extract components to form complexes with ferrous ions. Furthermore, our finding indicates that the both PLE and AP have a stronger tendency for chelating iron than ascorbic acid ($p<0.05$), while, no statistic significant were noted in this effect between PLE and AP. Our results are compared to those of[70] who used one concentration and different solvent system and[13] who used root juice of parsley in contrast to our study using 70% ethanol.

Table 7. Fe^{+2} Chelating Ability (%) of PLE and Purified AP.

concentrations (ppm)	PLE	AP	AA
17.42	$37.8^{bc} \pm 0.88$	$37.88^{bc} \pm 1.76$	$26.67^{a} \pm 4.58$
34.84	$46.15^{cd} \pm 1.01$	$44.69^{cd} \pm 2.83$	$44.61^{cd} \pm 6.59$
52.3	$65.27^{e} \pm 1.54$	$69.23^{e} \pm 2.81$	$63.96^{e} \pm 9.5$

The numbers in the identical column and raw accompanied by similar alphabetical characters, mean no significant changes (P > 0.05).

Scavenging of Superoxide Anion by PLE and Purified AP

Table 8 summarizes the percentage of inhibition of superoxide radical by PLE and purified AP comparing to ascorbic acid as standards. The results clearly indicated the contribution of both, PLE and AP in the scavenging of superoxide radicals in a dose-dependent way. While, the purified AP has stronger scavenging effect than PLE with a significant difference ($p<0.05$) and both have a weaker inhibition than ascorbic acid. The reason behind this scavenging effect might be due to the parsley contents of kaempferol, quercetin as well as glycosylated flavones type compounds, which all possesses antioxidant properties or to the ability of AP to bind with superoxide anion[13]. Nevertheless, research done by[71] investigated the effect of parsley leaves for enhancement of antioxidant enzymes activities; specifically, erythrocyte glutathione reductase (GR) as well as superoxide dismutase (SOD) and discovered that apigenin is mostly accountable for this effect. At pathological level, the super oxide anion considered harmful, as they can induce apoptosis, necrosis, and autophagic cell death[72]. Thus, our data concerning superoxide scavenging by PLE and AP may shed a light on the possible therapeutic uses of these compounds against several pathological events.

Table 8. Superoxide Anion Radical Scavenging Activity of PLE and Purified AP (% Inhibition).

concentrations (ppm)	PLE	AP	AA
50	$33.02^{a} \pm 1.1$	$44.77^{c} \pm 2.7$	$52.7^{d} \pm 2.82$
100	$38.85^{b} \pm 2.83$	$51^{d} \pm 0.67$	$67.7^{e} \pm 4.62$
200	$49.72^{cd} \pm 0.93$	$65.21^{e} \pm 1.01$	$82.39^{f} \pm 3.26$

The numbers in the identical column and raw accompanied by similar alphabetical characters, mean no significant changes (P > 0.05).

Suppressing of DPPH Radicals by PLE and Purified AP

The DPPH free radical capturing assay is an effective technique used in this research to assess scavenging potential of both PLE and purified AP compared to ascorbic acid. In the current work, the reduction in

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Baghdad Science Journal

the optical density was induced by both compounds, and thus, serves as a measure of the effectiveness of both specimens in the removal of harmful radicals. Our data showed also that increasing the concentration of PLE and AP in the reaction mixture accompanied with the elevation of the scavenging activity (dose dependent) Table 9. This effect might be ascribed to the phytochemical nature of specimens, which facilitated their ability to perform as hydrogen donors and quenchers of singlet oxygen. The mechanism of apigenin action includes: chelating of metals; scavenging of free radicals and amplification enzymatic and non- enzymatic antioxidants [61]. Data established in Table 9 indicated that PLE has stronger and significant extent to reduce DPPH when compared with purified AP ($P < 0.05$). Apparently, this might be associated with the need to a variety of antioxidant species for scavenging of DPPH radicals rather than AP alone, compared with ascorbic acid, PLE and AP showed lessened abilities for changing the stable DPPH radicals to DPPH-H form. Our results compared to studies of[73, 74] which also discovered the same finding but in methanolic and aqueous extract of parsley leaves.

Table 9. The Percentage of DPPH Radicals Inhibition (%) by PLE and Purified AP.

concentrations (ppm)	PLE	AP	AA
36.49	$5.18^{b} \pm 0.55$	$1.52^{a} \pm 0.7$	$97.56^{g} \pm 0.7$
72.99	$14.61^{d} \pm 0.46$	$11.11^{c} \pm 2.3$	$98.33^{g} \pm 0.35$
145.98	$40.94^{f} \pm 1.62$	$22.91^{e} \pm 1.73$	$98.71^{g} \pm 0.86$

The numbers in the identical column and raw accompanied by similar alphabetical characters, mean no significant changes ($P > 0.05$).

Conclusion

The findings of this research concluded that parsley leaves are considered as an excellent source of antioxidants (phenolic, flavonoid, and vitamin C contents). Regarding to trace elements analysis of parsley leaves, both calcium and sodium gave the highest concentrations followed by magnesium and iron while copper and manganese exhibited the lowest concentrations. Furthermore, this study demonstrated that apigenin (a major flavonoid) from parsley leaves is effectively purified using column chromatography, as evidenced by a single peak product in an HPLC chromatogram with a high yield and purity. By using non-enzymatic methods, purified AP had more scavenging activity for reducing power, total antioxidant, nitric oxide, and superoxide scavenging than ethanolic parsley leaf extract. In contrast, the ethanolic PLE displayed stronger antioxidant activity than AP towards reducing the DPPH and hydroxyl radicals. Therefore, there is need to confirm these findings by doing *in vivo* animal model research to evaluate the role of this plant and its purified compound, apigenin in the scavenging of free radicals related diseases. As a result, it is necessary to better understanding and to screen medicinal plants for bioactive compounds as a basis for future pharmacological research.

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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.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Zakho.

Authors' Contribution Statement

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Baghdad Science Journal

A. A. M. and L. Y. M. contributed to the concept, design, and implementation of the research, and to the revision and proofreading of the manuscript. A.A. I. performed the experiments, acquisition of data, analysis and interpretation of the research, to write the draft of the manuscript.

References

1. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. Int J Environ Res Public Health. 2020; 17(10): 3376. https://doi.org/10.3390/ijerph17103376
2. Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S. Therapeutic and pharmacological efficacy of selective Indian medicinal plants– A review. Phytomed Plus. 2021; 1(2): 100029. https://doi.org/10.1016/j.phyplu.2021.100029
3. Nazir N, Rahman A, Uddin F, Khan Khalil AA, Zahoor M, Nisar M, et al. Quantitative Ethnomedicinal Status and Phytochemical Analysis of Berberis lyceum Royle. J Agron. 2021; 11(1): 130. https://doi.org/10.3390/agronomy11010130
4. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021; 20(3): 200-216. https://doi.org/10.1038/s41573-020-00114-z
5. Okaiyeto K, Oguntibeju OO. African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. Int J Environ Res Public Health. 2021; 18(11): 5988. https://doi.org/10.3390/ijerph18115988
6. Karipçin MZ. Hydrogels improved parsley (Petroselinium crispum (Mill) Nyman) growth and development under water de fi cit stress. Peer J. 2023; 11: e15105. https://doi.org/10.7717/peerj.15105
7. Kustiawan PM, Siregar KAAK, Saleh LO, Batistuta MA, Setiawan IM. A review of botanical characteristics, chemical composition, pharmacological activity and use of scorodocarpus borneensis. Biointerface Res Appl Chem. 2022; 12(6): 8324-8334. https://doi.org/10.33263/BRIAC126.83248334
8. Fernandes Â, Polyzos N, Petropoulos SA, Pinela J, Ardohain E, Moreira G, et al. Phytochemical composition and nutritional value of pot-grown turnip-rooted and plain and curly-leafed parsley cultivars. J Agron. 2020; 10(9): 1416-page.https://doi.org/10.3390/agronomy10091416
9. Bommakanti V, Puthenparambil Ajikumar A, Sivi CM, Prakash G, Mundanat AS, Ahmed F, et al. An Overview of Herbal Nutraceuticals, Their Extraction, Formulation, Therapeutic Effects and Potential Toxicity. Separations. 2023; 10(3): 177.https://doi.org/10.3390/separations10030177
10. Pajević S, Mimica-Dukić N, Nemeš I, Župunski M, Simin N, Watson M, et al. Arsenic content and phenolic compounds in parsley (Petroselinum Crispum (Mill) Fuss) and celery (Apium Graveolens L.) cultivated in vojvodina region, Serbia. Food Res. 2021; 48(2): 213-225. https://doi.org/10.5937/ffr48-34625
11. El-Sayed MM, Metwally NH, Ibrahim IA, Abdel-Hady H, Abdel-Wahab BSA. Antioxidant Activity, Total Phenolic and Flavonoid Contents of Petroselinum crispum Mill. J Appl Life Sci Int. 2018; 19(2): 1-7.https://doi.org/10.9734/JALSI/2018/45113
12. Aishwaya J. Nutraceuticals Potential of Petroselinum Crispum: A Review. J Complement Med Altern Healthc. 2018; 7(2): 1-6. https://doi.org/10.19080/jcmah.2018.07.555707
13. Papuc C, Predescu C, Nicorescu Va, Stefan G, Nicorescu I. Antioxidant properties of a parsley (Petroselinum crispum) juice rich in polyphenols and nitrites. Curr Res Nutr Food Sci J. 2016; 4: 114-118.
14. Al-Khayri JM, Sahana GR, Nagella P, Joseph B V, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022; 27(9): 2901. https://doi.org/10.3390/molecules27092901
15. Salehi B, Venditti A, Sharifi-Rad M, Kręgiel D, Sharifi-Rad J, Durazzo A, et al. The Therapeutic Potential of Apigenin. Int J Mol Sci. 2019; 20(6): 1305. https://doi.org/10.3390/ijms20061305
16. Majma Sanaye P, Mojaveri MR, Ahmadian R, Sabet Jahromi M, Bahramsoltani R. Apigenin and its dermatological applications: A comprehensive review. Phytochem. 2022; 203: 113390.
17. Yoon JH, Kim MY, Cho JY. Apigenin: A Therapeutic Agent for Treatment of Skin Inflammatory Diseases and Cancer. Int J Mol Sci. 2023; 24(2): 1498. https://doi.org/10.3390/ijms24021498
18. Radovanović B, Mladenović J, Radovanović A, Pavlović R, Nikolić V. Phenolic composition, antioxidant, antimicrobial and cytotoxic activites of Allium porrum L.(Serbia) extracts. J Food Nutr Res. 2015; 3(9): 564-569.
19. Turatbekova A, Mirzarakhmetova D, Jumaniyozov J, Khudayberganov E, Toshpulatov N, Rakhmatov A, et al. A brief overview on the methods for extraction and identification of flavonoids. 4th International Conference on Energetics, Civil and Agriculture Engineering. 2023; 434: 1 -8. https://doi.org/10.1051/e3sconf/202343403037
20. Tavakoli S, Khalighi SF, Khosravi DN,Yaghoob M, Hajiaghaee R, Gholami A, et al. Isolation and purification of apigenin, quercetin and apigenin 7-O-glycoside from Apium graveolens L., Petroselinum

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https://doi.org/10.21123/bsj.2024.9622
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Baghdad Science Journal

crispum (Mill.) Fuss, Allium cepa L., respectively . J Med Plants. 2022; 21(83): 72-86. https://doi.org/10.52547/jmp.21.83.72
21. Nayaka HB, Londonkar RL, Umesh MK, Tukappa A. Antibacterial Attributes of Apigenin Isolated from Portulaca oleracea L. Int J Bacteriol. 2014; 2014: 1-8. https://doi.org/10.1155/2014/175851
22. Chuljerm H, Paradee N, Katekaew D, Nantachai P, Settakorn K, Srichairatanakool S, et al. Iron Chelation Property, Antioxidant Activity, and Hepatoprotective Effect of 6-Gingerol-Rich Ginger (Zingiber officinale) Extract in Iron-Loaded Huh7 Cells. Plants. 2023; 12(16): 2936. https://doi.org/10.3390/plants12162936
23. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 2007; 101(1): 140-147. https://doi.org/10.1016/j.foodchem.2006.01.014
24. Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. J Univ Chem Technol Metall. 2011; 46(1): 81-88.
25. Gouveia S, Castilho PC. Antioxidant potential of Artemisia argentea L'Hér alcoholic extract and its relation with the phenolic composition. Food Res Int. 2011; 44(6): 1620-1631.https://doi.org/10.1016/j.foodres.2011.04.040
26. Ceccanti C, Brizzi A, Landi M, Incrocci L, Pardossi A, Guidi L. Evaluation of Major Minerals and Trace Elements in Wild and Domesticated Edible Herbs Traditionally Used in the Mediterranean Area. Biol Trace Elem Res. 2021; 199(9): 3553-3561. https://doi.org/10.1007/s12011-020-02467-3
27. Riaz M, Nawaz M, Qadir R, Hussain S, Roheen T, Afzal M, et al. Characterization and antioxidant potential of white mustard (Brassica hirta) leaf extract and stabilization of sunflower oil. Open Chem. 2023; 21(1): 20230175.https://doi.org/10.1515/chem-2023-0175
28. El Khomsi M, Kara M, Hmamou A, Assouguem A, Al kamaly O, Saleh A, et al. In Vitro Studies on the Antimicrobial and Antioxidant Activities of Total Polyphenol Content of Cynara humilis from Moulay Yacoub Area (Morocco). Plants. 2022; 11(9): 1-12. https://doi.org/10.3390/plants11091200
29. Ezeanyika LUS, Anosike CA, Oji CN, Chibuogwu CC. Phytochemistry, micronutrient composition, and antioxidant potentials of Citrus maxima (Shaddock) fruit juice. J Pharmacogn Phytochem. 2022; 11(5): 20-23.https://doi.org/10.22271/phyto.2022.v11.i5a.14498
30. Saravanakumar A, Periyasamy P, Jang HT. In vitro assessment of three different artemisia species for their antioxidant and anti-fibrotic activity. Biocatal Agric Biotechnol. 2019; 18: 101040. https://doi.org/10.1016/j.bcab.2019.101040
31. Amudha M, Rani S. Evaluation of in vitro antioxidant potential of Cordia retusa. Indian J Pharm Sci. 2016; 78(1): 80-86. https://doi.org/10.4103/0250-474x.180253
32. Oboh G, Puntel RL, Rocha JBT. Hot pepper (Capsicum annuum, Tepin and Capsicum chinese, Habanero) prevents Fe^{2+}-induced lipid peroxidation in brain – in vitro. Food Chem. 2007; 102(1): 178-185. https://doi.org/10.1016/j.foodchem.2006.05.048
33. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971; 44(1): 276-287. https://doi.org/10.1016/0003-2697(71)90370-8
34. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995; 28(1): 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
35. Mensor LL, Menezes FS, Leitão GG, Reis AS, SantosTC, Coube CS, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001; 15(2): 127-130. https://doi.org/10.1002/ptr.687
36. Ma Y, Meng A, Liu P, Chen Y, Yuan A, Dai Y, et al. Reflux Extraction Optimization and Antioxidant Activity of Phenolic Compounds from Pleioblastus amarus (Keng) Shell. Molecules. 2022; 27(2): 362. https://doi.org/10.3390/molecules27020362
37. Dini I, Laneri S. Nutricosmetics: A brief overview. Phytother Res. 2019; 33(12): 3054-3063. https://doi.org/10.1002/ptr.6494
38. Ibrahiem AA, Mosa AA, Mohammed LY. Estimation of oxidant , antioxidant and metabolic biomarkers in sera of diabetic rats administrated with parsley leaves extract and its isolated apigenin. Clin Nutr Open Sci. 2024; 54: 113-126. https://doi.org/10.1016/j.nutos.2024.02.003
39. Kowalska T. Thin Layer Chromotography (TLC) in the screening of Botanicals – Its Versatile Potential and Selected Applications. Molecules. 2022; 27(19): 6607. https://doi.org/10.3390/molecules27196607
40. Derouich M, Bouhlali EDT, Hmidani A, Bammou M, Bourkhis B, Sellam K, et al. Assessment of total polyphenols, flavonoids and anti-inflammatory potential of three Apiaceae species grown in the Southeast of Morocco. Sci Afr J. 2020; 9: 0-15. https://doi.org/10.1016/j.sciaf.2020.e00507
41. Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, et al. Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. Front Pharmacol. 2022; 13: 283. https://doi.org/10.3389/fphar.2022.806470
42. El-Houseiny W, Algharib S, Mohamed E, Metwally M, Mahmoud Y, Alghamdi Y. Dietary parsley seed mitigates methomyl-induced impaired growth

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.9622
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Baghdad Science Journal

performance, hemato-immune suppression, oxidative stress, hepato-renal damage, and pseudomonas aeruginosa susceptibility in Oreochromis niloticus. Antioxidants. 2022; 11(6): 1-21. https://doi.org/10.3390/antiox11061185

43. Yi X, Dong M, Guo N, Tian J, Lei P, Wang S, et al. Flavonoids improve type 2 diabetes mellitus and its complications: a review. Front Nutr. 2023; 10: 1192131. https://doi.org/10.3389%2Ffnut.2023.1192131
44. Sitarek-Andrzejczyk M, Przybył J, Gajewski M. The effect of post-harvest treatment and storage conditions on vitamin C content in two leafy parsley cultivars. Proceedings of the 8th International Scientific Conference Rural Development. 2017; 108-113. https://doi.org/10.15544/RD.2017.018
45. Amirpasha V, Kulieva T, Hernández E, Jukka M, Suomela P. Non -destructive assessment of vitamin C in foods : a review of the main findings and limitations of vibrational spectroscopic techniques. Eur Food Res Technol. 2022; 248(8): 2185-2195. https://doi.org/10.1007/s00217-022-04023-w
46. Dobričević N, Šic Žlabur J, Voća S, Pliestić S, Galić A, Delić A, et al. Bioactive compounds content and nutritional potential of different parsley parts (Petroselinum crispum Mill). J Cent Eur Agric. 2019; 20(3): 900-910. https://doi.org/10.5513/JCEA01/20.3.2417
47. Mazurek A, Włodarczyk-Stasiak M. A New Method for the Determination of Total Content of Vitamin C, Ascorbic and Dehydroascorbic Acid, in Food Products with the Voltammetric Technique with the Use of Tris (2-carboxyethyl) phosphine as a Reducing Reagent. Molecules. 2023; 28(2): 812. https://doi.org/10.3390/molecules28020812
48. Didier AJ, Stiene J, Fang L, Watkins D, Dworkin LD, Creeden JF. Antioxidant and Anti-Tumor Effects of Dietary Vitamins A, C, and E. Antioxidants. 2023; 12(3): 632. https://doi.org/10.3390/antiox12030632
49. Cerullo G, Negro M, Parimbelli M, Pecoraro M, Perna S, Liguori G, et al. The Long History of Vitamin C: From Prevention of the Common Cold to Potential Aid in the Treatment of COVID-19. Front Immunol. 2020; 11(10): 1-16. https://doi.org/10.3389/fimmu.2020.574029
50. Ustianowski Ł, Ustianowska K, Gurazda K, Rusiński M, Ostrowski P, Pawlik A. The Role of Vitamin C and Vitamin D in the Pathogenesis and Therapy of Periodontitis-Narrative Review. Int J Mol Sci. 2023; 24(7): 6774. https://doi.org/10.3390/ijms24076774
51. Sadee BA, Ali RJ. Determination of essential and trace elements in various vegetables using ICP-MS. Baghdad Sci J. 2023; 20(3): 715-725. https://doi.org/10.21123/bsj.2022.7253
52. Najmi A, Albratty M, Al-Rajab AJ, Alhazmi H, Javed S, Ahsan W, et al. Heavy Metal Contamination in Leafy Vegetables Grown in Jazan Region of Saudi Arabia: Assessment of Possible Human Health Hazards. Int J Environ Res Public Health. 2023; 20(4): 2984. https://doi.org/10.3390/ijerph20042984
53. Weyh C, Krüger K, Peeling P, Castell L. The role of minerals in the optimal functioning of the immune system. Nutrients. 2022; 14(3): 644. https://doi.org/10.3390/nu14030644
54. Jomova K, Makova M, Alomar SY, Alwasel S, Nepovimova E , Kuca K , et al. Essential metals in health and disease. Chem Biol Interact. 2022; 367: 110173.https://doi.org/10.1016/j.cbi.2022.110173
55. Bernal A, Zafra MA, Simón MJ, Mahía J. Sodium Homeostasis, a Balance Necessary for Life. Nutrients. 2023; 15(2): 395. https://doi.org/10.3390/nu15020395
56. Ishfaq M, Wang Y, Yan M, Wang Z, Wu L, Li C, et al. Physiological essence of magnesium in plants and its widespread deficiency in the farming system of China. Front Plant Sci. 2022; 13: 802274. https://doi.org/10.3389/fpls.2022.802274
57. Tardy AL, Pouteau E, Marquez D, Yilmaz C, Scholey A. Vitamins and minerals for energy, fatigue and cognition: a narrative review of the biochemical and clinical evidence. Nutrients. 2020; 12(1): 228. https://doi.org/10.3390%2Fnu12010228
58. Chase C, Lunney JK. Immune System. 10 th edition. Hoboken, New Jersey: John Wiley and sons, Inc. 2019. Ch 16, Diseases of Swine. p. 264- 290. https://doi.org/10.1002/9781119350927
59. Zheng YZ, Deng G, Zhang YC. Multiple free radical scavenging reactions of flavonoids. Dye Pigment. 2022; 198: 109877. https://doi.org/10.1016/j.dyepig.2021.109877
60. Mara de Menezes Epifanio NM, Cavalcanti LRI, Dos Santos KF, Soares Coutinho Duarte P, Kachlicki P, Ożarowski M et al. Chemical characterization and in vivo antioxidant activity of parsley (Petroselinum crispum) aqueous extract. Food Funct. 2020; 11(6): 5346-5356. https://doi.org/10.1039/d0fo00484g
61. Kashyap P, Shikha D, Thakur M, Aneja A. Functionality of apigenin as a potent antioxidant with emphasis on bioavailability, metabolism, action mechanism and in vitro and in vivo studies: A review. J Food Biochem. 2022; 46(4): e13950. https://doi.org/10.1111/jfbc.13950
62. Dzoyem JP, Donfack ARN, Tane P, McGaw LJ, Eloff JN. Inhibition of Nitric Oxide Production in LPS-Stimulated RAW 264.7 Macrophages and 15-LOX Activity by Anthraquinones from Pentas schimperi. Planta Med. 2016; 82(14): 1246-1251. https://doi.org/10.1055/s-0042-104417
63. Fitton R, Sweetman J, Heseltine-Carp W, van der Feltz-Cornelis C. Anti-inflammatory medications for the treatment of mental disorders: A scoping review. Brain Behav Immun Health. 2022; 26: 100518. https://doi.org/10.1016/j.bbih.2022.100518
64. Gandhimathi C. Nutritional Evaluation, in vitro Free Radical Scavenging and in vivo Anti-inflammatory Effects of Gisekia pharnaceoides and Identification of

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.9622
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Kaempferol as a Nutraceutical Agent. Br Biotechnol J. 2011; 1(3): 113-135. https://doi.org/10.9734/bbj/2011/597
65. Shantabi L, Jagetia GC, Ali MA, Singh TT, Devi SV. Antioxidant potential of Croton caudatus leaf extract in vitro. Tranl Med Biotech. 2014; 2(1): 1-15.
66. Lalrinzuali K, Vabeiryureilai M, Jagetia GC, Lalawmpuii PC. Free radical scavenging and antioxidant potential of different extracts of Oroxylum indicum in vitro. Adv Biomed Pharm. 2015; 2(3): 120-130. https://doi.org/10.19046/abp.v02i03.02
67. Sanna D, Fadda A. Role of the Hydroxyl Radical-Generating System in the Estimation of the Antioxidant Activity of Plant Extracts by Electron Paramagnetic Resonance (EPR). Molecules. 2022; 27(14): 4560. https://doi.org/10.3390/molecules27144560
68. Gulcin I, Alwasel SH. Metal Ions , Metal Chelators and Metal Chelating Assay as. Processes. 2022; 10(132): 1-16. https://doi.org/10.3390/pr10010132
69. Al-Muwaly KY, Al-Flayeh KA, Ali A, Younus K. Antioxidant and free radical scavenging effects of Iraqi sumac (Rhus coriaria L). Baghdad Sci J. 2013; 10(3): 921-933. https://doi.org/10.21123/bsj.2013.10.3.921-933
70. Kuźma P, Drużyńska B, Obiedziński M. Optimization of extraction conditions of some polyphenolic compounds from parsley leaves (Petroselinum crispum). Acta Sci Pol Technol Aliment. 2014; 13(2): 145-154. https://doi.org/10.17306/J.AFS.2014.2.4
71. Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P, Sandström B, et al. Effect of parsley (Petroselinum crispum) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. Br J Nutr. 1999; 81(6): 447-455. https://doi.org/10.1017/s000711459900080x
72. Andrés CMC, Pérez de la Lastra JM, Andrés Juan C, Plou FJ, Pérez-Lebeña E. Superoxide anion chemistry—Its role at the core of the innate immunity. Int J Mol Sci. 2023; 24(3): 1841-page. https://doi.org/10.3390%2Fijms24031841
73. Tian C, Liu X, Chang Y, Wang R, Lv T, Cui C, et al. Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. S Afr J Bot. 2021; 137: 257-264. https://doi.org/10.1016/j.sajb.2020.10.022
74. Gnintoungbe GS, Medehouenou TCM, Adounkpe F, Akpovi C, Loko F. Phytochemical Screening, Antioxidant Activity and Safety of Petroselinum crispum (Mill.) AW Hill Apiaceae Leaves Grown in Benin. Open J Appl Sci. 2023; 13(1): 36-50. https://doi.org/10.4236/ojapps.2023.131004

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تقييم محتوى البوليفينولات، فيتامين ج، المحتويات المعدنية بالإضافة الى الفعالية المضادة للأكسدة لمستخلص أوراق البقدونس الخام (*Petroselinum crispum*) بالمقارنة مع الأبيجينين المعزول منه

أفين أكرم ابراهيم[1]، عزام عبدالستارموسى[2] ، لينا يوسف محمد[3]

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الخلاصة

البقدونس أو *Petroselium crispum* هو خضار طهي تقليدي يستخدم بشكل كبير كمكمل غذائي ، كمنكهات اومضافات معطرة للأطعمة. تركزت اهداف الدراسة الى تنقية الفلافونيد الرئيسي الموجود في أوراق البقدونس,(الأبجنين) وتقييم محتوى البوليفينول, فيتامين ج والعناصرالمعدنية في مستخلص أوراق البقدونس بالاضافة الى مقارنة الأنشطة المضادة للأكسدة لمستخلص أوراق البقدونس الأيثانولي مع مركب الأبيجينين المنقى باستخدام طرق تحليلية مختلفة. علاوة على ذلك, اجراء تحليل كامل للمركبات الحيوية الأكثر وجودا في أوراق البقدونس باستخدام تحليل كروماتوكرافيا السائل العالي الأداء (HPLC).

أظهرت نتائج الدراسة الى امكانية تنقية مركب الأبيجينين من أوراق البقدونس باستخدام كروماتوغرافيا العمود بكفاءة ونقاوة عالية وذلك من خلال الحصول على حزمة واحدة نقية في كروماتوغرام ال HPLC. اشارت نتائج التحليل أيضا أن محتوى الفينول الكلي ومحتوى الفلافونيدات في مستخلص أوراق البقدونس الكحولي كان بحدود 3.33 ±75.91 ملغ/ غم, 0.18l±15.61ملغم/ غم على التوالي. بلغ محتوى فيتامين ج 4.01 ملغم /غم.

كما دلت نتائج تحليل المعادن النادرة، أن مستوى الكالسيوم والصوديوم كانتا الأعلى من بين المعادن المقاسة في أوراق البقدونس , بقيم بلغت 14.13 ملغ/غم و 8.51 ملغ/غم على التوالي بينما كان مستوى كل من عنصري النحاس و المنغنيز الاقل قيمة (0.02 ملغم/ غم) و(0.012 ملغم/ غم) على التوالي. وأخيرا, وجد من خلال النتائج أن للأبجنين المنقى خواص مضاد اكسدة أعلى من المستخلص الكحولي للبقدونس من ناحية القوة الاختزالية، الفعالية المضادة للأكسدة الكلية ، والقابلية على اكتساح جذورأوكسيد النتريك و السوبراوكسيد الفعالة. وعلى العكس من ذلك, كان للمستخلص الكحولي للبقدونس نشاط مضاد أكسدة اعلى من الأبجنين المنقى في تثبيط جذور DPPH وجذورالهيدروكسيل.

الكلمات المفتاحية: مضادات الأكسدة , أبجنين, كروماتوغرافيا, البقدونس, فيتامين ج .