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Antioxidant Activity, Mineral Absorptivity and Chemical Analysis of P. Graveolens

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Abstract

This article focuses on Pelargonium graveolens, a fragrant medicinal plant from the Geraniaceae family. The study examines the plant's phytochemical composition, antioxidant activity, and mineral absorptivity, with the plant being grown indoors. The study also examined the plant's ash content and antioxidant activity using a variety of techniques, and the results demonstrated that P. graveolens is effective at absorbing lead. The plant contains eight different minerals, including Cu, Mn, Co, Ni, Pb, Mg, Fe, and Ca. Statistical analysis was used to determine the level of antioxidants present, using DPPH, reducing power, and total antioxidant capacity methods. The extraction process used a mixture of water, 70% ethanol and absolute ethanol solvents in varying ratios. To conclude, the study examines Pelargonium graveolens, a fragrant medicinal plant, for its antioxidant activity and mineral absorptivity.

Keywords: Antioxidants, DPPH, Heavy metals, IC_{50}, Pelargonium graveolens, Phytochemical screening.

Introduction

Natural products are materials that are found in nature and have not undergone chemical modification. They are found in many different industries, including food, cosmetics, and medicine, and can be derived from minerals, plants, and animals. Because science has not yet identified the active elements in natural products, which have been used for generations, it is thought that these items are more effective than synthetic ones. Because they think they are safer than prescription medications, some people are choosing to treat their illnesses with natural products. Nevertheless, some natural products can be harmful and cause allergic reactions in some people, and not all of them are safe for human consumption. Manufacturers of natural products should strive to make their goods safer by making sure that no dangerous materials, including pesticides or heavy metals, are present in their ingredients [1–4].

A chemical process called oxidation occurs when electrons or hydrogen are transferred from one material to an oxidizing agent. Extremely reactive free radicals may be produced by this reaction, and they may set off damaging chain reactions in cells. Free radicals are chemicals that have the potential to

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harm cells and induce illnesses. On the other hand, the body can fight these free radicals with the help of a class of natural compounds called antioxidants [5–7].

Natural products like antioxidant-rich foods, herbs, and supplements can be helpful for people who want to improve their health. The most common natural product is vitaminC[8], flavonoids[7], carotenoids[9], and polyphenols[10]. Antioxidants terminate chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidants to detoxify the reactive intermediates or to repair the damage caused by these intermediates[5–7].

The DPPH assay was used to assess the antioxidant activity of the phenols and flavonoids content. The DPPH assay, also known as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, is a free radical scavenging assay that can be used to measure the antioxidant activity of a given compound[11,12]. The capacity of an antioxidant is measured by its ability to neutralize free radicals. The greater the capacity of an antioxidant, the better it will be at protecting you from damage caused by free radicals[13,14].

Pelargonium graveolens is a perennial herb that thrives in damp regions and has a potent, pungent odor that some individuals find soothing. It is utilized in perfumes and serves as an organic insecticide. *P. graveolens* has been employed in medicine for many years to manage respiratory infections, bronchitis, and other respiratory ailments. Scientists have identified the chemical compounds and antidiabetic properties of *P. graveolens* essential oil and have detected 36 chemical components present in it [15]. On the other hand, Džamić et al. reported 56 chemicals; antifungal and antioxidant activity of *P. graveolens* essential oil [16].Other researchers discovered 15 extra chemicals in *P. graveolens* essential oil and they measured the antioxidants activity by DPPH assay[17,18] .Okla et al. studied the effect of laser on mineral content of *P. graveolens* and they found 7 minerals including zinc and iron[17]. Boukhris et al. investigated the chemical compounds and biological functions of polar extracts and essential oil and showed that β-citronellol as the primary constituent of the oil part and nine flavonoids were identified as well[19]. Ouadi et al. studied the *P. graveolens* extract by using diethyl ether and ethyl acetate as solvent for this purpose [5].

The objective of this research is to examine the mineral content and evaluate the antioxidant potential of extracts of Pelargonium graveolens (obtained using D.I.W., 70% ethanol, and 100% ethanol), which may serve as a natural antioxidant source in the pharmaceutical or food sectors (Fig. 1).

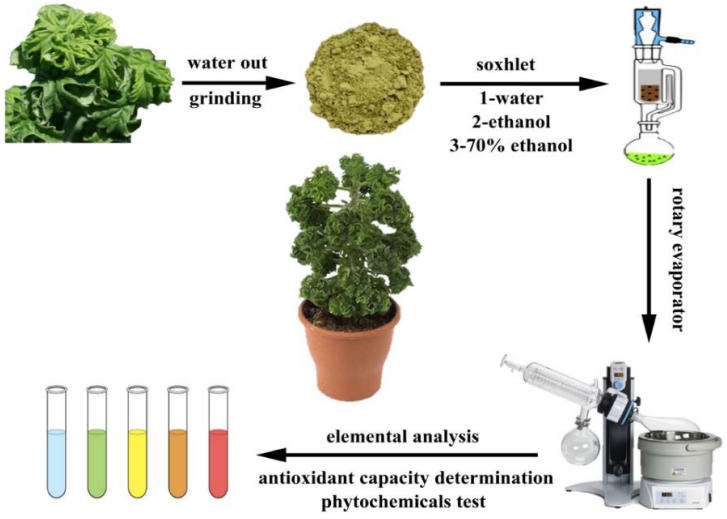

Figure 1. Schematic diagram illustrated the practical work

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Materials and Methods

Plant Material and Reagents:

In September 2021, indoor-cultivated plant material of *P. graveolens* was obtained. The stems and leaves were isolated, then the leaves were left to dry at room temperature, away from sunlight. A voucher was utilized, which was authenticated by a taxonomist affiliated with the College of Agriculture, Duhok University. The reagents employed in the study were of the highest possible purity and were procured from the Sigma Aldrich Chemical Company.

Instrument

The equipment used in this study included: spectrophotometer (Perkin Elmer Lambda 35 UV/VIS), Centrifuge (Model–, Eltek Equipment Pvt. Ltd., Vasai (E), Thane, India. Absorption Spectrophotometer (AAS) type Perkin Elmer PinAAcle 900T, USA (PinAAcle 900T Atomic Absorption Spectrophotometer (AAS) Tandem Flame and THGA Zeeman Furnace with Hydride Vapour and Water Purification System) and a rotary vacuum evaporator (Model Heidolph, Malaysia)

Preparation of P. graveolens Extract:

Using the method described by Zaini [20] with some modification, three distinct *P. graveolens* extracts were prepared. 10 g of *P. graveolens* fine powder was loaded into a thimble and placed in the extraction chamber of Soxhlet and the sample soaked overnight, then extracted with 100 ml of solvent. A 1:10 *P. graveolens* leaves fine powder and three different solvents were selected for this extraction process, including water (D.I.W.), 70% ethanol, and 100% ethanol. After this stage, the solvent was recovered using a rotary evaporator.

Determination of Antioxidant Capacity of P. graveolens Extracts:

100 mg of each extract were dissolved in 100 ml of deionized water for determination the antioxidant activity and to conduct a qualitative test.

Determination of Reducing Power: To determine the reducing power of the *P. graveolens* extracts, a method described by Mathew and Abraham was followed[21]. Known volumes (100-500 µl) of each extract were added to clean test tubes, which were then made up to 1.0 ml with deionized water. Potassium ferricyanide solution (2.5 ml, 1% w/v) and phosphate buffer solution (2.5 ml, 0.2 M, pH 6.6) were added to these tubes and thoroughly mixed. The mixtures were incubated at 50°C for 20 minutes, and then 2.5 ml of trichloroacetic acid solution (10% w/v) were added to each mixture. After centrifuging the tubes at 5,000 rpm for 10 minutes, 2.5 ml of each supernatant was taken in another test tube, and 0.5 ml of ferric chloride solution (0.1% w/v) and 1 ml of [21]deionized water were added and mixed. The absorbance was then measured at 700 nm. Ascorbic acid was used as a reference standard, and its stock solution was prepared with the same concentration as the *P. graveolens* extract. The increase in absorbance of the reaction mixture indicated an increase in the reducing power of the extracts.

Determination of Total Antioxidant Capacity: The phosphomolybdenum method described by Kumaran and Karunakaran [22], was used to measure the total antioxidant capacity of each *P. graveolens*. Each *P. graveolens* was introduced at known volumes (0.1–0.3 ml) before being diluted with DIW. to a consistent volume (0.3 ml). Each tube received three milliliters of the reagent solution, which consisted of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4.0 mM ammonium molybdate. After thoroughly mixing each tube, it was incubated at 95 °C for 90 minutes. The standard ascorbic acid was used as a control and compared with the extract of *P. graveolens.* Then absorbance was recorded at 695nm as a function of antioxidant capacity.

Assay of DPPH Radical Scavenging Activity: Each *P. graveolens* extract was tested for its capacity to scavenge the stable DPPH free radical in order to measure its antioxidant activity. The method used was described by Lee et al [23]. In this method, 100-500 µl of each *P. graveolens* extract were added to individual test tubes, then brought to 1 ml with D.W. To each tube, 1.0 ml of DPPH solution (0.2 mM in ethanol) was added, and the tubes were mixed and left to incubate at room

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temperature for 30 minutes. The absorbance of the solution was then measured at 517 nm, and the IC50 was calculated from the percent of inhibition. Ascorbic acid was used as a reference standard at the same concentration as the *P. graveolens* extract. A control sample was also prepared without any extract or ascorbic acid, and the percent of scavenging of the DPPH free radical was measured.

Determination of Vitamin C Content: The method described by Benderitter and colleagues in 1998 was applied to determine the content of vitamin C in water extracts. The reaction mixture consisted of 400 µL of sample solution (300 µL of extract and 100 µL of 13.3% trichloroacetic acid), to which 75 µL of DNPH (a mixture of dinitrophenyl hydrazine, thiourea, and copper sulfate in 5 mol/L sulfuric acid) was added. The mixture was incubated at 37°C for 3 hours, then 0.5 ml of 65% sulfuric acid was added. The vitamin C content was then measured at 520 nm and expressed as ascorbic acid equivalent [24,25].

Total Flavonoids Content: The flavonoid content of the plant extracts was determined using a spectrophotometric technique involving aluminum chloride. Initially, 0.1 ml of each extract was dissolved in methanol and then complete the volume to 100ml using a volumetric flask. After that, 500 µl of each extract was mixed with 2.8 ml of distilled water, 2 ml of 95% methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 0.1 ml of 10% aluminum chloride. After the reaction mixture had been incubating at room temperature for 30 minutes, a spectrophotometer was used to measure the absorbance at 415 nm. Quercetin, a flavonoid standard at a concentration of 0.1 mg/ml, was used to calculate the total flavonoid content of the extracts in mg/g dry weight, following the method described by Mahae and Chaiseri in 2009[26].

Total Phenolic Content: To determine the total phenolic content (TPC) of *P. graveolens*, a spectrophotometric method using the Folin-Ciocalteu reagent was employed, following the protocol described by Gao et al.[27]. In this method, 100 ml of *P. graveolens* extract was mixed with 0.2 ml of Folin-Ciocalteu reagent, 2.0 ml of water, and 1.0 ml of a 7.5% Na_2CO_3 solution. The blue color absorption was measured at 765 nm against a blank after 2 hours at room temperature. A standard curve was created using serial concentrations of a tannic acid solution (50-500 mg/mL) prepared in the same manner to determine the TPC concentration in each extract.

Moisture Constants

A heating method was applied for determination the test material's moisture content. The sample was weighed after being added to a ceramic crucible in amounts of two grams. After that, the crucible was heated overnight in an air oven at a temperature of 100–110 °C. The crucible was then allowed to cool in a desiccator at ambient temperature before being weighed once more. This method was repeated until three successive weights were reached. The moisture content was computed using the weight difference, following the method described by Alasmary et al [28].

Ash Content

The methods used to determine the sulfated ash, total ash, acid-insoluble ash, and water-soluble ash contents were designated in studies by Pareek and Bhatnagar (2020), Ks, P, and S (2017), and Pande et al. (2018)[29–31]

Determination of Metals in Both Plant and Soil

Samples of soil were digested using a concentrated solution of analytical-grade HNO_3 and $HClO_4$ in order to determine the metal content of the samples. Approximately 0.5 g of dry soil sample and 5 ml of pure HNO_3 were added to a glass jar. A further 5 milliliters of concentrated HNO_3 and 3 milliliters of concentrated $HClO_4$ were added, and the mixture was kept at 150 oC for three hours on an electrothermal board until it had almost dried up. The digested sample was decanted into a glass tube and diluted with 2% HNO_3. The metal content of *P. graveolens* was determined using the method described by Hseu [32], which involved digesting 0.5 g of powdered dry leaves with concentrated H_2SO_4 and H_2O_2, then measuring the metal concentration using atomic absorption spectroscopy.

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Qualitative Phytochemical Analysis

Qualitative analysis was conducted to identify the presence of flavonoids [33-35], saponins, carbohydrates, tannins, phenolics [35-37], alkaloids, amino acids [38-39], , sterols [35,40] , terpenoids [41] , emodins, anthocyanins, leuconthocyanins, quinones, and anthraquinones [42-44] in the sample.

Statistical Analysis

The measurements were conducted in triplicates, and the mean value with standard deviation error (SE) was reported. Analysis of variance was used to evaluate the data, with a significance level of 0.05., and Duncan's multiple range test was applied to separate the means. The statistical analysis was performed using SPSS version 21. However, the graph was generated using Origin Lab Pro (Massachusetts, USA), and IC50 was determined using Quest Graph software [45].

Results and Discussion

Phytochemical Screening of P. graveolens leaves

Table 1 summarizing the phytochemical analysis of various extracts of *P. graveolens* indicates the presence of flavonoids, carbohydrates, tannins, phytosterols, and coumarins in all extracts. However, proteins and amino acids were not detected in any of the extracts. Alkaloids, resins, and triterpenoids were only found in the 70% ethanol and ethanolic extracts, while cardiac glycosides, saponins, and anthraquinones were present in 70% ethanol and water extracts. Phlobatannins and terpenoids were exclusively found in the water extract. The results indicated that the 70% ethanol extract contained a higher number of constituents than the other two extracts. The study was conducted using qualitative methods.

Table 1. Qualitative analysis of *P. graveolens* leaves extracts.

Phytochemicals	Test	Aqueous	70 % Ethanol	Absolute Ethanol
Flavonoids	Alcoholic KOH reagent	+++	++	+
	Lead acetate reagent	+++	++	+
	Ammonia test	+	++	+++
	Conc.H_2SO_4 test	+	++	+++
Carbohydrates	Molisch's reagent	+++	++	+
	Seliwanoff's reagent	+++	+	++
	Bial's reagent	-	-	-
	Iodine reagent	+	-	-
	Benedict's reagent	+++	++	-
Cardiac glycosides	Keller kiliani's test	+++	+	-
Tannins	Braymer's reagent	++	+	+
	Lead acetate reagent	+++	++	+
Alkaloids	Wagner's reagent Dragendroff's reagent	-	+	-
		-	+	++
	Hager's reagent	-	+	+
	Tannic acid test	-	+	++
Proteins and Amino acids	Ninhydrin test	-	-	-
Saponin	Aqueous Mercury chloride	++	+	-
	Foam teat	+	-	-
Phytosterols	Salkowski's reagent Liebermann-	+	++	+++
	Burchard's reagent	-	+	++
	Phlobatannins (Olive oil test)	+	-	-
Terpenoids	Chloroform test	+	-	-
Tri-terpenoids		-	+	++
Resins	Turbidity	-	+	++

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Coumarins	Sodium hydroxide test	+++	++	+
Phenolic				
Anthocyanins	Hydrochloric acid test	-	-	-
Quinones	Conc.H_2SO_4	+	++	+++
Anthraquinone	Ammonia solution	+	++	-
Emodins test:		-	-	-
Leuconthocyanins test		-	-	-

Note: +present in low quantity, ++ present in moderate; +++ present in more quantity; - Absent.

Physiochemical Characterization of P. graveolens Leaves

Physical parameters of *P. graveolens* showed that the values of the total ash, water-soluble ash, acid-soluble ash, and sulphated ash were 11.919±0.15 %, 2.667±0.033%, 10.742±0.06% and 10.583±0.073% respectively, as shown in Table 2.

The moisture content of *P. graveolens* was 5.783±0.067% and the solid content was 94.217±0.067%. The moisture content percentage of the *P. graveolens* leaves was within the acceptable range (5%–8%), which suggests that the formulation can be kept for a long time and won't be readily infected by bacteria [46].

Table 2. Physiochemical properties of *P. graveolens* leaves.

Constituent %	N	Mean ± S. E
Ash content (%w/w)	3	11.919±0.15
Water soluble ash (%w/w)	3	2.667±0.033
Acid soluble ash (%w/w)	3	10.742±0.06
Sulphated ash (%w/w)	3	10.583±0.073
Solid content (%w/w)	3	94.217±0.067
Moisture content(%w/w)	3	5.783±0.067

Minerals Analysis and Metal Absorptivity

Table 3 showed the findings of the analysis of eight mineral elements (Ca, Co, Cu, Fe, Mg, Mn, Ni, and Pb) in both P. graveolens leaves and the soil. The data indicated that the highest concentration of calcium was found in the soil 48.540 mg/g and in the leaves 16.675 mg/g. On the other hand, the magnesium content recorded 0.017 mg/g in the leaves and 8.225 mg/g in the soil. Therefore, this may indicated that P. graveolens has a higher affinity for calcium than magnesium.

The iron concentration in the leaves of *P. graveolens* was significantly higher at 0.500 mg/gm compared to the iron content found in the soil, which was 36.248 mg/gm. However, trace amounts of copper, manganese, cobalt, and nickel were present in the plant leaves, but their levels were below what is considered normal for physiological functions. Additionally, the lead content in the leaves of *P. graveolens* was 2.6 times higher than the lead content in the soil, suggesting that *P. graveolens* has a strong affinity for absorbing lead.

Table 3. Mineral elements contents in *P. graveolens* leaves and soil

Mineral	Concentration (mg/gm)	
	Plant (leaves)	Soil
Ca	16.675	48.540
Co	0.069	0.027
Cu	0.028	0.046
Fe	0.500	24.040
Mg	0.017	8.225
Mn	0.072	0.533
Ni	0.080	0.166
Pb	0.092	0.035

Total Phenolic, Total Flavonoid Contents and Vitamin C Determination

The determination of total phenols and flavonoids in three fractions of aqueous, 70% ethanol, and ethanolic extracts of Pelargonium graveolens was conducted using colorimetric methods (Folin-Ciocalteux/standard tannic acid and trichloride

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aluminum ($AlCl_3$)/quercetin as standards, respectively). According to the findings, *P. graveolens* leaves contain a significant (P<0.0001) amount of phenolic, flavonoid, and vitamin C compounds, as shown in Table 4. Also, we found that a high amount of phenolic was in the aqueous extract (662.668 ± 15.084 mg/g), but the highest quantity of the flavonoid compound was found in the ethanolic extract (107.429 ± 5.378 mg/g).

On the other hand, the total vitamin C is determined by using the 2, 4-dinitrophenylhydrazine spectrophotometric method. The current results showed that 70% ethanolic extract had the highest vitamin C content than water extract and 100% ethanolic extract, following the order: 70% ethanol (36.994 ± 0.138 mg/g) > water (20.444 ± 0.079 mg/g) > absolute ethanol (14.151 ± 0.25 mg/g). The standard calibration curves of TFC, TPC, and vitamin C are shown in figure 2 (A, B, and C, respectively).

Table 4. Total phenol, flavonoid and Vitamin C contents in *P. graveolens* leaves

Sample	N	Phenolic content (mg/g)	Flavonoids content (mg/g)	Vitamin C (mg/g)
Aqueous	6	662.668 ± 15.084[b]	30.655 ± 0.736[a]	20.444 ± 0.079[b]
70 % Ethanol	6	371.537 ± 5.55[a]	43.274 ± 0.828[b]	36.994 ± 0.138[c]
Absolute ethanol	3	386.064 ± 7.252[a]	107.429 ± 5.378[c]	14.151 ± 0.25[a]
P-value		<0.0001	<0.0001	<0.0001

- Values are represented as Mean ± SE Differences between numbers in the same column and following the same letter are not significantly different at P < 0.05.

Antioxidant Activity of P. graveolens Leaves

To determine the antioxidant capacity of *P. graveolens* leaves, multiple methods were employed, including the DPPH radical method, the reducing power assay, and the total antioxidant capacity. Using a single test is insufficient to establish the antioxidant capacity of *P. graveolens* extracts because each technique is based on a distinct mechanism that can produce different findings.

DPPH• Radical Scavenging Activity

Antioxidant activity of *P. graveolens* leaves for each extract was estimated by the method (1,1-diphenyl-2-picrylhydrazyl). A stable free radical, DPPH• must receive an electron or hydrogen radical in this process to transform into a stable molecule.

The activity percentage of extracts increases as their concentration increases, as more antioxidants (DPPH) are reduced. This phenomenon is due to the ability of the tested samples to donate hydrogen to the DPPH, causing it to change color from violet to yellow and absorb less light. This decrease in light absorption is more pronounced at higher concentrations because there are more antioxidants available to reduce the DPPH, as noted by Yassir [47].

Based on the information provided in Table 5, it has been observed that extracts of *P. graveolens* possess the ability to reduce the DPPH radical. The results indicate that at a concentration of 250.0 ppm, ascorbic acid exhibited the highest radical scavenging effect of 90.763%, while the aqueous extract of *P. graveolens* demonstrated a slightly lower effect of 89.449%. Similarly, the ethanol and 70% ethanol extracts showed radical scavenging effects of 87.489% and 86.962%, respectively.

In Table 5, the study presented the outcomes regarding the effectiveness of different solvent fractions of *P. graveolens* in scavenging the DPPH radical. The results showed that the scavenging ability of these fractions was influenced by their concentration, which was measured using IC50 values. The IC50 value indicates the concentration of the sample required to reduce the initial concentration of DPPH• by 50%. A lower IC50 value indicates a higher antioxidant activity. The findings demonstrated that the aqueous fraction of *P. graveolens* displayed the greatest radical

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scavenging activity, with a moderate IC50 value of 243.5 µg/g. Conversely, the 70% ethanol fraction had a higher IC50 value of 376.7 µg/ml, indicating a lower scavenging capacity.

Table 5. Scavenging activity of *P. graveolens* leaves extracts and ascorbic acid against DPPH radical

P. graveolens Conc.(ppm)	Scavenging activity (%)				P-value
	Aqueous	**70 % Ethanol**	**Absolute ethanol**	**Ascorbic acid(standard)**	
50	85.284 ^{f} ± 0.0774	72.397 ^{a}± 0.14083	74.358 ^{b}± 0.12804	90.007^{l}± 0.25256	<0.0001
100	86.017 ^{g}± 0.0242	78.446^{c} ± 0.031	78.422 ^{c}± 0.28356	90.445^{m} ± 0.00671	
150	86.927 ^{h}± 0.11415	81.456 ^{d}± 0.14114	83.331 ^{e}± 0.07071	90.616 ^{m}± 0.0795	
200	88.376^{j} ± 0.02013	83.219 ^{e}± 0.02357	85.036 ^{f}± 0.15971	90.744^{m} ± 0.01025	
250	89.449^{k} ± 0.00387	86.962 ^{h}± 0.04366	87.489 ^{i}± 0.0205	90.763^{m} ± 0.00387	
IC_{50}	243.5909	376.7252	132.5845	69.807	

Values are means of three replicates Mean ± SE Numbers followed by the same letter are not significantly different at P < 0.05

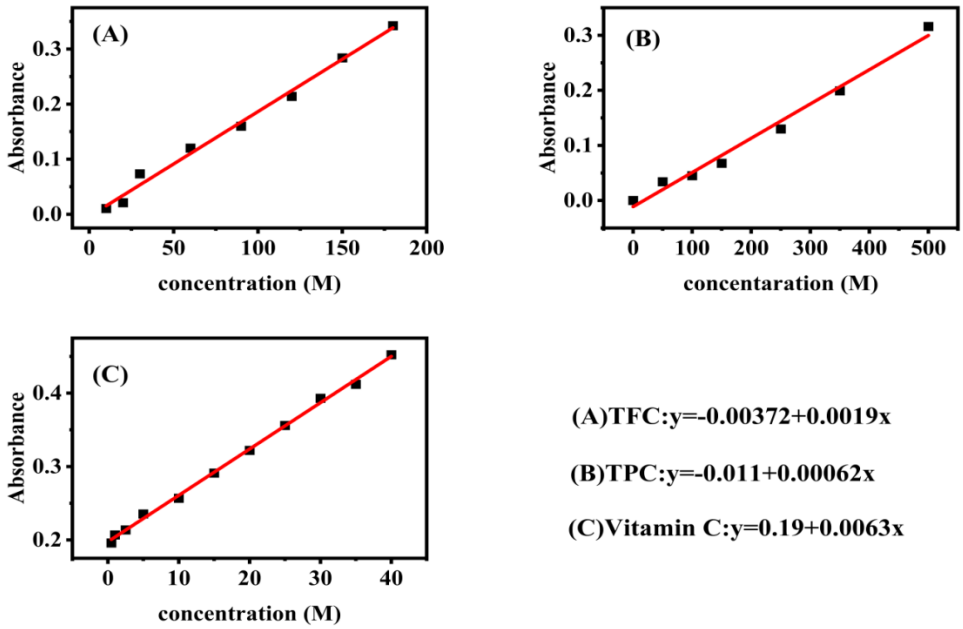

Figure 2. Calibration curves with regression equations of (A) TFC, (B) TPC and (C) ascorbic acid.

Reducing Power Assay

Reducing power activities showed that there is a significant (p<0.0001) direct relationship between concentration and absorbance. Correspondingly, it showed that the extracts have a higher reduction capacity than the reference standard ascorbic acid compared to the same concentration. On the other hand, our study showed that the ethanolic fraction has the highest absorbance at 58.82 ppm and the lowest IC50 (6.7138) value than the other extract, in the order ethanolic >70 % ethanol >aqueous > ascorbic acid, as presented in Table 6.

Antioxidants are known to neutralize and deactivate oxidants by functioning as reductants [48]. Previous findings have been proposed that the reducing power of a substance can act as an important indicator of its powerful antioxidant activity, and it has been suggested that *P. graveolens* may possess

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such reducing power [49]. In order to estimate the antioxidant potential of various extracts of *P. graveolens* leaves, their ability to reduce potassium ferricyanide (Fe^{+3}) to potassium ferrocyanide (Fe^{+2}) was measured. In order to form a ferric-ferrous complex, which has a maximum absorption at a wavelength of 700 nm, the resultant Fe^{+2} reacts with ferric chloride.

Table 6. Total Reduction power of *P. graveolens* leaves extracts and Ascorbic acid

P. graveolens Conc.(ppm)	Reducing power (O.D 700 nm)				
	Aqueous	**70 % Ethanol**	**Ethanolic**	**Ascorbic acid**	**P-value**
11.76	0.237^{cd}± 0.00381	0.262^{efg} ± 0.00552	0.271^{gh} ± 0.0054	0.178^{a}± 0.00275	<0.0001
23.53	0.232^{cd}± 0.00299	0.265^{fg}± 0.01039	0.3^{i}± 0.00462	0.182^{a}± 0.00448	
35.29	0.228^{c}± 0.00394	0.268^{g}± 0.00766	0.304^{i} ± 0.00757	0.185^{a}± 0.00547	
47.06	0.244^{cde}± 0.00437	0.287^{hi}± 0.00964	0.323^{j} ± 0.00629	0.21^{b} ± 0.00518	
58.82	0.248^{def} ± 0.0048	0.301^{i} ± 0.0091	0.321^{j}± 0.00318	0.194^{ab}± 0.00551	
Ic_{50}	45.2958	45.9481	6.7138	36.3883	

Values are means of three replicates Mean ± SE Numbers followed by the same letter are not significantly different at P < 0.05

Total Antioxidant Capacity

The total antioxidant capacity method was used to evaluate the potential reduction of different extracts of *P. graveolens* leaves using different concentrations (30.3 ppm – 151.52 ppm) in comparison with ascorbic acid as a standard. Determination the antioxidant activities of different extract were achieved by the formation of a green phosphomolybdenum complex. Formation of this complex is monitored by measuring the absorbance's intensity of each extract as shown in Table 7.

In the grading of the total antioxidant capacity obtained by this method, at the highest concentration (151.52 ppm) the aqueous extract of *P. graveolens* leaves showed higher phosphomolybdenum reduction (0.225± 0.00022) compared to standard ascorbic acid is less 0.191± 0.00034 in the same concentration followed by 70% ethanol (0.171± 0.00171). This might be explained by the fact that antioxidants' ability to transfer electrons and hydrogen depends on their structures [50]

This method depends on reducing of Mo^{+6} to Mo^{+5} at an acidic pH to produce green phosphate/Mo(V). The test was expanded to include plant polyphenols since it was easy to use and independent of other antioxidant measures that are often used [51].

Table 7. Total antioxidant capacity of *P. graveolens* leaves extracts and Ascorbic acid

P. graveolens Conc.(ppm)	Total antioxidant capacity (O.D 695nm)				P-value
	Aqueous	**70 % Ethanol**	**Ethanolic**	**Ascorbic acid**	
30.3	0.043^{c} ± 0.00039	0.034^{b} ± 0.00009	0.03^{a} ± 0.00006	0.156^{l} ± 0.00004	<0.0001
60.61	0.078^{f} ± 0.00016	0.062^{e} ± 0.00006	0.053^{d} ± 0.00021	0.191 ± 0.00015	
90.91	0.122^{j} ± 0.00023	0.097^{g} ± 0.00041	0.062^{e} ± 0.00056	0.195^{o} ± 0.00015	
121.21	0.171^{m} ± 0.00011	0.129^{k} ± 0.00013	0.1^{h} ± 0.0008	0.198^{p} ± 0.00007	
151.52	0.225^{p} ± 0.00022	0.171^{m} ± 0.00171	0.106^{i}± 0.00061	0.191^{n} ± 0.00034	

*** Values are means of three replicates Mean ± SE Numbers followed by the same letter are not significantly different at P < 0.05**

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Conclusion

The study discovered that *P. graveolens* leaf extracts contain various secondary metabolites such as phenols, flavonoids, alkaloids, saponins, and terpenoids. The quantitative analysis demonstrated that the absolute and 70% ethanol extract had the highest level of flavonoid concentration, while the water extract had the highest total phenolic content. Therefore, the presence of phenols and flavonoids gives *P. graveolens* plants the ability to function as antioxidants. Out of the eight mineral elements examined, calcium, magnesium, and iron were identified as having the most abundant concentrations. Moreover, the plant exhibited a greater capacity to absorb calcium and lead compared to the levels present in the soil. Additionally, the study revealed that *P. graveolens* extracts possess a significant activity of antioxidants, as demonstrated by different methods such as DPPH, reducing power, and total antioxidant capacity. Therefore, the plant may have potential applications in the dietary supplements and pharmaceutical industries. However, it is important to remove lead from the plant before processing.

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Authors' Declaration

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

Authors' Contribution Statement

K H J. carried out the isolation and instrumental analysis of samples. Sh A I. conducted the writing of original draft. L Y M. contributed to all laboratory work. All authors contributed to data curation, analysis at supporting degree and guiding of the writing and data proof reading.

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نشاط مضادات الأكسدة وامتصاص المعادن والتحليل الكيميائي ل P. GRAVEOLENS

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الخلاصة

تركز هذا البحث على Pelargonium Gravolens ، وهو نبات طبي عطري من عائلة Geraniaceae. تفحص الدراسة التركيب الكيميائي النباتي للنبات المختار، ونشاط مضادات الأكسدة ، وامتصاص المعادن ، مع زراعة النبات في الداخل المنزل. أظهرت النتائج أن P. Gravolens فعال في امتصاص الرصاص ، كما قامت الدراسة بتحليل محتوى الرماد والنشاط المضاد للأكسدة في النبات باستخدام طرق مختلفة. وجد أن النبات يحتوي على ثمانية معادن مختلفة ، بما في ذلك النحاس ، والمنغنيز ، والكوبالت ، والنيكل ، والرصاص ، والمغنيسيوم ، والحديد ، والكالسيوم. تم استخدام التحليل الإحصائي لتحديد مستوى مضادات الأكسدة الموجودة ، باستخدام DPPH ، و القدرة المختزلة ، وطرق القدرة الإجمالية لمضادات الأكسدة. لغرض الحصول على المستخلص تم استخدام مزيجًا من الماء ومذيبات الإيثانول بنسب متفاوتة. في الختام ، تفحص الدراسة Pelargonium Gravolens ، وهو نبات طبي عطري ، لنشاطه المضاد للأكسدة وامتصاص المعادن. النبات فعال في امتصاص الرصاص ويحتوي على ثمانية معادن. تم استخدام طرق DPPH ، وتقليل الطاقة ، وإجمالي قدرة مضادات الأكسدة لتحديد مستويات مضادات الأكسدة.

الكلمات المفتاحية: مضادات الأكسدة، DPPH، المعادن الثقيلة، IC_{50}، قبور بيلارجونيوم، الفحص الكيميائي النباتي.