PHYTOCHEMICAL IDENTIFICATION OF *PELARGONIUM GRAVEOLENS* L. AND STUDYING ITS BIOLOGICAL ACTIVITY

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ABSTRACT

This study was aimed to determine the active compounds in alcoholic extracts (methanolic 75% and ethanolic 75%) of *Pelargonium gravolens* leaves and flowers as well as their antibacterial and antioxidant activities. The methanolic extract of dry leaves and fresh flowers (30 g each) gave 4.8650, 2.8015 g respectively. While ethanolic extract for the same parts gave 5.5787, 2.1832g respectively. Spectrophotometric analysis showed that, the flavonoids content in leaves and flowers methanol extract were 20.38, 70.90 µg rutin equivalent/g of extract, respectively, as for ethanol extract those values were 21.89, 71.63 µg rutin equivalent/g of extract, respectively. According to the above findings, it has been noticed that the flowers ethanol extract recorded the highest value for flavonoids (71.63 µg rutin equivalent /g of extract). The results indicate that the free radical scavenging activity (RSA) of ethanolic extract of the flowers and leaves was lower than that of methanolic extract. Where the RSA percentages of the leaves methanolic extract were (12, 13, 14, 16, 19 %) at a concentration (0.25, 0.50, 0.75, 1.00, 1.25 mg/ml) respectively, and for the flowers were (142, 162, 162) 164, 166%) at the same concentrations respectively.

Keyword: Active compounds, antioxidant, extract. .
***Part of Ms. Dissertation of the 1st author.**

مجلة العلوم الزراعية العراقية- 2025 :56 (3):1242-1251 **ضفير و حبيب**

التحري الكيميائي لنبات العطرة ودراسة نشاطه البيولوجي.

سعاد فاضل ضفير **هديل مكي حبيب**
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المستخلص

هدفت هذه الدراسة إلى تحديد المركبات الفعالة في المستخلصات الكحولية (الميثانول 75٪ و الإيثانول 75٪) لأوراق وأزهار نبات العطرة، بالإضافة إلى دراسة نشاطها المضاد للبكتيريا ومضادات الأكسدة. أعطى المستخلص الميثانولي للأوراق الجافة والزهور الطازجة (30 جم لكل منهما) 4.8650 و 2.8015 جم على التتابع. بينما أعطى المستخلص الإيثانولي لنفس الأجزاء 5.5787، 2.1832 جرام على التوالي. أظهر تحليل مقياس الطيف الضوئي أن محتوى الفلافونويد في مستخلص الميثانولي للأوراق والازهار كان بواقع 20.38 ، 70.90 ميكروغرام مكافئ روتين/ غرام من المستخلص) على التوالي، أما بالنسبة للمستخلص الإيثانولي، فقد كانت تلك القيم 21.89، 71.63 ميكروغرام روتين مكافئ/ غرام من المستخلص على التوالي. وفقًا للنتائج المذكورة أعلاه ، فقد لوحظ أن مستخلص الإيثانول للزهور سجل أعلى قيمة لمركبات الفلافونويد (71.63 ميكروغرام روتين مكافئ/ جرام من المستخلص). تشير النتائج إلى أن نشاط إزالة الجذور الحرة (RSA) للمستخلص الإيثانولي للزهور والأوراق كان أقل من نشاط المستخلص الميثانولي. حيث كانت نسب RSA للمستخلص الإيثانولي للأوراق (12، 13، 14، 16، 19٪) بتركيز (0.25، 0.50، 0.75، 1.00، 1.25 مجم/ مل) على التوالي، وبالنسبة للزهور كانت (142، 162)،. 162) 164، 166٪) وعند نفس التراكيز، على التوالي.

الكلمات المفتاحية: المركبات الفعالة، مضاد للأكسدة، مستخلص.

البحث مستل من رسالة ماجستير للباحث الأول.

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INTRODUCTION

Medicinal plants have a long history of remediation of various types of diseases worldwide because they include several chemical substances that act to prevent, relieve and treat illnesses (2, 3, 6). *Pelargonium graveolens*, is an aromatic and medicinal plant belonging to *Geraniaceae* family. They are an erect, multi-branched shrub that grows up to 1.5 m and has a spread of 1 m. The leaves are deeply incised, velvety and soft to the touch. The flowers vary from pale pink to almost white. Some plants are strongly scented, and others have little or no scent (22 , 32). Flavonoids are a type of naturally organic compound found in plants. Flavonoids, as a group, comprise upwards of 8000 diverse compounds (27). From a chemical structure standpoint, all flavonoids stemmed from the main skeleton they share and differentiate from each other based on the substituent attached to any part of the structure. Flavonoids possess phenolic and pyrene rings in their structures and have many subclasses, such as flavonols, flavones, flavanones, chalcones, and anthocyanidins (20). The flavonoid skeleton comprises an aromatic ring linked on one side with a six-membered heterocyclic ring, which bears an oxygen atom instead of carbon next to the common side. The two rings connect to another aromatic ring to form the skeleton. Flavonoids have a wide range of pharmacological activities that include anti-oxidant, antimicrobial, anti-inflammatory, antimutagenic, antitumor, and their effects on human health are very often ascribed to their potential ability to act by diminishing free radical steady-state concentration in biological systems and so providing antioxidant protection (13 , 35). Antioxidant activity was displayed by a number of extracts of representative species and cultivars of *Pelargonium.* Miller (35) established that the flavonoids isolated from *P. reniforme* produced higher anti-oxidant activity than ascorbic acid. Potent anti-oxidant activity was observed for the extracts of *P. betulinum* (IC50: 4.13±0.14 µg/ml) and *P. crispum* (IC50 : 4.49±0.18 µg/ml). *P. cordifolium* and *P. scabrum* also showed potent radical scavenging activity. The antimicrobial activity of extracts of *Pelargoniums* and their constituents is reported against bacterial (*Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Haemophilus influenzae*), fungi (*Microsporum canis, Microsporum gypseum, Aspergillus fumigatus, Mucor racemosus, Rhizopus nigricans*) and pathogens as well as opportunistic yeasts such as *Candida albicans* (24 , 31). *Pelargonium glutinosum, Pelargonium pseudoglutinosum, Pelargoniums scabrum* and *Pelargonium sublignosum* exhibited considerable antimicrobial activity against the Gram-positive bacteria (*S. aureus* and *Bacillus cereus*) and Gram-negative bacteria (*K. pneumonia*) (21). This research aims to extract and purify flavonoids from the leaves and flowers of *Pelargonium graveolens* L. and study their biological activity as antioxidants and antibacterial.

MATERIALS AND METHODS

Plant collection; Plant was collected from Baghdad nurseries plant. The plant was classified by Dr. Zainab Abed Aoun Department of Biology / College of Science for Women / University of Baghdad. The leaves were dried in the shade at room temperature and ground by the electric grinder, and kept at the refrigerator temperature until use (while fresh (wet) flowers were used).

Extraction of the active compounds; The active compounds from leaves and flowers were extracted by Soxhlet using methanol (75%) and ethanol (75%) as extraction solutions. Mixing ratio 1:10 (weight / volume) at a temperature of 60-70 ° C for 6-8 hours. Then the extract was filtered and dried by a rotary evaporator at a temperature of 40 °C (4) (1).

Qualitative detection of the active compounds in the crude extract of *Pelargonium graveolens* L. leaves and flowers: The active compounds alkaloids, flavonoids, terpenes, tannins and saponins were detected according to Ali *et al*.(5). Each active compound was detected by using two different specific qualitative reagents (Table1).

Table 1. The types of detections and the reagents that used in the current study

N	Type of detection	Type of reagents
1	Alkaloids	A- Mayer reagent
2		B- Wagner reagent
3	Flavonoids	A- Magnesium crystals and 1% HCl
4		B- H2SO4 reagent
5	Terpenes	A- Chloroform and H2SO4
6		B- Anace aldehyde reagent
7	Tannins	A- FeCl3 reagent
8		B- Lead acetate
9	Saponins	A- Foam reagent
10		B- HgCl2 reagent

Total flavonoids content

The total flavonoids content in the crude extract was determined by the colorimetric method of aluminum chloride (7) . fifty µl of crude extract (one mg/ml ethanol) were made up to one ml with methanol, mixed with four ml of distilled water and then 0.3 ml of 5% $NaNO_2$ solution; 0.3 ml of 10% $AlCl_3$ solution was added after five min of incubation(20 C), and the mixture was allowed to stand for six min. Then, two ml of NaOH (1M) solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. Finally, the total flavonoid content was calculated from the standard curve equation, and the result was expressed as the equivalent of mg rutin per gram of dry weight.

Standard curve of Rutin

Fifty mg Rutin was dissolve in 25 ml distilled water, the dilutions from this standard solution was prepared as shown in the table (2).

Table 2. Concentration of rutin used in preparing the standard curve to estimate the content of flavonoids.

Tube No.	Rutin concentration (µl)	Distilled water (µl)	Concentration mg/ml	Absorbance at 510 nm
1	0	2000	0	0
2	50	1950	0.050	0.199
3	100	1900	0.100	0.28
4	150	1850	0.150	0.499
5	200	1800	0.200	0.63
6	250	1750	0.250	0.821
7	300	1700	0.300	0.96
8	350	1650	0.350	1.117
9	400	1600	0.400	1.28
10	450	1550	0.450	1.389
11	500	1500	0.500	1.43

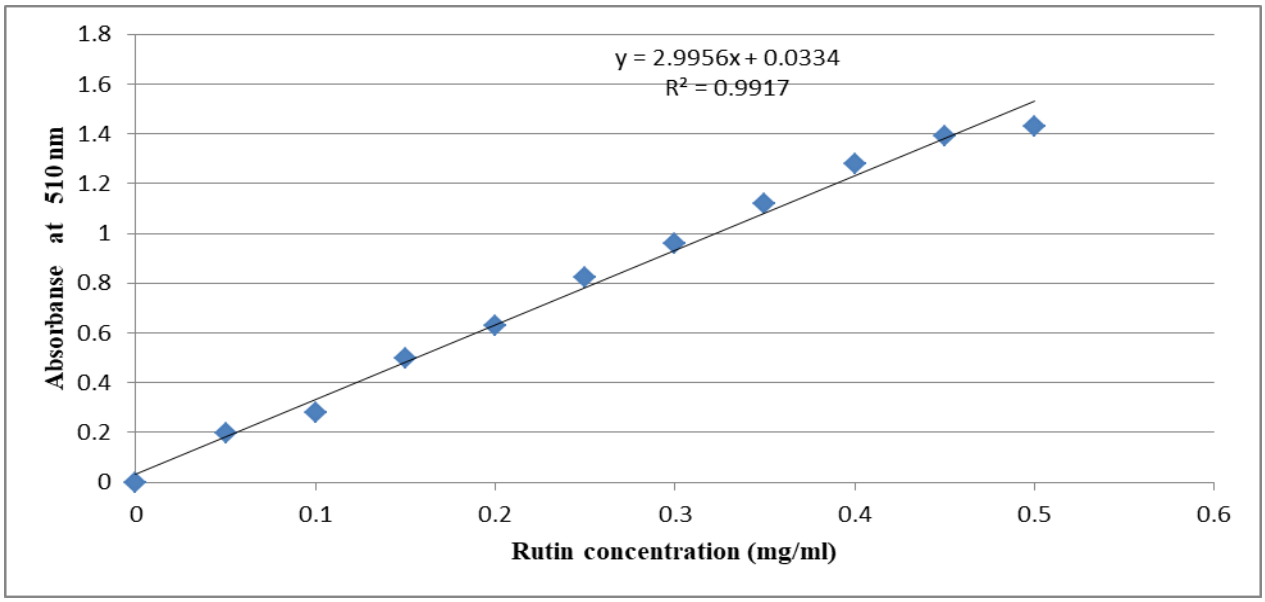

Fig 1. Standard curve of rutin concentration (mg / ml).

Purification of flavonoids

The crude extract was concentrated to 60 ml and transferred to a separating funnel; 30 ml of distilled water and 300 ml of ethyl acetate was added, and shake several times. Finally, the organic layer was separated from the aqueous layer and dried using a rotary evaporator at 40 °C. The dry matter, which represents the flavonoids, was collected (16 , 12).

High-performance liquid chromatography analysis (HPLC): Quantifying individual flavonoid compounds was performed by reversed-phase HPLC analysis, using an SYKAMN HPLC chromatographic system equipped with a UV detector, (Che station, and a Zorb ax Eclipse Plus-C18-OSD .25cm, 4.6mm column). The column temperature was 30ºC the gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was performed, as follows: initial 0-4 min, 40 % B; 4-10 min, 50 % B; and flow-rate of 0.7 ml/min. The injected volume of samples was 100 µl, .and the standard was 100 µL and was done automatically using an auto sampler. The spectra were acquired at 280 nm (30).

Determination of antioxidant activity: DPPH Radical-Scavenging Activity (RSA)

The RSA was measured according to Hammood *et al*. (15) with some modulations. First, one ml sample (1 mg/ml) was mixed with 1 ml DPPH solution (0.1 M). The mixture was kept in the dark at room temperature for 30 minutes and then centrifuged at 10,000x g for 5 min. The absorbency was measured at 517 nm. The percentage of the scavenging activity was calculated according to the following equation (15):

Radical Scavenging Activity = [C – (B–A)/ C] x100

A (Absorbency) = Spectrophotometer reading of the tested sample at517 nm wavelength.

B = the Absorbance of the control sample at 517 nm (prepared by mixing 1 ml of ethyl alcohol with 1 ml of the sample under study).

C = Absorbance of the positive control at 517 nm (obtained from mixing 1 ml of DPPH with 1 ml of distilled water).

RESULTS AND DISCUSSION

Crude extraction: Table 3. shows the extraction yield of active compounds from leaves and flowers of *Pelargonium graveolens*. The active compounds were extracted by two methods. The first method 75% methanol was applied at 70°C. The results showed that 30 g of dry leaves gave 4.8650 g of active compounds, while 30 g of fresh flowers gave 2.8015 g of active compounds. For the second method, ethanol (75%) was used at 70°C, When 30 g of dry leaves gave 5.5787 g of active compounds, while 30 g of wet flowers gave 2.1832 g of active compounds.

Table 3 .The yield of active compounds extracted from leaves and flowers of *Pelargonium graveolens* plant

Extraction solution	Leaves	flowers	L.S.D
Methanol (75%)	16.2166 % (4.8650 g)	9.3383 % (2.8015 g)	3.81*
Ethanol (75%)	18.5956 % (5.5787 g)	7.2773 % (2.1832 g)	4.06*
L.S.D	2.75 NS	2.29 NS	-------

Mizzi *et al.* (25), studied two types of *Pelargonium*, found that *Geranium atlanticum* contained a higher amount of crude alcoholic extract (19.05%) compared to *Geranium lucidum*, whose content was (16.95%).

Detection of the active compounds of *Pelargonium graveolens:* The qualitative detection of some active compounds from four plant extracts of *Pelargonium graveolens* showed that the plant contained alkaloids, terpenes, saponins, tannins and flavonoids, as shown in table 3. These results are consistent with Robert and Philip 2003, findings which mentioned to the presence of terpenes, saponins, tannins and flavonoids in the extracts of *Pelargonium graveolens*. While Saraswathi *et al.* (33) referred to the presence of terpenes, tannins and flavonoids in the aqueous and alcoholic extracts of the aerial parts (flowers, stems and leaves) of the plant *Pelargonium graveolens*. Boukhris *et al.* (9), reported that the aqueous and alcoholic extract of the leaves and flowers of this plant contain flavonoids and phenols. The variance in the published results may be due to genetic factors and environmental factors such as soil components, pH, temperature, light intensity and photoperiod that affect the type and quantity of these compounds which affect the metabolic pathways of the active compounds (10) .

Table 4. Qualitative detection of active compounds of alcoholic extract of *Pelargonium graveolens*

Active compounds	Methanol extract 75% (Leaves)		Methanol extract 75% (Flowers)		Ethanol extract 75% (Leaves)		Ethanol extract 75% (Flowers)	
	Reagent A*	Reagent B*	Reagent A*	Reagent B*	Reagent A*	Reagent B*	Reagent A*	Reagent B*
Alkaloid	(+) White precipitate	(+) Brown precipitate	(+) White precipitate	(+)Brown precipitate	(+) White precipitate	(+)Brown precipitate	(+) White precipitate	(+)Brown precipitate
Saponins	(+) Thick foam	(+) White precipitate	(+) Thick foam	(+) White precipitate	(+)thick foam	(+) White precipitate	(+)thick foam	(+) White precipitate
Terpenes	(+) Brown - reddish	(+)Brown precipitate	(+)Brown – reddish	(+)Brown precipitate	(+)Brown - reddish	(+)Brown precipitate	(+)Brown – reddish	(+)Brown precipitate
Tannins	(+) Green - bluish	(+)Yellow precipitate	(+) Green – bluish	(+)Yellow precipitate	(+) Green - bluish	(+)Yellow precipitate	(+) Green – bluish	(+)Yellow precipitate
Flavonoid	(+)Red-orange	(+) Red	(+)Red-orange	(+) Red	(+)Red-orange	(+) Red	(+)Red-orange	(+) Red

(+) Presence of the active compound. *The reagents mentioned in Table (1).

Determination of total Flavonoids

Fig 2. shows the total flavonoid extracted (µg of Rutin equivalent/g of extract) by methanol (75%) and ethanol (75%) form *Pelargonium graveolens* leaves and flowers. The data show that total flavonoid concentrations ranged from 21.89 to 71.63 (µg Rutin equivalent/g of extract). The high concentration of total flavonoids was observed in flowers ethanol extract (71.63 µg rutin equivalent/g of extract), followed by flowers methanol extract (70.90 µg rutin equivalent /g of extract) as compared to the leaves extracts.Pradeepa *et al.* (29) found that the ethanol extract of *Pelargonium gravolens* leaves exhibited high amounts of flavonoid content. While Hsouna and Hamdi (17) found that the total flavonoid content ranged from 10.90 to 21.75 mg quercetin equivalent /g, for *Pelargonium tomb* leaves extract. The aqueous extract had the highest level, 21.75 mg kaempferol equivalent /g, while hexane extract had the lowest amount 10.90 mg quercetin equivalent /g . As can be seen, a high phenol content was not always accompanied by high concentrations of flavonoids. These results proved that methanol is the most suitable solvent for the extraction of phenolic compounds (8).

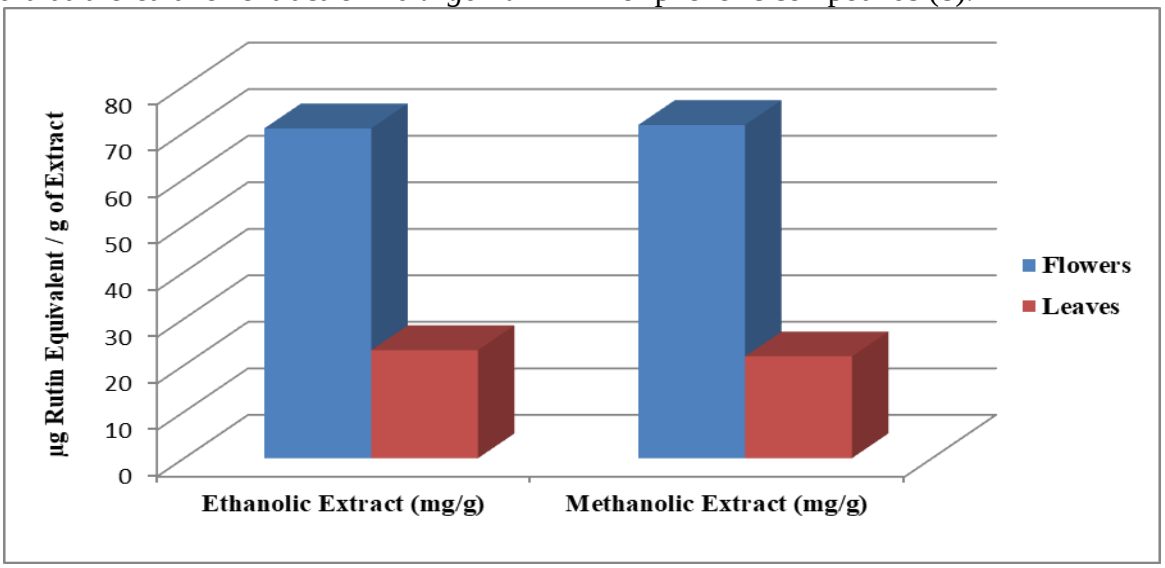

Fig 2. Total flavonoid extracted (µg of Rutin equivalent/g of extract) by methanol (75%) and ethanol (75%) from leaves and flowers of *Pelargonium graveolens* L

Quantitative analysis of *Pelargonium graveolens* flavonoid: HPLC technique is widely applied to quantify and separate antioxidants, mainly phenolic and flavonoid compounds in recent years (25 , 19). Table 5. shows that six types of available standard flavonoid compounds were used, and compared to what is available in the *Pelargonium graveolens* alcoholic extract, namely, Catechin, Quercetin, Rutin, Galic acid, Kaempferol and Apigenin.

Table 4. Quantitative analysis of flavonoids in *Pelargonium graveolens* alcoholic extract using HPLC.

Flavonoids compounds	Retention time (min) of standard flavonoid compounds	Methanol (75%) extract		Ethanol (75%) extract		L.S.D
		Leaves Concentration (µg/g)	Flower Concentration (µg/g)	Leaves Concentration (µg/g)	Flower Concentration (µg/g)	
Catechine	3.15	103.1727	38.5018	50.4578	50.0293	8.03*
Quercetine	4.78	55.1714	28.6243	36.3421	12.6263	7.94*
Rutin	8.74	-	250.7024	-	99.9849	23.57*
Galic acid	10.10	127.7065	44.8720	62.6096	100.1716	21.08*
Kaempferol	6.69	-	35.1521	-	12.2676	8.52*
Apigenin	12.68	177.5652	119.800	104.2250	104.2100	21.07*

Table 4. also shows the concentrations of flavonoids extracted from *Pelargonium **graveolens*** leaves and flowers by methanol and ethanol. The results of HPLC analysis elucidated that the flowers alcoholic extract contained the following compound (catechin, quercetin, rutin, gallic acid, kaempferol and apigenin) and their concentrations were (38.5018, 28.6243, 250.7024, 44.8720, 35.1521, 119.800 µg /gm) respectively. Rutin recorded the highest concentration (250.7024 µg /g), and quercetin recorded the lowest concentration in comparison to the other (28.6243 µg /g). While in the ethanolic extract of the flowers, all flavonoids were appeared at concentrations of (50.0293, 12.6263, 99.9849, 100.1716, 12.2676, 104.2100 µg/g), respectively. It is obvious that the apigenin gave the highest value in this extract (104.2100 µg/g), where as kampferol was the lowest (12.2676 µg/g). From the above results, the methanol extract of the leaves showed fewer active compounds represented by catechin, quercetin, gallic acid and apigenin at concentrations of (103.1727, 55.1714, 127.7065, 177.5652 µg/g), respectively. Apigenin recorded the highest concentration among the active compounds (177.5652 µg/g) while quercetin recorded the lowest concentration (55.1714 µg/g). The ethanol extract of the leaves showed the same types of active compounds but with different concentrations (50.4578, 36.3421, 62.6096, 104.2250 µg/g) respectively. Similarly apigenin recorded the highest concentration among the active compounds (104.2250 µg/g) while quercetin recorded the lowest concentration (36.3421 µg/g). Boukhris *et al.* (9) identified nine flavonoids by high-performance liquid chromatography in aqueous and methanolic extracts of Pelargonium graveolin leaves and flowers. The concentration of flavonoids ranged between 29.9 and 78.2 mg/g in the aqueous and alcoholic extracts of the flowers, respectively, while the concentration of these compounds was 22.5 and 71.2 mg/g in the aqueous and alcoholic extracts of the leaves, respectively. From the results of the previous table, it can be concluded that the flowers and leaves of the *Pelargonium graveolens* plant are promising sources for flavonoid compounds and effective antioxidants. Furthermore, the flowers have a higher content of active flavonoid compounds compared to the leaves (Figures 3 , 4 , 5 , 6).

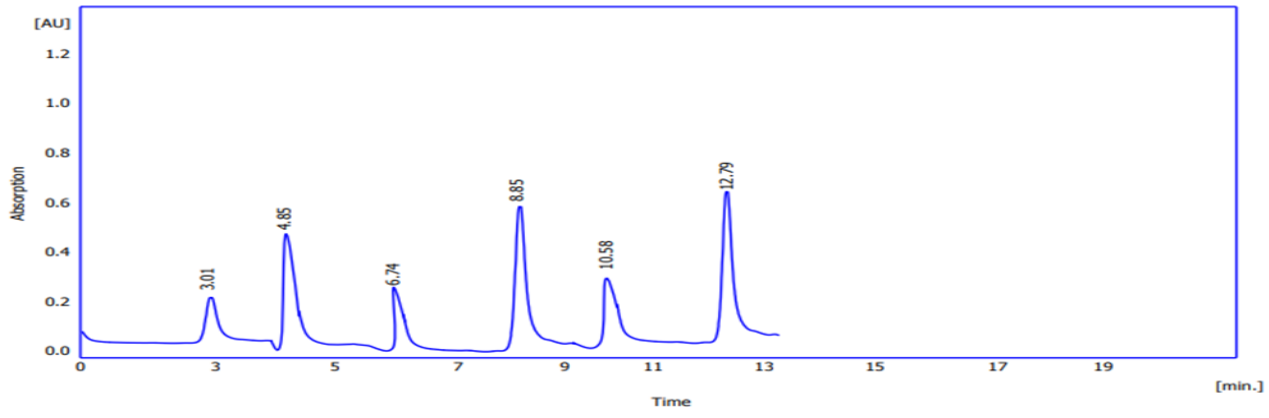

Figure 3. Quantitative analysis of flavonoids in Pelargonium graveolens flowers methanolic extract using HPLC

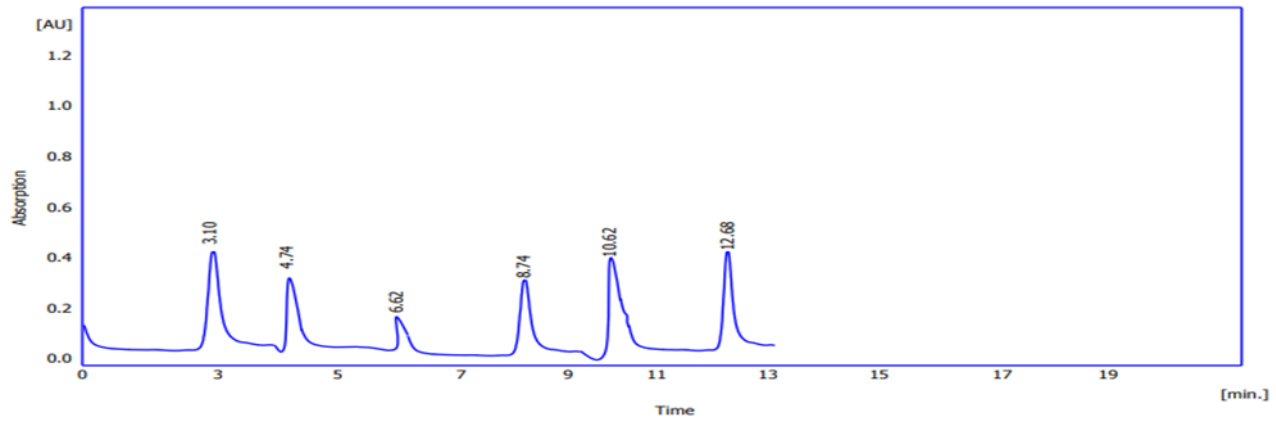

Figure 4. Quantitative analysis of flavonoids in Pelargonium graveolens flowers ethanolic extract using HPLC

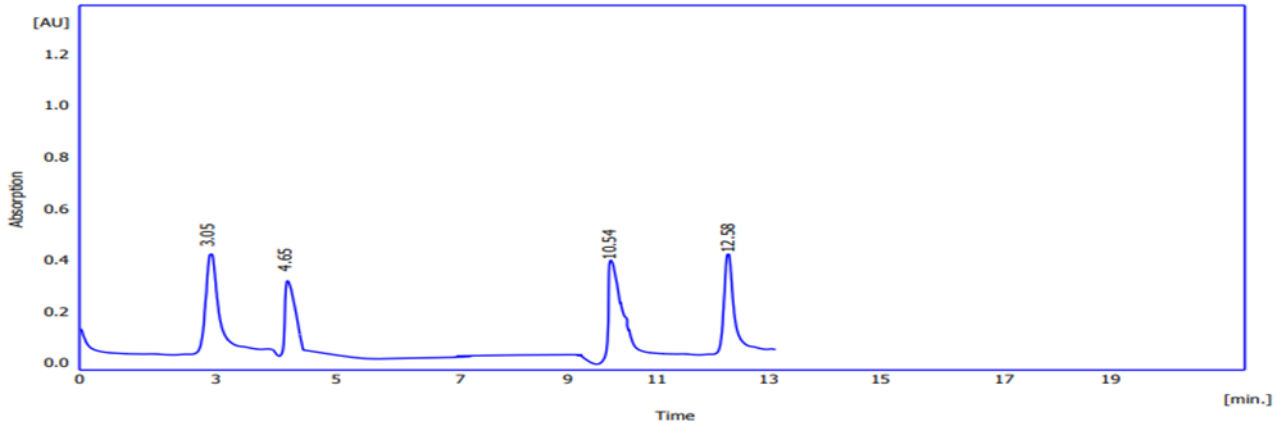

Figure 5. Quantitative analysis of flavonoids in Pelargonium graveolens leveas methanolic extract using HPLC

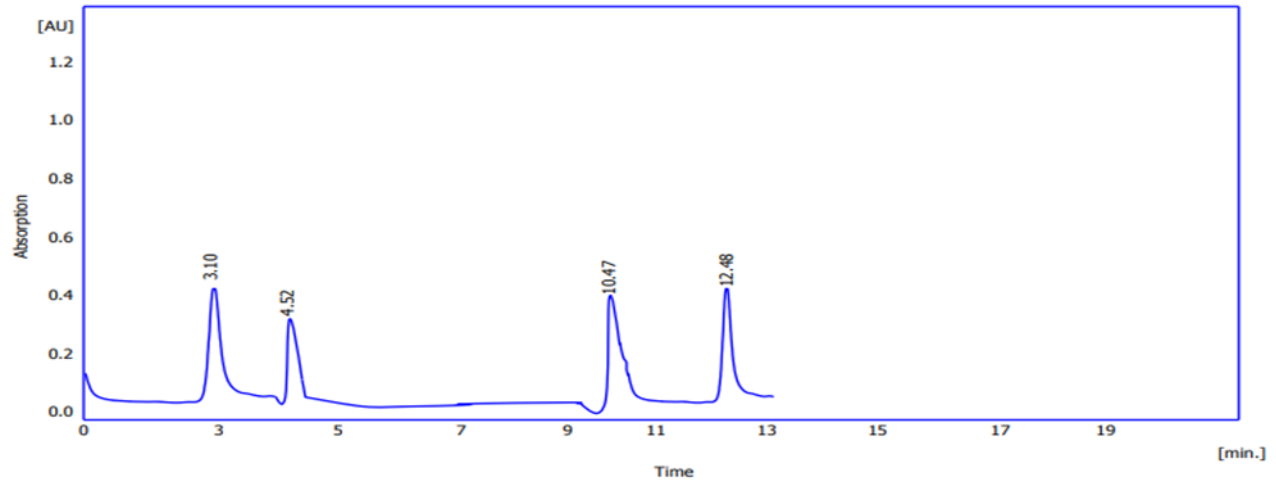

Figure 6. Quantitative analysis of flavonoids in Pelargonium graveolens flowers ethanolic extract using HPLC

Radical-Scavenging Activity (RSA)

Figure 7. shows radical scavenging activity (using DPPH) for extracted flavonoids of leaves and flowers of *Pelargonium graveolens* by methanol (75%) and ethanol (75%). Flavonoids are natural compounds in the plant system that inhibit oxidation activity, and play a significant role in absorbing, decomposing the free radicals and neutralizing and quenching (11, 18). Therefore, the DPPH radical has been used widely to test the potential compounds as free radical hydrogen donors and to investigate plant extracts' antioxidants compounds. The results indicate that the free radical scavenging activity of methanolic extract of the flowers and leaves of the *pelargonium graveolens* plant was lower than that of ethanolic extract. Where the effect

of scavenging free radicals for the methanolic extract of leaves were 16, 18, 19, 17, 20 %, at concentrations 0.25, 0.50, 0.75, 1.00, 1.25 mg/ml, respectively, and for flowers were 46, 84, 122, 144, 170 % at the same concentrations. The effectiveness of ethanolic extract for leaves were 12, 13, 14, 16, 19 at concentrations 0.25, 0.50, 0.75, 1.00, 1.25 mg/ml, respectively, and for flowers were 142, 162 ,162, 164, 166 % , at the same concentrations. The higher activity in the resulting methanol extract compared with ethanol extract may be attributed to the polarity indicated by the published literature (28). As the effectiveness of free radical scavenging for flowers extracts (in both ethanol and methanol) were higher than that of leaves extracts, so the flowers contain higher percentage of flavonoids as compared to leaves. Previous literature studied *P. graveolens* essential oil found significant antioxidant and biological activities (26) . Maria et al. (23) reported that the ethanol extract of the aerial parts of this plant gave a high potency as an antioxidant.

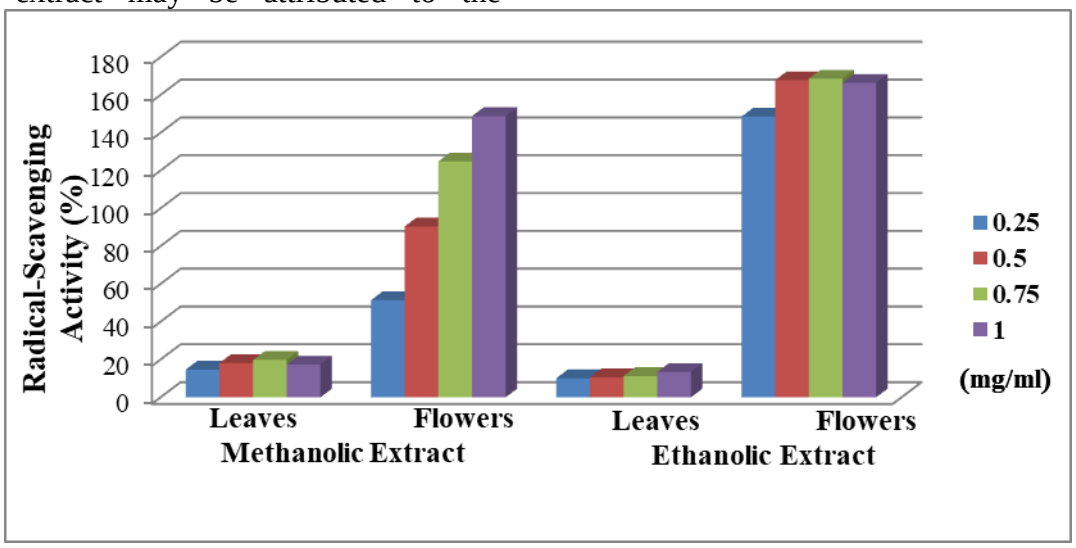

Figure 7. Radical scavenging activity (using DPPH) of flavonoids extracted from leaves and flowers of *Pelargonium graveolens* by methanol (75%) and ethanol (75%).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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