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GC/MS analysis, antioxidant and anti-inflammatory activity of *Pelargonium graveolens*

[Análisis GC/MS, actividad antioxidante y antiinflamatoria de *Pelargonium graveolens*]

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Abstract: One strategy used to discover new drugs for therapeutic use is the study of natural products. Rose Geranium, *P. graveolens*, is a plant that has long been used in traditional medicine for its many medicinal benefits. In this study, we present the first phytochemical study of *Pelargonium graveolens* cultived in Souk Ahras, Algeria. The volatile profile of the plant's leaves was analysed by GC/MS. More than 59 compounds were identified in the essential oils obtained from the leaves, representing 100% of the total oil weight. The main essential oil compounds were oxygenated monoterpenes, of which citronellol (26.69%) and geraniol (13.83%) were the main representatives. Radical neutralisation activity was measured by the DPPH method and IC_{50} values and the result for iron-reducing activity was from 22.93 ± 8.08 µg/mL and 18.79 ± 2.89 µg/mL (leaves) for the essential oil respectively. Considering these findings, *Pelargonium graveolens* essential oils have the potential to be a good source of natural antioxidants.

Keywords: GC/MS; Essential oil; *Pelargonium graveolens*; Anti-inflammatory activity; Souk-Ahras

Resumen: Una estrategia utilizada para descubrir nuevos medicamentos para uso terapéutico es el estudio de productos naturales. El geranio rosa, *P. graveolens*, es una planta que se ha utilizado durante mucho tiempo en la medicina tradicional por sus numerosos beneficios medicinales. En este estudio, presentamos el primer estudio fitoquímico de *Pelargonium graveolens* cultivado en Souk Ahras, Argelia. El perfil volátil de las hojas de la planta se analizó mediante GC/MS. Se identificaron más de 59 compuestos en los aceites esenciales obtenidos de las hojas, representando el 100% del peso total del aceite. Los principales compuestos del aceite esencial fueron los monoterpenos oxigenados, de los cuales el citronelol (26.69%) y el geraniol (13.83%) fueron los principales representantes. La actividad de neutralización de radicales se midió mediante el método DPPH y los valores de IC_{50}, y el resultado para la actividad reductora de hierro fue de 22.93 ± 8.08 µg/mL y 18.79 ± 2.89 µg/mL (hojas) para el aceite esencial, respectivamente. Considerando estos hallazgos, los aceites esenciales de *Pelargonium graveolens* tienen el potencial de ser una buena fuente de antioxidantes naturales.

Palabras clave: GC/MS; Aceite esencial; *Pelargonium graveolens*; Actividad antiinflamatoria; Souk-Ahras

INTRODUCTION

Aromatic and medicinal plant essential oils are one of the various types of natural substances that are receiving particular attention as potential natural agents have been used in food and medical agricultural and cosmetics industries because of their nutritional, food preservation and therapeutic value. Therefore, the essence of studies on essential oils resides not only in their chemical characterisation, but also in the possibility of linking their chemical content to specific bioactive functional properties (Ćavar & Maksimovićm, 2012).

Pelargonium graveolens L. Her. ex-Ait. (synonym *P. roseum* Willd.) is a species of the genus *Pelargonium*. *P. graveolens* is an important and valuable perennial aromatic shrub that can reach a height of 1.5 metres. The plant can reach a height of 1.3 m and a spread (lateral growth) of 1 m (Sharopov *et al.*, 2014). It is a climbing, semi-succulent, enduring plant, trailing through different trees and bushes in its environment (Seidel, 2002).

P. graveolens belong to the Geraniaceae family, most of which are native to Africa and comprise 11 genera, including the genus *Pelargonium*, which comprises more than 7500 species (Jeiter *et al.*, 2017; Alonso *et al.*, 2022).

Pharmacological studies indicate that Pelargonium oil has powerful antioxidant activity, immune modulating effects and antibacterial and antifungal properties. This is why it is normally used in the treatment of inflammation, haemorrhoids, dysentery, heavy menstruation and even cancer (Jaggali *et al.*, 2011; Ali *et al*., 2018).

Among the natural products found in plants, polyphenols and their glycosides constitute one of the largest classes of natural compounds known. The beneficial effects of polyphenols on health are becoming increasingly important. In fact, as natural antioxidants, they are playing an increasingly important role in the prevention and treatment of cancer (Younas *et al.*, 2018), inflammatory (Boo, 2019), cardiovascular and neurodegenerative diseases (Abbas *et al.*, 2017). Based on the above-mentioned information related to *P. graveolens, i*n this study we examined, for the first time, the chemical composition of the essential oil of *Pelargonium graveolens* L. cultivated in Souk ahras district. The biological activities of the essential oil, including antioxidant activity, were tested by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and iron reducing antioxidant power (FRAP).

MATERIALS AND METHODS

Plant collection

The *Pelargonium graveolens* samples come from the Souk ahras region (Algeria) and were harvested in May 2023 (Figure No. 1). The plant was identified by Dr. Ketfi Louiza, botanist, and a voucher specimen (V. PG-34) was deposited in the herbarium of the Department of Botany, at the Faculty of Science, University of Mohamed Cherif Messaadia, Souk Ahras, Algeria. The aerial parts were dried at room temperature (20-25°C) for 14 days.

Isolation of essential oil

A quantity of 200 g of the dried plant was transferred to hydrodistillation and one litre of distilled water was added. The oil was extracted using a Clevenger-type apparatus for 4 hours. Liquid oil was collected, dried with sodium sulphate to remove all traces of water, then collected and stored in an amber bottle at 4°C until use.

The essential oil yield is the ratio between the weight of the extracted oil and the weight of the plant's dry matter.

GC - MS *analyses*

A SHIMADZU GCMS- QP2020, fitted with a fused Rxi®-5 ms capillary column (30 m × 0.25 mm i.d. 0.25 µm film thickness A sample volume of 0.5 µL of net essential oil was injected in split mode (1:80). The injector and detector temperatures were maintained at 250°C and 310°C, respectively. Program temperature at: 50°C (2 min), 50- 310°C at 3°C/min, 310°C (2 min). Helium (99.995% purity) was used as carrier gas at a flow rate of 1 mL/min. Conditions for the mass spectrometer were as follows: ionisation voltage 70 eV, ion source temperature 200°C, mass range 45-600 a.m.u.

Component identification of the essential oil

Individual compound linear retention indices (LRIs) were calculated using a series of homologous n-alkanes (C8-C33). Compounds were identified by comparing their calculated LRIs with those reported in the literature (Adams, 2008; Babushok *et al.*, 2011) and their mass spectra compared with those recorded by the NIST (National Institute of Standards and Technology) and Wiley libraries "NIST17.lib, W11N17MAI and FFNSC1.2.lib".

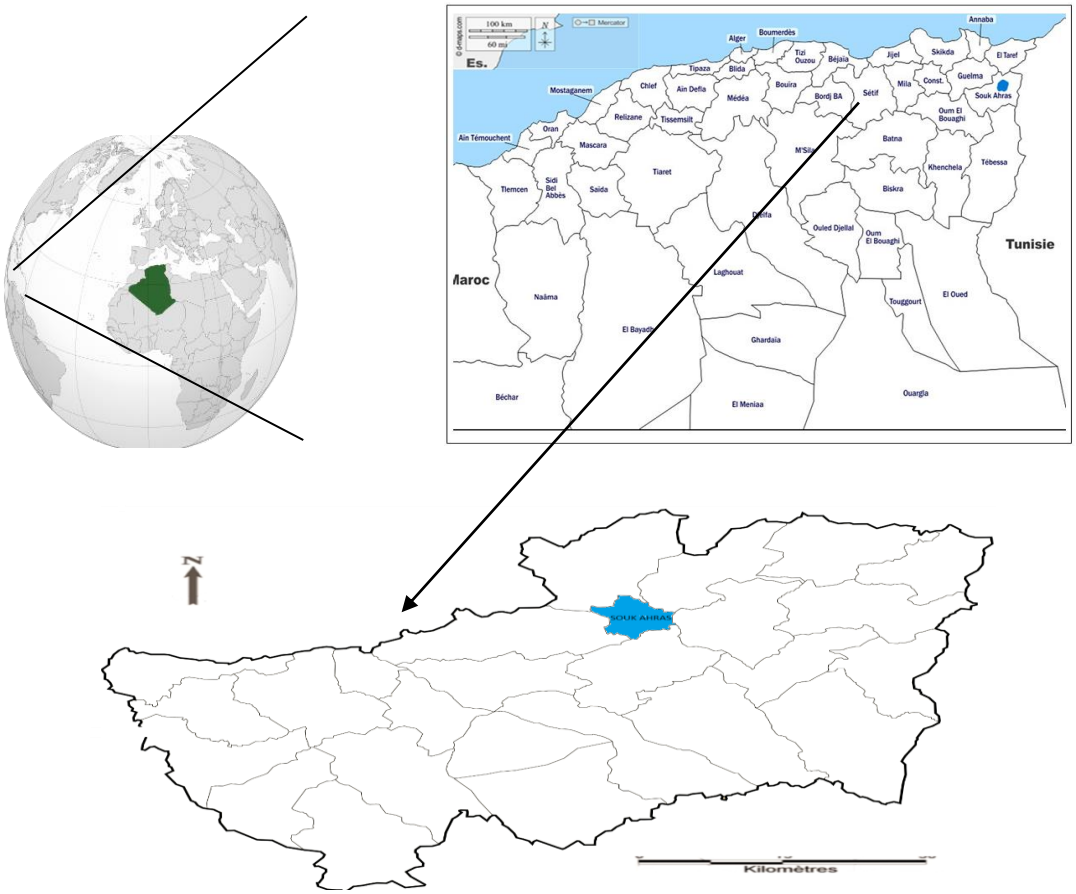

Figure No. 1
Map of the study area, of Souk Ahras

Antioxidant activity

To investigate the anti-free radical activity of the extract, the method described by Brand-Williams *et al.* (1995), was followed.

A solution of 100 µL of essentiel oil sample was added to 2 mL of DPPH (2.4 mg prepared in 100 mL of methanol). Simultaneously, a negative control was prepared by combining 100 µL of methanol with 2 mL of DPPH methanol solution. The solution absorbance was measured against a reference solution at 517 nm after a 30-minute incubation at room temperature in the dark. Positive control was expressed as a solution of a standard antioxidant; ascorbic acid absorbance was measured under the same conditions as the samples. The assay was repeated 3 times for each concentration. The scavenging activity of free radicals was estimated according to the equation below:

$$\mathbf{I\% = Ac - At/AC \times 100} \qquad \text{(Eq.2)}$$

Antioxidant ferric reducing power (FRAP) test

The iron reductive activity of phenolic extracts was assessed according to the methodology described by Mohamadi *et al.* (2023). About 500 µL of a sample with a concentration of approximately 10 µg/mL was combined with 1250 µL of phosphate buffer (pH 6.6) and 1250 µL of potassium ferrocyanide ($K_3Fe(CN)_6$) at a concentration of 1%. These solutions were then incubated at 50°C for 20 minutes. The reaction was stopped by allowing the mixture to cool to room temperature before being treated with 2500 µL of a 10% trichloroacetic acid solution. The mixture was then centrifuged at 3000 rpm for 10 minutes. After centrifugation, 1250 µL of the resulting supernatant

was combined with an equal volume of distilled water and 250 µL of a 0.1% $FeCl_3$ solution. Spectrophotometric absorbance of the resulting solution was measured at a wavelength of 700 nm, with a control sample prepared in the same way serving as a reference. Furthermore, the same experimental procedure was carried out for ascorbic acid at different concentrations (ranging from 0 to 100 µg/mL in distilled water). The experiments were repeated three times for all the compounds tested.

Evaluation of anti-inflammatory activity

The anti-inflammatory activity of the sample was evaluated according to the protein thermal denaturation inhibition assay described by Karthik *et al.* (2022**).** Concentrations in different ranges, from 0.0625 to 1 g/mL, were prepared for use in these assays. Solutions composed of 1 mL of each dilution and 1 mL of 0.2% ovalbumin (diluted in phosphate-buffered saline [PBS]) were incubated for 5 min at 72°C. After vortexing and cooling, absorbance was measured at a wavelength of 660 nm against a blank sample free of extract. The reference anti-inflammatory agent, diclofenac, was prepared using the same procedures as the extract. The inhibition level (T%) of protein denaturation was calculated using the following formula:

$$\%=100 - ODs / ODc \times 100$$

where ODs: Optical Density of extract ODc: Ovalbumine solution without extract

Data analysis

For all experiments, the analyses were performed in triplicate, and the values are reported as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Chemical composition

A yellow to brownish oil was obtained from the hydrodistillation *of P. graveolens* leaves. GC-MS analysis of the essential oil resulted in the identification of 59 different compounds, representing 100% of the overall chemical composition listed in Table No. 1 and Figure No. 2, according to their order of elution on the column. The essential oil contained a complex mixture of mainly monoterpene and other essential phytochemicals compounds.

The yield of *Pelargonium graveolens* essential oil extracted, as studied, was 0.37%. Almost identical results were found in the literature, particularly for *Pelargonium graveolens* essential oils from Morocco (M'Hamdi *et al.*, 2022) and Egypt (Fayed, 2009) respectively.

Nevertheless, in our study, the yield is considerably high compared to the results of Rana *et al.* (2002) and Fayed (2009) with a yield of about 0.22% (v/w) and 0.26% (v/w) respectively. However, our results are consistent with those of M'Hamdi *et al.* (2022) for *Pelargonium graveolens* essential oil from Er-Rachidia, Tétouan, and Meknès, Morocco, with yields of 0.308% and 0.381%, respectively.

In contrast, Afifi *et al.* (2014), and Jaradat *et al.* (2022), reported a 1.5% yield for the extraction of PGEO from its air-dried leaves collected in Amman, Jordan and 1.01% yield for the extraction of PGEO from its air-dried leaves collected in Tulkarem, Palestine.

According to the results available, the differences in yield are mainly due to variations depending on the morphology of the plant and the type of soil, as well as the species, the harvest period, the cultivation practices involved and techniques used for extraction (Elshafie & Camele, 2017).

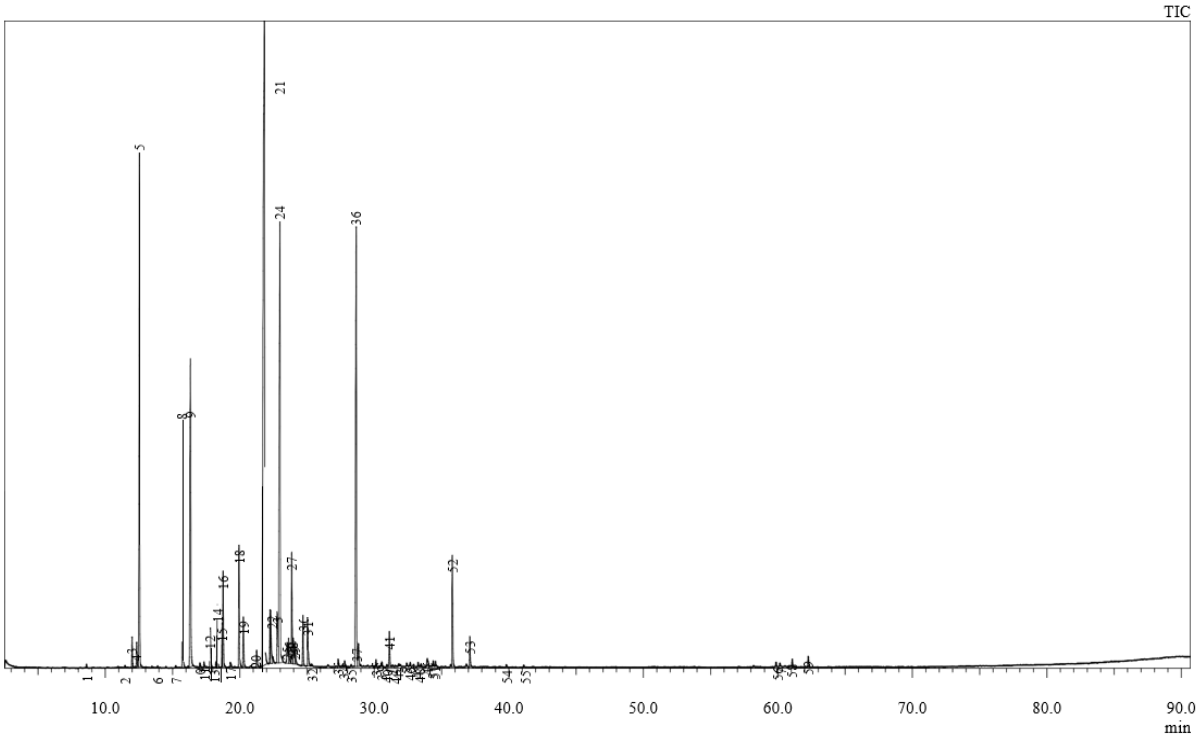

Figure No. 2
Chromatographic profile of the essential oil of *Pelargonium graveolens* harvested in the Souk Ahras region, Algeria, analysed by GC/MS

Although a comparison of the compounds identified in this study with the preceding reports highlights some similarities, there are nevertheless quantitative and qualitative differences between these samples.

The elements of citronellol (26.69%) and geraniol (13.83%), were the main monoterpene alcohols representatives. According to an earlier report, the leaves produced 0.17-0.2% light yellow volatile oil, composed mainly of geraniol and citronellol (Anon, 1991). Both citronellol and geraniol alcohols are derived from geranyl pyrophosphate, but the variability in their concentration is thought to be due to production by different enzymes (Verma *et al.*, 2010).

Pelargonium graveolens essential oil contains other components such as geranyl acetate (10.86%), limonene (10.08%), phenylethyl alcohol (7.36%), linalool (5.33%), α -Terpineol (2.52%), Geranyl butanoate (2.24%), methyl ether, Borneol (2.14%), Geranyl (1.60%), butanoate (1.47%), Isoborneol (1.28%), Neral (1.07%) and γ-Terpineol (1.01%).

Most of the constituents of essential oil of *Pelargonium graveolens* are monoterpene oxygenated derivatives (52.54%), monoterpene hydrocarbons make up 10.17%, sesquiterpene hydrocarbons account for 25.42%, oxygenated sesquiterpenes represent 1.69%, and other compounds constitute 10.17%. However significant variations in the chemical composition can be noted, particularly depending on the origin of the plant used for EO extraction.

According to the study of Bigos *et al.* (2012), the primary constituents of *Pelargonium graveolens* Ait. were found to be citronellol (26.7%) and geraniol (13.4%). Other common compounds found in geranium EO included nerol (8.7%), citronellyl formate (7.1%), isomenthone (6.3%), linalool (5.2%), and 10-epi-γ-eudesmol (4.4%), among the sixty-seven constituents discovered.

Notably, *Pelargonium graveolens* essential oil (PGEO) obtained from Palastine exhibited Citronellol (24.44%), citronellyl formate (15.63%), γ-

eudesmol (7.60%), and iso-menthone (7.66%) were the dominant chemical markers and there were 70 chemicals found in the GCMS analysis, and oxygenated terpenoids were the most abundant group of the total PGEO.

On the other hand, thirty-two compounds constituting 99.23% of geranium essential oil from Egypt have been identified. The major components were citronellol (29.90%), trans-geraniol (18.03%), 10-epi-γ-eudesmol (8.27%), isomenthone (5.44%) and linalool (5.13%) with geranium yield of 0.26%(v/w).

Reported by Boukhris *et al.* (2013), the Tunisian PGEO, by contrast, showed significant concentrations of β-citronellol (21.9%), citronellyl formate (13.2%), geraniol (11.1%), 10-epi-γ-eudesmol (7.9%), geranyl formate (6.2%) and (l)-linalool (5.6%). Additionally, according to Boukhatem *et al.* (2013), a total of 45 compounds representing 94.2% of the essential oil were identified. The main constituents were citronellol (30.2%), citronellyl formate (9.3%) and geraniol (7.6%).

Table No. 1
Chemical composition of *Pelargonium graveolens* essential oil from Souk Ahras, Algeria

Peak	Retention Time (min)	Area%	Similarity	Index	Name of compound
1	8.607	0.06	95	*926*	α-Pinene
2	11.469	0.02	93	998	α-Phellandrene
3	11.992	0.51	96	1010	α-Terpinene
4	12.331	0.44	96	1018	ρ-Cymene
5	12.529	10.08	97	1022	Limonene
6	13.897	0.01	89	1052	γ-Terpinene
7	15.233	0.04	84	1082	cis-Sabinene hydrate
8	15.761	5.33	95	1094	Linalool
9	16.330	7.36	97	1106	Phenylethyl alcohol
10	17.041	0.08	95	1122	trans-Rose oxide
11	17.342	0.09	93	1128	Terpin-3-en-1-ol
12	17.813	0.92	91	1138	Isopulegol
13	18.225	0.10	93	1147	Menthone
14	18.333	1.28	96	1149	Isoborneol
15	18.694	0.58	27	1157	iso-Menthone
16	18.758	2.14	88	1158	Borneol
17	19.291	0.12	90	1170	Phenethyl formate
18	19.942	2.52	94	1184	α -Terpineol
19	20.264	1.01	95	1191	γ-Terpineol
20	21.251	0.42	93	1212	iso-Dihydrocarveol
21	21.833	26.69	94	1225	Citronellol
22	22.285	1.07	84	1235	Neral
23	22.776	1.60	95	1246	Carvacrol methyl ether
24	22.996	13.83	95	1251	Geraniol
25	23.518	0.58	93	1262	Carvacrol
26	23.636	0.56	94	1265	Geranial
27	23.864	2.09	95	1270	Citronellyl formate
28	23.966	0.72	94	1272	p-Cymen-2-ol
29	24.111	0.48	93	1276	Thymol
30	24.691	0.97	97	1288	Menthyl acetate
31	25.063	0.98	93	1296	Geranyl formate

32	25.340	0.06	94	1303	α-Terpinyl acetate
33	27.309	0.15	95	1348	Citronellyl acetate
34	27.792	0.11	94	1359	Neryl acetate
35	28.284	0.03	93	1371	alpha-Copaene
36	28.652	10.86	96	1379	Geranyl acetate
37	28.819	0.67	90	1383	β-Elemene
38	30.128	0.16	95	1414	β-Caryophyllene
39	30.557	0.11	95	1424	β-Ylangene
40	30.934	0.05	95	1433	α-Guaiene
41	31.120	0.75	93	1438	Guaia-6,9-diene
42	31.354	0.04	88	1443	Cedrane
43	31.546	0.02	89	1448	α-Humulene
44	31.823	0.06	95	1454	Alloaromadendrene
45	32.412	0.05	84	1469	γ-Muurolene
46	32.670	0.07	94	1475	Germacrene D
47	33.259	0.09	93	1489	Viridiflorene
48	33.479	0.07	89	1494	α-Muurolene
49	33.943	0.29	90	1506	γ-Cadinene
50	34.379	0.10	89	1517	δ-Cadinene
51	34.546	0.08	95	1521	Citronellyl isobutyrate
52	35.801	2.24	96	1554	Geranyl butanoate
53	37.099	0.60	97	1587	~~nd~~
54	39.820	0.05	95	1660	(E)-Citronellyl tiglate
55	41.113	0.04	90	1694	Geranyl tiglate
56	59.868	0.09	89	2285	Isopimaric acid
57	60.150	0.07	74	2295	Methyl isopimarate
58	61.078	0.17	88	2329	Methyl dehydroabietate
59	62.264	0.25	90	2372	Methyl abietate
Oxygenated monoterpenes: 52.54%					
Sesquiterpene hydrocarbures: 25.42%					
Monoterpene hydrocarbures: 10.17%					
Oxygenated sesquiterpenes: 1.69%					
Others: 10.17%					

The antioxidant capabilities of the PGEO employed in this investigation were higher than those of PGEO from other countries. Fayed (2009) reported that the EC_{50} (geranium from egypt) = 66.45 µg/mL and EC_{50} and (ascorbic acid) = 38.49 µg/mL.

Our findings are much lower than those reported for other *Pelargonium* (Kačániová *et al.*, 2023), which showed the ability of PGEO to neutralize stable DPPH radicals and ABTS radicals was evaluated based on IC_{50} and TEAC values. In the DPPH assay, the IC_{50} value was found to be 1.14 ± 0.08 mg/mL, while the TEAC value was evaluated at 0.0040 ± 0.0002. Also, the antioxidant capabilities of the PGEO employed in this investigation were lower than those of PGEO from Palestine where the EO showed remarkable antioxidant properties, with an IC_{50} dose of 3.88 ± 0.45 µg/mL and an antioxidant activity of 48.45% compared to Trolox (IC_{50} = 1.88 ± 0.45 µg/mL) (Jaradat *et al.*, 2022).

Nevertheless, considerable variations in constituent profile of essential oil might be influenced by the phenological stages and geographical conditions of the growing region (Zomorodian *et al.*, 2013), environmental conditions, plant nutritional status, season, and others (Rathore *et al.*, 2023).

Similarly, for the antioxydant activity, the moderate phenols and flavonoids contents could enhance hydrogen proton donation to the unpaired electrons of the radical, chelating of transition metals thus neutralize its pathological damage.

However, Al-Mijalli *et al.* (2022), reported in his research that the essential oil at the full flowering stage showed the best antioxidant activity, with using DPPH (IC_{50} = 83.26 ± 0.01 µg/mL) and FRAP (IC_{50} = 116.42 ± 0.07 µg/mL) methods and Dzamic *et al.* (2014), reported that the oil of *Pelargonium graveolens* essential oil obtained from the Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, showed a slightly lower antioxidant activity (IC_{50} = 0.802 mg/mL) than the synthetic antioxidant BHT (IC_{50} = 0.328 mg/mL).

According to Ming, (2001), as the scavenging activity of DPPH depends on the hydrogen donation capacity of the compound tested, the comparisons are not quantitative because the reaction with DPPH depends on the structural conformation of this compound (Fukumoto & Mazza, 2000). Substitution of the ortho position of monophenols with a methoxy group, which acts as an electron donor, increases the percentage of inhibition. Substitution with a hydroxyl group was more effective than the methoxy group of the phenols in trapping DPPH. Antioxidant activity is reduced by the presence of a glycoside (Gadow *et al.*, 1997), which is probably due to steric hindrance (Fukumoto & Mazza, 1997; Ming, 2001).

For the FRAP method, the blue Fe (III) tripyridyltriazine ferric complex is decolourised into a dark blue Fe (II) ferrous complex in the presence of an antioxidant (Azrina *et al.*, 2010). Chelating activity of metals is based on the quantitative chelation of Fe^{2+} ions by the reagent ferrozine, which leads to the formation of a complex with Fe^{2+} ions. (Saritha *et al.*, 2010).

The result for iron-reducing activity was 18.79 ± 2.89 µg/100 mL for the essential oil of *Pelargonium graveolens* and 0.50 ± 0.0 µg/mL for ascorbic acid. The reference drug (Ascorbic acid) showed greater antioxidant activity in both DPPH and Ferric reducing antioxidant power (FRAP) assays compared to the essential oil (Table No. 2).

Table No. 2
Antioxidant activity of *Pelargonium graveolens* essential oil from Souk Ahras, Algeria

Species	**DPPH** IC_{50} in µg.mL^{-1}	**FRAP** µg.100mL^{-1}
Pelargonium graveolens	22.93 ± 8.08	18.79 ± 2.89
***Ascorbic acids**	1.309 ± 0.012	0.5 ± 0.00

[a]Antiradical DPPH activities are expressed as IC50 in µg/mL^{-1} for sample and compounds; [b] Reducing powder mesured as the ability to reduce ferric ions in µg.100 mL^{-1}. Data are the mean of three replicates (n=3) and represented as mean±SD * Used as standard antioxidants

Reducing power is expressed when an antioxidant acts as an electron donor that reduces the intermediate oxidised substances produced by lipid peroxidation. Therefore, the reducing power component acts as a primary and secondary antioxidant (Yen & Chen, 1995). Therefore, chelation capacity influences other free radical scavenging activities that protect organisms against oxidative damage (Kumar *et al.*, 2017).

It could be inferred from this study that essential oil of *Pelargonium graveolens* could serve as a potential source of natural antioxidant agent against radical related diseases.

Additionally, one of the largest and most widely distributed classes of phytochemicals in the plant kingdom are polyphenols. More than 8,000 polyphenolic compounds have been identified in various plant species (Saboon *et al.*, 2019).

Due to their robust antioxidant capabilities, potential health benefits, wide variety of biological activities, and strong antioxidant qualities, phenolic compounds are substances that have drawn considerable attention in recent years (Hemmami *et al.*, 2023).

In plants, phenolics are ubiquitous secondary metabolites. They are aromatic compounds synthesised by the phenylpropanoid pathway and believed to be participative in adapting the plants in a stressed situation due to environmental changes (Zhao, 2015).

The well documented fact that most medicinal plants are enriched in phenolic compounds has revealed biological and pharmacological properties such as antimicrobial, antiviral, antioxidant, anti-inflammatory and cytotoxic activity that could validate the use of the plant in ethnomedicine (Lee *et al.*, 2013).

Numerous studies suggest a direct correlation between phenolics and antioxidant activity, so estimating total phenolics is very important. A good indicator of the antioxidant capacity of the extracts studied could be the phenolic compound content. Plant phenolics include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans (Dai & Mumper, 2010). Because they contain hydroxyl groups, phenols are compounds that have the ability to destroy radicals. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals; they therefore play an important role in antioxidant activity. Determining the amount of phenolic compounds is therefore very important in determining the antioxidant capacity of plant extracts. The research on *P. graveolens* oil of Ćavar & Maksimović (2012), revealed that the content of phenolic compounds in corresponding hydrosols as 34.88 ± 2.00 mg GAE/g in leaves and 102.44 ± 1.63 mg GAE/g in stems including flavonoid compounds of 32.35 ± 0.81 mg GAE/g to 101.87 ± 1.03 mg GAE/g. Morever, the results showed that the methanolic extract contained the highest amounts of total phenolic, flavonoids, flavonols, and condensed tannins 381.25 ± 2.65 µg GAE/mg, 330.08 ± 10.88 µg QE/mg, 181.52 ± 2.35 µg QE/mg and 246.62 ± 3.26 µg CE/mg of extract, respectively.

The quantitative and qualitative composition of the polyphenolic fraction determines fresh plant materials' sensory quality and biological value (Nurzyńska-Wierdak, 2023).

The phenolic compound content varies from one country to another According to (Haddouchi *et al.*, 2021). The synthesis and concentration of the accumulated phenolics depend on many internal and external factors such as plant physiology, age, development stage, climate, and the type of pathogen attack (Ozyigit *et al.*, 2007; Pratyusha, 2022).

Anti-inflammatory activity

Proteins denaturation is a known source of inflammation. Therefore, as part of the investigation to assess the anti-inflammatory mechanism of EO, its inhibitory ability on BSA denaturation was calculated. Table No. 3 shows the inhibitory action of EO on BSA denaturation.

Table No. 3
Anti-inflammatory activity of *Pelargonium graveolens* oil from Souk Ahras, Algeria

Species	**IC_{50} (µg/mL)**
Pelargonium graveolens	**44.06 ± 0.8 µg/mL**
Diclofenac sodium	**11.55 ± 0.403**

Data are the mean of three replicates (n=3) and represented as mean ± SD

*Pelargonium graveolen*s essential oil was found to effectively inhibit BSA denaturation in a dose-dependent manner, achieving maximum inhibition at 44.06 ± 0.8 µg/mL. In contrast, diclofenac sodium, a standard anti-inflammatory drug, displayed an IC_{50} value of 11.55 µg/mL, corresponding to a 64.44% inhibition rate. It's noted that plant essential oils (EOs) like *Pelargonium graveolens* oil are known to prevent protein denaturation, a mechanism crucial to the action of NSAIDs.

According to Boukattem *et al.* (2022), their study highlights EO's remarkable anti-inflammatory effect, reporting a significantly lower IC_{50} value of 4.63 ± 0.3 µg/mL. This suggests that EO, likely *Pelargonium graveolens* oil as well, demonstrates greater potency in inhibiting inflammation compared to diclofenac sodium. Additionally, Boukattem *et al.* (2022), underscore EO's role as an effective stabilizer of erythrocyte membranes, which was not specifically addressed in the investigation. Thus, Boukattem *et al.* (2022), findings suggest EO's

potential broader therapeutic benefits beyond its direct anti-inflammatory action.

CONCLUSION

The purpose of this research was to describe the chemical composition and antioxidant activity of *Pelargonium graveolens* essential oil. 58 constituents were found by GC-MS analysis of the essential oils. Assessment of antioxidant activity *in vitro* demonstrated anti-inflammatory activity and an antioxidant capacity. These numerous results show that *Pelargonium graveolens* essential oil are an interesting natural alternative for aromatherapy and medicinal use.

For future research in this field, it is crucial to focus on studying the variability in chemical composition of essential oils, considering factors such as plant age, harvest period, and geographical location. This approach will allow researchers to observe qualitative and quantitative changes in essential oils, thereby determining optimal conditions for achieving satisfactory yield or desirable biological activity.

Furthermore, expanding this study could involve deeper investigations into the tested essential oils. Specifically, researchers should aim to identify specific biological activities associated with their unique compositions. Additionally, studying cytotoxicity and biological tolerance will be essential to assess their potential for enhancing traditional medicines, potentially including the development of new contraceptives. Moreover, exploring the individual and synergistic effects of these oils' chemical compounds on various biological activities will provide valuable insights into their therapeutic potential across medical and pharmaceutical applications. These efforts will contribute to a better understanding of essential oils' mechanisms and pave the way for their innovative use in healthcare.

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CONFLICT OF INTERESTS

All authors state that the research was conducted in the lack of any commercial or financial conflicts.

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