CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *Pelargonium graveolens* OIL

S. H. Obeid **B. M. Jaber**
Researcher **Assistant Prof.**
Department of Biology ,Collage of Science for Women ,University of Baghdad, Iraq.
sabahussein2015@yahoo.com

ABSTRACT

A comparison was conducted between volatile oil of the plant parts leaves and stems of *Pelargonium graveolens* as antioxidant by using the compound DPPH as a free radical. This study aimed at isolation and diagnosis the active compounds of the volatile oil .The volatile oil was extracted by using the water distillation Clevenger .Volatile oils were detected by using the liquid gas chromatography. The results showed that 100 g of leaves and steams produced a volatile oil estimated by 5% and 2.3% respectively. The study has shown that volatile oil of leaves contains 12compounds and the highest was Terpineol reaching 31.52% , Camphor 7.06 % and Cis-alpha-Bisabolene 4.38%. The study showed that volatile oil in steams contains 10 compounds and Linalool represented the highest (3.00%), followed by Camphor (2.76 %), then (E) –B-Farnesene (2.31 %). The results also showed high volatile oil antioxidant activity in both leaves and steams at the concentration 125 µg ml^{-1} reached 100 % compared with Ascorbic Acid when studying the effect of their oxidative effect on the free radical DPPH, the results also indicate significant differences between the volatile oil concentrations in both leaves and steams.

Keywords: Geranium, volatile oil, Ascorbic acid

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التركيب الكيميائي والفاعلية المضادة للأكسدة لزيت نبات العطرة

صبا حسين عبيد بشرى محمد جابر
باحث استاذ مساعد
قسم علوم الحياة /كلية العلوم للبنات /جامعة بغداد/ العراق
sabahussein2015@yahoo.com

المستخلص

أجريت مقارنة بين الزيت الطيار للأجزاء النباتية الاوراق والسيقان لنبات العطرة ***Pelargonium graveolens*** كمضاد للأكسدة باستخدام المركب (DPPH)، كجذر حر .هدفت هذه الدراسة إلى عزل وتشخيص المركبات الفعالة من الزيت الطيار. تم أستخلاص الزيت الطيار باستخدام طريقة التقطير المائي بواسطة جهاز (Clevenger) ،وقدرت مكونات الزيت الطيار باستخدام تقنية كروموتوغرافيا الغاز السائل – مطياف الكتلة Gass Chromatography Mass. أظهرت النتائج أن 100 غرام من الأوراق والسيقان انتجت مايقارب 5% و2.3 % على التوالي. وقد أوضحت الدراسة أن الزيت الطيارللأوراق يحتوي على 12 مركب ،وقد اظهر Terpineol أعلى تركيزكانت نسبته المئوية (31.52%) وCamphor (7.06 %) ثم Cis– alpha–Bisabolene (4.38 %)، أما زيت السيقان فمكون من 10 مركبات، وقد احتل Linalool أعلى تركيز في هذا الزيت حيث بلغت نسبته المئوية (3.00%) ثم Camphor بتركيز (2.76%)، ويليه (E –B–Farnesene) بتركيز (2.31%)، أظهرت النتائج الفعالية العالية للزيت الطيار لكل من الاوراق والسيقان عند تركيز 125 مايكروغرام مل$^{-1}$ كمضاد للأكسدة بنسبة 100% مقارنة مع معامل السيطرة (حامض الاسكوربك) عند دراسة تأثير فعاليته التأكسدية على الجذر الحر (DPPH)، حيث لوحظ وجود فروق معنوية بين تراكيز الزيت الطيار لكل من الاوراق والسيقان.

الكلمات المفتاحية: نبات العطرة، الزيت الطيار، حامض الاسكوربيك.

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INTRODUCTION

The term Alternative Medicine has become very common these days and is based on the idea of using plants for medical therapeutic purposes . Medicinal plants are used as crud or raw material to extract the active ingredients involved in the manufacture of various medicinal drugs , blood thinners, laxatives , antibiotic ,anti malaria and others (20).Herbal treatment derived from medicinal plants have been categorized by the World Health Organization (WHO) to be the most widely used around the world ,and encouraged the use of herbal drugs which being safe and easy to store and more effective and side effects are almost none compared to chemical treatments (23).The existence of dictionaries of medicines (Pharmacopia) ,which is an important source of knowledge of medicinal plant species and their active ingredients ,alkaloids ,phenols and terpenoids are the main groups for the classification of secondary compounds responsible for the therapeutic properties of the plants that present in them (7) Aromatic oils were used in the manufacture of perfumes and as flavors for food, drink or healing for many diseases for thousands of years (12). Essential oils in Mesopotamia ,ancient Egypt ,China ,India and Persia have been known in many treatments and in various forms (5).At present time ,peppermint oil, Lavender oil, Geranium oil, Eucalyptus oil, Rose oil and Chamomile oil are the most widely traded (15).Geranium oil is an important oil because it is used in the pharmaceutical industry and herbal treatment. It possesses therapeutic properties as it can be used as sedatives, antidepressants, infections, diarrheal diseases, diabetes, stomach ulcers, kidney stones, urinary incontinence, preventing fluid retention in the body, in addition to its use in the cosmetics industry (8, 18, 22). The plant has an aromatic smell that is similar to the scent of roses, this aromatic smell of the plant is due to its richness in aromatic essential oil. It is made up of several compounds, the most important of which are the main compounds responsible for aromatic smell which are linalool, geraniol, pinene, myrcene, citronellol, limonene and menthone (24).The essential oil of the plant is a nature source of antioxidant compounds, including, linalool, farnesene, pinene, camphor, myrcene, terpineol and camphene, the scientists pointed that these compounds have a clear effect as antioxidant (2, 9, 16,19,22).

MATERIALS AND METHODS

The fresh parts of *Pelargonium graveolens*(Geranium) were collected during the growing period from nurseries of Zayuna in Baghdad .The plant was classified in the Iraqi National Herb /Ministry of Agriculture .The plant cleaned well from the dust and separated leaves ,steams and kept in the refrigerator at 4^0c for the purpose of oil extracting. 100 g of fresh leaves and steams of the plant were used for extraction volatile oils by hydro distillation using a Clevenger type apparatus .The oils was collected in dark sealed vial and kept in refrigerator at 4^0c until use (26). The extracted volatile oils volumes and percentage were calculated according to the following equation :

Extracted oil ratio $\frac{Oil\ volume}{sample\ fresh\ weight} \times 100$

The volatile oil of the plant parts (leaves ,steams) were analyzed by GC-Mass spectra at the conditions according to Table 1 and compared with the library of National Instituts of Standards and Technology (NIST) (14). The hydrogen electron donating ability of the corresponding volatile oils was measured from the bleaching of the purple colored methanol solution of 2,2-diphenyl – picrylhydrazyl (DPPH) (1) .This assay uses the free radical DPPH , as a reagent, which prepared by adding 1.97 mg of DPPH to 50 ml of absolute methanol to prepare solution with 0.1 mmol concentration .Five concentrations were uses (25,50,75,100,125 µg ml^{-1}) of the essential oil (leaves ,steams) , 3 ml of oil was added to 1 ml of methanolic solution of DPPH. The samples leaved in incubation at 37^0 c (310 K) for 30 minutes, then measured by spectrophotometer ,the absorbance was read against blank at 517 nm .Ascorbic acid was used as standard control .The antioxidant capacity to scavenge the DPPH radical for the oils was calculated by the following equation :

Scavenging activity % = $\frac{\text{A 517 nm control} - \text{A517nm sample}}{\text{A517nm control}} \times 100$

Where A 517 nm control is the absorbance of control reaction (containing methanol only), and A517 nm sample is the absorbance of samples.

Table. 1. The conditions of GC-Mass

Temperature program	**Rate temperature (^{0} c)**		**Hold time (min)**
		50 ^{0} c	**3**
	10 ^{0} c	**300 ^{0} c**	**10**
Stationary phase	**Medium non polar**		
Mobile phase	**Carrier gas (Helium split ratio 1.53 ml min^{-1})**		
Detector	**Detector mass**		
Flow rate	**8 meter /min.**		
Sample size	**1 µ L**		

The results were analyzed statically using the statical program Statical Analysis System (25), to study the effect between the concentration and absorption of the volatile oil for leaves and steams ,and the effect between the concentration and percentage of antioxidant activity ,compare the significant differences between means by choosing Least Significant Difference [LSD] in probability ≤0.05 % (25).

RESULTS AND DISCUSSION

The water distillation *of P.graveolens* leaves and steams yield 5% and 2.3% (v/w) respectively. The GC-Mass analysis of leaves and steams essential oils are presented in Table 2 and Table 3, respectively. The results showed that volatile oil of leaves contains 12 compounds and the highest was Terpineol reaching 31.52% followed by Camphor 7.06 %, followed by Cis-alpha-Bisabolene 4.38% and the volatile oil in steams contains 10 compounds and Linalool represented the highest (3.00%), followed by Camphor (2.76 %), then(E) –B-Farnesene(2.31 %),while Fayed (3) pointed out that the components of the volatile oil of *P.graveolens* which grown in Egypt contained: citonellol 29.90 %trans-geranoil 18.03 % and 10-epi-γ-eudesmol 8.2% . Boukhris et al.,(17) indicated that the main composition of the volatile oil of *P.graveolens* grown in Tunisia were beta-citronellol 21.9%, citronellylformate 13.2% and geraniol 11.1%. The reason for the difference in the main components of the extracted volatile oil is due to the difference in the cultured area of plant where the change in the geographical nature and environmental conditions of the region has a significant impact on the quality and quantity of extracted volatile oil from the same species of the plant and cultured in different areas (8),the methods used to extract the volatile oil have an effect on the quality and quantity of the produced volatile oil (11).As well as the plant part used in extraction and the location of the leaves on the steams also have impact on the quality and quantity of the volatile oil (14). The DPPH free radical scavenging activities of leaves and steams volatile oils at various concentrations were determined and compared with that of the ascorbic acid as a standard antioxidant (Table 4).The results showed high volatile oil antioxidant activity in both leaves and steams at the concentration 125 µg ml^{-1} reached 100 % compared with antioxidant activity percentage of Ascorbic Acid at 125 µg ml^{-1} was 98.35% .The percentage of antioxidant activity of the steams oil were 98.28%, 88.66% ,67.61% and 50.12% at 100,75,50,25 µg ml^{-1}, respectively while the percentage of antioxidant activity of the leaves oil were 100%,93.55%,82.82% and 70.61% at 100,75,50,25 µg ml^{-1} ,respectively. The percentage of antioxidant activity of the ascorbic acid were 93.25%,85.62%,63.98% and 51.30% at 100,75,50,25 µg ml^{-1} , respectively. The results indicate significant differences between the volatile oil concentrations in both leaves and steams at ≤0.05 probability. The results also indicate significant differences between the volatile oil of the leaves and the volatile oil of the steams at probability ≤0.05. The results also indicate significant differences between the volatile oils and the ascorbic acid as a standard antioxidant at ≤0.05 probability. The results of the current study proved the high efficiency of leaves and steams oils of *P.graveolens* as antioxidant reached to 100% which agreed with other researcher (10). The current study proved that the activity of the volatile oil of *P.graveolens* steams and leaves as antioxidant increased with increased concentration and also proved that the activity of the volatile oil of leaves as antioxidant for different concentration was the highest . The reason for this was the oil contain active compounds that characterized by high effectiveness as antioxidants and accounted for about 59.37% of the components of the volatile oil ,as evidenced by the analysis of GC-Mass as

shown in (Table 2) .The volatile oil of the steams as these components accounted for about 14.62 % of the components of oil and this is comfirmed by the analysis of GC-Mass and as shown in (Table 3) and the most important of these compounds are linalool, farnesene ,pinene, camphor ,myrcene, terpineol ,camphene which have a clear effect as antioxidants (2, 9,16,19,21). In conclusion The *P.graveolens* plant contained a volatile oil in leaves and steams with the amount of the leaves oil higher than the oil of steams. The volatile oil of the leaves and steams contained similar compounds but differed in quantity and have a great economic importance such as Linalool ,Camphor , Pinene and Myrcene .The results showed that the volatile oil extracted from the leaves was highly effective as an antioxidant higher than the volatile oil of the steams .

Table .2. Compounds of the volatile oil of *P. graveolens* Leaves by GC- Mass technique

NO.	Name of compound	Retation time	Area %
1	Bicyclo[3,1,1]hept-2-ene,2,6,6-trimethyl	5.929	2.47
2	Bicyclo[4.1.0]heptane ,7-(1-methylethylidene)	6.193	2.48
3	beta-pinene	6.667	3.50
4	beta-Myrcene	6.816	1.69
5	1,7-Octanediol ,3,7-dimethyl	8.178	0.48
6	beta-Linalool	8.659	3.94
7	Camphor	9.491	7.06
8	Terpineol	7.586	31.52
9	alpha-Santoline alcohol	9.874	0.30
10	Myrcenol	10.218	1.48
11	Cis-.alpha-Bisabolene	13.769	4.38
12	beta-Ocimene	14.237	0.85

Table.3. Compounds of the volatile oil of *P. graveolens* steams by GC-Mass Technique

NO.	Name of compound	Retation time	Area%
1	beta-Myrcene	3.378	0.87
2	6,6-Dimethyl-2-methylenebicyclo [3.1.1] heptanes	5.916	0.60
3	Bicyclo[4.1.0]heptane,7-(1-methylethylidene)	6.179	0.69
4	beta-Pinene	6.653	0.87
5	beta-Linalool	7.376	3.00
6	Camphor	8.423	2.76
7	Santolina alcohol	9.477	1.26
8	alpha –linalool	9.580	0.54
9	(E)-B-farnesene	13.611	2.31
10	alpha.-Farnesene	13.756	1.72

Table .4.Percentage of the volatile oil efficiency of *P.graveolens* Leaves and Steams as an antioxidant compared with ascorbic acid

Concentration Microgram/ milliliter	Antioxidant activity %			LSD
	Oil of Leaves	Oil of Steams	Ascorbic acid (Ve+ control)	
25	70.61±2.94 c	50.12 ± 0.20 d	51.30±2.11 d	*4.426
50	82.82 ±2.52c	67.61 ±0.26d	63.98 ± 1.69d	* 4.281
75	93.55 ± 0.30 b	88.66 ± 0.29 c	85.62 ± 1.07c	* 3.178
100	100.00 ±0.00 a	98.28 ± 0.31b	93.25 ± 0.85b	* 4.517
125	100.00 ± 0.00 a	100.00 ±0.00a	98.35± 0.64a	2.974NS
LSD	* 3.576	* 0.708	* 3.067	---
.(P≤0.05) * The arithmetic averages of different letters within the same column differ significantly different .				

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