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ABSTRACT

Pelargonium graveolens (Family: Geraniaceae) was considered a medicinal with various medicinal, pharmaceutical and food applications. The ethanolic extract from this plant exhibited antimicrobial activities against different groups of test strains including *Staphylococcus aureus* (Gram-positive bacterium), *Escherichia coli* (Gram-negative bacterium), Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* (yeast) as well as the food-borne bacterial strains *Salmonella typhimurium* and *Listeria monocytogenes*. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extract were evaluated against all test microbes and results indicated that the MIC values were between 19.53 and 78.125µg/ml. the ethanolic extract also had antibiofilm activities against all test microbes especially *E. coli* and *S. typhimurium* (78.125 µg/ml). Antioxidant and phenolic content of the extract were also investigated revealing that it had a high level of phenolic content (1160.62µgAAE/g dry extract) leading to high total antioxidant activity (1104.46 µgGAE/g dry extract). GC/MS analysis revealed that compounds Tartaric acid, bis-O-(trimethylsilyl)-, bis (trimethylsilyl) ester (15.52%), Spiro[cyclopropane-3-(3,4-dihydro-1h-2-thianaphthalene)] (10.60%), Isoquinoline, 3,4-dihydro-6,7-dimethoxy-1-(methoxymethyl)- (10.34%), Myo-inositol, 1,2,3,4,5,6-hexakis-o-(trimethylsilyl)- (8.21%), 8,9-Di(p-methoxyphenyl)-7,10-dimethyltricyclo[4.2.0.2(2,5)] deca-7,9-diene

(6.05%), and Butanedioic acid, [(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester (5.08%) were the dominant components in the extract. HPLC phenolic contents showed that gallic acid, catechin, Naringenin and chlorogenic were the major constituents in the extract. Cytotoxic studies of the extract against a normal cell line exhibited a high IC_{50} value (179.55 µg/ml) leading to the concept that this extract is safe to be used as a food additive. The addition of *P. graveolens* extract affected the sensory characteristics of ice cream. It makes a difference to the control's overall acceptability or appearance, flavor, texture, or color. Ice cream supplemented with 10% and 15% *P. graveolens* extract scored excellent overall acceptability in all sensory attributes. In addition, the color showed excellent acceptance over the control and 5%.

Keywords: *Pelargonium graveolens, antimicrobial, antioxidant, phenolics, cytotoxicity, GC/Ms, sensory evaluation.*

المنتجات الطبيعية ذات القيمة الغذائية من نبات العطر

المستخلص:

يعتبر نبات العطر (العائلة: الجيرانية) بمثابة دواء له العديد من التطبيقات الطبية والصيدلانية والغذائية. أظهر المستخلص الإيثانولى من هذا النبات نشاطًا مضادًا للميكروبات ضد مجموعات مختلفة من السلالات موضع الاختبار والتى تشمل المكورات العنقودية الذهبية بالإضافة إلى السلالات البكتيرية المنقولة بواسطة الغذاء مثل السالمونيلا والليستيريا. تم تقييم الحد الأدنى للتركيز المثبط والحد الأدنى للتركيز المبيد للجراثيم للمستخلص ضد جميع الميكروبات المختبرة وأشارت النتائج إلى أن قيمه MIC تقع بين ١٩,٥٣ و٧٨,١٢٥ ميكروغرام/مل. كما كان للمستخلص الإيثانولى نشاط مضاد للأغشية الحيوية ضد جميع الميكروبات المختبرة وخاصة الاشريكية القولونية والسالمونيلا (٧٨,١٢٥ ميكروجرام/مل). تم أيضًا تقدير مضادات الأكسدة والمحتوى الفينولى، في المستخلص موضع الاختبار وجد مستوى عالٍ من المحتوى الفينولي (١١٦٠,٦٢ ميكروجرام مكافئ حمض الجاليك/ جرام مستخلص جاف) مما ادى ذلك ارتفاع النشاط المضاد للأكسدة (١١٠٤,٤٦ ميكروجرام مكافئ حمض الاسكوربيك جرام مستخلص جاف). كشف تحليل كروماتوغرافيا الغاز – مطياف الكتلة أن المركبات السائدة فى المستخلص هى حمض الطرطريك، – –بيز–o–(تريميثيلسيليل)–، بيز (تريميثيلسيليل) إستر (١٥,٥٢%)، سبيرو [سيكلوبروبان–٣–(٣,٤–ثنائي هيدرو–١هـ–٢–ثيانافثالين)] (١٠,٦٠%)، إيزوكينولين، ٣،٤–ثنائي هيدرو–٦،٧–ديميثوكسي–١– (ميثوكسي ميثيل) – (١٠,٣٤%)، ميو–إينوزيتول، ١،٢،٣،٤،٥،٦–هيكساكيس–o–(ثلاثي ميثيل سيليل) – (٨,٢١%)، ٨,٩–داى(ب–ميثوكسى فيتيل)–٧,١٠–داى ميثيل تراى سيكلو[٤,٢,٠,٢(٢,٥)] ديكا–٧,٩–دين

(٦,٠٥%)، وحمض البيوتانديويك، [(تريميثيلسيليل) أوكسي] ، (تريميثيلسيليل) إستر (٥,٠٨٪). أظهرت نتائج الفصل الكروماتوجرافى عالى الكفاءة أن المركبات الفينولية الرئيسية فى المستخلص هى حمض الجاليك، الكاتكين، النارينجينين والكلوروجينيك. أظهرت دراسات السمية الخلوية للمستخلص مقابل خط الخلايا الطبيعية قيمة IC_{50} عالية (١٧٩,٥٥ ميكروجرام/مل) مما يدل على أن هذا المستخلص آمن للاستخدام كمادة مضافة غذائية. إضافة مستخلص نبات العطر أدى إلى حدوث تغيرات فى الخصائص الحسية فى جميع المنتجات. وأظهر الآيس كريم المدعم بمستخلص نبات العطر بتركيز ١٠ % و١٥% درجة تقبل عام ممتاز. وبالإضافة إلى ذلك، أظهرت خاصية اللون قبولا ممتازا مقارنة بالعينة المرجعية وتركيز ٥٪.

الكلمات المفتاحية: نبات العطر، مضاد للميكروبات، مضاد للأكسدة، الفينولات، السمية الخلوية، الكروماتوغرافيا الغازية – قياس الطيف الكتلي، التقييم الحسي

1. Introduction

Plants are considered as profound sources for many medicinal, pharmaceutical as well as food purposes **(Saraswathi *et al.*, 2011)**. Medicinal plants are still used in many countries as remedies for different human diseases as they contain many chemical groups of therapeutic importance **(Derwich *et al.*, 2010)**. Huge populations throughout the world still use traditional medicine due to the scarcity and cost of manufactured medicine **(Ayo, 2020 and Balunas and Kinghorn, 2005)**. Medicinal plants exhibited wonderful applications in agriculture, human and veterinary drugs, foods as well as perfume industry **(Butles, 2004)**. *Pelargonium graveolens*, a member of the family Geraniaceae, is known to grow in temperate regions around the world **(Charwood and Charlwood, 1991)**. This plant is considered an evergreen flowering plant generally acknowledged for its rose-like smell as well as its essential oil. Due to its aroma, it is usually called rose fragrant geranium and/or rose geranium. About 300 geranium species are commonly known. *P. graveolens* exhibited numerous therapeutic and fragrant values of marketable significance (**Brian *et al.*, 2010**). Traditionally, geranium (*P. graveolens*) was applied for healing wounds, ulcers as well as skin syndromes. Additionally, it was used to treat diarrhea, staunch bleeding, dysentery, and colic **(Matthews, 1995)**. *P. graveolens* exhibited unique use in the food and beverages industries **(Dzamic *et al.*, 2014)**. Researchers focused on the plant essential oils, revealed that the plant exhibited antimicrobial, and antimalarial activities **(Lalli, 2005)**, in addition to its applications as antiasthmatic, antidiarrhoeic, antihepatotoxic, and antiallergic **(Boukhris *et al.*, 2012)**. The work is

undertaken to use natural extract from natural healthy sources to treat enriched foods. To achieve this goal *P. graveolens* was selected and extracted with ethanol. The produced extract was evaluated for its antimicrobial, antioxidant, and safety to be used as a food additive.

Material and methods

Preparation of plant leaves:

In this study *Pelargonium graveolens* green leaves were used. Leaves were washed with tap water to diminish the particulate materials as well as dust. Washed leaves were instilled between two sheets of filter paper to remove excess water. leaves are now ready to be extracted.

Extraction:

P. graveolens leaves were cut into tiny parts and to 500g of them 1500ml of ethanol (absolute) was added and kept at ambient temperature for 24h. The filtrate was cleaned to get rid of any residual plant parts. The rotary evaporator was used to evaporate the ethanol solvent using the rotary evaporator model Model Heidolph tell dryness and the obtained greenish extract (this extract is free from any solavent) was kept at 4^{o}C for further studies.

GC- Mass spectrometry (MS) analysis:

The chemical constituent of the ethanolic extract from *P. graveolens* was quantitated with Thermo Scientific/Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). Timorous documentation of the current compounds was achieved by the valuation of the retention time and mass spectra against those of the NIST and WILLY library data of the GC-MS system **(Abdel-Aziz *et al.*, 2021)**.

Antimicrobial activity of ethanolic extract:

The obtained greenish extract was assessed for its antimicrobial activity against Gram-positive bacteria (*S. aureus* ATCC 6538-P, L. ATCC19117, and MRSA), Gram-negative bacteria (*E. coli* ATCC 25933 and *S. typhimurium* ATCC14028 as well as the yeast strain (*C. albicans* ATCC 10231). A cup agar plate diffusion protocol was used to evaluate the antimicrobial activity of *P. graveolens* extract. Plates having nutrient agar medium were inoculated by 10^6 cells/ml from each test strain. The antimicrobial activity was detected by evaluating the clear zone values (mm). Results are means of twofold readings (**Rayes Kamel *et al.*, 2022 and Abd El Salam *et al.*, 2024**)

MIC and MBC of ethanolic extract of *P. graveolens* green leaf extract:

The minimum inhibitory concentration (MIC) of *P. graveolens* was detected against, *S. aureus* ATCC 6538-, *L. monocytogenes*

ATCC19117, and MRSA as Gram-positive test bacterial strains *E. coli* ATCC 25933, and *S. typhimurium* ATCC14028 were selected as Gram-negative bacterial test strains. Additionally, *C. albicans* ATCC 10231 was used as a yeast test microbe. Nutrient broth medium was used in this test after collecting them by centrifugation under sterile conditions in a concentration of $5x10^6$ CFU (stock absorbance of 0.5-1Au). Resazuin reagent was prepared as previously described **(Sarker *et al.*, 2007)**. Twofold dilution was done of the ethanolic extract of *P. graveolens* dissolved in dimethyl sulfoxide (DMSO) in 96 wells microplate containing nutrient broth medium in all wells and sequentially resazurin and microbial cell (10µl from each) were added. The cultivated plates were kept at 35ºC overnight. Any change in the original resazurin colour (purple) to red or colourless is considered a positive result. The minimum bactericidal inhibitory effect of the extract (MBC) was known as the concentration of the extract which didn't show any microbial growth by cultivating them on the newly prepared nutrient agar plates **(Abo-Salem *et al.*, 2024)**.

Biofilm inhibition of ethanolic extract from *P. graveolens*:

The minimum biofilm inhibitory concentration (MBIC) was tested against biofilm formatting bacterial test strains namely, *S. aureus* ATCC 6538-, *L. monocytogenes* ATCC19117 and MRSA as Gram-positive test bacterial strains *E. coli* ATCC 25933 as well as *S. typhimurium* ATCC14028 were selected as Gram-negative bacterial test strains. Additionally, *C. albicans* ATCC 10231 was used as a yeast test microbe ***(Abo-Salem et al., 2021and Ceri et al., 2006)***. In the 96-well microplate, 100µl of nutrient broth was distributed in all wells. Additionally, 100 µl from ethanolic extract from *P. graveolens* was dropped into the first raw of wells then serial dilution (2-fold) was achieved excepting the last one raw that left as controls. 10µl of each microbial culture ($5x10^5$ CFU/ml) was dispensed to every well. After 24h of incubation at 35ºC, cultures were lightly poured, and the plates were washed using saline phosphate buffer (PBS). After dryness of the plates for 30min, crystal violet (200 µl of 0.1%) was added to all wells for 30min. The excess crystal violet solution was decanted and washed three times with distilled water and left to dry for 30 minutes. 200µl of ethanol (95%) was poured into each well.

Antioxidant activity using phosphomolybdate technique:

The total antioxidant of the ethanolic extract of *P. graveolens* was assessed using the phosphomolybdate method **(Prieto *et al.*, 1999 and Elsemelawy and Tag Al-Deen 2020)**. In details, 300µL of the extract was mixed with 2700µL of phosphomolybdate reagent consisting of

0.6M H_2SO_4, 28mM NaH_2PO_4 and 4mM $(NH_4)_6Mo_7O_{24}$, and a blank using methanol (solvent) was constructed at the same time. The reaction mixture was incubated at 90°C for 90 minutes. After cooling at room temperature, the absorbance was distinguished at 695nm (Shimadzu UV1024-PC). A standard curve of ascorbic acid was done.

Antioxidant activity using DPPH free radical scavenging capacity:

2,2- Diphenyl–10 picrylhydrazyl (DPPH) free radical scavenging capacity of the ethanolic extract from *P. graveolens* was assessed according to **Wu *et al.*, (2019)**. In detail, 50µL from each concentration (1000-31.25µg/mL) of the extract was added to 1950 µL of 100µM DPPH made by dissolving 4mg of DPPH in 100ml methanol. The mixture was stirred toughly and kept in dark at ambient temperature for 30 minutes **(Ennaji *et al.*, 2020)**. The absorbance was dignified at 517nm. The DPPH activity was designed rendering to the following calculation: DPPH SCA % = A0-AE/A0×100. In which A0 and AE are the optical density of the control and extract, respectively. IC_{50} was designed for tested extract and standard (ascorbic acid) as well.

Total Phenolic content (TPC) determination:

The ethanolic extract from *P. graveolens* was assessed for its phenolic content was measured as mentioned by **Kupina *et al.*, (2017)** using Folin reagent. 100µl from the extract was mixed vigorously with 1900µl followed by the addition of 500µl of FCR and 2.5 ml of Na_2CO_3 (20%) solution was added and the mixture was kept at bench temperature until the colour was developed (40 min). The gallic acid standard curve was constructed at the same time. Total phenolic contents (µg/g) in the extract were considered as gallic acid equivalent (GAE).

Effect of extract on Cell Viability by MTT assay:

Ethanolic extract of *P. graveolens* and its effect on normal cell viability was studied **(Thabrew *et al.*, 1997)**. MTT study was done under aseptic situations in a laminar flow cabinet using bio-safety class II level model Baker, SG403INT, and Sanford, ME, USA). 24h-old cells achieved by inoculating $2x10^4$ cells/well using *Human Fetal Lung Fibroblast* (Wi38 cell line) in 96-well plates. 100µg/ml of the extract was supplemented to the prepared medium (in triplicates) for 48 h. Doxorubicin (100 µM) was considered as positive control whereas DMSO (0.5 %), was used as negative control. MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) procedure was applied to assay the cell viability **(Mosmann, 1983)**. Cytotoxicity (%) was considered using this equation: % *cytotoxicity = [1- (AVx / AVNC)] x 100* considering Av is the average, X is the absorbance of the sample, and NC is the absorbance of the negative control.

HPLC for polyphenols outlines ethanolic extract of *P. graveolens*:

Phenolics and other associated compounds were evaluated by using HPLC RP (reverse phase) with diode array detector (DAD) Model Hewlett Packard (HP1050) containing C18 Alltima column. Nineteen standard polyphenols were used for comparison. polyphenolic compounds were assessed at 280nm and expressed in µg/100ml **(Goupy *et al.*, 1999)**.

Preparation of ice cream:

Setting up the conditions for the ice cream process. Using a mixer model Yasuda Corporation, Japan, the materials were first combined and dissolved in water at 70°C, following Table (1). Next, the composition was put on a plate pasteurizer and heated to 95°C for 30 seconds, using the high-temperature, short-time (HTST) method. The produced fat globules were then homogenized. In a two-step homogenization model Sanmaru Machinery Co., Ltd. (Japan). The cooled blend was reserved in refrigerator at 5°C for ageing for 24h. The ice cream was put in freezer to be used **(Keisuke *et al.*, 2012)**.

Table (1): Ingredient of ice cream

Ingredients (g)	C. I.C	I.C.P. E 5%	I.C.P. E 10 %	*I.C.P. E 15%
Skim milk powder (g)	20	20	20	20
Unsalted butter (g)	10	10	10	10
Water (ml)	100	95	90	85
High-fructose corn syrup (ml)	15	15	15	15
Vanilla extract (g)	1	1	1	1
Sugar powder (g)	15	15	15	15
High-fructose corn syrup (g)	15	15	15	15
P. graveolens extract (ml)	0	5	10	15
Emulsifier (g)	0.5	0.5	0.5	0.5
Stabilizer (g)	0.5	0.5	0.5	0.5

C.I.C= Control ice cream I.C.P. E = ice cream with *P. graveolens* extract

Organoleptic evaluation of ice cream:

For the sensory evaluation, a nine-point hedonic scale was utilized **(Watts *et al.*, 1989 and Mohamed, 2024)**. The examination was done by well-qualified persons (30 made) from the postgraduate and researchers in Chemistry of Natural Compounds Department, National Research Center in Dokki, Giza, Egypt. They were given a 9-point Hedonic scale to rate the mackerel on, where 1 mean "dislike extremely" and 9 signified "like extremely."

Statistical analysis:

The results were statistically analyzed by IBM SPSS 23 program mentioned by **Kirkpatrick and Feeney (2012)**. For each measurement on each sample, analyses were performed in triplicate. At a 5% significance level, mean differences were compared using the Duncan test. The model's significance was determined using ANOVA.

Results and discussion:

Figure (1) a showed part of the used plant whereas Figure 1b revealed the plant leaves. Figure 1c shows the green produced ethanolic extract. Moreover, Figure (2) revealed the maximum absorbance of ethanolic extract. It had been found that the extract exhibited maximum absorbance at 450-550 nm.

Figure 1: A part used plant *P. graveolens* (a), plant leaves ready for extraction and ethanolic extract produced

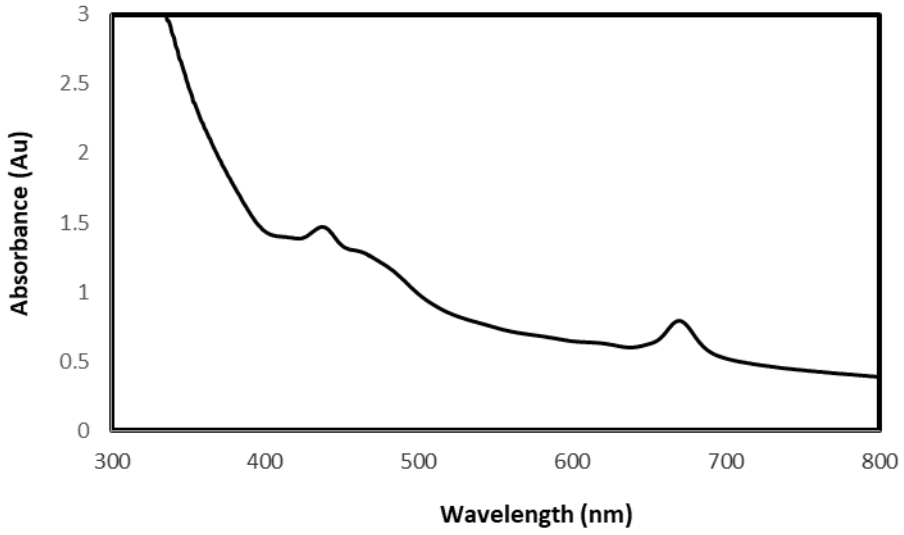


Figure 2: The maximum absorbance of the obtained ethanolic extract (λ_{max})

GC- Mass spectrometry investigation of *P. graveolens* ethanolic extract:

GC-MS examination of *P. graveolens* comprises 30 compounds Figure (3). The total peak areas of the identified compounds constitute

94.18%, the prospects of the chemical structures of the identified compounds are summarised in Table (2). The main detected compounds include Tartaric acid, bis-O-(trimethylsilyl)-, bis(trimethylsilyl) ester (15.52%), Spiro[cyclopropane-3-(3,4-dihydro-1h-2-thianaphthalene)] (10.60%), Isoquinoline, 3,4-dihydro-6,7-dimethoxy-1-(methoxymethyl)- (10.34%), Myo-inositol, 1,2,3,4,5,6-hexakis-o-(trimethylsilyl)- (8.21%), 8,9-Di(p-methoxyphenyl)-7,10-dimethyltricyclo [4.2.0.2(2,5)] deca-7,9-diene (6.05%), and Butanedioic acid, [(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester (5.08%). The compound identification was attained through computer search compared to libraries incorporating mass spectra **(Shawky *et al.*, 2019)**. The methanolic extract from leaves of *P. graveolenes* exhibited the presence of the following major compounds (%): Aspidospermidin-17-ol, 1-acetyl19,21-epoxy-15,16-dimethoxy (0.26), Glycol-D-asparagine (0.23), 3,5-heptadienal,2-ethylidene-6- methyl (0.65), Tetradecane,2,6,10-trimethyl (0.64), 3,7,11,15-Tetramethyl-2- hexadecen-1-ol (1.16), Geranyl isovalerate (2.94), Hexadecanoic acid, methyl ester (4.94), n-Hexadecanoic acid (6.70), Trans-13-Octadecenoic acid, methyl ester (12.54), Heptadecanoic acid, 16-methylmethyl ester (2.53), Ethyl 3,7,12-trihydroxycholan-24-oate (2.49) **(Makanyane *et al.*, 2019)**.

Table (2): The chemical composition of ethanolic extract of *P. graveolens* as analyzed by GC/ Mass spectrometry

No.	R_t	Area%	Identified compounds	SI	M.W.	M.F.
1	3.95	2.23	(1,2,3,4-Tetrahydro-naphthalen-2-yloxy)-acetic acid benzo[1,3]dioxol-5-ylmethylene-hydrazide	545	352	$C_{20}H_{20}N_2O_4$
2	11.85	3.02	Cholesta-5,23-dien-3-ol, 23-methyl-, (3á,23z)-	577	398	$C_{28}H_{46}O$
3	17.32	5.08	Butanedioic acid, [(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	618	350	$C_{13}H_{30}O_5Si_3$
4	18.12	0.72	3-Methoxy-11h-11-carbomethoxybenzo[b]fluorene	705	304	$C_{20}H_{16}O_3$
5	19.19	0.88	D-Glucitol, 6-deoxy-1,2,3,4,5-pentakis-O-(trimethylsilyl)-	643	526	$C_{21}H_{54}O_5Si_5$
6	21.00	15.52	Tartaric acid, bis-O-(trimethylsilyl)-, bis(trimethylsilyl) ester	764	438	$C_{16}H_{38}O_6Si_4$
7	22.85	0.56	5á-Cholestane-3à,7à,12à,24à,25-pentol TMS	573	812	$C_{42}H_{88}O_5Si_5$
8	24.66	6.05	8,9-Di(p-methoxyphenyl)-7,10-dimethyltricyclo[4.2.0.2(2,5)] deca-7,9-diene	659	372	$C_{26}H_{28}O_2$
9	24.73	2.83	á-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(trimethylsilyl)-	625	540	$C_{21}H_{52}O_6Si_5$
10	24.89	4.69	4-isoquinolineacetic acid, à-(1,3-benzodioxol-5-ylmethylene)-1,2-dihydro-3,7-dimethoxy-2-m ethyl-1-oxo-, ethyl ester, (e)-	581	437	$C_{24}H_{23}NO_7$
11	24.98	4.81	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-	520	540	$C_{21}H_{52}O_6Si_5$
12	26.51	10.34	Isoquinoline, 3,4-dihydro-6,7-dimethoxy-1-(methoxymethyl)-	778	235	$C_{13}H_{17}NO_3$
13	26.64	0.71	1H-Indole, 6-methoxy-5-(phenylmethoxy)-1-(trimethylsilyl)-	680	325	$C_{19}H_{23}NO_2Si$
14	28.26	10.60	Spiro[cyclopropane-3-(3,4-dihydro-1h-2-thianaphthalene)]	784	204	$C_{13}H_{16}S$

15	28.77	0.68	Hexadecanoic acid, trimethylsilyl ester	836	328	$C_{19}H_{40}O_2Si$
16	29.39	0.67	3-Buten-2-one, 4-(2,2,6,7-tetramethyl-7-azabicyclo[4.1.0]heptan-1-yl)-	642	221	$C_{14}H_{23}NO$
17	30.24	8.21	Myo-inositol, 1,2,3,4,5,6-hexakis-o-(trimethylsilyl)-	715	612	$C_{24}H_{60}O_6Si_6$
18	31.86	1.42	5á-Cholestane-3à,7à,12à,24à,25-pentol TMS	507	812	$C_{42}H_{88}O_5Si_5$
19	32.21	0.85	2-(4'-Hydroxyphenyl)-1-acetyl-3-phenylindolizine	664	327	$C_{22}H_{17}NO_2$
20	34.20	0.91	Nonadecanoic acid, trimethylsilyl ester	611	370	$C_{22}H_{46}O_2Si$
21	35.10	2.31	2-t-Butyl-N,N'-diheptyl-N,N'-dimethyl-malonamide	443	382	$C_{23}H_{46}N_2O_2$
22	35.28	0.55	Glucosamine, n-acetyl-, o-methyloxime, tetrakis-o-(trimethylsilyl)-	511	538	$C_{21}H_{50}N_2O_6Si_4$
23	36.29	0.98	N-Cyclohexylidenecyclododecanamine	567	263	$C_{18}H_{33}N$
24	42.23	0.66	13-Docosenamide, (Z)-	560	337	$C_{22}H_{43}NO$
25	43.14	2.42	4-Methoxycarbonyl-5-methyl-2,3-dioxo-2,3-dihydrofuran	677	232	$C_{12}H_8O_5$
26	44.49	2.41	6,7-Dihydroxycoumarin-á-d-glucopyranoside, penta-tms	557	700	$C_{30}H_{56}O_9Si_5$
27	47.74	0.62	1H-Indole-2-carboxylic acid, 1-methyl-, trimethylsilyl ester	629	247	$C_{13}H_{17}NO_2Si$
28	49.15	0.96	4-Methylbenzo-1-thiopyrylium	517	237	$C_{16}H_{13}S$
29	50.11	1.18	2-O-Glycerol-à-d-galactopyranoside, hexa-TMS	692	686	$C_{27}H_{66}O_8Si_6$
30	52.19	1.31	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	531	430	$C_{27}H_{42}O_4$
		94.18%				

Rt: Retention time; **M.W.:** Molecular weight; **M.F.:** Molecular formula.

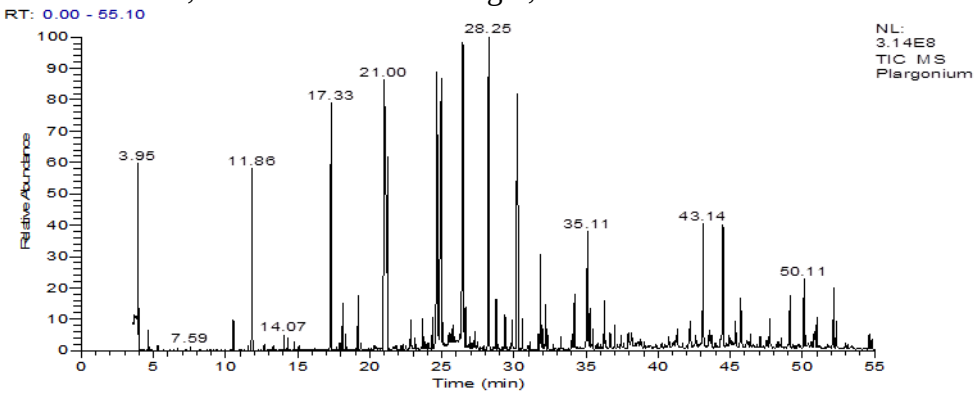


Figure 3: GC-MS chromatogram of *P..graveolens* green leaves ethanolic extract

The antimicrobial activity of *P. graveolens* ethanolic extract:

The ethanolic extract from *P. graveolens* had been assessed for its antimicrobial activities against different test microbes including *Staphylococcus aureus* ATCC 6538-P and the food-born *Listeria monocytogenes* ATCC19117 as Gram-positive representatives, whereas *Escherichia coli* ATCC 25933 and *Salmonella typhimurium* ATCC14028 as food bornt strain as Gram-negative test microbe in addition to the yeast strain Candida albicans ATCC 10231. Results in Table (3) and Figure (4) exhibited that the ethanolic extract of *P. graveolens* showed antimicrobial activities against all test microbes with inhibition values of 19, 18,15,22, 16 and 13mm against *S. L. monocytogenes E. coli, S. typhimurium, C. albicans* and MRSA,

respectively. Ethanolic extract from *P. graveolenes* exhibited higher antibacterial activity against *L. momocytogenes* (G+ve bacteria) than other test bacterial strains namely: *S. aureus* (G+ve), *E. coli* (G-ve) and *S. entrica* (G-ve) as mentioned by **Dimitrova *et al.*, (2015)**. The antimicrobial activity of methanolic extract from *P. graveolenes* was assessed against 8 test strains namely: *S. aureus* ATCC 25923, *L. innocua* CECT433, *B. subtilis* DSM 6633, *E. coli* ATCC 25922, *E. coli* K12 CECT433, *P. aeruginosa* CECT 118, *C. albicans* 10231 and *C. neoformans* with inhibition zones of 13.47, 14.27, 12.67, 11.20, 10.23, 9.43, 13.60 and 10.83mm, respectively **(El Aanachi *et al.*, 2020)**

Table (3): The antimicrobial activity of the ethanolic extract from *P. graveolens* against different test microbes

Test microorganism	Inhibition zone (mm)
***Staphylococcus aureus* ATCC 6538-P**	19
***Listeria monocytogenes* ATCC19117**	18
***Escherichia coli* ATCC 25933**	15
***Salmonella typhimurium* ATCC14028**	22
***Candida albicans* ATCC 10231**	16
MRSA	13

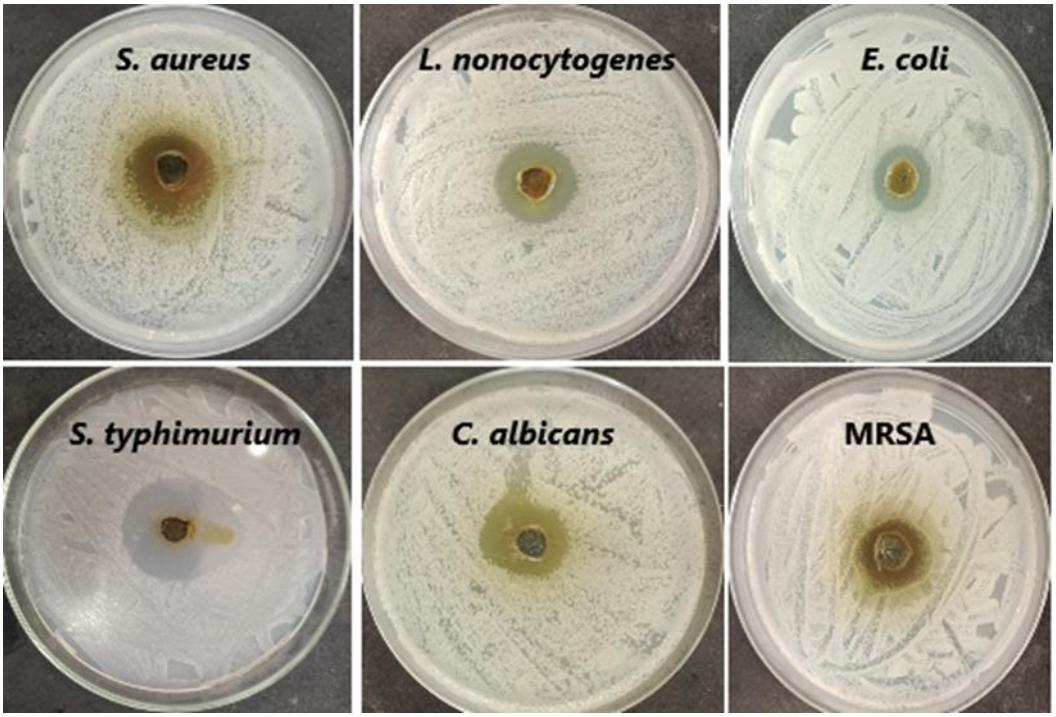


Figure 4: The antimicrobial activity of *P. graveolens* ethanolic extract

Minimum inhibitory and bactericidal concentrations (MIC and MBC) of *P. graveolens* ethanolic extract:

The MIC values of the ethanolic extract from *P. graveolens* are represented in Table (4) and Figure (5). Results indicated that ethanolic

extract from *P. graveolens* exhibited potentially low MIC and MBC values of 19.53 and 79.125 µg/ml against *S. typhimurium* followed by *E. coli* (39.06 and 156.25 µg/ml for MIC and MBC, respectively). The extract exhibited moderately low MIC values of 78.125 µg/ml for the rest of the test microbes (*S. aureus*, *L. monocytogenes*, *C. albicans*, and MRSA). The MBC values for the ethanolic extract exhibited MBC values of 312.5, 625, 156.25, 156.25 and 312.5 µg/ml against *S. aureus*, *L. monocytogenes*, *E. coli*, *C. albicans* and MRSA, respectively. The minimum inhibitory concentrations of the methanolic extract from *P. graveolenes* were 470, 940, 470, 940, 1870, 470, 470 and 1870µg/ml against *S. aureus* ATCC 25923, *L. innocua* CECT433, *B. subtilis* DSM 6633, *E. coli* ATCC 25922, *E. coli* K12 CECT433, *P. aeruginosa* CECT 118, *C. albicans* 10231 and *C. neoformans*, respectively **(El Aanachi *et al.*, 2020)**. Phenolic compounds derived from plants, that include phenolic acids, flavonoids, tannins, and stilbenes, have the potential to inhibit the growth of microbial organisms, including food-borne diseases, pathogenic fungi, bacteria and protozoa **(Daglia, 2012; Schmidt *et al.*, 2012; Li *et al.*, 2014).**

Table (4): The MIC and MBC of the ethanolic extract from *P. graveolens* against different test microbes

Test organism	MIC (µg/ml)	MBC (µg/ml)
Staphylococcus aureus	78.125	312.5
Listeria monocytogenes	78.125	625
Escherichia coli	39.06	156.25
Salmonella typhimurium	19.53	79.125
Candida albicans	78.125	156.25
MRSA	78.125	312.5

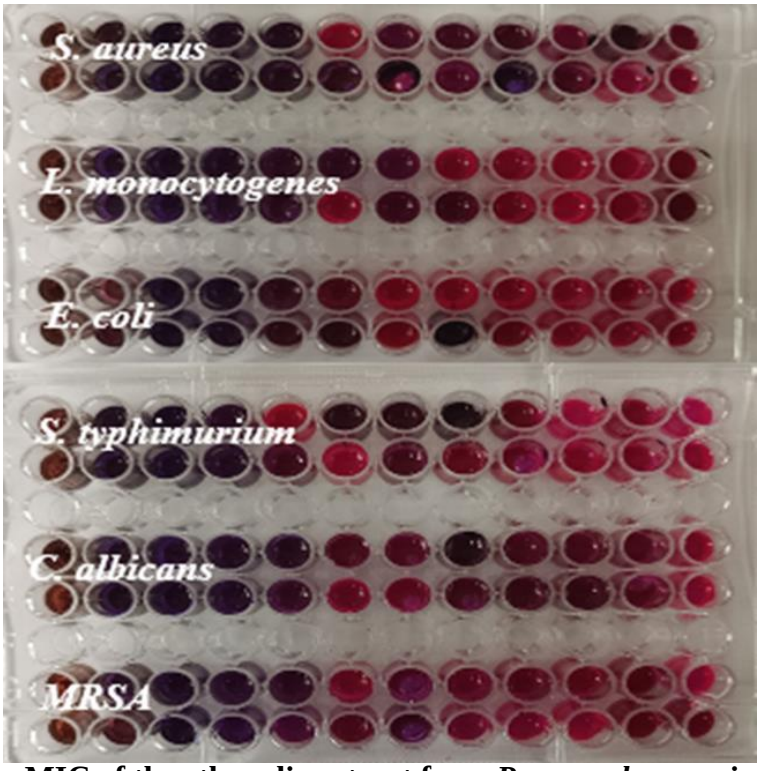


Figure 5: the MIC of the ethanolic extract from *P. graveolens* against different test microbes

Antibiofilm formation of ethanolic extract of *P. graveolens*:

The minimum biofilm inhibitory concentrations (MBIC) of the ethanolic extract of green leaves from *P. graveolens* were evaluated against different test microbial strains namely: *S. aureus, L. monocytogenes, E. coli, S. typhimurium, C. albicans* and MRSA The MBIC values were varied from test microbe to another one results obtained from Table (5) and Figure (6) showed that the MBIC was low with *E.coli* and *S. typhimurium* with values of 78.125 and 78.125 µg/ml respectively, followed by *S. aureus* and *C. albicans* with MBIC values of 156.25 and 156.25, respectively. *L. monocytogenes* and MRSA both exhibited MBIC values of 312.5 µg/ml Essential oil from *P. graveolenes* exhibited remarkable antibiofilm activity against *S. aureus* and *C. albicans* **(Abu El Wafa *et al.*, 2023)**.

Table (5): The minimum biofilm inhibitory concentration (MBIC) of ethanolic extract from *P. graveolens* against different test strains

Test microbe	MIC of Biofilm inhibition (µg/ml)
Staphylococcus aureus	156.25
Listeria monocytogenes	312.5
Escherichia coli	78.125
Salmonella typhimurium	78.125
Candida albicans	156.25
MRSA	312.5

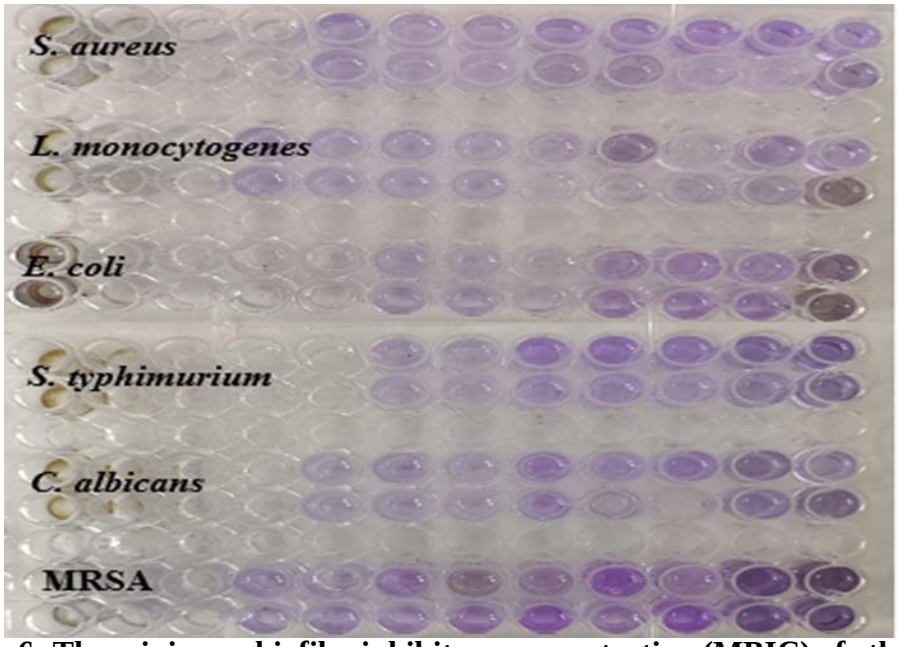


Figure 6: The minimum biofilm inhibitory concentration (MBIC) of ethanolic extract from P. graveolens against different test strains

Total antioxidant and total phenolic contents:

The total free-radical scavenger antioxidant activity of the ethanolic extract from *P. graveolens* was assessed using the phosphatomolybdate method. Results in Table (6) revealed that the tested extract exhibited antioxidant activity of 1104.46 µgAAE/ g dry extract. Folin-ceaucateu's method was used for the assessment of the total phenolic content of ethanolic extract from *P. graveolens* green leaves. Results in Table (6) revealed that the ethanolic extract exhibited a total phenolic content of 1160.62 µgGAE/g dry extract. ethanolic extract from *P. graveolens* exhibited total phenolic content of 381 µgGAE/g dry extract as postulated by **El Aanachi *et al.*, (2020)**. Moreover, the water leaf extract of *P. graveolenes* had a high level of polyphenolic compounds (142.71mg/g dry extract) **(Ali *et al.*, 2020)**.

Table (6): Total antioxidant and total phenolics of the ethanolic extract from *P. graveolens*

Item	Value±SD
Total antioxidant (µgGAE/g dry extract)	1104.46±6.86
Total phenolics (µgAAE/ g dry extract)	1160.62±19.25

DPPH scavenging activity of ethanolic extract of *P. graveolens*:

Results in Table (7) and Figure (7) revealed the percentage of DPPH scavenging activities of the ethanolic extract of *P. graveolens* green leaves compared to ascorbic acid as standard. Results indicated the extract exhibited promising DPPH scavenging activity compared to the standard (ascorbic acid). It had been found that the ethanolic extract of *P. graveolens* green leaves exhibited a potent IC_{50} of 128.656 µg/ml compared to ascorbic acid (121.068 µg/ml). The DPPH scavenging

activity of essential oil and extracts from *P. graveolens* having IC_{50} ranges from 711 to 01280 µg/ml for oils and 12.24 to 44.24 µg/ml for extracts **(El Aanachi *et al.*, 2020)**. Additionally, **Al-Saffar *et al.*, (2017)** reported that the ethanolic extract from *P. graveolens* had DPPH with an IC_{50} of 484 µg/ml.

Table (7): DPPH scavenging activities of ethanolic extract from *P. graveolens* compared to ascorbic acid

Concentration (µg/ml)	Ascorbic acid		Ethanolic extract of *P. graveolins*	
	DPPH scavenging activity (%)	IC_{50} (µg/ml)	DPPH scavenging activity (%)	IC_{50} (µg/ml)
15.63	5.945 ±0.309	121.068 ±1.343	7.223 ±0.722	128.656±0.6535
31.25	8.815 ±0.1888		17.765± 0.833	
62.5	15.170 ± 0.309		35.871 ± 0.387	
125	28.126 ± 1.032		65.983 ± 0.471	
250	57.155 ± 1.675		76.720 ± 0.439	
500	95.899 ± 0.432			

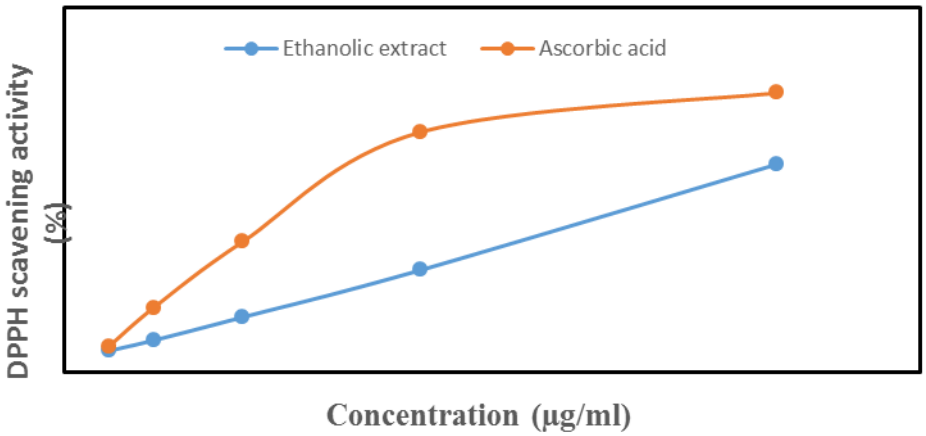


Figure 7: DPPH scavenging activities of ethanolic extract from *P. graveolens* compared to ascorbic acid

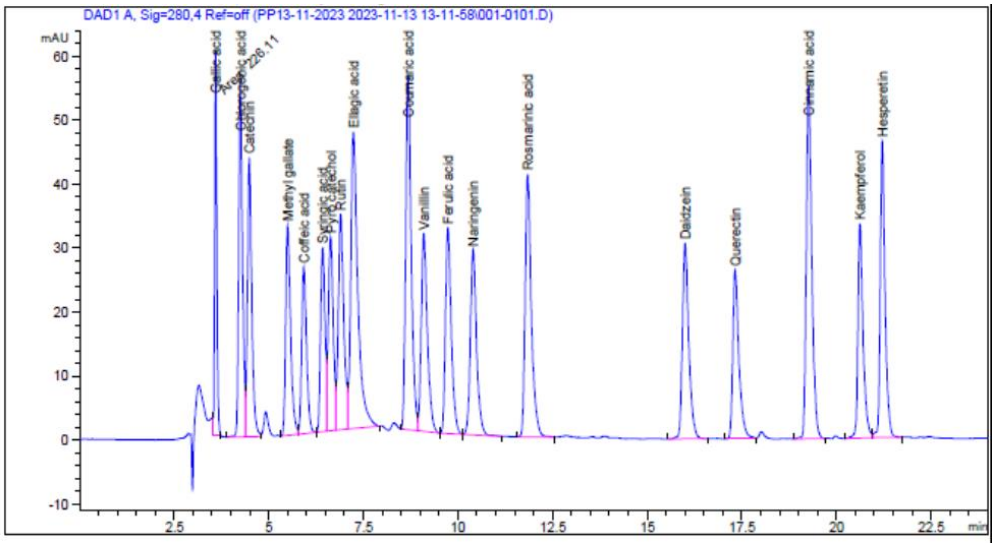
HPLC fingerprint of phenolic compounds present in *P. graveolens* ethanolic extract:

Ethanolic extract from *P. graveolens* leaf extract was assessed using high-performance liquid chromatographic fingerprint studies. The chromatograms presented by HPLC studies (Table 8 and Figure 8 –a and b) showed the *phenolic compound* contents of the tested extract as compared to 19 *phenolic compound* standards. HPLC results revealed that gallic acid and catechin are the major phenolic content present in the ethanolic extract of *P. graveolens* extract (2154.63 and 1712.25 µg/g extract). Naringenin and chlorogenic acid were also present in the extract with values of 874.19 and 710.42 µg/g, respectively. Considerable concentrations of ferulic acid, quercetin, caffeic acid and rutin were observed in this ethanolic extract with values of 470.20, 443.73, 434.41 and 305.0742 µg/g extract, respectively. The ethanolic extract from *P.*

graveolens leaf extract exhibited acceptable quantities of rosmarinic acid, syringic acid, methyl galate and Kaempferol (174.46, 92.91, 88.41 and 77.41 µg/g extract, respectively). the ethanolic extract possessed lower phenolic contents than coumaric acid, hesperetin, ellagic acid, diadzein, cinnamic acid and vanillin (39.83, 33.08, 23.01, 18.93, 11.57 and 2.35 µg/g extract, respectively). The phenolic member Pyro catechol was not found in this extract. The main compounds of the ethanolic extract of *P. graveolenes* were rutin and quercetin **(Angelis *et al.*, 2013)**.

Table (8): Phenolic compounds in the tested extract compared to nineteen standard phenolic compounds

Standard			*P. graveolins* ethanolic extract		
Standard name	**Conc. (µg/ml)**	**Area**	**Area**	**Conc. (µg/ml)**	**Conc. (µg/g)**
Gallic acid	20	226.11	487.18	43.09	2154.63
Chlorogenic acid	50	385.33	109.50	14.21	710.42
Catechin	75	347.64	158.73	34.25	1712.25
Methyl gallate	15	297.70	35.10	1.77	88.44
Caffeic acid	18	232.60	112.27	8.69	434.41
Syringic acid	17.2	235.18	25.41	1.86	92.91
Pyro catechol	40	277.46	0.00	0.00	0.00
Rutin	50	338.96	41.36	6.10	305.07
Ellagic acid	60	600.66	4.61	0.46	23.01
Coumaric acid	20	561.98	22.38	0.80	39.83
Vanillin	12.9	347.12	1.26	0.05	2.35
Ferulic acid	20	344.29	161.88	9.40	470.20
Naringenin	30	328.22	191.28	17.48	874.19
Rosmarinic acid	50	466.34	32.54	3.49	174.46
Daidzein	20	356.62	6.75	0.38	18.93
Quercein	40	296.35	65.75	8.87	443.73
Cinnamic acid	10	558.41	12.93	0.23	11.57
Kaempferol	20	317.06	24.55	1.55	77.41
Hesperetin	20	406.79	13.46	0.66	33.08



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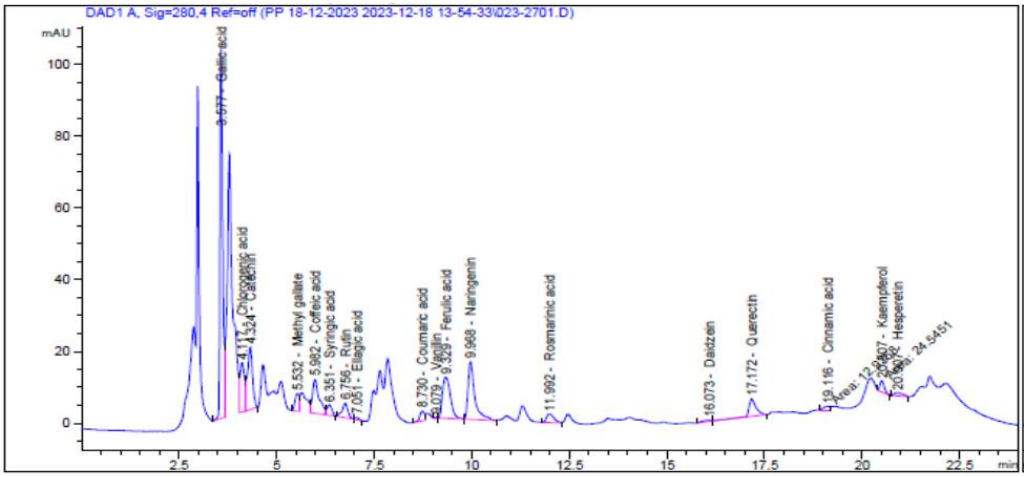

B

Figure 8: HPLC chromatogram of standard phenolics (a) and phenolics of *P. graveolens* ethanolic extract (b)

Effect of ethanolic extract from *P. graveolenes* on cell viability by MTT assay:

The cytotoxic activity of the ethanolic extract from *P. graveolens* against the human foetal lung fibroblast normal cell lines (Wi38) is illustrated in Table (9) and Figure (9). The extract was highly safe as it exhibited little cytotoxic activity on normal cell lines. The dose-response study had been studied according to **Elshahid *et al.*, (2021)**. Different concentrations (1000, 500, 250, 125, 62.5 µg/mL to reach 31.25 µg/mL). in triplicates, were tested for their cytotoxic effect against normal cell lines. The IC_{50} values presented in Table (9) were calculated through the concentration-response curve fit the non-linear regression model using Graph Pad Prism® v6.0 (GraphPad Software Inc., San Diego, CA, USA). Figure (9) shows the viability and cytotoxicity of the different concentrations of the extract on the normal cells. At very high

concentrations of the ethanolic extract of *P. graveolens* a little change in the normal cell had been noticed. According to the US NCI (United States National Cancer Institute) search database, it had been found that the crude extract that exhibited IC_{50} value located between 30–40µg/mL was considered promising and noncytotoxic. Considering our results, the ethanolic extract from *P. graveolens* showed an IC_{50} value ≥100 µg/mL, so it is considered to be safe **(Mahmoud *et al.*, 2022)**.

Table (9): Cytotoxicity, viability, and IC_{50} of the ethanolic extract of *P. graveolens* related to normal cells using the MTT method

Sample	Concentration ug/ml	Viability %	Toxicity %	IC_{50} ± SD
Wi38 control	--------	**100**	**0**	**Ug/ml**
Ethanolic extract of *P. graveolens*	**1000**	**3.941**	**96.059**	
	500	8.623	91.377	
	250	25.591	74.409	
	125	64.395	35.605	**179.55 ± 1.59**
	62.5	99.907	0.0927	
	31.25	99.954	0.0463	

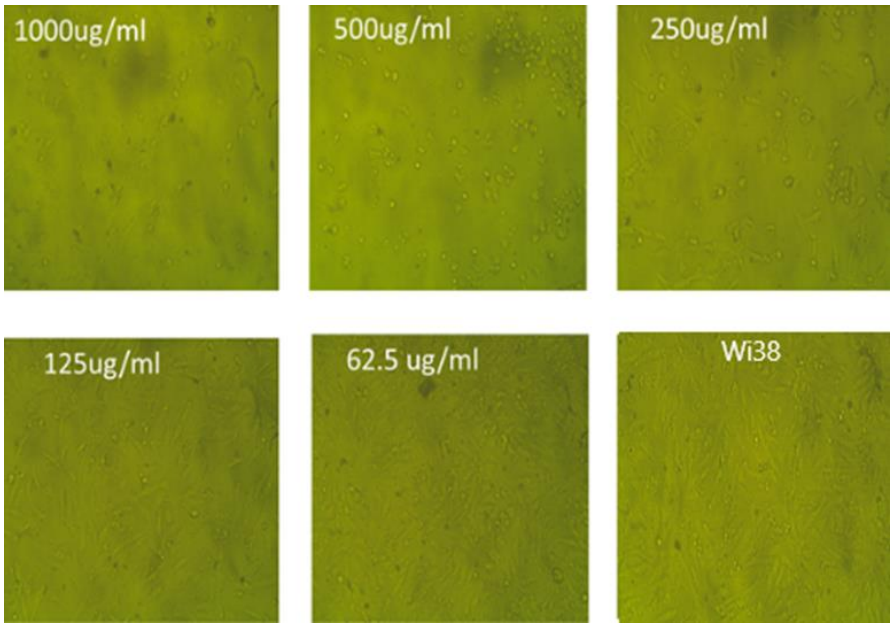

Figure 9: The effect of different concentrations of *P. graveolens* ethanolic extract on the normal cell compared to control (Wi38)

Sensory evaluation of ice cream with *P. graveolens* extract:

The results in Table (10) and Figure (10) show that the addition of *P. graveolens* extract affected the sensory characteristics of ice cream. It makes a difference to the control's overall acceptability, appearance, color and odor. The overall sensory characteristics score (varied between 7. 71 to 8.43) for ice cream. Table (10) shows a significant difference when compared with the control and the other treatments. Ice cream

supplemented with 10% and 15% *P. graveolens* extract scored an excellent 8.43 overall. In addition, the color received excellent acceptance over the control and 5%.

Table (10): Sensory evaluation of ice cream with *P. graveolens* extract (n = 30)

Sample	Appearance	Taste	Texture	Color	odor	Acceptability
C. I.C	$7.80^{b}\pm0.48$	$7.90^{a}\pm0.66$	$8.03^{a}\pm0.41$	$7.77^{b}\pm0.73$	$7.57^{b}\pm0.57$	$7.\ 71^{b}\pm0.56$
I.C.P. E 5%	$8.10^{ab}\pm0.76$	$7.97^{a}\pm0.81$	$8.07^{a}\pm0.79$	$8.13^{a}\pm0.82$	$8.0^{a}\pm0.79$	$8.\ 23^{a}\pm0.81$
I.C.P. E 10%	$8.30^{a}\pm0.70$	$8.07^{a}\pm0.74$	$8.20^{a}\pm0.66$	$8.40^{a}\pm0.72$	$8.13^{a}\pm0.86$	$8.\ 31^{a}\pm0.71$
I..C.P. E 15%	$8.40^{a}\pm0.62$	$8.13^{a}\pm0.63$	$8.23^{a}\pm0.68$	$8.43^{a}\pm0.57$	$8.33^{a}\pm0.76$	$8.\ 43^{a}\pm0.72$
(F)	4.97	0.634	0.68	5.58	5.60	14.24
(P)	0.001	0.595	0.564	0.001	0.001	0.001

* C. I.C = Control ice cream, I.C.P. E = Ice cream with *P. graveolens* extract *Values are expressed as means ± SE Mean values and significantly different ($p < 0.05$)

(1) C. I.C (2) I.C.P. E 5% (3) I.C.P. E 10% (4) I.C.P. E 15%

Figure 10: Sensory evaluation of ice cream with *P. graveolens* extract

* C. I.C = Control ice cream, I.C.P. E = Ice cream with *P. graveolens* extract

Conclusion

Pelargonium graveolens (family: geraniaceae), is known for its rose-like smell as well as its essential oil. This plant and/or its extracts exhibited many applications in the food, cosmetic, medicinal and pharmaceutical industries. Ethanolic extract from its green leaves exhibited potential antimicrobial and antioxidant activities as it is rich in many phenolic constituents. The ethanolic extract from *P. graveolens* didn't exhibit cytotoxic activity on normal cell lines which makes it a potential source in food applications. Ice cream supplemented with 10% and 15% *P. graveolens* extract scored excellent overall. In addition, the color received excellent acceptance over the control and 5%.

References

Abd El Salam, H. A., Abo-Salem, H. M., Kutkat, O., Abdel-Aziz, M. S., Montaser, A. S. and El-Sawy, E. R. (2024). Synthesis of 5-heptadecyl-4H-1,2,4-triazole incorporated indole moiety: Antiviral (SARS-CoV-2), antimicrobial, and molecular docking studies, *Journal of Molecular Structure*, 1303:137517.

Abdel-Aziz, M. S., Ghareeb, M. A., Hamed, A.A., Rashad, E. M., El-

Sawy, E. R., Saad, I. M. and Ghoneem, K. M. (2021). Ethyl acetate extract of Streptomyces spp. isolated from Egyptian soil for management of Fusarium oxysporum: The causing agent of wilt disease of tomato, *Biocatalysis and Agricultural Biotechnology*, 37:102185.

Abo-Salem, H. M., Ali, E. A., El-Mowafi, S. A., Abdel-Aziz, M. S., El-Sawy, E. R. and Abd El Salam, H. A. (2024). New sulfonamide-tethered coumarin derivatives as potential DNA gyrase inhibitors: Design, synthesis, antimicrobial evaluation, and in silico study, *Journal of Molecular Structure*, 1296 (1): 136860.

Abo-Salem, H.M., Abd El Salam, H.A., Abdel-Aziem, A.M., Abdel-Aziz, M.S. and El-Sawy, E.R. (2021). Synthesis, Molecular Docking, and Biofilm Formation Inhibitory Activity of Bis (Indolyl) Pyridines Analogues of the Marine Alkaloid Nortopsentin. *Molecules*. 26 (14):4112.

Abu El Wafa, S. S., El Ashmawy, A. A., Kassem, H. A. H., Eissa, I. H., Abu Elghait, M. Younis, N. A. and Younis, I. Y. (2023). Optimization of oil yield of *Pelargonium graveolens* L'Hér using Box Behnken design in relation to its antimicrobial activity and in silico study. *Scientifc Reports* 13:19887

Ali, I.B.E., Tajini, F., Boulila, A., Jebri, M. A.b., Boussaid, M.a., Messaoud, C. and Sebaï, H. (2020). Bioactive compounds from Tunisian *Pelargonium graveolens* (L'H′er.) essential oils and extracts: α-amylase and acethylcholinesterase inhibitory and antioxidant, antibacterial and phytotoxic activities. *Industrial Crops and Products* 158:112951

Al-Saffar, A. Z., Al-Shanon, A. F., Al-Brazanchi, S. L., Sabry, F. A., Hassan, F. and Hadi, N. A. (2017). Phytochemical Analysis, Antioxidant and Cytotoxic Potentials of *Pelargonium graveolens* Extract in Human Breast Adenocarcinoma (MCF-7) Cell Line. *Asian J. Biochem*., 12 (1): 16-26.

Angelis, A., Boukhris, M., Aligiannis, N., Bouaziz, M., Sayadi, S. and Skaltsounis, A. (2013). Phytochemical analysis of biological active methanolic extract of cultivated *Pelargonium graveolens* using countercurrent chromatography conjugated with sephadex column. *Planta Med* 2013; 79 - PI7

Ayo, R.G. (2010). Phytochemical constituents and bioactivities of the extracts of Cassia nigricans Vahl. *J. Med. Plant Res*., 14: 1339-1348.

Balunas, M.J. and Kinghorn, A.D. (2005). Drug Discovery from Medicinal Plants. *Life Sciences*, 78, 431-441.

Boukhris, M., Bouaziz, M., Feki, I., Jemai, H., Feki, A. E. and Sayadi, S. (2012). Hypoglycemic and antioxidant effects of leaf essential oil of *Pelargonium graveolens* L'Hér. In alloxan induced diabetic rats. *Lipids in Health and Disease*, 11:81.

Brian, A. K., Brian, E. W., Ingram, M. and Brenda, C. (2010). Geranium leaf tissue nutrient sufficiency ranges by chronological age. *J. Plant Nutr*. 33(3):339-350.

Butles, M.S. (2004). The role of natural product chemistry in drug discover. *J. Nat. Prod*., 67: 2141-2153.

Ceri, H., Olson, M.E., Morck, D.V. and Storey, D.G. (2006). Minimal biofilm eradication (MBEC) assay: susceptibility testing for biofilms. In: Pace JL, Rupp ME, Finch RG, editors. Biofilms, infection, and antimicrobial theraphy. *CRC Pres, Boca Raton*, pp. 257-269.

Charlwood, B. V. and Charlwood, K.A. (1991). Pelargonium spp. (Geranium): in vitro culture and the production of aromatic compounds. In: Bajaj, Y.P.S. (Ed.).*Biotechnol Agric* For: Med Arom Plant, 15:339-352.

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol*. 23: 174–181.

Derwich, E., Benziane , Z. and Boukir, A. (2010). Chemical composition of leaf essential oil of Juniperus phoenicea and evaluation of its antibacterial activity. Int. *J. Agric. Biol*., 12: 199-204.

Dimitrova, M., Mihaylova, D., Popova, A., Alexieva, J., Sapundzhieva, T. and Fidan, H. (2015). Phenolic profile, antibacterial and antioxidant activity of Pelargonium graveolenes leaves' extracts. Scientific Bulletin. Series F. *Biotechnologies*, Vol. XIX,

Džamić, A. M., Soković, M. D., Ristić, M. S., Grujić, S. M., Mileski, K. S. and Marin, P. D. (2014). Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. *J App Pharm Sci*. 4(3): 1-5.

El Aanachi, S., Gali, L., Nacer, S. N., Bensouici, C., Dari, K. and Aassila, H. (2020). Phenolic contents and in vitro investigation of the antioxidant, enzyme inhibitory, photoprotective, and antimicrobial effects of the organic extracts of *Pelargonium graveolens* growing in Morocco. *Biocatalysis and Agricultural Biotechnology*. 29 101819

Elsemelawy, S. A. and Tag Al-Deen, R. I. (2020). Bioactive Effects of Annona Muricat Juice on Side Effects of Aspirin Induced Hyperuricemia in Experimental Rats. *Journal of Research in the fields of Specific Eudcation*. 6(2), 230 246.

EL-Shahid, Z.A., Abd EL-Hady, F.K., Walid, F. W, AbdelAziz, M.S., Abd EL-Azeem, E.M. and Ahmed, E.K. (2021). Antimicrobial, Cytotoxic, and α-Glucosidase Inhibitory Potentials Using the One Strain Many Compounds Technique for Red Sea Soft Corals Associated Fungi' Secondary Metabolites and Chemical Composition Correlations, *J. Biol. Active Prod*. From Nat., 11:5-6, 467-489

Ennaji, H., Chahid, D., Aitssi, S., Badou, A. and Khlil , N. (2020). Phytochemicals screening, cytotoxicity and antioxidant activity of the Origanum majorana growing in Casablanca, Morocco. Open *J Biol Sci*. 5(1): 053-059.

Goupy, P., Hugues, M., Boivin, P. and Amiot, M.J. (1999). Antioxidant composition and activity of barley (Hordeum vulgare) and malt extracts and of isolated phenolic compounds *Journal of the Science of Food and Agriculture*, 79 (12):1625-1634.

Keisuke, I., Yosuke, I., Makiko, I., Masayoshi, T., Nobuo, I., Keiji, I. and Kiyoshi, T. (2012). Effects of Manufacturing Process Conditions on Sensory Attributes and Microstructure of Ice Cream. Sensors and Materials, Vol. 24, No. 5 .245–260

Kirkpatrick, L. and Feeney, B. (2012). A simple guide to IBM SPSS: for version 20.0: Nelson Education: USA.

Kupina, S., Fields, C., Roman, M.C. and Brunelle, S.L. (2017). Determination of Total Phenolic Content Using the Folin-C Assay: Single-Laboratory Validation, *J AOAC Int*. 1;101(5):1466-1472.

Lalli J. Y. (2005). In vitro pharmacological properties and composition of leaf essential oils and extracts of selected indigenous Pelargonium (Geraniaceae) species. PhD Thesis, Faculty of Health Sciences. University of the Witwatersrand, Johannesburg.

Li, A.-N., Li, S., Zhang, Y.-J., Xu, X.-R., Chen, Y.-M. and Li, H.-B. (2014). Resources and biological activities of natural polyphenols. Nutrients. 6:6020–6047.

Mahmoud, K., Mohammed, A., Salem, D., Ibrahim, K., Shawky, M., Osama, M., Rageh, M., Tarek, R. and El Shahed, Z. (2022). Cytotoxic, Genotoxic and pro-apoptotic effect of some medicinal plants on the expression of colon cancer-related genes (P53 and Bcl2) in the colorectal cell line and syngenic animal cancer model. *Egyptian Journal of Chemistry*. 65(11): 761 – 777.

Makanyane, D. M., Ejidike, I. P., Ssemakalu, C. C., Mtunzi, F. M., Pakade, V. E., Klink, M. J. and Lebelo, R. S. (2019). GC/MS analysis and extraction optimization of bioactive compounds from Pelargonium graveolenes L'HER methanolic extract and their activities as pharmacological agents. *Int. Res. J. Pharm*. 2019, 10 (9)

Matthews, A. J. (1995). Geranium leaves for cracked nipples. Aust. *J. Hosp. Pharm.* 25:538-539.

Mohamed, S. E. (2024). Quality Attributes of Cake Made with Green Banana Fruit Peels. *Journal of Research in the fields of Specific Eudcation*, 10(1), 94-10.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 16; 65 (1-2):55-63

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1,269 (2):337-41

Rayes, K. A., Selim, A., Mohammed, S., Al Sayed Abdel Ghaffar, D. N. A. L., Mohammad, A. and Batoul, N. (2022). Chemical, Nutritional Analysis, Quality of Essential Oil of Eruca sativa and It s Potential Antimicrobial Activity. *Journal of Research in the fields of Specific Eudcation*, 8(4), 804-832

Saraswathi, J., Venkatesh, K., Baburao, N., Hilal, M. H. and Rani, A. R. (2011). Phytopharmacological importance of Pelargonium species. *Journal of Medicinal Plants Research*. 5(13), pp. 2587-2598, 4

Sarker, S.D., Nahar, L., Kumarasamy, Y. (2007). Microtiter plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 42: 321-324.

Schmidt, T. J., Khalid, S. A., Romanha, A. J., Alves, T., Ma Biavatti, M. W., Brun, R., Costa, F. B., Da Castro, S. L. de Ferreira, V. F., de Lacerda, M. V. G., Lago, J. H. G., Leon, L. L., Lopes, N. P., Neves Amorim, R. C., das Niehues, M., Ogungbe, I. V., Pohlit, A. M., Scotti, M. T., Setzer, W. N., Soeiro, M. de N. C., Steindel, M., Tempone, A. G. (2012). The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases-part II. *Curr. Med. Chem*. 19: 2176–2228.

Shawky, B. T., Nagah, M., Ghareeb, M. A., El-Sherbiny, G. M., Moghannem, S. A. M. and Abdel-Aziz, M. S. (2019). Evaluation of Antioxidants, Total Phenolics and Antimicrobial Activities of Ethyl Acetate Extracts from Fungi Grown on Rice Straw. *Journal of Renewable Materials*. 7(7):667-682

Thabrew, M.I., Hughes, R.D. and McFarlane, I.G. (1997). Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *J Pharm Pharmacol*. 49(11):1132-5.

Watts, B. M., Ylimaki, G., Jeffery, L. and Elias, L. G. (1989). Basic sensory methods for food evaluation: IDRC, Ottawa, ON, CA

Wu, G., Chang, C., Hong, C., Zhang, H., Huang, J., Jin, Q. and Wang, X. (2019). Phenolic compounds as stabilizers of oils and antioxidative mechanisms under frying conditions: A comprehensive review. Trends in Food Science and Technology 92: 33-45.