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Chemical Composition and *In vitro* Studies of the Essential Oil and Aqueous Extract of *Pelargonium graveolens* Growing in Jordan for Hypoglycaemic and Hypolipidemic Properties

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FUA, RAD and VK designed the study and wrote protocol. Authors FUA and IMA evaluated phytochemical analyses. Author VK performed the statistical analysis. All authors wrote first draft and managed samples for phytochemical and biological analyses. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study aimed to analyze the chemical composition of essential oil of *Pelargonium graveolens* L. Her. ex Ait. growing in Jordan and to test the efficacy of the leaves aqueous extract and essential oil against pancreatic triacylglycerol lipase (PL), α-amylase and α-glucosidase.
Study Design: GC-MS analysis of the essential oil obtained by hydrodistillation and Solid Phase Microextraction (SPME) methods as well as *in vitro* enzymatic investigations.
Place and Duration of Study: Faculty of Pharmacy, The University of Jordan, between November 2012 and August 2013.
Results: The hydrodistilled oil of *P. graveolens* fresh leaves yielded twenty eight components, accounting for 95.83 % of the total oil content, while thirty seven components were detected from the fresh leaves by SPME (98.86%). Twenty six and thirty one components were identified in the hydrodistilled and SPME oils of the dried leaves amounting to 96.08 % and 97.83 %, respectively. Oxygenated monoterpenes

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predominated the volatile fractions of the leaves of both methods with citronellol, citronellyl formate and menthone/isomenthone as the major constituents. Similar to orlistat (PL IC_{50} of 114.0 ± 4.0 ng/mL), *P. graveolens* extract and volatile oil as well as their purified phyto-constituents inhibited highly substantially in a dose dependent trend PL *in vitro* (n=3). The *P. graveolens* extract PL- IC_{50} was 207.4±15.2 µg/mL. As for their volatile oils' components, PL- IC_{50} (%) (V/V) in an ascending order were: menthone; 0.01±0.0 <geraniol; 0.34±0.02 < linalool; 0.7 ± 0.0 < caryophyllene; 1.17±0.12 <*P. graveolens* oil; 2.93 ± 0.27. Comparable to acarbose, *P. graveolens* leaves aqueous extracts (AEs) were identified as *in vitro* potent and efficacious dual inhibitors of α-amylase and α-glucosidase with IC_{50}: 4.6±0.1 mg/mL ($p<0.001$, n=3).

Conclusion: Taken together, *P. graveolens* leaves, as a nutraceutical modulating gastrointestinal carbohydrate and lipid digestion and absorption, maybe advocated as candidate for obesity-diabetes/metabolic syndrome management.

Keywords: Pelargonium graveolens L. Her.ex Ait.; Geraniaceae; SPME; GC-MS; pancreatic lipase; α-amylase; α-glucosidase; Jordan.

1. INTRODUCTION

Type 2 diabetes and obesity, referred to as diabesity, comprise global health threats with rising prevalence [1]. Recently, the high prevalence of dyslipidemia, obesity and diabetes in Jordan was marked as being alarmingly worrisome [2-6]. Plants have been long used for the ethnomedical integrative/complementary treatment of obesity-diabetes in various systems of medicine [7-8]. Among many, rose-scented geranium, *Pelargonium graveolens* L.Her.ex Ait (known also as *Geranium graveolens*), (Geraniaceae) is used traditionally for the treatment of hyperglycaemia in multiple folk medicine systems. In Jordan, *Geranium* spp. are used as astringent, diuretic, antidiabetic, antispasmodic for stomach troubles, and as gargle for throat and tonsils [9]. FDA (US Food and Drug Administration) classified geranium oils as GRAS (Generally Recognised As Safe) for food use [10]. Hence, geraniums are promoted as important aromatic plants and flavoring agents in perfumery, cosmetic, food and pharmaceutical industries [11-12]. Reports discussed *P. graveolens* antidiabetic propensities in addition to plausible action mechanisms [13-16]. Additionally, antimicrobial, antifungal, antioxidative, insecticidal, and antitumor as well as immunomodulatory activities were attributed to *P. graveolens* [14,17-20]. Based to low LD_{50} values, *P. graveolens* oil has been suggested as a source of safe anti-inflammatory agents [21]. Phytochemically, *P. graveolens* growing in different geographies has been investigated for identification of its essential oil composition [21-24].

Plant-based inhibitors of hydrolysing enzymes in carbohydrates and lipids digestion and absorption can offer an attractive combinatorial therapeutic strategy for the management of postprandial dysglycemia and dyslipidemia. Various studies were conducted to explore medicinal plants as potential therapeutic agents for dual management of diabetes and hyperlipidemia via digestive enzymes' inhibition, namely pancreatic alpha-amylase, intestinal alpha-glucosidase and pancreatic triacylglycerol lipase (PL) [25-27]. Hence, the purpose of the present study was to investigate the inhibitory effects of leaves aqueous extract and essential oil of *P. graveolens* on these digestive enzymes *in vitro.*

2. MATERIALS AND METHODS

2.1 Equipments, Chemicals and Biochemicals

Unless stated otherwise, all reagents and chemicals were from Sigma (Dorset, UK). GC-grade hexane and analytical reagent grade anhydrous Na_2SO_4 were purchased from Scharlau (Barcelona, Spain) and UCB (Bruxelles, Belgium), respectively. Reference substances for GC-MS were obtained from Fluka, Buchs, Switzerland. In UV determinations; UV-VIS spectrophotometer from SpectroScan 80D (UK) was used. Sonicator (Bandelin Sonorex, Bandelin electronics, Germany) and rotary evaporator (Laborota 4000-efficient, Heidolph, Germany) were also used. Glucose GOD-PAP kit was obtained from BioLabo Reagents, France. Trinder method (GOD-POD) is for quantitative determination of glucose. Glucose is oxidized by GOD (glucose oxidase) to gluconic acid and hydrogen peroxide which is in conjunction with POD (peroxidase) reacts with chloro-4-phenol and PAP (para-aminophenazone) to form a red quinoneimine. The absorbance of the colored complex, proportional to the concentration of glucose in the specimen is measured at 500 nm [28].

2.2 Plant Material

Leaves of *P. graveolens* were collected from Amman during the flowering period late summer 2012. The plant was identified by one of the authors (F.U. Afifi). The leaves were air dried at room temperature (RT) in the shade until constant weight, and subsequently assayed for essential oil composition as the fresh leaves. A voucher specimen (GER 001/FMJ) has been deposited in the Department Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman, Jordan.

2.3 Hydrodistillation of Leaves

Each 200 g of fresh and air dried leaves (about 150 g equivalent to 300 g of fresh leaves) were coarsely powdered and then hydro-distilled using a Clevenger apparatus for 3 h. The extraction was repeated three times and the obtained oils were pooled separately, dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4ºC in amber glass vials until analysis.

2.4 Solid Phase Micro Extraction (SPME) of Volatile Oils

The SPME experiments were performed using the fiber assemblies (PDMS/DVB; d_f 65 μm, length 1 cm) for manual sampling (Supelco, USA). About 0.2 g of fresh leaves and about 0.1 g freshly powdered dried leaves were put into 5.0 mL amber glass vials, tightly capped with PTFE-coated septa, and SPME extraction was performed for 2 min at RT. Desorption of the analytes was carried out at 240ºC for 1 min. Extraction was repeated three times.

2.5 GC-MS and GC-FID Analysis

The GC-MS analysis was performed using Varian chrompack CP-3800 GC/MS/MS-200 (Saturn, Netherlands) fitted with DP-5 (5% diphenyl, 95% dimethyl polysiloxane) GC capillary column (30 m ×0.25 mm i.d., 0.25 μm film thicknesses). The MS ionization source was 180ºC and the ionization voltage was 70 eV. The column temperature was kept at 60ºC for 1 min (isothermal), and rammed from 60ºC to 246ºC at a rate of 3ºC/min, and then held constant at 246ºC for 3 minutes (isothermal). Helium was used as a carrier gas at a flow rate of 0.9 mL/min. A hydrocarbon mixture of n-alkanes (C_8-C_{20}) was analyzed separately by

GC/MS under the same chromatographic conditions using the same DB-5 column. About 1 µL aliquot of each oil sample, appropriately diluted to 10 µL in GC grade n-hexane, was subjected to GC/MS analysis. The temperature in MS source reached 180ºC, the ionization voltage was 70 eV. The column temperature was kept at 60ºC for 1 min (isothermal), and then programmed to 246ºC at a rate of 3ºC/min, and kept constant at 246ºC for 3 min (isothermal). A hydrocarbon mixture of n-alkanes (C_8-C_{20}) was analyzed separately by GC/MS under the same chromatographic conditions using the same DP-5 column. Identification of compounds was based on the built in libraries (NIST Co and Wiley Co, USA) and by comparing their calculated retention indices (RI) relative to (C_8-C_{20}) n-alkanes literature values measured with columns of identical polarity [29], or with authentic samples. α- and β-pinenes, citronellol, limonene, linalool, geraniol, menthol, menthon and caryophyllene were used as reference substances in GC/MS analysis.

For the quantitative analysis (% area), a Hewlett-Packard HP-8590 gas chromatograph equipped with a split-splitless injector (split ratio 1:50) and an FID detector was used. The column was an optima-5 (5% diphenyl, 95% dimethyl polysiloxan) fused silica capillary column calculate the concentration of the detected compounds. (30 m × 0.25 mm, 0.25 µm film thickness). The temperature of the oven was increased at a rate of 10ºC/min form 60ºC to 250ºC and then held constant at 250ºC for 5 min. The temperatures of the injector and detector were maintained at 250ºC and 300ºC, respectively. The relative peak areas of the oil components were measured and then used to

2.6 Preparation of the *P. graveolens* aqueous extracts (AEs)

AEs were prepared by refluxing each 10 g of the dried coarsely powdered plant material with 100 ml tap water for 15 min. The overnight kept extracts were filtered twice through filter paper and the volume of the filtered solution was increased to 100 mL with tap water to obtain 10% (equivalent to 100 mg/1 mL) crude aqueous solutions [30]. Sonication of stock crude extract or testing concentrations was performed before implementation of investigations. For pancreatic lipase experimentation; water was evaporated under vacuum at 40^{o}C using a rotary evaporator. The solid residues were collected and stored in dry conditions until analysis.

2.7 Preparation of *P. graveolens* Leaves Aqueous Extract, Volatile Oil and Pure Volatile Constituents for *In vitro* Pancreatic Triacylglycerol Lipase (PL) Activity Assay

Tested aqueous extracts were initially dissolved in Tris-HCl buffer (2.5 mM (Promega, USA), pH 7.4 with 2.5 mM NaCl) to give five initial stock solutions with a concentration range displayed in Table 1. Subsequently, 20 µl aliquot of each stock solution was used in the reaction mixture to give a final concentration range illustrated in the same table. Extracts were prepared according to the traditional indications of use, thus DMSO or any other organic solvent, even to the minimum concentration was avoided [31]. Also, citronellol, menthone, geraniol, linalool, caryophyllene and *P.graveolens* oil (dissolved in DMSO) were prepared into five stock solutions with a concentration range as in Table 1. Thereafter, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range as in the same table. Finally, orlistat the reference drug (in DMSO; 1 mg/mL), was prepared in six different stock solutions with a concentration range of 0.625 - 20 µg/ mL [32]. Thereafter, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 0.0125 – 0.4 µg/ mL.

Table 1. Initial and final tested concentration ranges of *Pelargonium graveolens* leaves AEs, and its volatile constituents in pancreatic triacyglycerol lipase bioassay *in vitro*.

	Initial stock concentration	Final concentration
P. graveolens leaves AE	25 mg/mL	250 µg/mL
	25 mg/mL	500 µg/mL
	50 mg/mL	1000 µg/mL
	100mg/mL	2000 µg/mL
	200 mg/mL	4000 µg/mL
geraniol % (V/V)	0.097656	0.001953
	0.390625	0.007813
	1.5625	0.03125
	6.25	0.125
	25	0.5
menthone % (V/V)	0.09765625	0.001953
	0.390625	0.00781
	1.5625	0.03125
	6.25	0.125
	25	0.5
caryophyllene, citronellol, linalool and *P. graveolens* oil % (V/V)*	6.25	0.125
	12.5	0.25
	25	0.50
	50	1
	100	2

**Each of these compounds and P. graveolens oil was separately prepared in the given initial and final concentrations for PL testing.*

2.8 Spectrophotometric Quantification of PL Inhibition by Test Extracts and Compounds

In vitro enzymatic PL activity was assayed according to Al-Hallaq et al. [33]. Subsequent determinations were undertaken for the tested extracts and pure compounds in comparison to control evaluations, to calculate the concentration required for PL 50% inhibition (IC_{50}).

2.9 *In vitro* Enzymatic Starch Digestion Assay

In vitro enzymatic starch digestion was assayed with acarbose, as the reference drug [33]. The extent of polysaccharide breakdown into glucose was evaluated in a concentration range of plant aqueous extract 1, 5,10,12.5,25,50 and 100 mg/mL. The effects of acarbose at 1000 µg/ mL concentration were evaluated as well. Control (tap water only) samples contained neither acarbose nor plant extract.

2.10 Statistical Analysis

The values are presented as mean ± S.E.M. (Standard Error of the Mean) of 3-4 independent experiments. Statistical differences between control and different treatment groups were determined using Graphpad Prism one way analysis of variance (ANOVA) followed by Dunnett post test whenever appropriate (version 3.02 for windows; Graph Pad Software, San

Diego, CA, USA). Values were considered significantly different if P<0.05 and highly significantly different if P<0.01 and P<0.001.

3. RESULTS AND DISCUSSION

3.1 Identification of the Chemical Composition of the Volatile Oil of *P. graveolens*

The composition of the volatile fraction of *P. graveolens* was determined by hydrodistllation and SPME method. The hydrodistillation of the fresh and ground air dried leaves of *P. graveolens* afforded colorless oil (1.7% and 1.5 %, v/w, respectively). The essential oil components were identified in GC-MS analysis based on the comparison of the obtained RI and MS fragmentation patterns to those of standard compounds and on computer matching with the built-in libraries [28]. The obtained results are presented in Table 2.

Table 2. Composition of *Pelargonium graveolens* essential oil using two extraction methods

RI Lit[a].	RI Exp[b].	Compound	Oil 1	Oil 2	Oil 3	Oil 4
802	801	hexenal	1.49	-	7.41	-
859	857-862	hexenol<3Z >	3.28	1.46	1.38	0.89
939	937	pinene <alpha->	0.40	0.69	0.10	0.30
950	954	citronellene<beta->	-	-	-	1.63
991	989-991	myrecene	-	0.38	-	1.50
1003	1005	phellandrene<alpha>	-	0.72	-	-
1026	1029	cymene<ortho->	-	-	-	1.01
1030	1032	phellandrene<beta>	-	0.53	-	-
1031	1033	carene<delta-3->	-	0.83	-	-
1043	1041	rose oxide<cis-dihydro->	-	-	-	0.10
1073	1076	rose oxide<trans-dihydro->	-	-	-	0.72
1097	1095-1100	linalool	0.48	0.73	0.57	0.56
1108	1107-1115	rose oxide<cis>	-	1.18	0.48	5.61
1126	1130	rose oxide<trans>	-	0.64	0.31	2.40
1135	1136	linalool<dihydro->	-	-	-	0.52
1153	1154	citronellal	0.46	0.21	-	0.75
1153	1156	menthone	12.95	15.21	8.07	6.31
1162	1163	menthone<iso->	-	-	9.01	28.85
1172	1174	menthol	2.15	-	3.15	3.25
1215	1217	dihydro carveol<iso->	-	-	-	0.72
1226	1230	citronellol	27.45	8.15	27.70	6.73
1243	1244	carvone	0.52	-	0.49	-
1258	1253	geraniol	8.04	4.40	9.15	1.81
1274	1277	citronellyl formate	13.57	15.41	15.11	13.33
1302	1298-1301	geranylformate	3.98	4.24	1.15	1.27
1338	1334	elemene<delta->	0.19	0.17	-	-
1351	1349	cubebene<alpha->	-	0.91	-	0.38
1353	1352	citronellylacetate	-	0.24	-	
1377	1378	copaene<alpha->	0.47	1.51	0.59	0.91
1388	1386	bourbonene<beta->	0.92	1.05	-	2.96
1388	1390	cubebene<beta->		0.54	-	0.48
1391	1392	elemene<beta->	0.71	1.01	-	-

1419	1422	caryophyllene<(E)->	1.49	6.61	-	2.34
1437	1438	elemene<gamma>	0.54	2.12	-	0.64
1440	1444	guaiene<alpha->	8.35	20.91	4.60	8.12
1454	1452	muurola-3,5-diene<trans>	0.43	0.50	0.16	0.35
1455	1458	humulene<alpha>	-	1.16	-	-
1485	1485	germacrene D	3.06	4.25	1.01	1.18
1490	1493	selinene<beta->	-	0.24	-	-
1500	1499	bicyclogermacrene	0.51	0.52	0.27	-
1500	1501	muurolene<alpha->	0.18	0.40	0.41	-
1502	1502	guaiene<trans-beta->	-	0.20	-	-
1514	1513	cadinene<gamma->	0.42	0.20	0.16	-
1523	1524	cadinene<delta->	0.82	0.64	0.63	0.29
1531	1533	bisobolene<(E)-gamma	-	0.18	0.60	-
1564	1560	geranylbutanoate	1.15	0.60	1.38	-
1583	1588	caryophyllene oxide	1.11	-	1.21	0.92
1701	1699	caryophyllene acetate	0.71	0.12	0.98	-
		Terpenoids				
	Monoterpenes					
		Monoterpene hydrocarbons	0.40	3.15	0.10	4.44
		Oxygenated monoterpenes	69.60	50.17	75.19	72.93
	Sesquiterpenes					
		Sesquiterpene hydrocarbons	18.09	43.36	8.43	18.65
		Oxygenated sesquiterpenes	2.97	0.72	3.57	0.92
	Aliphatic compounds		4.77	1.46	8.79	0.89
	Non-terpenoid aromatic compounds		-	-	-	-
	Total identified		95.83	98.86	96.08	97.83

Oil 1: Hydrodistilled (fresh leaf); Oil 2: SPME (fresh leaf); Oil 3: Hydrodistilled (dry leaf); Oil 4: SPME (dry leaf).

[a]RI Lit., Reported Retention Index [29].

[a]RI Exp., Retention index relative to (C_8-C_{20}) n-alkanes

[c]The percentage composition based on the GC peak areas

GC/MS analysis of the hydro-distilled oil obtained from fresh and dried leaves led to the identification twenty eight and twenty six components, accounting for 95.83 % and 96.08 %, respectively, of the total oil content. Both oils were strongly characterized by the presence of oxygenated monoterpenes, comprising 69.60% of the oil content of fresh leaves and 75.19% of the oil obtained from the dried leaves with citronellol, citronellyl formate and menthone/iso-menthone dominating this fraction. Monoterpene hydrocarbons were poorly represented. Alpha pinene was detected in fresh and dried leaves as the solo representative of this class in concentrations of 0.40% and 0.10%, respectively. Sesquiterpenoids (21.06 % and 12.00% in the oils of fresh and dry leaves, respectively) appeared as hydrocarbons rather than oxygenated derivatives, with alpha guaiene as the main component.

Analysis of the SPME obtained volatile oil of *P. graveolens* using fresh and dried leaves resulted in the identification of thirty seven and thirty one compounds amounting to 98.86 % and 97.83% of the total oil content, respectively. Both SPME oils were characterized again by the occurrence of high concentration of oxygenated monoterpenes. Total identified monoterpenoids in the oil of fresh leaves constituted 54.72%, composed mainly of oxygenated monoterpenes (51.57%) with citronellyl formate (15.41%), menthone (15.21%) and citronellol (8.15%). The SPME oil of the dried leaves revealed again the presence of higher concentration of oxygenated monoterpenes with the same major components. Also in the SPME oils sesquiterpenes were detected in much lower concentrations as compared to monoterpenoids, again with alpha guaiene as the main component. The composition of the SPME volatile oil showed quantitative and qualitative differences as compared to the composition of the hydro-distilled oil. The number of different constituents identified in SPME oil was also higher compared to the hydrodistilled oil, which indicates the sensitivity of the SPME.

Prior to the present study the composition of *P. graveolens* oil obtained by hydrodistillation was studied by several researchers and the findings indicate that *β*-citronellol is a prominent part of volatile oil as in the present study [14,17,22, 34-36]. Another *Pelargonium* species, *P. asperum*, grown in Japan has exhibited the same tendency [37]. Our findings in applying hydrodistillation are in agreement with these previous studies. Quantitative differences were observed with regard to minor oxygenated monoterpenoids and sesquiterpenoid hydrocarbons. Among the different studies, including the present study, the greatest fluctuation was observed with the two biogenetically parent compounds of the monoterpenoids, namely geraniol and linalool. This indicates that the plant materials were collected at different stages of the flowering period as well as from different geographical regions, such as Tunisia, Iran, India and Jordan. Influence of climatic and environmental conditions may contribute to the observed qualitative and quantitative fluctuations in composition.

SPME reflects the actual composition of the volatile substances without being exposed to heat. Since the present study, with the best of our knowledge, is the first evaluation of *P. graveolens* essential oil using this method, no similar data are available to compare our findings. But interestingly in this method citronellyl formate and menthone/isomenthone replace citronellol as the major component in the fresh and dried leaves.

3.2 *In vitro* Extrapancreatic Inhibitory Effects of *P. graveolens* AE, Volatile Oil and Volatile Constituents on PL Activity

In this current study, the PL inhibitory profiles of the aqueous extract of leaves of *P. graveolens,* its essential oil and volatile constituents are shown in Figs. 1A-B, respectively. Orlistat PL IC_{50} of 114.0 ± 4.0 ng/mL, equivalent to 0.2 ± 0.0 µM, is comparable to reported PL IC_{50} values elsewhere [32] (Table 3). Comparable to orlistat performance, a marked concentration dependent PL inhibition trend was obtained per tested extracts (the same figures) as well as their volatile components. PL IC_{50} values obtained for a minimum of triple separate determinations are also illustrated (Table 3).

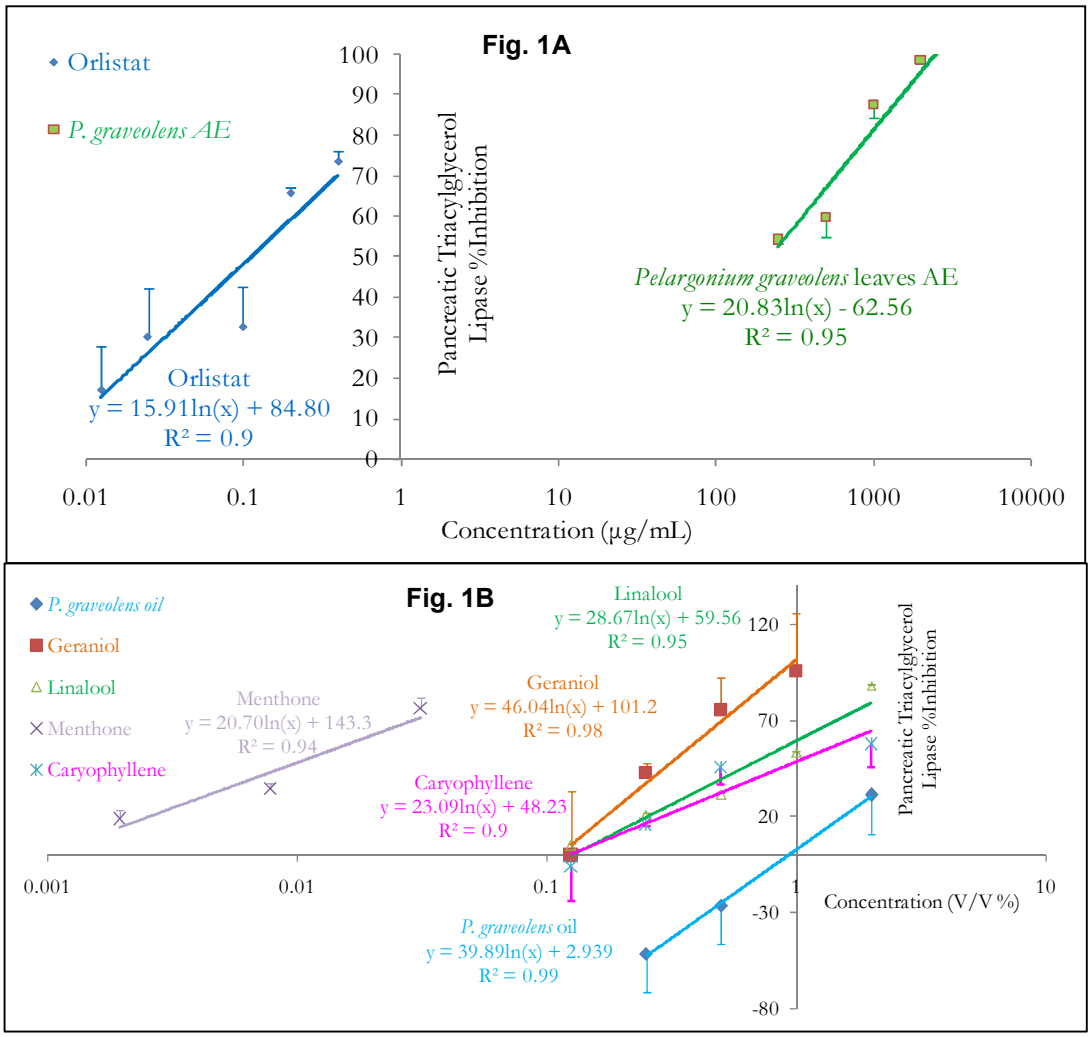

Fig. 1. A-B. ***In vitro*** **inhibitory effects of leaves AE of *P. graveolens*, its essential oil and volatile principles, as well as orlistat on PL activity**.

Results per each test extract, oil or compound are the mean ±SEM (n=3 independent replicates).

Table 3. Pancreatic Lipase IC_{50} values for tested concentrations of *P. graveolens* leaves AEs, volatile oil and its principles and orlistat

Extract/pure compounds	PL-IC_{50}
P. graveolens leaves AE	207.4 ± 15.2 µg/mL
menthone	0.01± 0.0 % V/V
geraniol	0.34±0.02 % V/V
linalool	0.7 ± 0.02 % V/V
caryophyllene	1.17±0.12 % V/V
citronellol	Inactive
P. graveolens oil	2.93 ± 0.27 % V/V
orlistat	0.114 ± 0.0 µg /mL

Results are mean ± SEM (n = 3 independent replicates).

3.3 *In vitro* Inhibitory Effects of *P. graveolens* Leaves AEs on Enzymatic Starch Digestion

With acarbose (0.1 mg/mL) as the reference drug, glucose liberation from starch was inhibited by 97.6% highly substantially (p<0.001, vs. drug-free control incubations, n=3, Fig. 2). Furthermore, Fig. 2 demonstrates that *P. graveolens* leaves AEs concentrations 0.1-10 mg/mL had highly substantial dose-related reductions in aldohexose release from culinary polymeric cornstarch (p<0.001 vs. plant-free control determinations, n=3). With IC_{50} of *P. graveolens* leaves; 4.6±0.1 mg/mL, the highly significant dose related (p<0.001) % decreases in enzymatic starch hydrolysis by *P. graveolens* leaves dosage gradient (0.1-10 mg/mL) are summarized in Table 4. Earlier, Boukhris et al. (2012) demonstrated antioxidative and hypoglycemic effect of *P. graveolens* essential oil in alloxan-diabetic rats in comparison to glibenclamide [14]. Our findings, on the other hand, emphasize the enzymatic inhibitory activities of the study plant aqueous extracts in linkage to starch hydrolases, namely alpha amylase and alpha glucosidase. This combinatorial efficacy illustrated in the present study is based on a different mode of action than that of glibenclamide, a hypoglycemic insulin secretagogue sulfonylurea.

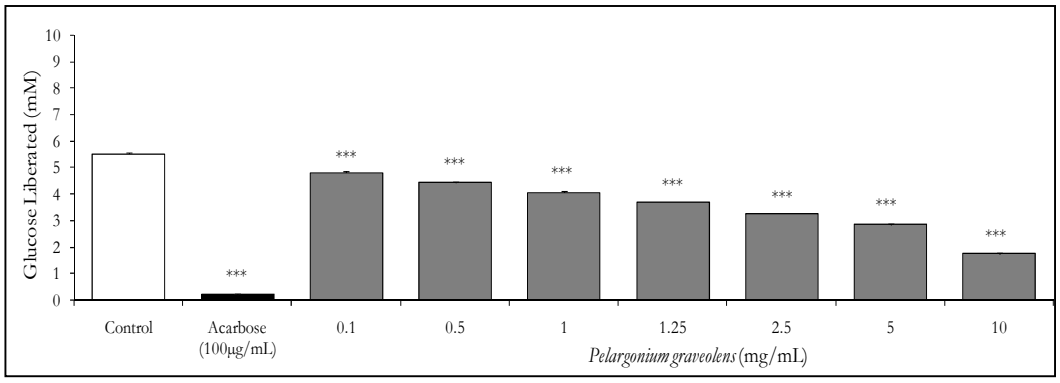

Fig. 2. *In vitro* inhibitory effects of *P. graveolens* leaves aqueous extracts on enzymatic starch digestion

*Results are mean ± SEM (n = 3 independent replicates). Statistical significance of difference from corresponding control incubations' values: ***P<0.001*

Table 4. **Effects of *Pelargonium graveolens* leaves AE concentrations (mg/mL) on % reduction of enzymatic starch digestion *in vitro*.**

Extract (mg/mL)	0.1	0.5	1	1.25	2.5	5	10
P. graveolens Leaves AE	12.9 ± 0.7 ***	19.6 ± 0.6 ***	26.3 ± 0.9 ***	32.9 ± 0.5 ***	40.9 ± 0.3 ***	48.5 ± 1.0 ***	68.4 ± 0.1 ***

*Results expressed as % decrease in control values are mean ± SEM (n = 3 independent replicates). ***P<0.001 compared to control (drug-free or plant-free) determinations*

4. CONCLUSIONS

The SPME technique is found to be more sensitive in identification of terpenoid compounds, since high temperature of the Clevenger apparatus may affect the stability of these components. SPME is a very simple, rapid, solvent-free and inexpensive extraction method that deserves to be considered as an alternative technique in analysis of volatile compounds from plants. Succinctly *P. graveolens* phytochemicals can inhibit crucial gastrointestinal enzymes involved in carbohydrate and lipid digestion and absorption thus advocating a dual-target management strategy in metabolic syndrome and obesity-diabetes (diabesity).

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest concerning this article.

REFERENCES

1. Tschop MH, DiMarchi RD. Outstanding Scientific Achievement Award Lecture 2011: defeating diabesity: the case for personalized combinatorial therapies. Diabetes. 2012; 61(6):1309-14.
2. Abu Baker NN, Daradkeh SM. Prevalence of overweight and obesity among adolescents in Irbid governorate, Jordan. East Mediterr Health J. 2010;16(6):657-62.
3. Khader YS, Batieha A, El-Khateeb M, Al Omari M, Ajlouni K. Prevalence of dyslipidemia and its associated factors among Jordanian adults. J Clin Lipidol. 2010; 4(1):53-8.

4. Khader YS, Batieha A, Jaddou H, Batieha Z, El-Khateeb M, Ajlouni K. Metabolic abnormalities associated with obesity in children and adolescents in Jordan. Int J Pediatr Obes. 2011;6(3-4):215-22.
5. Khattab M, Khader YS, Al-Khawaldeh A, Ajlouni K. Factors associated with poor glycemic control among patients with type 2 diabetes. J Diabetes Complications. 2010;24(2):84-9.
6. Al-Nsour M, Zindah M, Belbeisi A, Hadaddin R, Brown DW, Walke H. Prevalence of selected chronic, noncommunicable disease risk factors in Jordan: results of the 2007 Jordan Behavioral Risk Factor Surveillance Survey. Prev Chronic Dis. 2012; 9:E25.
7. Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of Iranian medicinal plants useful in diabetes mellitus. Arch Med Sci. 2008;4(3):285-92.
8. Hasani-Ranjbar S, Nayebi N, Larijani B, Abdollahi M. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. World J Gastroenterol. 2009; 15(25):3073-85.
9. Oran SA, Al-Eisawi DM. Check-List of medicinal plants in Jordan. Dirasat. Med Biol Sci.1998;25(2):84-112.
10. Lis-Balchin M: Geranium and Pelargonium: the genera Geranium and Pelargonium. 2002. London: CRC Press, Taylor & Francis
11. Verma RS, Verma RK, Yadav AK, Chauhan A. Changes in the essential oil composition of rose-scented geranium (*Pelargonium graveolens* L'Herit. ex Ait.) due to date of transplanting under hill conditions of Uttarakhand. Indian J Nat Prod Res. 2010;1(3):367-70.
12. Verma RK, Verma RS, Chauhan A, Singh A, Rahman L, Kalra A. Biomass yield, essential oil yield and oil quality of rose-scented geranium (*Pelargonium graveolens* L.), intercropped with vegetables. Int J Agric Res. 2011;6(12):830-9.
13. Afifi FU, Kasabri V. Pharmacological and phytochemical appraisal of selected medicinal plants from Jordan with claimed antidiabetic activities. Sci Pharma. ACCEPTED; 2013.
14. Boukhris M, Bouaziz M, Feki I, Jemai H, El Feki A, Sayadi S. Hypoglycemic and antioxidant effects of leaf essential oil of Pelargonium graveolens L'Hér. in alloxan induced diabetic rats. Lipids Health Dis. 2012;11:81-91.
15. Kasabri V, Afifi FU, Hamdan I. Evaluation of the acute antihyperglycemic effects of four selected indigenous plants from Jordan used in traditional medicine. Pharm Biol. 2011;49(1): 687-95.
16. Kasabri V, Abu-Dahab R, Afifi FU, Naffa R, Majdalawi L, Shawash H. *In vitro* effects of *Geranium graveolens, Sarcopoterium spinosum* and *Varthemia iphionoides* on pancreatic MIN6 proliferation and insulin secretion and on extrapancreatic glucose diffusion. Int J Diabetes Dev Ctries. In Press DOI 10.1007/s13410-013-0131-5; 2013.
17. Bigos M, Wasiela M, Kalemba D, Sienkiewicz M. Antimicrobial activity of geranium oil against clinical strains of *Staphylococcus aureus*. Molecules. 2012;17(9):10276-91.
18. Sabzghabaee AM, Shirdare Z, Ebadian B, Aslani A, Ghannadi A. Clinical evaluation of the essential oil of *Pelargonium graveolens* for the treatment of denture stomatitis. Dent Res J (Isfahan). 2011; 8(Suppl 1):S105-8.
19. Zhou ZL. Studies on the antitumor constituents of *Pelargonium graveolens*. Zhong Yao Tong Bao. 1982; 7(4):31-2.

20. Zhuang SR, Chiu HF, Chen SL, Tsai JH, Lee MY, Lee HS, Shen YC, Yan YY, Shane GT, Wang CK. Effects of a Chinese medical herbs complex on cellular immunity and toxicity-related conditions of breast cancer patients. Br J Nutr. 2012; 107(5):712-8.
21. Boukhatem MN, Kameli A, Ferhat MA, Saidi F, Mekarnia M: Rose geranium essential oil as a source of new and safe anti-inflammatory drugs. Libyan J Med. 2013; 8: 22520.
22. Boukhris M, Simmonds MS, Sayadi S, Bouaziz M. Chemical composition and biological activities of polar extracts and essential oil of rose-scented geranium, *Pelargonium graveolens*. Phytother Res. 2013; 27(8): 1206-13.
23. Bouzenna H and Krichen L. *Pelargonium graveolens* L'Her. and *Artemisia arborescens* L. essential oils: chemical composition, antifungal activity against *Rhizoctonia solani* and insecticidal activity against *Rhysopertha dominica*. Nat Prod Res. 2013; 27(9):841-6.
24. Slima AB, Ali MB, Barkallah M, Traore AI, Boudawara T, Allouche N, Gdoura R. Antioxidant properties of *Pelargonium graveolens* L'Her essential oil on the reproductive damage induced by deltamethrin in mice as compared to alpha-tocopherol. Lipids Health Dis. 2013;12(1):30.
25. Rani N, Sharma SK, Vasudeva N. Assessment of antiobesity potential of *Achyranthes aspera* Linn. seed. Evid Based Complement Alternat Med. 2012;2012: ID 715912, 7 pages.
26. Roh C, Jung U. Screening of crude plant extracts with anti-obesity activity. Int J Mol Sci. 2012;13(2):1710-9.
27. Sun X, Zhang K, Ji X, Wang Y, Jeffrey Z, Tong Y, Gao H, Zhang J, Wang Z. Screening of pancreatic lipase and alpha-glucosidase inhibitors from Chinese dietary herbs. Zhongguo Zhong Yao Za Zhi. 2012; 37(9):1319-23.
28. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 1969; 6:24-27.
29. Adams RP. Identification of essential oil components by Gas Chromatography /Mass Spectrometry. 2001. Illinois: Allured Publishing Corporation.
30. Hamdan II, Afifi FU. Studies on the *in vitro* and *in vivo* hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. J. Ethnopharmacol. 2004; 93(1):117-21.
31. Gurbuz I, Ustun O, Yesilada E, Sezik E, Kutsal O. Anti-ulcerogenic activity of some plants used as folk remedy in Turkey. J Ethnopharmacol. 2003; 88(1):93-7.
32. Habtemariam S. The antiobesity potential of sigmoidin A. Pharma Biol. 2012;50 (12):1519-22.
33. Al-Hallaq EK, Kasabri V, Abdalla SS, Bustanji YK, Afifi FU. Anti-obesity and antihyperglycemic effects of *Crataegus aronia* extracts: *In vitro* and *in vivo* evaluations. Food Nutrition Sci. 2013; 4(9):972-83.
34. Ben Hsouna A, Hamdi N. Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from *Pelargonium graveolens* growing in Tunisia. Lipids Health Dis. 2012;11:167-74.
35. Ghannadi A, Bagherinejad MR, Abedi D, Jalali M, Absalan B, Sadeghi N. Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L'Her and *Vitex agnus-castus* L. Iranian J Microbiol. 2012;4(4):171-6.

36. Rana VS, Juyal JP, Blazquez MA. Chemical constituents of essential oil of *Pelargonium graveolens* leaves. Int J Aromather. 2002;12(4): 216-8.

37. Inouye S, Takizawa T, Yamaguchi H: Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother. 2001;47(5):565-73.

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