

Chemical Characterization and Antidiabetic Activity of Essential Oils from *Pelargonium graveolens* Leaves

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Abstract—*Pelargonium graveolens* (Geranium) is a source of finest quality of fragrance and its essential oils (EOs) are used as antibacterial and antifungal agent. The aim of the current research is to determine chemical constituents in EO of *P. graveolens* by gas chromatography–mass spectrometry (GC–MS) and evaluate its antidiabetic activity through α -glucosidase inhibition assay. The chemical composition of *P. graveolens* EO was determined by GC/MS and its antidiabetic activity was assessed through inhibition of α -glucosidase enzyme in *in vitro* models. GC–MS analysis determines 36 chemical components in the EO of *P. graveolens* leaves, and citronellyl isovalerate (10.41%), menthol (9.61%), linalool (8.63%), *p*-menthone (6.31%), and geranyl tiglate (4.99%) were recorded as major constituents. The EO of *P. graveolens* leaves showed concentration-dependent inhibition of α -glucosidase enzyme ranging from 28.13 ± 1.41 to $74.24 \pm 2.53\%$ for concentration ranging from 31.25 to 1000 $\mu\text{g/mL}$, respectively. The IC_{50} values for of *P. graveolens* and acarbose were found as 93.72 ± 4.76 and 80.4 ± 2.17 $\mu\text{g/mL}$, respectively, against the α -glucosidase enzyme. The study finding explores the chemical components of *P. graveolens* growing in Iraqi Kurdistan region and scientifically supported its possible use in diabetic patients for controlling postprandial hyperglycemia.

Index Terms—*Pelargonium graveolens*, Geranium, Geraniaceae, Diabetes, Gas chromatography–mass spectrometry, α -Glucosidase enzyme.

I. INTRODUCTION

Medicinal plants have emerged as major source drugs for the treatment of chronic diseases such as cancer, diabetes, and cardiovascular complications. Natural products derived from medicinal plants are considered safe and effective compared

to synthetic modern drugs based on the long history of use by humans as food and medicine (Ahamad, et al., 2019). Diabetes is a chronic disorder of metabolism caused by an absolute or relative lack of insulin and characterized by hyperglycemia, glycosuria, and hyperlipidemia (Gin and Rigalleau, 2000). The number of people in the world with diabetes has increased dramatically over recent years. Postprandial hyperglycemia (PPHG) is one of the key risk factors associated with diabetes. PPHG results due to the fast uptake of glucose in the intestine under the influence of hydrolyzing enzymes α -amylase and α -glucosidase that change polysaccharides through oligosaccharides to monosaccharides (Subramanian, Asmawi and Sadikun, 2008). Inhibition of α -amylase and α -glucosidase enzymes leads to a reduction in their hydrolysis and thereby controlled blood glucose levels (Dong, Li, Zhu, Liu and Huang, 2012). Therefore, an important strategy for managing PPHG is to inhibit α -amylase and α -glucosidase enzymes (Scheen, 2003). The α -amylase and α -glucosidase enzyme inhibitors as therapeutic agents are being increasingly pursued. These enzymes are preferred targets for the management of PPHG particularly in pre-diabetics or those with impaired glucose tolerance (Ahamad, Naquvi, Mir and Ali, 2011). Natural products as α -amylase and α -glucosidase inhibitors have attracted considerable interest in the drug discovery endeavors (Ahamad, Hasan, Amin and Mir, et al., 2016; Ahamad, Alkefai, Amin and Mir, 2020; Alkefai, Ahamad, Amin and Mir, et al., 2018; Alkefai, Ahamad, Amin and Mir, 2018). The clinically used inhibitors of carbohydrate metabolizing enzymes such as acarbose, voglibose, and miglitol are non-specific in their action. This results in side effects such as flatulence and distension caused due to bacterial fermentation of undigested carbohydrates. Thus, the search for selective inhibitors of these enzymes is still on and more and more medicinal plants are being screened continuously. Screening of traditionally used plants for the drug discovery process is often advocated as the chances of success using this approach are more (Fabricant and Farnsworth, 2001).

Pelargonium genus belonging to the family Geraniaceae and contains about 250 species (such as *Pelargonium*

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graveolens, *Pelargonium capitatum*, *Pelargonium zonale*, *Pelargonium roseum*, and *Pelargonium odoratissimum*). *Pelargonium* is distributed throughout Mediterranean region but most profoundly present in South Africa (Miller, 2002). *P. graveolens* is a source of high-quality essential oil (EO) that is rich in citronellol and geraniol. *P. graveolens* EO is commonly known as geranium oil and is extensively used as perfumes and in skin care products. *P. graveolens* EO is also used in aromatherapy, and pharmacologically, it acts as antibacterial, antifungal, insect repellent, and has skin cleansing properties (Pattnaik, Subramanyam and Kole, 1996; Pattnaik, Subramanyam, Bapaji and Kole, 1997; Rao Rajeswara, Kaul, Mallavarapu and Ramesh, 1996; Lis-Balchin, Buchbauer, Ribisch and Wenger, 1998; Lis-Balchin, Steyrl and Krenn, 2003). In the present research work, an attempt has been made to determine the chemical composition of EO from leaves of *P. graveolens* by gas chromatography–mass spectrometry (GC–MS). The present study was also designed to evaluate its antidiabetic activity through inhibition of α -glucosidase enzyme.

II. MATERIALS AND METHODS

A. Plant Materials and Chemicals

The fresh leaves of *P. graveolens* (500 g) were collected in the month of March 2020 from local market of Erbil, Kurdistan Region, Iraq. The authenticity of all the accession was ascertained by Taxonomist. The plant sample was kept in the Faculty of Pharmacy, Tishk International University, Erbil, Iraq. For future reference (voucher number: PRL/2020/07), *p*-Nitrophenyl- α -D-glucopyranoside (PNPG), 3,5-dinitrosalicylic acid (DNS), acarbose, and α -glucosidase enzyme were brought from Subra Scientific Company, Chennai, India. All other solvents and chemicals used in the study were of analytical grade

B. Isolation of EO from Leaves of *P. graveolens*

The fresh *P. graveolens* leaves (500 g) were hydrodistilled for 6 h in Clevenger apparatus. After isolation of EO, the volatile oil was collected in the graduated tube and dried over anhydrous sodium sulfate and stored at 4°C in a refrigerator for further use.

C. GC–MS Analysis and Identification of Chemical Constituents

The chemical composition of *P. graveolens* EO was determined by the GC–MS method. Agilent Bench Top GC–MS (Agilent Technologies, Wilmington, DE, USA) equipment with a capillary column of DB-5 glass (specifications 30 m \times 2.5 mm i.d.; film thickness of 0.25 μ m) was applied for test sample analysis. Helium was used as carrier gas at flow rate of 1 mL/min. The oven temperature was set at 50°C for 1 min and then holds isothermally for 2 min at 320°C. The temperature of injector port was maintained at 250°C, and ion source temperature was kept at 200°C, and interface temperature was maintained at

300°C. The EO (1 μ L) was injected and the split ratio was kept at 1:5. Data capture took place at 70 eV using scanning times of 1.5 s in the mass range of 50–1000 amu and run time was kept up to 38 min. The chromatography and mass spectra were handled with ChemStation software (Agilent Technologies, Wilmington, DE, USA).

The individual peaks/constituents were identified by comparison of their Kovats index (K.I.) with those of literature in close agreement to K.I. Further identification of EO constituents was made by comparison of the fragmentation pattern of mass spectra obtained by GC/MS analysis with those stored in the spectrometer database of NIST, NBS 54 K.L, WILEY8 libraries, and published literature (Adams, 2007; Ali, 2001; Rana, et al., 2002; Bhattacharya, Kaul, Rao, Ramesh and Mallavarapu, 1993; Abd El-Kareem, Rabbih, Elansary and Al-Mana, 2020). The percent composition of the EO compounds was calculated. The qualitative analysis was based on the percent area of each peak of the sample compounds.

D. In vitro α -Glucosidase Inhibitory Activity

Antidiabetic activity of EO of *P. graveolens* was assessed through α -glucosidase inhibition assay. The assay method briefly summarized as 60 μ L of EO of *P. graveolens* in DMSO with concentrations ranging from 31.25 to 1000 μ g/mL and 0.1 M phosphate buffer (50 mL; pH 6.8) containing α -glucosidase solution (0.2 U/mL) was incubated at 37°C for 20 min in 96-well plates. PNPG (50 mL; 5 mM) solution in a 0.1 M phosphate buffer (pH 6.8) was applied to each well after pre-incubation and further incubated for 20 min at 37°C. Then, the reaction was stopped by adding 160 mL of 0.2 M NaCO₃ into each well. Absorbance (A) was reported at 405 nm with microplate reader and compared to a control which had 60 μ L of buffer solution in place of the test sample. Acarbose was used as a standard drug and evaluated same way as the test sample (Ahamad, Alkefai, Amin and Mir, 2020). The % inhibition of α -glucosidase enzyme was calculated using the following formula:

$$\% \text{Inhibition} = \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \times 100$$

where, Abs is absorbance of the control and absorbance of the sample

III. RESULTS AND DISCUSSION

A. GC–MS Analysis of EO of *P. graveolens*

The EO from leaves of *P. graveolens* was isolated by the hydrodistillation method. The hydrodistillation yielded 0.45% of EO from leaves of *P. graveolens*. The chemical composition of EO of *P. graveolens* was determined by the GC–MS method and results are presented in Table I and Fig. 1. Thirty-six components representing 99.17% of the total detected constituent were identified. The major constituents in the EO of *P. graveolens* include

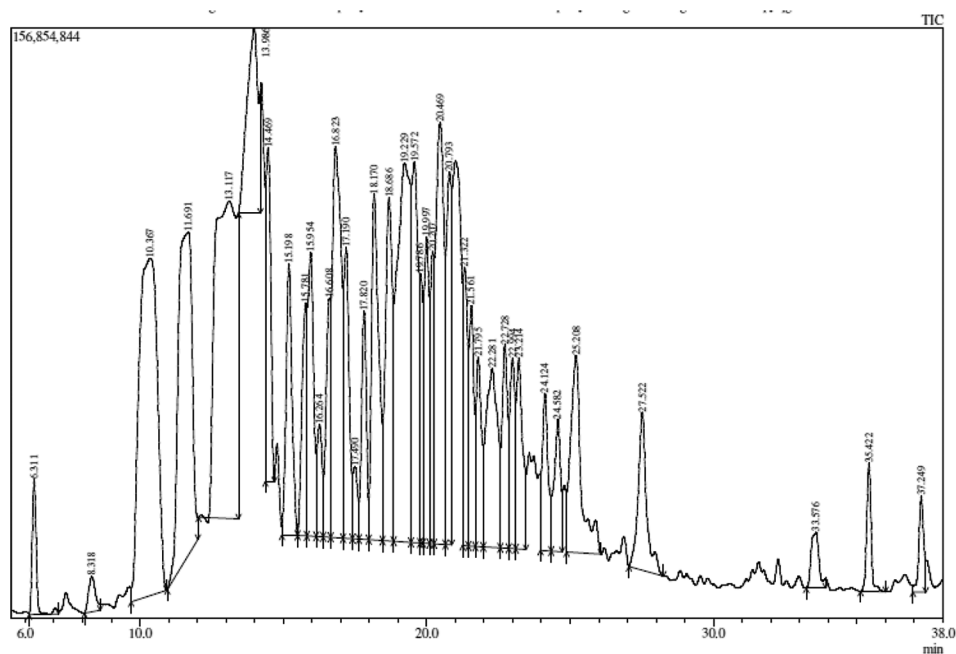


Fig. 1. Gas chromatography–mass spectrometry chromatogram of essential oil of *Pelargonium graveolens*.

citronellyl isovalerate (10.41%), menthol (9.61%), linalool (8.63%), *p*-menthone (6.31%), geranyl tiglate (4.99%), neryl propionate (4.79%), isocaryophyllene (3.77%), and caryophyllene epoxide (3.63%). The other components of *P. graveolens* EOs are geranyl butyrate (3.50%), neryl hexanoate (2.98%), geranyl heptanoate (2.93%), heptaminol (2.92%), citronellyl hexanoate (2.64%), neryl acetate (2.32%), myrtanol (2.23%), citronellol (2.22%), and nerolidyl acetate (2.18%). Other minor chemical components (<2%) of EO of *P. graveolens* are also presented in Table I. Rana, et al. studied the EO composition of *P. graveolens* leaves from Uttarakhand, India. The results show that citronellol (33.6%), geraniol (26.8%), linalool (10.5%), citronellyl formate (9.7%), and *p*-menthone (6.0%) were major chemical components in EO (Rana, et al., 2002). Bhattacharya, et al. (1993) studied volatile oil components of rose-scented *Geranium* and found that citronellol (25.44%), geraniol (21.87%), and linalool (7.87%) as major chemical compounds. In another study, EO components of *P. graveolens* from Egypt were studied by GC–MS. The study also shows citronellol (27.67%), *cis*-Menthone (10.23%), linalool (10.05%), eudesmol (9.40%), geraniol formate 6.87%, and rose oxide (5.77%) as major constituents (Abd El-Kareem, Rabbih, Elansary and Al-Mana, 2020). The present research is the first study on EO composition of *Geranium* growing in Iraqi Kurdistan. The study shows the presence of derivatives of citronellyl and geraniol, the major components already reported by other researchers such as citronellyl isovalerate (10.41%) and geranyl tiglate (4.99%). The present study finding is also comparable with the previous findings as study shows the presence of menthol (9.61%), linalool (8.63%), and *p*-menthone

(6.31%) as other major components. The variation in the chemical composition of *Geranium* is maybe due to variations in geographical location, temperature, rainfall, soil, etc. (Ahmad, Uthirapathy, Ameen and Anwer, 2019).

B. α -Glucosidase Inhibition Activity of EO of *P. graveolens*

The EO of *P. graveolens* showed concentration-dependent α -glucosidase enzyme inhibition that varies from 28.13 ± 1.41 to $74.24 \pm 2.53\%$ for concentration ranging from 31.25 to 1000 $\mu\text{g/mL}$ (Table II and Fig. 2). Acarbose was used as positive standard and it also showed concentration-dependent inhibition of α -glucosidase enzyme inhibition ranging from 31.94 ± 2.45 to $81.26 \pm 3.21\%$ for the same concentration as test sample above. The IC_{50} values for *P. graveolens* and acarbose were found as 93.72 ± 4.76 and $80.4 \pm 2.17 \mu\text{g/mL}$, respectively, against α -glucosidase enzyme.

The results of enzyme inhibition assay indicate that the EO of *P. graveolens* inhibits α -glucosidase enzyme in *in vitro* study. The results also show that the EO of *P. graveolens* was found comparable with standard acarbose in inhibition of α -glucosidase enzyme. α -Amylase and α -glucosidase enzymes are present in brush border of gastrointestinal tract and responsible for breakdown of polysaccharides and disaccharides, respectively. Inhibition of these enzymes leads reduction of monosaccharides available for absorption in blood and ultimately it controls sudden rise of blood glucose level after meals. The increased blood sugar level after a meal is known as PPHG and controlling it by inhibiting α -glucosidase enzyme is a new strategy in management of type 2 diabetes. The inhibitors of α -amylase and α -glucosidase enzymes such as acarbose and miglitol are non-specific in their action, the strong inhibition of both enzymes leads decreased metabolism of polysaccharides which causes

TABLE I
CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *P. GRAVEOLENS*

Chemical constituents	RT	KI	Concentration (%)
<i>p</i> -Cymene	6.311	1026	0.78
Limonene	8.318	1031	0.34
Linalool	10.367	1097	8.63
<i>p</i> -Menthone	11.691	1153	6.31
Menthol	13.117	1173	9.61
Heptaminol	13.986	1188	2.92
Citronellol	14.469	1226	2.22
Myrtanol	15.198	1259	2.23
Citronellyl acetate	15.781	1353	1.57
Neryl acetate	15.954	1365	2.32
α -Copaene	16.264	1376	0.79
Isocaryophyllene	16.608	1413	3.77
Neryl propionate	17.190	1428	4.79
Epoxy cholesterol	17.490	-	0.48
α -Himachalene	17.820	1447	1.57
Geranyl butyrate	18.170	1562	3.50
Citronellyl isovalerate	19.229	1563	10.41
Caryophyllene epoxide	19.572	1581	3.63
Epicubenol	19.786	1613	0.99
Nerolidyl acetate	19.997	1632	2.18
Cubenol	20.207	1641	1.44
Geranyl tiglate	20.469	1700	4.99
Citronellyl hexanoate	20.793	1702	2.64
Citronellyl cinnamate	21.322	-	1.78
Geranyl hexanoate	21.561	1725	1.65
Kauren-19-yl-acetate	21.795	-	1.43
Geranyl heptanoate	22.281	1824	2.93
1-Hexadecanol	22.728	1868	1.58
3,7-Dimethyloct-6-en-1-yl heptanoate	22.994	-	1.40
2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate	23.214	1831	1.68
Citronellyl valerate	24.124	1880	1.31
Hexadecanoic acid	24.582	1972	1.18
Neryl hexanoate	25.208	2031	2.98
Methyl linolenate	27.522	2098	1.87
2-Methylhexacosane	33.576	2663	0.61
Tetrapentacontane	35.422	-	0.66

Where, RT: Retention time, and KI: Kovats index

TABLE II
A-GLUCOSIDASE ENZYME INHIBITORY ACTIVITY OF EO FROM LEAF OF
P. GRAVEOLENS

Conc. (μ g/mL)	Acarbose	<i>P. graveolens</i>
31.25	31.94 \pm 2.45	28.13 \pm 1.41
62.5	45.44 \pm 2.63	41.72 \pm 0.93
125	57.23 \pm 3.31	55.17 \pm 3.46
250	63.82 \pm 2.47	59.03 \pm 2.45
500	76.81 \pm 2.96	63.28 \pm 2.45
1000	81.26 \pm 3.21	74.24 \pm 2.53
IC ₅₀ values	80.4 \pm 2.17	93.72 \pm 4.76

Data were presented as mean of % inhibition in triplicate determinations \pm SD

flatulence and distension as side effects due to bacterial fermentation from undigested carbohydrates (Subramanian, Asmawi and Sadikun, 2008; Dong, Li, Zhu, Liu and Huang, 2012). The present study finding supports the potential antidiabetic effects of *P. graveolens* EO in controlling PPHG through inhibition of α -glucosidase enzyme.

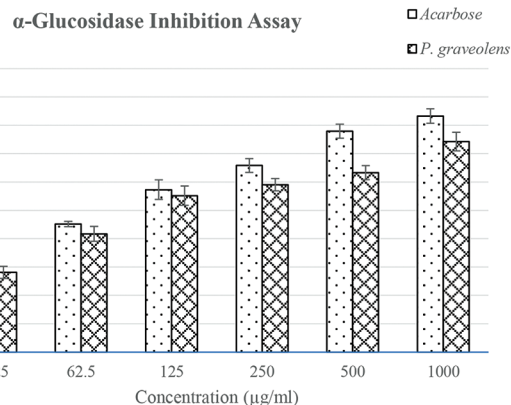


Fig. 2. α -Glucosidase enzyme inhibitory activity of essential oils from the leaf of *Pelargonium graveolens*.

IV. CONCLUSION

GC-MS analysis of Kurdish *P. graveolens* EO yielded citronellyl isovalerate, menthol, linalool, *p*-menthone, and geranyl tiglate as major constituents. *P. graveolens* EO also found potent inhibitors of α -glucosidase enzyme. The current research is first reports on the chemical composition of *P. graveolens* growing in Iraqi Kurdistan region. The findings of the present study also provide a scientific basis for its use in diabetic patients for controlling PPHG.

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