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Chemical composition and antimicrobial activity of *Pelargonium roseum* essential oil from Southwest of Iran

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ABSTRACT

In this work the chemical composition and antimicrobial activity of *Pelargonium roseum* leaves essential oil that grown in southwest of Iran were determined. The hydrodistilled volatile components were identified by GC/MS analysis. A total of 33 components have been identified in the oil, representing 99.38% of the total oil and citronellol (44.63%), citronellyl formate (14.22%), isomenthon (6.34%), geraniol (5.30%) and caryophyllene (3.55%) were the main components, respectively. Antimicrobial property of essential oil was investigated by disc diffusion method against three Gram positive (*Staphylococcus aureus*, *Bacillus ceruse*, *Streptococcus faecium*) and four Gram negative (*Escherichia coli*, *Salmonella typhi*, *Shigella dysenteria*, *Pseudomonas aeruginosa*) bacteria and a yeast (*Sacharomyces cervisiae*) in comparable with the standard antibiotics. The essential oil of *P. roseum* showed inhibitory activity against all the bacteria and yeast tested to varying degree. *B. cereus* and *S. faecium* were found as the most sensitive and resistant bacteria, respectively. According to these results, it can be concluded that the essential oil of *P. roseum* has suitable spectrum antimicrobial activity and can be used as natural preservatives in food industry.

Keywords: *Pelargonium roseum*, essential oil, antimicrobial, GC-MS

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1. Introduction

Growth of microorganisms in food stuff cause spoilage and reduction of food quality (Celiktas et al., 2007). It has been estimated, every year in industrialized countries as many as 30% of people suffer from food borne diseases and according to world health organization, in 2000, at least two millions of people around the world died from diarrhea disease (WHO, 2010). Increasing consumer demand on organic foods from one side and growing up on cases of microbial resistance to existing antibiotics on the other hand because rising up attempts to seek new natural and safe substances with antimicrobial property to eliminate synthetic preservatives (Bakkali et al., 2008; Owlia et al., 2010).Essential oils are natural, volatile and complex components of secondary metabolism of aromatic plants which are concentrated in special organs of plant like flowers, leaves, rhizomes, root, stems, seed, barks or fruits for protection against bacteria, viruses, fungi and pests (Bakkali et al., 2008; Rota et al., 2008). Essential oils derived from aromatic plants have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant activities and they are used in different industries (Bakkali et al., 2008; Burt, 2004). Plants essential oils are rich sources of biologically active compounds which are important both for food conservation and the control of human and plant diseases that are of microbial origin (Brul & Coote 1999; Owlia et al., 2010; Rusenova & Parvanov, 2009). *Pelargonium roseum*, commonly known as geranium, belongs to Geraniaceae family and is indigenous to South Africa. This aromatic plant is extensively cultivated in Reunion Island, Madagascar, Egypt, China, India, Australia, Portugal, and recently in Iran for their beauty and scents (Dabiri et al., 2011; Ghannadi et al., 2012; Gomes et al., 2004; Southwell & Stiff, 1995). Geranium essential oil is one of the most important materials in the perfumery, cosmetic, pharmaceutical and food industries and often used as a substitute for rose essential oil, (Gomes et al., 2004; Verma et al., 2010). *Pelargonium* aerial parts are used in folk medicine of Iran as a food and tea drinks additive and for relieving of some disorders (Ghannadi et al., 2012).Although the chemical composition of the volatile oils of this herb is well studied and volatile components of *Pelargonium* are responsible for its biological activities (Elmann et al., 2010), but to the best of our knowledge, no work has so far been conducted on *P. roseum* which

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grown in southwest of Iran. The aim of this study was to evaluate the chemical component and antimicrobial activity of essential oil from the leaves of *P. roseum* collected in the southwest of Iran.

2. Material and Methods

P. roseum were collected at flowering phase from the greenhouses in Khouzestan province in southwest of Iran in May 2013. Voucher specimen of this plant was deposited in Herbarium of the faculty of Agriculture at Ramin Agriculture and Natural Resources University.

2.1. Essential oil extraction

The leaves of *P. roseum* dried at room temperature for five days and ground to powder before subjected to hydro distillation using a clevenger-type apparatus for four hours. Then the oils collected were dried over anhydrous sodium sulphate and stored at 4℃ before experiments.

2.2. GC-MS analysis

The chemical analyses of the essential oils were performed using Agilent Technologies GC-6890A and GC-MSD5975 with quadruple detector operating at 70 eV in electron ionization mode. Analyses were carried out using helium as the carrier gas at a flow rate of 1 mL/min in a split ratio of 1:20 on HP-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Oven temperature programmed to 60ºC for 1 min, then raised to 250ºC at rate of 5ºC/min, with a final hold for 1 min. Injector and detector temperatures were 240ºC and 300ºC, respectively and 0.5 µL of the extracts were injected. All experiments were conducted in duplicate.

The compounds of essential oils were identified according to GC-MS retention times (authentic chemicals), Kovats indices (KI) in reference to n-alkanes (C_8–C_{24}), and mass spectra (authentic chemicals and NIST05 spectral library collection) (NIST 2011). Identification was considered tentative when it was based on mass spectral data only. The essential oil components were quantified using percentage peak area calculations by means of a gas chromatograph, Agilent 6890A, with a flame ionization detector (GC-FID). The column and chromatographic conditions were the same as previously described for the GC-MS analysis. The injector temperature was 250ºC and helium was used as the carrier gas (1 mL/min).

2.3. Microbial strain

Three Gram positive (*Staphylococcus aureus* ATCC 25923, Bacillus ceruse PTCC 1154, *Streptococcus faecium* ATCC 10541), four Gram negative (*Escherichia coli* ATTC 25992, *Salmonella typhi* PTCC 1609, *Shigella dysenteria* PTTC 188, *Pseudomonas aeruginosa* ATTC 85327) bacterial species and one yeast (*Sacharomyces cervisiae* ATTC 9763) used in this study were obtained from the Institute culture collection of Iranian Research Organization for Science and Technology. Microbial strains were kept on beads at -20°C. Prior to use, they were grown freshly on tryptone soya blood broth (Merck, Darmstate, Germany) at 37 ºC for 24 h. Also, *S. cervisiae* was cultured in yeast extract glucose chloramphenicol agar (YGCA) (Sigma-Aldrich, Minnesota, USA) at 28 ºC for 5 days.

2.4. Antimicrobial screening

Disc diffusion method was carried out to determine antimicrobial activity of the essential oil according to Ghanadi et al. (2012) and Owlia et al. (2010) with some modification. Briefly, one hundred microliter broth culture of each microorganism containing approximately 108 cfu/mL of the Mc Farland scale was spread over the surface of sterile solid Mueller-Hinton agar and YGCA plates using a sterile cotton swab in order to get a uniform microbial growth for bacteria and yeast, respectively. Essential oil was sterilized by passing through 0.22 mm pore size membrane filters and then 10, 20 and 20 µL of the essential oil were pipetted into the center of each empty sterilized blank discs (6.4 mm diameter, Padtan, Iran).

Sterile forceps were used to place the antibiotic-impregnated discs onto the plates. The discs were then tapped lightly with the forceps to insure better contact with the agar. The plates were left for 30 min at room temperature to allow the diffusion of oil and then they were incubated at 37°C for 24 h and 28°C for 5 days for bacteria and yeast, respectively. In this study, chloramphenicol (30 µL/disc) and nystatin (30 µL/disc) were used as antibacterial and antifungal positive control, respectively. After overnight incubation, the diameter of the zone of inhibition around each disc was measured in cm. All the experiments were performed in aseptic conditions and the tests were carried out in triplicate. The mean value of each test was calculated and the results were expressed as mean±SD.

3. Results and Discussion

3.1. GC-MS analysis

Chemical composition of Essential oil the oil yield obtained from the hydro-distillation of the *P. roseum* leave was 0.19 % (w/w) on a dry weight basis. The oil was characterized by a pale-greenish color and refreshing and pleasant odor. The essential oil yield (0.19%) in the present study was comparable to reports by Dabiri et al. (2011), who found the yield of the essential oils from the *P. roseum* leaves by steam distillation to be 0.2%. Gomes et al. (2004) reported the yield of the *P. roseum* leaves essential oils from plants grown in Portugal to be 0.1-0.2 %.

The components, retention time, kovats indices and their percentages of hydro distilled essential oil by GC-MS are shown in Table 1. Thirty-three compounds were tentatively identified and quantified in the leaves of *P. roseum* oil, representing 99.38 % of the total volatile compounds extracted. The oil was found rich in oxygenated monoterpenes type constituents (76.23%), followed by sesquiterpene hydrocarbons (10.18%), monoterpene hydrocarbons (8.39%), and oxygenated sesquiterpenes (1.06%). As shown in Table 1, the main compositions of the essential oil were beta citronellol (44.63%), citronellyl format (14.22%), Isomenthon (6.34%), Geraniol (5.30%) and Caryophyllene (3.55%).

Table 1. Volatile compound of essential oil of *pelargonium roseum* leaves from southwest of Iran.

Pe	Compound	RTa (min)	KIb	%c
1	alpha pinene	8.342	938	1.01
2	p-cymene	11.375	1024	0.13
3	Limonene	11.497	1028	0.33
4	1,8 cineole	11.708	1034	0.36
5	Linalool	13.885	1099	2.15
6	cis rose oxide	14.252	1111	2.78
7	trans rose oxide	14.758	1128	0.99
8	Menthone	15.563	1158	0.91
9	isomenthone	15.896	1163	6.34
10	p- menthanol	16.43	1173	0.29
11	alpha terpineol	16.685	1190	0.23
12	beta citronellol	17.829	1233	44.63
13	Pulegone	18.152	1243	0.39
14	Carvone	18.263	1244	0.20
15	Geraniol	18.529	1252	5.30
16	citronellyl formate	19.074	1281	14.22
17	geranyl formate	19.807	1304	1.4
18	alpha cubebene	21.073	1344	0.16
19	citronellyl acetate	21.173	1356	0.65
20	alpha copaene	21.784	1379	0.49
21	beta bourbonene	22.029	1392	1.33
22	Caryophyllene	22.49	1416	3.55
23	citronellyl propanoate	23.484	1445	0.98
24	alpha humulene	23.806	1454	1.17
25	aromadenderene	23.984	1459	0.16
26	germacrene D	24.484	1479	1.95
27	bicyclogermacrene	24.873	1494	0.33
28	delta cadinene	25.495	1519	1.04
29	citronellyl butanoate	25.562	1532	1.33
30	caryophyllene oxide	26.339	1581	0.85
31	Spathulenol	26.85	1600	0.20
32	phenyl ethyl Tiglate	26.995	1603	2.67
33	Geranyl tiglate	29.539	1705	0.85
	Monoterpene hydrocarbons			8.39
	Oxygen-containing monoterpenes			76.23
	Sesquiterpene hydrocarbons			10.18
	Oxygen-containing sesquiterpenes			1.06
	Other			3.52
	Total			99.38

[a]Retention Time; [b]Kovats Indices calculated for HP-5MS column and literature values were obtained from NIST05; [c]Relative percentage obtained from peak area.

The chemical composition of the essential oil of *P. roseum* described in this study agreed well with those previously reported in the literature; however, there were differences in relative quantities of volatile compounds. Citronellol was also found to be the main component of the geranium essential oil, similar to those reported in other studies from Iran, Portugal, Australia, and India (Dabiri et al., 2011; Gomes et al., 2004; Southwell & Stiff 1995; Verma et al., 2010).

Also, comparing our results with a sample from northern Iran showed the amount of citronellol and citronellyl from the Southwest of Iran were more than North, while the amount of carophyllene, delta cadinene and carophyllene oxide were less (Dabiri et al., 2011).

3.2. Antimicrobial activity

In this study disc diffusion method was applied to determine in vitro antimicrobial property of the essential oil against eight microorganisms in three levels of 10, 15 and 20 µL/disc and compared with standard antibiotics. The obtained data were summarized in Table 2. The results indicated that all tested microbial strains were susceptible against *P. roseum* essential oil. It inhibited the growth of the tested microorganisms by producing a zone of inhibition from 1.03 to 3.66 cm in diameter.

Data on the antibacterial activity of the geranium essential oil indicated *B. cereus* and *S. faecium* were the most susceptible and resistant bacterial strain in all levels of essential oil, respectively.

This result disagree with previous study which introduced S. aureus (4.2 ± 0.32 cm) as the most sensitive bacterium against neat essential oil of P. geraveulenc LHer (Ghannadi et al., 2010). The difference in plant species, bacterial strain, amount of the components and specially the variety in quantity and quality of the main composition of essential oils can be responsible for their different antimicrobial activity.

On the basis of the results given in Table 2, it is concluded that level of 20 µL had the strongest inhibition zone for all tested bacteria, also, by increasing in amount of essential oil from 10 to 15 µL/disc, the diameter of inhibition zones were decreased (the diminution is not significant for most of the tested bacteria) with the exception of *S. typhi* and *S. cervisiae* that their inhibition zones were raised up from 10 to 15 µL/disc which can be seen in Table 2.

Decreasing size of the inhibition zones might be caused by the ability of some bacteria to breakdown monoterpens, because some microorganisms have potential to degrade terpenoid compounds, therefore they can use them as sole carbon source and live on them (Del Rosario et al., 1992; Tozoni et al., 2010).

Table 2. Antimicrobial activity of the *pelargonium roseum* leaves essential oils and standard antibiotics against tested microorganisms using disc diffusion method (zone of inhibition in cm)[a,b]

microorganisms	E	Essential oil amount (µL/disc)		Standard antibiotics (30 µL/disc)	
	10	15	20	chloromphenicol[1]	nystatin[2]
B. cereus	3.33^{bA} ±0.31*	2.96^{cA} ± 0.31	3.66^{aA} ±0.31	3^{c}±0.11	-
S. aureus	2.56^{bBC} ±0.52	2.20^{bB} ± 0.52	3.30^{aAB} ±0.52	2.5^{b}±0.23	-
S. faecium	1.20^{bD} ± 0.48	1.03^{bF} ± 0.48	1.73^{bD} ± 0.48	3.5^{a}±0.29	-
E. coli	1.56^{cD} ± 0.82	1.10^{cEF} ± 0.82	2.80^{bBC} ±0.82	3.5^{a}±0.33	-
S. typhi	2.33^{bC} ± 0.58	2.80^{abA} ± 0.58	3.43^{aAB} ±0.58	3^{ab}±0.23	-
S. dysenteria	2.86^{bB} ± 0.75	1.76^{cC} ± 0.75	3.43^{aAB} ± 0.75	3^{b}±0.33	-
P. aeruginosa	1.63^{cD} ± 0.43	1.40^{cDE} ±0.43	2.26^{bCD} ±0.43	3.5^{a}±0.26	-
S. cervisiae	1.23^{cD} ± 0.32	1.60^{bCD} ±0.32	1.93^{aD} ± 0.32	-	4.15^{a}±0.21

[a]values represent mean ± standard deviation of three replicates

[b]values followed by the same small and caps letter at the same row and column, are not significantly diferent ($p < 0.05$).

But increasing in amount of essential oil from 15 to 20 µL/disc increased the inhibition zones in all of the cases. It can be explained by toxin effects of higher concentration of monoterpens which are known as antimicrobial compounds (Tozoni et al., 2010). Skočibušić et al. (2006) also reported that by increasing in amount of essential oil from 10 to 20 µL/disc diameter of inhibition zones were increased (Skočibušić et al., 2006).

The essential oil in level of 20 µL/disc was more effective than the reference antimicrobial substance positive control for four bacterial strains. It is noticed that the antifungal activity of essential oil was in average more than two times lower compared with nystatin.

It has been introduced oxygenated monoterpens as compounds with high antibacterial activity (Kotan et al., 2007), so the higher oxygenated monoterpenic content of studied essential oil might have contributed to its antimicrobial activity.

Although the major component of essential oils determine their biological property, the minor materials play an important role in this activity (Burt, 2004). On the other hand, synergistic of trace materials of essential oils such as linalool, alpha pinene, p-cymene, and 1,8-cineol have an undeniable role in their biological activity (Cosentino et al., 1999).

The mechanism of essential oil revenue is not completely found, but some papers reported that the hydrophobicity of essential oils enables them to partition the lipids of the cytoplasmic membrane and mitochondria, rendering cell membranes permeable and leading to leakage of cell contents (Burt, 2004; Knobloch et al., 1989; Sikkema et al., 1994).

4. Conclusion

In conclusion, our study showed the essential oil of *P. roseum* has antimicrobial activity which can be useful in the treatment of various infectious diseases caused by bacteria and fungi. Nevertheless, further studies are required to understand the action mechanisms against microorganisms, and acquire more information on the safety, efficacy and toxicity of this essential oil for use as antimicrobial agents and natural preservatives in different products such as food.

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