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Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil

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ABSTRACT

The present study describes the chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. The essential oil profile was determined by GC and GC-MS. The main compounds were citronellol (24.54%), geraniol (15.33%), citronellyl formate (10.66%) and linalool (9.80%). Minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) were recorded using the microdilution and macrodilution methods. Commercial antimycotic bifonazol was used as a control. The concentration of 0.25-2.5 mg/ml showed fungicidal activity. The most resistant fungi were *Mucor mucedo* and *Aspergillus* species. The antioxidant activity of pure essential oil was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical assay. The essential oil of *P. graveolens* was able to reduce DPPH radicals into the natural DPPH-H form, and this activity was dose-dependent. The oil exhibited antioxidant activity and reduced DPPH to 50% at EC_{50} value of 0.802 mg/ml of oil solution.

INTRODUCTION

Pelargonium graveolens L' Herit is an aromatic and hairy herbaceous shrub, up to 1 m high. Leaves are prickly and carved, flowers are small, usually pink. *P. graveolens* (geranium) is native to South Africa (Comoros Islands) and it is widely cultivated in Russia, Egypt, Algeria, Morocco, Congo, Japan, Central America and Europe (Spain, Italy, France). There are three main regions for the production of *P. graveolens* oil: Reunion, Egypt, Russia (Lawless, 2001). Essential oil of *P. graveolens* is used as a fragrant component in perfumery, food and beverages industry, also as antidepressant and antiseptic remedy. It has an astringent and chemostatic effect, also it regulates the bloodstream, stimulates the adrenal glands and lymphatic system which in combination with diuretic properties makes this essential oil excellent in the fight against cellulite and fluid retention in the body. Due to the antiseptic effect it is used for the hygiene of the oral cavity and for treatment of various skin problems (Lavabre, 1998; Lawless, 2001). Lis-Balchin *et al* (1996, 2003) studied the biological activity of few commercial geranium oil samples. They have been tested for their possible application in the food industry.

According to available data on chemical composition of *P. graveolens* essential oil, dominant volatiles were citronellol, geraniol and citronellyl formate (Jirovetz *et al*., 2006; Verma *et al*., 2010; Ghannadi *et al*., 2012). Through several studies it was shown that essential oil and extracts of *Pelargonium graveolens* possess antibacterial and antifungal activity (Baratta *et al*., 1998; Dorman and Deans, 2000). Antimicrobial and antimalarial activity of *P. graveolens* extracts can be attributed to significant cytotoxic effect which this extracts provided and probably flavonoid derivatives have positive contribution to this biological activity (Lalli, 2005). Antioxidant and antitermitic activity of *P. graveolens* has been reported as well (Zheng and Wang, 2001; Fayed, 2009; Seo *et al*., 2009; Čavar and Maksimović, 2012).

The purpose of this study was to determinate chemical composition of *P. graveolens* essential oil and to evaluate its antifungal and antioxidant activity due to its commercial usage.

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MATERIAL AND METHODS

Essential oil sample

The sample of *Pelargonium graveolens* essential oil was obtained from the Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade. The referent oil was provided from Haarmann & Reimer, Holzminden, Germany.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

Qualitative and quantitative analyses of the essential oil were performed using GC and GC-MS. The GC analysis of the oil was carried out on a GC HP-5890 II apparatus, equipped with split-splitless injector, attached to HP-5 column (25 m x 0.32 mm, 0.52 µm film thickness) and fitted to FID. Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250 °C, detector temperature 300 °C, while column temperature was linearly programmed from 40-240 °C (at rate of 4°/min). The same analytical conditions were employed for GC-MS analysis, where HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used. Transfer line was heated at 260 °C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-400. Identification of the individual oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with MS libraries (NIST and Wiley 275.l) using a computer search and literature (Adams, 2007). For the purpose of quantitative analysis, area percents obtained by FID were used as base.

Tested fungal strains

The fungi used in this study were: *Alternaria alternata* (ATCC 13963), *Aspergillus flavus* (ATCC 9170), *A. niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *A. terreus* (ATCC 16792), *A. versicolor* (ATCC 11730), *Aureobasidium pullulans* (ATCC 9348), *Candida albicans* (clinical isolate), *Cladosporium cladosporioides* (ATCC 13276), *C. fulvum* (TK 5318), *Fusarium sporotrichoides* (ITM 496), *F. tricinctum* (CBS 514478), *Mucor mucedo* (SBR 2000), *Penicillium funiculosum* (ATCC 10509), *P. ochrochloron* (ATCC 9112), *Phoma macdonaldii* (CBS 38167), *Phomopsis helianthi* (ATCC 201540), *Trichoderma viride* (IAM 5061) and *Trichophyton menthagrophytes* (human isolate). The moulds were obtained from culture collection of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Researches "Siniša Stanković", Belgrade. The fungi were maintained on malt agar (MA) and Sabouraud dextrose agar (SDA) (Booth, 1971).

Microdilution method

Modified microdilution technique was used to investigate the antifungal activity of essential oil (Hanel and Raether, 1988; Daouk *et al*., 1995). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). Spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^6 in a final volume of 100 µl per well. The inocula were stored at 4 °C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum. The minimal inhibitory concentration (MIC) determination was performed by a serial dilution technique using 96-well microtitre plates. The investigated EO was dissolved in malt broth with fungal inoculum in a concentration of 0.25-5.00 mg/ml. The lowest concentrations without visible growth (under a binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 µl of tested essential oil dissolved in medium and inoculated for 72 h, into microtiter plates containing 100 µl of broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating a 99.5% killing of the original inoculum. DMSO was used as a negative control, and commercial fungicide, bifonazole (Srbolek, Belgrade, Serbia) was used as a positive control (0.1–0.25 mg/ml). The test was done in triplicate and repeated two times.

Macrodilution method

In order to investigate antifungal activity of *P. graveolens* essential oil, the modified mycelial growth method was used **(**Ishii, 1995). Tested micromycetes were grown on malt agar (MA) in Petri dishes at room temperature for 21 day and after that the inoculation of fungi was done (inoculum density was 10^6 spores per ml) (Booth, 1971). Different concentrations of *P. graveolens* essential oil were diluted in molten malt agar and poured into Petri dishes. The minimal inhibitory concentrations (MICs) of tested essential oil were determined (concentrations of geranium oil which achieve the complete inhibition of the mycelial growth of fungi). Minimal fungicidal concentrations (MFCs) were determined by monitoring the growth zones of re-inoculated mycelial peripheral parts in pure medium (concentrations of geranium oil at which the re-growth of the inoculum did not occur were taken as MFCs). The tested fungi were inoculated at the centre of the plates. Plates were incubated for three weeks at room temperature and after this period MICs were determined. The test was done in triplicate and repeated two times. Commercial fungicide bifonazole was used as control and the following concentrations of this synthetic antimicotic were prepared: 10, 15, 20 µl/ml, representing 0.1, 0.15 and 0.2 mg/ml of active substance in solution.

Antioxidant activity

The antioxidant activity of EO was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method. This spectrophotometric assay uses stable DPPH radical as reagent (Blos, 1958). The methanol solution of the investigated EO (200 µl) (with starting concentrations of 200, 400, 600, 800 µl/ml of solution) was added to an 1800 µl methanol solution of DPPH radical (concentration of 0.04 mg/ml) and after shaking, the reaction mixture was left to react in the dark for 30 minutes at room temperature. The absorbance of the remaining DPPH radical was measured at 517 nm after that time (A_1) on JENWAY 6305

UV-VIS spectrophotometer. Every concentration was done in triplicate and the same was done with Trolox and BHT, known commercial antioxidants. The same procedure was used for extracts. Blank probes were done in the same way, using methanol instead of the investigated solution (A_0). The decrease in the absorption of DPPH solution is calculated by the following equation:

$$I\ (\%) = (A_0\text{-}A_1) \times 100/A_0$$

Concentrations which reduce the absorption of DPPH solution by 50% (EC_{50}) were obtained from the curve dependence of absorption of DPPH solution on 517 nm from the concentration for essential oil and standard antioxidant. Microsoft Excel was used to calculate these values. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

The results of chemical analysis of *Pelargonium graveolens* essential oil are presented in **Table 1**. It can be seen that tested oil contain a significant amount of citronellol (24.54%), geraniol (15.33%), citronellyl formate (10.66%) and linalool (9.80%). Also, in noticeable amounts were present 6,9-guaiadiene, geranyl formate, menthone and isomenthone. Other constituents of tested oil were present in small quantities (less than 1%). In total, 55 compounds were identified representing 99.32% of the total oil weight. Oxygenated monoterpenes were the most dominant group of oil constituents representing 59.74% of the total oil **(Table 1)**, while monoterpene hydrocarbons were present with two compounds representing only 0.49% of the oil yield**.**

Table. 1: Chemical composition of *Pelargonium graveolens* essential oil.

Constituents	KIE	KIL	%
α-Pinene	924.5	932	0.41
2,2,6-Trimethyl-6-vinyltetrahydropyran	963.9	972	0.03
Limonene	1022.0	1024	0.08
cis-Linalool oxide (furanoid)	1067.0	1067	0.09
trans-Linalool oxide (furanoid)	1082.7	1088	0.04
Linalool	1094.0	1095	9.80
cis-Rose oxide	1103.8	1106	1.17
trans-Rose oxide	1120.3	1122	0.37
Menthone	1145.0	1148	4.33
Isomenthone	1155.5	1158	2.86
Menthol	1176.2	1167	0.08
α-Terpineol	1185.6	1186	0.36
Citronellol	1224.2	1223	**24.54**
Neral	1236.5	1235	0.68
Geraniol	1249.9	1249	**15.33**
Geranial	1266.3	1264	0.06
Citronellyl formate	1268.6	1271	10.66
Neryl formate	1274.5	1280	0.41
Geranyl formate	1295.1	1298	5.61
α-Cubebene	1339.6	1345	0.07
Citronellyl acetate	1348.6	1350	0.28
α-Copaene	1365.1	1374	0.46
β-Bourbonene	1373.8	1387	1.38
β-Elemene	1382.3	1389	0.25
β-Caryophyllene	1407.5	1417	1.33
trans-α-Bergamotene	1425.8	1432	0.10
α-Guaiene	1427.9	1437	0.55
6,9-Guaiadiene	1432.7	1442	7.08
Aromadendrene	1441.4	1439	0.95
α-Humulene	1445.9	1452	0.42
allo-Aromadendrene	1453.0	1458	0.14
cis-Muurola-4(14),5-diene	1466.6	1465	0.09
γ-Muurolene	1465.9	1478	0.18
Geranyl propionate	1472.5	1476	0.86
γ-Gurjunene	1474.7	1475	0.35
β-Selinene	1484.1	1489	0.16
α-Muurolene	1493.7	1500	0.19
γ-Cadinene	1507.0	1513	0.16
Geranyl isobutanoate	1510.1	1514	0.28
δ-Cadinene	1516.8	1522	1.16
Citronellyl butanoate	1524.5	1530	0.68
Furopelargone A	1536.9	1538	0.50
Geranyl butanoate	1557.5	1562	0.76
Neryl isovalerate	1574.9	1582	0.05
Caryophyllene oxide	1578.8	1582	0.43
5,5,9,10-Tetramethyltricyclo [7.3.0.0(1,6)]dodecan-11-one**	1577.6	n/a	0.46
2-Phenyl ethyl tiglate	1586.5	1584	0.24
Geranyl isovalerate	1601.9	1606	0.33
Humulene epoxide II	1605.2	1608	0.09
1,10-di-epi-Cubenol	1611.5	1618	0.20
1-epi-Cubenol	1624.9	1627	0.12
Cubenol	1636.4	1645	0.15
α-Cadinol	1649.4	1652	0.13
cis-Citronellyl tiglate	1662.9	1666	0.31
Geranyl tiglate	1697.8	1696	1.52
Grouped constituents			
Monoterpene hydrocarbons			0.49
Oxygenated monoterpenes			59.74
Sesquiterpene hydrocarbons			15.02
Oxygenated sesquiterpenes			6.01
Others			18.06
Total			**99.32**

n/a – not available

Pelargonium graveolens oil showed effectiveness at concentrations of 0.25-2.5 mg/ml by serial dilution method, while according to results of macrodilution method, geranium oil was effective at concentrations of 0.5-5 mg/ml. When comparing the results of both, micro- and macrodilution tests, the most resistant fungi in the presence of geranium oil were *Mucor mucedo* and *Aspergillus flavus.* The most susceptible strains in this study were *Cladosporium fulvum, Phoma macdonaldii* and *Trichophyton menthagrophytes.* The sensitivity of tested micromycetes was more pronounced in microdilution method (**Table 2**). *P. graveolens* oil exhibited quite stronger antifungal potency comparing to bifonazole (**Table 2**). Free radical scavenging capacity of the tested oil was measured by DPPH assay and results are shown in **Fig 1**.

According to presented results, *P. graveolens* oil was found to possess slightly lower antioxidant activity (IC_{50} = 0.802 mg/ml) than synthetic antioxidant BHT (IC_{50} = 0.328 mg/ml). The yield of *P. graveolens* essential oil (approximately 0.15%), composition and constituent amounts may differ depending on the type and source of plant material (Lavabre, 1998; Lawless, 2001). It was observed by Verma *et al* (2010) that under the same climatic conditions, the composition of rose-scented geranium essential oil was significantly affected by crop duration and length of vegetation period. Lawrence (1999) provides a detailed research overview of the authors who analysed the geranium essential oil. Based on this study, it can be observed that the main components of *P. graveolens* essential oil are geraniol and citronellol, while linalool, isomenthone, citronellyl formate and linalyl formate are also present in significant percentages.

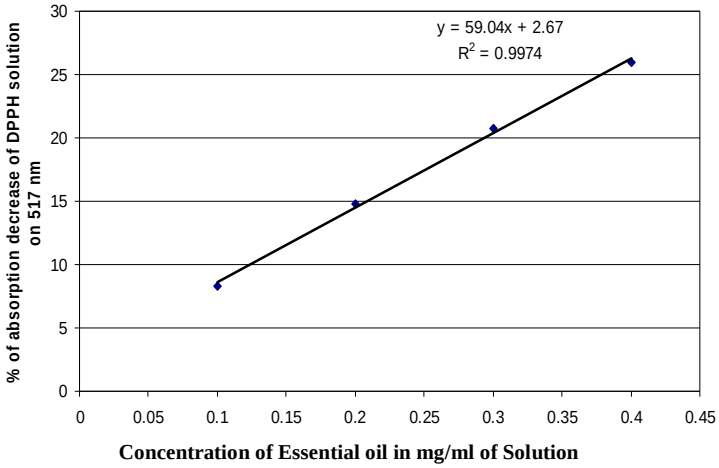

Fig. 1: Antioxidant activity of *Pelargonium graveolens* essential oil using DPPH test (EC_{50} = 0.802 mg/ml).

According to **Table 1** this data is in complete compliance with results of our chemical analyses. *Cis*- and *trans*-rose oxides are often identified as geranium oil constituents, although present in smaller amounts (Lawrence, 1999). In our study only *cis*–rose oxide was identified but in irrelevant amount (0.76%). The results on chemical analyses for Bourbon geranium oil were similar (Yan *et al*., 1994; Lis-Balchin *et al*., 1996). Alcohols geraniol and citronellol both originate from geranyl pyrophosphate, but the reason for their concentration variability is assumed to be production operated by different enzymes (Verma *et al*., 2010). Recent research on *P. graveolens* oil revealed that it manifests a strong inhibitory effect on Gram-positive bacterial strains such as *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* (Silva and Fernandes, 2010). *In vitro* synergism was demonstrated between *Pelargonium graveolens* oil and synthetic agent norfloxacin against *B. cereus* and *S. aureus*: oil increased the antimicrobial activity of this synthetic agent (Rosato *et al*., 2008).

Galea and Csedo (2007) have observed a complete inhibition of *Candida albicans* growth regarding to anticandidal activity of *P. graveolens* oil. The same results were achieved in our study with concentration of 0.50 mg/ml in microdilution method and 1.00 mg/ml for geranium oil in macrodilution method (**Table 2**), with conclusion that tested oil exhibited strong activity on pathogenic yeast *C. albicans*. It was found that a combination of *P. graveolens* oil and amphotericin B in treatment of *Candida* species can reduce the required dose of amphotericin B and thereby, minimize its negative effects (Rosato *et al*., 2008). Similar results were obtained by examining the effect of geranium oil on the growth of *Aspergillus* species when it was observed that the best activity had combination of *P. graveolens* oil, amphotericin B and ketoconazole (Vuuren *et al*., 2009). Still, according to the results that we obtained, *P. graveolens* oil itself has very strong fungicidal effect against all five *Aspergillus* strains that we used. Čavar and Maksimović (2012) published radical-scavenging activity of extracts and essential oils of *P. graveolens*. They measured antioxidant capacity by the DPPH method and get values ranged from 63.70 mg/ml (leaves) to 64.88 mg/ml (stems) for essential oils. In recent study antiradical activity of the geraniol oil was significantly stronger than activity of acetone extract of the plant (EC_{50} value of 14.49 mg/ml, EC_{50} = 66.45 µg/ml, respectively) (Fayed, 2009). Antioxidant effect of geranium oil was in positive correlation with anticancer activity which was determinated in the same research and both these properties could be attributed to the main ingredients of geranium oil, citronellol and *trans*-geraniol (Fayed, 2009). Citronellol is an oil soluble component with anticancer and anti-inflammatory properties. It was found that geraniol achieved significant inhibition (60-90%) of the growth of tumor cells of pancreas (Fayed, 2009).

Table. 2: Antifungal activity of *Pelargonium graveolens* essential oil (MICs and MFCs mg/ml) using microdilution and macrodilution methods.

Fungi	Microdilution method		Macrodilution method		Bifonazole	
	MIC	MFC	MIC	MFC	MIC	MFC
Alternaria alternata	0.25	0.50	0.50	1.00	0.10	0.10
Aspergillus niger	0.50	0.50	0.50	2.50	0.10	0.10
Aspergillus ochraceus	0.50	1.00	5.00	5.00	0.10	0.15
Aspergillus flavus	2.50	2.50	2.50	5.00	0.10	0.15
Aspergillus terreus	1.00	1.00	2.50	5.00	0.10	0.15
Aspergillus versicolor	0.50	1.00	0.50	1.00	0.10	0.10
Aureobasidium pullulans	0.50	0.50	0.50	1.00	0.05	0.10
Cladosporium cladosporioides	0.50	1.00	0.50	1.00	0.10	0.10
Cladosporium fulvum	0.25	0.50	0.50	0.50	0.05	0.10
Fusarium tricinctum	1.00	2.50	2.50	2.50	0.15	0.20
Fusarium sporotrichioides	1.00	2.50	1.00	2.50	0.15	0.20
Mucor mucedo	1.00	2.50	2.50	5.00	0.15	0.15
Penicillium funiculosum	0.50	1.00	1.00	2.50	0.15	0.20
Penicilium ochrochloron	1.00	2.50	1.00	2.00	0.15	0.20
Phomopsis helianthi	0.25	0.50	0.50	1.00	0.10	0.10
Phoma macdonaldii	0.25	0.25	0.50	1.00	0.10	0.15
Trichoderma viride	1.00	2.50	1.00	2.50	0.15	0.20
Trichophyton menthagrophytes	0.25	0.25	0.50	1.00	0.10	0.15
Candida albicans	0.25	0.50	1.00	1.00	0.10	0.15

CONCLUSION

The current study indicates that chemical composition of geranium oil is of high quality with alcohols citronellol and geraniol as dominant compounds. The oil expressed stronger antifungal activity compared to antioxidant results obtained for tested oil. We can report that *P. graveolens* essential oil exhibited high antifungal activity against various fungal strains which can be profitably explored. The biological properties manifested by geranium essential oil in this study substantiate its use in numerous health problems and medical conditions and validates its commercial exploitation in many industry branches.

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