

## PHYTOCHEMICAL PROFILE OF TWO CULTIVATED *PELARGONIUM* (*GERANIACEAE*) SPECIES

CRISTINA ELENA IANCU, OANA CIOANĂ\*, MONICA HÂNCIANU, CORNELIA MIRCEA

“Gr. T. Popa” University of Medicine and Pharmacy Iași, Faculty of Pharmacy, Department of Pharmacognosy, 16 Universității, Iași, Romania

\*corresponding author: oana.cioanca@gmail.com

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### Abstract

The current study is part of a broader research that evaluated the chemical and biological properties of some *Pelargonium* species cultivated in Romania. The selected samples, *P. hispidum* and *P. radens*, were obtained from the “Anastase Fătu” Botanical Garden, Iași, Romania. Our aim was to evaluate the chemical profile of the essential oils and hydro-alcoholic extracts isolated from these samples. The biological activity was assessed by two tests: iron chelating assay and superoxide scavenging assay. The ethanolic extracts were analysed by UHPLC, whereas the essential oil profile was established with GC-MS-FID. Both chosen samples represent aromatic species, but their organoleptic aspect and flavour are different. The GC profile indicated few common characteristics, but the most important variation was the proportion of the main terpenes in the essential oil. Also, the UHPLC results indicated differences of the chemical composition between the samples. These variations were noted in the biological activity assays.

### Rezumat

Studiul de față este parte a unei cercetări extinse ce evaluează proprietățile chimice și biologice ale unor specii de *Pelargonium* cultivate în România. Probele selecționate, *P. hispidum* și *P. radens*, au fost obținute prin amabilitatea Grădinii botanice “Anastase Fătu”, Iași, România. Scopul acestui studiu a fost de a evalua profilul chimic al uleiurilor volatile și a extractelor hidroalcoolice. Activitatea biologică a fost studiată prin două teste: chelatarea ionului feros și activitatea de scavenger față de anionul superoxid. Spectrul polifenolilor a fost stabilit prin UHPLC, iar gaz-cromatografia cuplată cu detector MS și FID a fost folosită pentru uleiurile volatile. Ambele specii sunt aromate, dar organoleptic se observă o diferență clară între arome. Profilul gaz-cromatogramelor a evidențiat câteva elemente comune, dar raportul și cantitatea grupelor terpenoidice majore ale fracțiunilor volatile variază semnificativ de la o probă la alta. Diferențe importante între probe au fost observate și în cadrul analizei UHPLC, precum și în cadrul testelor biologice.

**Keywords:** geranium oil, polyphenols, antioxidant, UHPLC

### Introduction

Most of the *Geraniaceae* species come from South Africa, but nowadays they are cultivated for its ornamental purposes throughout the world. Almost all varieties of *Geranium* and *Pelargonium* species are small shrubs, annuals and herbaceous perennials. The scientific literature comprises mostly data from the traditional medicine of different countries and also some recent investigations regarding the medicinal properties for *P. graveolens*, *P. crispum*, *P. echinatum*, *P. grossalarioides*, *P. parviflorum* and some others.

On the other hand, some of the most used *Pelargonium* species (incorrectly named Geraniums) are currently the aromatic rose-scented ones. They represent an important raw material for perfume industry.

In the present study, we investigated the chemical profile of two scented-leaved species: *Pelargonium hispidum* (L.) Wild. and *Pelargonium radens* H. E.

Moore grown in Romania. Some aspects regarding their antioxidant properties were also assessed.

### Materials and Methods

#### *Plant material*

The samples were represented by the dried leaves of *Pelargonium hispidum* (L.) Wild. and *Pelargonium radens* H. E. Moore that were collected from plants cultivated in the Botanical Garden in 2015 in Iași, Romania. One voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, “Gr. T. Popa” University of Medicine and Pharmacy, Iași, Romania.

#### *Essential oil and alcoholic extract isolation*

For essential oil extraction, dried leaves were coarsely cut and then subjected to hydro-distillation for 2 hours using a Clevenger type apparatus. The essential oil was dried over anhydrous sodium sulfate and kept at -4°C until analysis.

The hydro-alcoholic extracts (ethanol 50%) were obtained from 2 g of powdered plant material in a

repeated reflux system. The final extracts were levelled up to 100 mL, and then they were concentrated to dry consistency.

#### The chemical analysis

**GC/MS/FID:** Analysis of the essential oils used an Agilent 6890 GC-MS and a SRI 8610C GC-FID system. The established temperature was 250°C and helium (1 mL/min) was the carrier gas. The capillary column used was DB 5MS (30 m × 0.25 mm; 0.25 µm; Agilent, Palo Alto, CA, USA). 0.30 µL of each sample were injected in a split ratio 100:1, for 32 min. Two replicates of oil were processed in the same way. Acquisition mass range was: 40 - 400 amu. The identification of the compounds was based on comparison of their retention indices (RI), their retention times (RT) and mass spectra with those obtained from authentic Wiley libraries (available through Hewlett Packard) and the literature [9].

**UHLPC:** The profile of phenolic compounds was established with a Thermo UltiMate 3000 gradient chromatograph equipped with a quaternary pump controlled by Chromeleon interface, an autosampler and multidiode array detector (DAD). Solvents were filtered using a Millipore system and analysis was performed on an Accucore XL C18 column (150 x 4.6 x 4). All parameters for the analysis were as follows: mobile phase - acetonitrile (A) and 0.1% acetic acid (B); gradient - 10% - 23% (A) in 5 min; 23% (A) isocratic for 10 min and then 23% - 35% (A) in 12 min; 35% - 70% (A) for 5 min; injection volume - 20 µL; flow-rate - 0.2 mL to 1 mL/min; absorbance wavelengths - 240 nm to 520 nm. Other details regarding the recognition and calculation of the compounds amount were the same to the aspects described in a previous article [6].

#### Quantification of flavonoids and total phenols

**Total flavonoids** were calculated by establishing the method that uses the reaction between sodium nitrite (5%), aluminium chloride (10%) and sodium hydroxide (1 M). The absorbance was measured at 510 nm [7]. Rutin equivalents (mg/100 g dry extract) were used for quantification.

**Total phenols** were quantified by Folin-Ciocalteu reaction, with a short initial incubation period (5 min) at 25°C, in the absence of light, prior to the addition of the reagents. The absorbance of the mixture was determined at 750 nm, after the usual incubation time (120 min). GAE (gallic acid equivalents, mg/100 g dry extract) were used for quantification [5, 6, 8].

#### Antioxidant assays

The screening of the antioxidant potential of the hydro-alcoholic extracts was based on two tests:

iron chelating activity and the scavenger capacity against superoxide anion.

**Iron chelating activity assay:** this test used the method of Dinis *et al.* [4] with minor changes. Briefly, several dilutions in DMSO were prepared from the dried extracts. All following steps were as described by Robu *et al.* [10]. The IC<sub>50</sub> value (µg/mL) was calculated through linear interpolation between values above and below 50% activity.

**Scavenger capacity against superoxide anion:** Superoxide radicals (O<sub>2</sub><sup>•-</sup>) are usually generated in PMS-NADH systems by oxidation of NADH. The changes in colour intensity are assessed by the reduction of nitro-blue tetrazolium (NBT) which indicates that the extract found in the mixture has scavenger activity against superoxide anion [7].

Well-known antioxidant compound such as gallic acid was used as standard. All results are expressed as mean of three determinations.

**Reagents:** All chemical and reagents were of analytical grade or of chromatographic quality and were purchased from Sigma Aldrich (Seelze, Germany) or Fluka (Buchs, Switzerland).

## Results and Discussion

The results for the GC-MS-FID analysis indicated that the essential oils isolated from the investigated samples contain some common compounds, such as menthone and isomenthone – considered marker compounds for geranium oils, p-cimene, and α-pinene. Moreover, a large difference in the compound spectra was noted for the investigated samples (Figure 1).

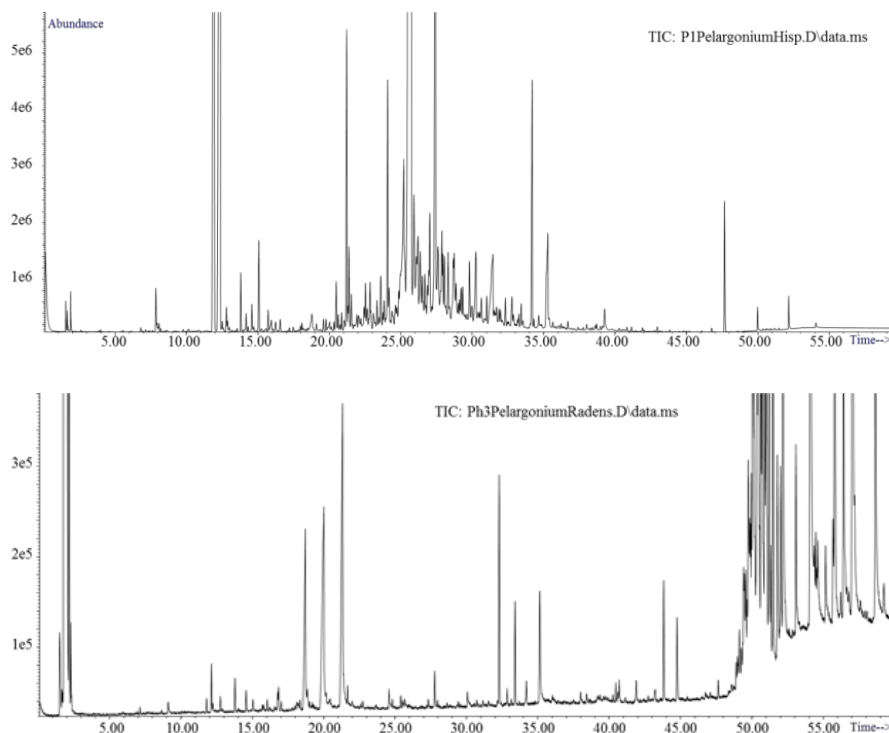
The major fraction of volatile substances is represented by the monoterpenes approx. 48% for *P. hispidum* and more than 90% for *P. radens*. Such variations are noticed from the different aroma the leaves have when the product is crushed. Moreover, the proportion and quantity of each compound varies greatly from one sample to the other: sabinene (0.49%), myrcene (0.57%), phellandrene (0.49%), carene (0.13%), terpinene (1.39) and limonene (0.40%) were present only in *P. radens* essential oil. On the other hand, rose oxides (0.64%) were detected only in *P. hispidum*. Sesquiterpenes (12%) were found in *P. hispidum* essential oil, amongst which, the highest concentrations were found, as follows: guajene (4.64%), patchoulene (1.88%), ylangene (1.88%), caryophyllene oxide (0.76%).

The samples were different in regard to their polyphenolic fraction as well.

**Table I**

The content of the active ingredients of *P. hispidum* and *P. radens*

| Samples            | Total phenols (mg% dry extract) | Flavones (mg% dry extract) |
|--------------------|---------------------------------|----------------------------|
| <i>P. hispidum</i> | 1691.31                         | 510.392                    |
| <i>P. radens</i>   | 1068.95                         | 311.540                    |



**Figure 1.**  
GC-MS chromatograms for the investigated samples

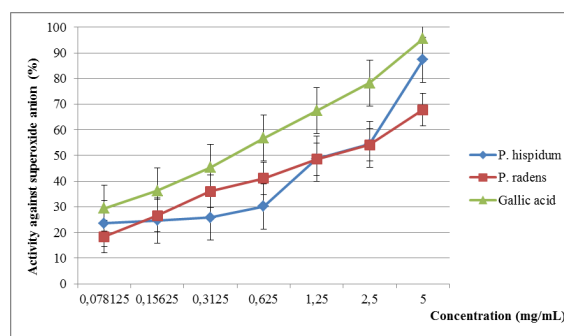
More specific characterization was possible by UHPLC, when the hydro-alcoholic extract of *P. hispidum* proved rich in rutoside (28.841  $\mu\text{g}/\text{mg}$ ), catechin (8.231  $\mu\text{g}/\text{mg}$ ) and quercetin-3-arabinoside (4.949  $\mu\text{g}/\text{mg}$ ). On the other hand, *P. radens* contained a double amount of quercetin-3-arabinoside (10.301  $\mu\text{g}/\text{mg}$ ) and a similar amount of catechin (8.041  $\mu\text{g}/\text{mg}$ ).

Among the polyphenolic acids, caffeic acid (2.263  $\mu\text{g}/\text{mg}$ ) was quantified in *P. radens* while rosmarinic (3.045  $\mu\text{g}/\text{mg}$ ) acid was noted in *P. hispidum*. These values confirmed partially other research data, but one should take into account that both species (*P. hispidum* and *P. radens*) were scarcely investigated so far. Most of the general literature concerning the chemical compounds found in geranium sustains the presence of flavonoid aglycones and glycosides [9, 11].

The iron chelating assay indicated that both investigated samples have a medium potential ( $\text{IC}_{50}$  *P. hispidum* =  $37.30 \pm 0.13$   $\mu\text{g}/\text{mL}$ ;  $\text{IC}_{50}$  *P. radens* =  $22.55 \pm 0.47$   $\mu\text{g}/\text{mL}$ ). These activities were in close relation to the total phenol concentration of each sample. Moreover, gallic acid (GA) used as a standard in our sample had a comparable activity ( $\text{IC}_{50}$  GA =  $19.14 \pm 0.09$   $\mu\text{g}/\text{mL}$ ) to *P. radens* sample. All these confirm once again that flavonoids and polyphenols have good chelating activity against

complex elements due to the presence of hydroxyl and ketone moieties on their structures [2, 5].

The results obtained in the second antioxidant test revealed that both samples have similar activity as scavengers against superoxide anion:  $\text{IC}_{50}$  *P. hispidum* =  $145.91 \pm 0.98$   $\mu\text{g}/\text{mL}$ ,  $\text{IC}_{50}$  *P. radens* =  $149.85 \pm 0.34$   $\mu\text{g}/\text{mL}$ . Still, for concentrations below 1.25 mg/mL the extract from *P. radens* is more active than the other sample, whereas concentrations over 2.5 mg/mL induce a higher potential for *P. hispidum* (Figure 2).



**Figure 2.**  
Scavenger activity against superoxide anion of the investigated samples

Our results, together with literature data prove that the vegetal material is highly important especially in regards to its chemical composition. The high

content in phenolic compounds modulates the antioxidant activity of each extract inducing a positive effect by improving the health status as usually indicated by literature [1, 3]. Although our registered values indicated a much lower quenching capacity against superoxide anion than that of iron chelating potential, the plant material remains a good source of active compounds.

### Conclusions

In summary, the present study indicated that *Pelargonium hispidum* and *Pelargonium radens* are important sources of flavonoids and essential oil. Although the antioxidant assays showed an important variation of the intensity of action, this only signifies that the extracts act by different mechanisms in a specific way. Also, further study aimed at the biological potential of certain isolated compounds or fractions will be taken into account.

### Conflict of interest

The authors declare that they have no potential conflicts of interest to disclose.

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