Phytochemical study of the plant *Pelargonium roseum* (*P*. *radula*)

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*Pelargonium roseum* Willd (*P*. *radula*) is a species originated in South-Africa and is spread especially in Cape Town region. For essential oil extraction, needed in the perfume industry, the species is cultivated in Reunion, Egypt, Algeria, Morocco, China, Spain and in southern France. Although the cold winters do not allow for the species *Pelargonium roseum* to be cultivated in perennial cultures in Romania, we tried to cultivate it in annual cultures. The plants spent the winters in hothouses and were propagated by cuttings. The essential oil was obtained by hydrodistillation, after which the oil was filtered in void on a stratum of waterless Na$_2$SO$_4$ in order to retain the water traces. The essential oil obtained between 2003-2006 was put into ampoules and eventually subjected to diverse physical and chemical tests.

1. In the first stage of the present study we cultivated the species *Pelargonium roseum* in the pedoclimatic conditions of the town Targu-Mures, from the point of view of the following aspects:

   - humidity
   - temperature
   - soil structure
   - luminosity.

Multiplication was made through cuttings, because the fruit does not reach maturity and the seeds show little vitality. The healthy plants were fragmented by cutting up to the height of 10-15 cm and 3-4 knots. When the branches reach the height of 1-3 cm, they are detached and planted into a layer containing 60 % sand and 40 % peat, at 3-5 cm depth. 16-20 days are needed for the creation of roots. At the beginning of spring the rooted cuttings are re-planted with distance between themselves, and at the beginning of May the whole culture is set up, with 80 cm distance between the rows and 50 cm between the plants of a row, obtaining a density of 25,000 plants / ha.

Harvesting was done in 3 stages. In July the ramifications of stalks with 3-5 leaves were harvested, in August the ramifications of well-developed stalks were harvested and the third harvest was obtained at the end of the vegetation period, in September.

Average green weight / plant was 520 g, and average dry drug weight / plant was 76 g. Total harvest / ha is 18500 kg.

From the harvested stalks and leaves, a part was dried in order to prepare infusions and for physical and chemical tests, and another part was subjected to distillation, in order to obtain essential oil.

2. The second stage of the study was destined to essential oil extraction. With this end in view both leaves and green stalks were subjected to direct water distillation. Essential oil content varies between 0.082 % and 0.16 %, being
dependent on the proportion of leaves-stalks subjected to distillation and is less for the dried drug as compared to the fresh plant.

A plant with an average weight of 520 g can yield about 0.624 g essential oil. The essential oil quantity yielded by the harvested green mass / ha is 22.2 kg.

The essential oil thus obtained, also called geranium oil, is a transparent, colourless, greenish liquid with a pleasant fragrance, reminiscent of that of roses.

3. Within the framework of the histo-anatomical analysis, plant sections were studied corresponding to root, stalk, leaf and flower. An important role was assigned to the essential oil secretory structure, the multicellular glandular hairs, situated on both (inferior and superior) leaf surfaces.

In this resume only elements characteristic for the species *Pelargonium roseum* are given relevance. Among these the presence of calcium – ursine oxalate crystals is especially relevant. These are visible at the main root, at the rhizomes, the stalk and the leaves.

On the surface of the above-ground organs – stalks, petioles, leaves – the indumentum consists of a mixture of hairs (trichomes), respectively glandular and non-glandular hairs.

The majority of non-glandular hairs are bicellular, simple (without ramifications), uniserial. The secretory (glandular) hairs are of two types: globular and bulb-shaped, corresponding to the different stages of leaf maturation. The bulb-shaped ones are unicellular, and eventually will become globular hairs. The globular hairs are tricellular, without ramifications, uniserial. The stalk of the hairs consists of a larger, cone-shaped cell and a smaller, flattened one. The head is unicellular, globular.

4. The analysis of the essential oil was accomplished in two stages. In the first one merely a screening using thin layer chromatography was done, which permitted the identification of only a few components. In the second stage the phytochemical analysis of the essential oils was achieved using gas chromatography with flame ionization detection (GC-FID) for the qualitative analysis, and gas chromatography with mass spectrometry (GC-MS) for the quantitative one, respectively. Four samples of essential oil yielded by *Pelargonium roseum* (3 ones obtained through cultivation in the Herbal Garden of U.M.F. Targu-Mures, the fourth being from Reunion) and 2 samples of essential oil from *Pelargonium graveolens* (Egypt and China) were analyzed.

Thin layer chromatography gave us approximate information, showing only few qualitative items. Chromatograms showed the presence of geranoil, citronellol and linalool, possibly nerol, and a series of other components, which could not be identified by comparing the Rᵣ values with the colours. Out of the samples analyzed, 3 have an extra compound at the Rᵣ value 0.53 and the other 3 have an extra compound at the Rᵣ value 0.30.

Gas chromatography helped to solve the problems posed by thin layer chromatography. In the structure of the essential oil a number of 61 components were detected, out of which 43 were identified. Citronellol (39.97 – 43.67 %) is the main component of all essential oils, except for that coming from Reunion (20.46 %). Citronellol is followed by geraniol (2.57 – 9.66 %), linalool (1.41 – 4.77 %), and their
esters: citronellyl formate (11.23 – 13.55 %), geranyl formate (1.15 – 2.21 %), citronellyl butyrate (1.01 – 1.54 %) and geranyl tiglate (1.22 – 1.75 %). It is necessary to mention that the Reunion essential oil reaches the highest concentration of geranyl (22.27 %). Significant concentrations are also attained by isomenthone (4.61 – 6.14 %), guaia-6,9-diene (1.06 – 7.06 %) and gamma-eudesmol (3.28 – 5.62 %).

The two unknown compounds that were not identified by thin layer chromatography are guaia-6,9-diene in the essential oils yielded by *Pelargonium graveolens* (China) and in 2 samples of essential oils yielded by *Pelargonium roseum* (France and Reunion) and 10-epi-gamma-eudesmol, respectively, present in the essential oil from *Pelargonium roseum* obtained in Targu-Mures and in that yielded by *Pelargonium graveolens* (Egypt).

5. Analyzing the polyphenols of the species *Pelargonium roseum* Willd, the study started by a screening using thin layer chromatography, similar to that used in case of the essential oils. At these analyses methanolic extracts of leaves and roots were used, having been harvested in different years.

By comparing the R_f values and the fluorescence of separate bands to that of the standards, the presence of ruthoside and hyperoside in the leaves of *Pelargonium roseum* was discovered. Using the same procedure, we can assert that free flavonoid aglycones (apigenine, luteoline, myrcetol, quercetol) can not be found in the leaves and root of the plant being studied.

In order to settle these questions, high performance liquid chromatography, coupled with mass spectrometry was used. The method can be applied for both qualitative (18 compounds) and quantitative analysis (14 compounds). Qualitative identification can be done both by MS detection and UV detection. Quantitative determination was done by UV detection in the concentration segment 0.5 – 5 microg/ml.

Since in these chromatographic conditions there are two pairs of substances that are incompletely separated (caftaric acid – gentisic acid, and caffeic acid – chlorogenic acid, respectively), for these compounds only a qualitative determination was done, on the basis of MS information.

Two samples of every infusion were analyzed in parallel, one as such and another after subjected to hydrolysis. Hydrolysis was necessary due to the presence of flavonoid aglycones in bound form (glycosides, esters) and of some polyphenolcarboxylic acids. Acid hydrolysis results in the release of these compounds from their bound form and offers more information as to the chemical composition of the polyphenolic content of the product under study.

In qualitative terms, 11 polyphenolic compounds were identified both in the leaf infusion and that made of stalks of *Pelargonium roseum*: caftaric acid, caffeic acid, chlorogenic acid, para-coumaric acid, ferulic acid, hyperoside, isoquercetrine, ruthoside, myrcetol, quercetol, käempferol.

6. Some trace elements in the root, stalk, leaves and flower of *Pelargonium roseum* were studied, with the purpose of a possible utilisation of the plant as a nutritional supplement. Its content of trace elements was analyzed on the basis of the ICP-MS method and the green mass was processed by the wet digestion method. The
elements whose concentration was studied are: Li, Be, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Se, Rb, Sr, Ag, Cd, In, Ba, Tl, Pb, Bi.

The values obtained in case of Fe are significant: in green stalks Fe concentration was found to be 1.21 g / kg, whereas in leaves it was 0.66 g / kg. Taking into account that in most European countries people’s need of Fe is not satisfied by nourishment, supplementing the Fe input is very timely.

*Pelargonium roseum* is a species rich in ferrum and as its tannine content is reduced, biodisponibility of Fe is not impeded. These are reasons for which the capsuled powder of this plant could be used as a nutritional supplement in case of iron deficiency.

7. The last chapter refers to the antibacterial and antifungal activity of the species *Pelargonium roseum* and *Pelargonium graveolens*. The microorganisms used in order to test the antimicrobial activity of the essential oils under discussion were:

Gram-negative bacteria: *Pseudomonas aeruginosa*
  *Proteus mirabilis*
  *Escherichia coli*

Gram-positive bacteria: *Staphylococcus aureus*
  *Enterococcus faecalis*

Fungi: *Candida albicans*

In case of *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) an inhibition comparable to that of microcapsules of antibiotics was recorded. The two types of essential oils under study did not show significant differences in their antimicrobial activity. As to their bioactivity against *Candida albicans*, the complete inhibition of the fungus’s development was pointed out. Further research based on the dilution method is needed in order to establish the minimal concentration in which geranium essential oil has an inhibitory effect against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, respectively.

The research undertaken within this thesis permits a many-sided characterization of the genus *Pelargonium*, including the identification, both qualitative and quantitative determination of the most relevant classes of active principles for the species *Pelargonium roseum*, being studied for the first time. The antibacterial and antifungal studies confirmed the potentiality of the species in this domain. This phytochemical study encourages further research for obtaining some products of dermatological use from the species *Pelargonium roseum*, taking into account the presence of ruthoside in high concentrations and of citronellol (as antibacterial agent) in the structure of geranium essential oil.