OBTAINING AND CHARACTERIZATION OF A SELECTIVE *PELARGONIUM GRAVEOLENS* L'HÉR. DRY EXTRACT WITH POTENTIAL THERAPEUTIC ACTIVITY IN METABOLIC DISEASES

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Abstract

The aim of our study was the obtaining, phytochemical characterization, evaluation of antioxidant activity and cytotoxic effects of a selective dry extract obtained from indigenous *Pelargonium graveolens* L'Hér (rose geranium, rose scented geranium) aerial parts, with potential therapeutic activity in metabolic diseases (diabetes mellitus, dyslipidaemia). Rose geranium flowers, leaves and aerial parts quality were determined by means of spectrophotometric methods (quantitative determination of anthocyanins, total phenolic content, flavones and phenolcarboxylic acids). The dry selective extract (obtained in 50% ethanol) contains: 0.45 g anthocyanins (expressed as cyanine chloride)/100 g dry extract and 45.73 g total phenolic content (expressed as tannic acid equivalents)/100 g dry extract. HPLC (high performance liquid chromatography) analysis revealed the presence of epigallocatechin and catechin derivatives. The dry extract antioxidant capacity was assessed using ABTS^{•+} (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenger activity and ferric reducing power assay. Antioxidant activity was expressed as EC₅₀ (μg/mL) (which represent the dry extract's concentration that inhibited 50% of ABTS^{•+} activity/ the concentration that provides 0.5 absorbance for ferric reducing power assay - EC₅₀ = 17.53 μg/mL/EC₅₀ = 74.43 μg/mL). The extract induced dose and time dependent cytotoxic effect against *Daphnia magna* invertebrates. Our preliminary results offer encouraging premises for obtaining a phytomedicine with potential therapeutic effect in metabolic diseases.

Rezumat

Scopul lucrării a constat în obținerea, caracterizarea fitochimică și evaluarea activității antioxidante a unui extract uscat, selectiv obținut din părți aeriene de *Pelargonium graveolens* L'Hér (mușcată), cu potențială activitate în tratamentul afecțiunilor metabolice (diabet zaharat, dislipidemie). Calitatea produselor vegetale (flori, frunze, părți aeriene) a fost determinată prin metode spectrofotometrice (determinări cantitative pentru polifenoli totali, antociani, flavone, acizi fenolcarboxilici). Extractul hidroalcoolic uscat (obținut în etanol 50%) conține 45,73 g polifenoli totali (exprimați în echivalenți de acid tanic)/100 g extract uscat și 0,45 g antociani (exprimați în echivalenți de clorură de cianidol)/100 g extract uscat. Analiza HPLC (cromatografie de lichide de înaltă performanță) a evidențiat prezența derivaților de catechol și a epigallocatecholului. Activitatea antioxidantă a extractului a fost determinată pe baza capacității de scavenger a radicalului liber ABTS^{•+} (acidul 2,2'-azinobis-3-etilbenzotiazolin-6-sulfonic) și pe baza capacității de reducere a ferului. Capacitate antioxidantă a fost evaluată pe baza EC₅₀ (μg/mL) (concentrația extractului care inhibă cu 50% activitatea radicalului liber/concentrația la care absorbanta are valoarea 0,5 pentru capacitatea de reducere a ferului - EC₅₀ = 17,53 μg/mL/EC₅₀ = 74,43 μg/mL). Toxicitatea extractului asupra nevertebratului *Daphnia magna* a fost dependentă de concentrația și timpul de incubare utilizat. Rezultatele obținute oferă premise încurajatoare pentru obținerea unui fitopreparat cu potențiala utilizare în tratamentul afecțiunilor metabolice.

Keywords: total phenolic content, rose geranium, catechin, antioxidant capacity

Introduction

The genus *Pelargonium* comprises over 270 species, native to southern part of the African continent. It includes annuals, herbaceous perennials, shrubs and deciduous plants [23]. *Pelargonium* species (*P. alpinum* Eckl. & Zeyh., *P. luteum* G. Don, *P. zonale* L'Hér ex Aiton, *P. reniforme* Curt., *P. sidoides* DC., etc.) are

traditionally used in the treatment of diarrhoea, wounds, respiratory tract infections, liver complaints, gastroenteritis [23] or as ornamental plants [22]. *Pelargonium graveolens* L'Hér, known as rose geranium or rose scented geranium, is one the most studied *Pelargonium* species, due to its essential oil (commercially known as geranium oil) [23]. Rose geranium leaves and aerial parts are an important source of essential oil, phenolic

compounds, coumarins and amines (methylhexanamine) [19, 23]. The essential oil has a complex chemical composition, the main constituents are: linalool (0.4 - 3.1%), citronellol (14.2 - 22.8%), citronellylformate (11.40%), mentone (2%), geraniol (26 - 50%), geranial (0.8%), geranyl valerate (0.4%), neral (0.3%), citral (0.65%), isomenthone (5.44%), γ -cadinene (0.5%), α -muurolene (0.2%), α -humulene (0.3%), α -pinene (1.08%), limonene (0.19%), γ -terpinene (2.41%), γ -eudesmol (11.23%), 1,8-cineole (0.6%), ledene (1.13%), germacrene (1.71%), β -elemene (0.6%), 6,9-guaiadiene (4.6%), decanoic acid (3.4%) and traces of camphene, *m*-cymene [4, 6, 9, 18]. The essential oil chemical composition is influenced by different phenological stages, the highest concentration of geraniol and linalool was found at flowering [5]. Regarding phenolic compounds, rose geranium leaves are a source of tannins (epigallocatechin gallate), phenolcarboxylic acids (protocatechuic acid, *o*-coumaric and *p*-coumaric acids), flavones (myricetin-3-O glucoside, quercetin-3-O-rhamnosyl (1 $\rightarrow$ 6) hexoside, quercetin-3-O-galactoside, kaempferol-3-O-glucoside) [2].

The essential oil and leaves have shown antibacterial, antifungal and antileishmanial effects [3, 8, 9, 21]. Geranium oil has anti-inflammatory effects due to its moderate anti-lipoxygenase activity [7, 16]. Recent research has shown that *Pelargonium* essential oil can effectively reduce anxiety during labour [10] and prevents oxidative testicular damage, similar to vitamin E, in mice exposed to deltamethrin pesticide [24]. Different extracts from rose geranium leaves/aerial parts showed hypoglycaemic, hypolipidemic [1], hepatoprotective [2] and anticholinesterase properties [15]. Taking into consideration the scientific data, the aim of our study was obtaining, phytochemical characterization, evaluation of antioxidant activity and cytotoxic effects of a selective dry extract obtained from indigenous rose geranium aerial parts, that might be used for the formulation of a phytomedicine with therapeutic activity in metabolic diseases (diabetes mellitus, dyslipidaemia).

Materials and Methods

Material

Leaves, flowers and aerial parts of *Pelargonium graveolens* were harvested from ecological crop, Romania, in 2017. The raw material was air-dried under shade and afterwards stored in laboratory conditions.

Reagents and solvents

All reagents and solvents were purchased from Roth (Germany), unless otherwise stated.

Methods

Our research was performed in several steps: 1) evaluation of herbal products (leaves, flowers and whole aerial parts) quality; 2) obtaining and phytochemical characterization of a selective dry extract; 3) evaluation of the extract's antioxidant capacity;

4) assessment of rose geranium dry extract toxicity upon *Daphnia magna* invertebrates.

The evaluation of herbal products quality (1) (leaves = PL, flowers = PF, whole aerial parts = PAP) was performed using spectrophotometric methods for determination of flavones, phenolcarboxylic acids, anthocyanins and total phenolic contents.

Preparation of samples for spectrophotometric determinations: 2 g of dried herbal products (flowers, leaves, whole aerial parts) was heated twice with 50 mL 50% ethanol (v/v) on a reflux condenser for 15 min. After cooling, the solutions were filtered in a 100 mL volumetric flask and filled to mark with the same solvent.

Spectrophotometric assay of flavones, phenolcarboxylic acids and total phenolic contents was carried out as previously described [11]. For anthocyanins content, the determination was performed according to European Pharmacopoeia 7th edition [26]. The following calibration curves have been used in order to determine the active substances content: rutin (5.0 - 35.0 μ g/mL, $R^2 = 0.9998$, $n = 11$), chlorogenic acid (0.0113 - 0.0527 mg/mL, $R^2 = 0.9998$, $n = 6$), tannic acid (2.0 - 12.0 μ g/mL, $R^2 = 0.9990$, $n = 10$) and cyanine chloride (linearity range: 1,63 - 9,79 μ g/mL μ g/mL, $R^2 = 0.9990$, $n = 11$).

Obtaining and phytochemical characterization of a selective rose geranium aerial parts dry extract (2) (PE). 800 g of whole aerial parts were heated twice with 50% ethanol (v/v) under a reflux condenser for 60 min. The drug solvent ratio was 1:10 for the first extraction and 1:5 for the second one. After cooling, the combined filtrates were concentrated using a rotary evaporator (Buchi R 210-215) and then freeze dried (Christ Alpha 1-2/B Braun, BiotechInt. lyophilizator) to yield 184 g dry extract. The extraction yield is expressed as the percentage of the total mass of the dry extract (Mext without water content) with respect to the mass of the raw material (Mpv) loaded onto to the flask for solvent extraction: $Y\% = (M_{ext}/M_{pv}) \times 100$ [25].

The same *spectrophotometric determinations* (as for step 1) have been used in order to determine the dry extract's chemical composition. *High performance liquid chromatography (HPLC) analysis* was carried out as previously described [11, 12]. Assays were performed using a 0.02% stock solution, prepared by dissolving the extract in 50% ethanol.

The dry extract's antioxidant capacity (3) was determined based on scavenger activity towards ABTS^{•+} (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) free radical and ferric reducing power, as previously described [11, 20]. The concentration range was 2 - 40 μ g/mL (for ABTS^{•+} method) and 65 - 3239 μ g/mL (for ferric reducing power). The antioxidant activity was expressed as EC₅₀ (μ g/mL), that represents the dry extract's concentration that inhibited 50% of ABTS^{•+} free radical activity or the concentration providing

0.5 absorbance (for ferric reducing power). EC_{50} ($\mu\text{g/mL}$) was determined graphically from the linear regression curve between percent (%) ABTS inhibition/Absorbance and concentration ($\mu\text{g/mL}$). Ascorbic acid was used as positive control.

The cytotoxicity of *Pelargonium* dry extract (4) was determined using *Daphnia magna* bioassay [11, 17]. The concentration range was 20 - 1500 $\mu\text{g/mL}$ and lethality percentages were determined at 24/48 h.

Statistical analysis

Data analysis was performed using Microsoft Excel 2007 (Microsoft Corp. USA) and GraphPad Prism v. 5.0. (GraphPad Software, USA).

Results and Discussion

Herbal products (flowers, leaves, whole aerial parts) quantitative analysis revealed that the highest phenolic

content was found for flowers followed by aerial parts (Table I). For PF and PAP, we also determined a moderate anthocyanins content. Our results are similar to other authors research, that identified flavones and phenolcarboxylic acids in rose geranium leaves extracts [2]. Regarding total phenolic content, Dimitrova M *et al* found 8.23 g tannic acid/100 g fresh aerial parts for an aqueous extract obtained using decoction, as extraction method [8]. However, the comparison of our results with scientific data literature was difficult, since differences can occur due to herbal product's time of harvest, raw material type (dried/fresh) and extraction solvent. Taking into consideration the similar total phenolic content of whole aerial parts and flowers and the anthocyanins presence in geranium flowers, we have chosen to obtain a selective dry extract from PAP. The extraction yield was 23%.

Table I
Spectrophotometric results for herbal products

Active substance	PF	PL	PAP
Total phenolic content (g tannic acid /100 g herbal product)	22.8150 $\pm$ 1.398	7.716 $\pm$ 0.326	16.1700 $\pm$ 1.4982
Flavones (g rutin/100 g herbal product)	nd	0.3902 $\pm$ 0.362	0.5136 $\pm$ 0.0981
Phenol carboxylic acids (g chlorogenic acid/100 g herbal product)	3.6910 $\pm$ 0.458	nd	1.307 $\pm$ 0.137
Anthocyanins (g cyanine chloride/100 g herbal product)	1.0666 $\pm$ 0.1548	nd	0.2798 $\pm$ 0.057

PF – rose geranium flowers; PL – rose geranium leaves; PAP – rose geranium aerial parts; nd – not determined; Results are mean $\pm$ standard deviation (SD) (n = 3)

The dry extract chemical composition was determined by means of spectrophotometric and HPLC methods. According to our results PE dry extract is a rich source of phenolic compounds and anthocyanins (Figure 1). Our results are lower compared to Pradeepa M. *et al* that found 123 g tannic acid/100 g dry ethanolic extract, obtained from rose geranium leaves [21]. Based on retention time, we have identified the presence of tannins in PE dry extract (epigallocatechin and catechin derivatives) (Table II).

Figure 1.

Quantitative analysis of PE dry extract

A – anthocyanins, TP – total phenolic content

Our results are similar to Al-Sayed E. *et al* that identified epigallocatechin, epigallocatechin (dimer), epigallocatechin (trimer) and monogalloyl glucose in an ethanolic dry extract obtained from *Pelargonium graveolens* dried leaves [2].

Table II
HPLC analysis for PE dry extract

Compound	R_t (min.)
Epigallocatechin	14.11
Catechin D ₁	2.81
Catechin D ₂	18.5
Catechin D ₃	19.1

Pelargonium dry extract has scavenger activity towards $ABTS^{++}$ free radical and ferric reducing power capacity (Table III). For $ABTS^{++}$ method the antioxidant capacity was higher compared to our positive control (ascorbic acid) (Table III). The antioxidant activity of rose geranium leaves/aerial parts/essential oil was also reported by other authors, who have used different methods (2,2-diphenyl-1-picrylhydrazyl free radical scavenger activity - DPPH assay) [6, 16, 18]. Important antioxidant activity was also determined for other *Pelargonium* species (*P. hispidum* and *P. zonale*) [14].

Table III
Antioxidant capacity evaluation

Sample	METHOD – EC_{50} ($\mu\text{g/mL}$)	
	$ABTS^{++}$	Ferric reducing power
PE dry extract	17.53 $\pm$ 0.3396	74.43 $\pm$ 0.468
Ascorbic acid	60 $\pm$ 0.009	21 $\pm$ 0.012

Results are mean $\pm$ standard deviation (SD) (n = 3)

Our extract showed a low toxicity on *Daphnia magna* after 24 h and a moderate to high toxicity after 48 h of exposure (Table IV, Figures 2 and 3). After 24 h, the

extract was cytotoxic only at high concentrations (1000 - 1500 µg/mL). However, after 48 h, toxicity was also observed for concentrations above 250 µg/mL.

Figure 2.

Lethality on *Daphnia magna* versus concentration of extract

Figure 3.

Concentration-lethality curves for cytotoxic activity on *Daphnia magna*

We assume that cytotoxic effects are correlated with the total phenolic content, since it is well known that high amounts of phenolic compounds act as pro-oxidants and induce apoptosis [13]. R^2 coefficients (> 0.8) are satisfactory, thus showing a good correlation between concentration and biological effect (Table IV).

Table IV

Cytotoxicity effect of PE on *Daphnia magna*

Sample	Time of determination	LC ₅₀ (µg/mL)	IC _{95%} (µg/mL)	r ²
<i>Pelargonium dry extract</i>	24 h	582.6	270.4 - 1255	0.8449
	48 h	203.3	137.8 - 299.9	0.9716

Conclusions

Pelargonium graveolens L'Hér aerial parts were phytochemically characterized by means of quantitative methods. The raw material was used to obtain a hydro-ethanolic (50% ethanol) dry extract rich in catechin and anthocyanins derivatives. The dry extract showed good antioxidant activity. *Pelargonium* dry extract induced time and dose dependent toxicity upon *Daphnia magna* invertebrates. Future pre-clinical research is needed in order to determine the pharmacotoxicological profile, in view of obtaining a phyto-medicine with potential therapeutic effect in metabolic diseases (diabetes mellitus, dyslipidaemia) treatment.

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