ORIGINAL ARTICLE

PELARGONIUM SP.: CHARACTERIZATION OF THE POLYPHENOLS AND THEIR BIOLOGICAL POTENTIAL

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Abstract

The current study regards the correlation between the biologic activity of two *Pelargonium* species and the spectra of the most important compounds of the samples. *Pelargonium zonale* and *P. hispidum* were not intensely studied, that is why the aim of the study was to correlate the chemical composition - antioxidant activity (DPPH, ABTS, reducing power assays) with the membrane stability of human blood cells. The methanolic extracts were spectrophotometrically analysed (polyphenols and flavonoids) and by using HPLC methods. We calculated the EC_{50} to evaluate the antioxidant activity. Although the differences of the chemical composition of the investigated extracts are not so obvious, *Pelargonium zonale* is twice more active than *Pelargonium hispidum*, fact also sustained by the HPLC results. The stability of the red blood cells proved that, at a low concentration, the extracts have a cytoprotective effect.

Rezumat

Studiul a urmărit evaluarea activităţii biologice a două specii de *Pelargonium* alături de spectrul celor mai importanţi compuşi prezenţi în probele investigate. *P. hispidum* şi *P. zonale* sunt specii puţin investigate pe plan internaţional, de aceea, scopul cercetării a constat în evidenţierea unor corelaţii compoziţie chimică-activitate antioxidantă (*scavenger* al radicalilor DPPH şi ABTS, capacitatea reducătoare) şi a influenţei asupra stabilităţii membranei eritrocitare. Extractele metanolice au fost analizate spectrofotometric (flavonoide şi polifenoli totali) şi prin tehnici HPLC. În vederea cuatificării acţiunii biologice s-a calculat CE_{50} pentru testele antioxidante. Deşi nu există diferenţe semnificative ale compoziţiei chimice a extractelor investigate, *P. zonale* prezintă acţiune biologică aproape de două ori mai intensă decât *P. hispidum*, susţinută şi de rezultatele analizei HPLC. Testul de stabilitate membranară a dovedit că la concentraţii mici, extractele prezintă un efect citoprotector.

Keywords: Pelargonium, polyphenols, antioxidant activity, HPLC

Introduction

Pelargonium species originating from South Africa are related to the *Geranium* oil-producing cultivars. The first *Pelargonium* species were brought since 1600 by various botanists, especially during Victorian times. While *Geranium* species are mostly used in herbal medicine, *Pelargonium sp*. are known due to their specific aroma included in perfumery, cosmetics and aromatherapy products. Leaves, tubers and roots were used in traditional medicine. The investigated *Pelargonium* sp. are mostly herbaceous, perennial and they all have alternate petiolate leaves with stipules. Some species have scented flowers and leaves, thus they can be easily identified [10]. Their water-soluble extracts have a high proportion of tannins that act as antimicrobial and antidiarrheal, whereas the lipophilic extracts have mainly a spasmolytic effect. Many scientists found a direct correlation between the pharmacological activity and the chemical composition of the volatile compounds, but there was still much controversy in this regard. According to the literature, various studies have focused on natural antioxidants. These antioxidants refer to a group of compounds that are able to inhibit or delay the oxidation of lipids or other biomolecules. Thus, they prevent or repair the damage of body cells caused by oxygen species.

These compounds should have, in their structure, hydroxyl groups in order to present a higher activity [4]. In addition, phenolic compounds are plant metabolites that possess an aromatic ring, bearing one or more hydroxyl substituents. We can divide these compounds into sub-groups such as: phenols, phenol acids, phenylpropanoids, flavonoids, flavones, glycoflavones, flavonones, isoflavones, hydrolysables and condensed tannins and quinines, etc. Our investigations showed a low extraction

yield regarding the essential oils; therefore the present paper includes antioxidant assays correlated to the phenolic content of the methanolic extracts.

The present study includes information about two *Pelargonium species*: *zonale* and *hispidum*. First we quantified the total phenolic content of the methanolic extract for each species and we also performed the HPLC analysis. We performed some assays in order to evaluate the antioxidant potential such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-*bis*-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), the reducing power capacity and the stability of the human blood cells assay.

Materials and Methods

Plant material

The subjects of the current research are two *Pelargonium* species*: hispidum and zonale*. The specimens were obtained from the Botanical Garden "Anastasie Fatu", Iasi, Romania. The plants were kept in similar growth conditions to provide a minimum environmental impact.

Preparation of the alcoholic extract

The vegetal extracts were obtained from the dried leaves, which were grounded prior to the extraction. 2 g of each sample were extracted three times with methanol at 85°C, on a thermostated water bath, and were brought to 100 mL in a volumetric flask. The vegetable extracts were dried at 40°C. We used the dried extract for the assays described below.

Determination of total flavonoids content

Total flavonoid concentration was calculated based on the reaction between 5% sodium nitrite, 10% aluminium chloride and 1 M sodium hydroxide with slight modifications. The absorbance was measured at 510 nm [6]. The results were expressed as rutoside equivalents (mg / 100 g dry extract).

Determination of total phenols content

Each sample was mixed with 1 mL Folin-Ciocalteu reagent, allowed to stand for 5 min at 25ºC before adding the rest of the reagents. For 120 min the samples were kept in the dark, before measuring the absorbance at 750 nm. Gallic acid was used as standard. The results were expressed as gallic acid equivalents (GAE) (mg / 100 g dry extract) [14].

Reducing power assay

The sample was mixed with a phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%), incubated at 50ºC for 20 min and trichloroacetic acid (10%) was added to the mixture. After centrifugation at 3000 rpm for 8 min, aliquots of the upper layer were mixed with distilled water and ferric chloride. The absorbance was measured at 700 nm. Gallic acid was used as a standard [11].

DPPH radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay is one of the most extensively used antioxidant assays for raw plant samples. DPPH is a stable free radical that reacts with compounds that can donate hydrogen. The absorption change was measured at 517 nm for 10 minutes. Gallic acid was used as a standard [8].

$ABTS^{\bullet+}$ radical scavenging assay

For the study of biologic activity, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30°C. After each sample extract was added, the absorbance was measured precisely 1 min after the initial mixing for up to 6 min. Gallic acid was used as a standard.

All determinations were carried out three times, and the results represent the mean value. The EC_{50} was calculated for a proper activity correlation [13].

Stability of the human blood cells assay

Heparinised blood was centrifuged at 2500 rpm for 10 min. The RBCs were separated from plasma and buffy coat was washed three times with 0.9% NaCl. The samples underwent the same preparation steps as described by Dzeletovic S. *et al.* Diclofenac was used as a standard [5].

HPLC Analysis

HPLC was performed with a Thermo UltiMate3000 gradient chromatograph controlled by Chromeleon interface, an autosampler, an Accucore XL C18 column (150x4, 6x4) and multidiode array detector (DAD). The mobile phase was made of acetonitrile (A) and water containing 0.1% acetic acid (B), in a linear gradient, increasing from 10% to 70% (A) in 25 min and a flow-rate of 1 mL / min, at 25ºC. The injection volume was 20 µL scanning absorbance wavelengths, from 240 nm to 520 nm, typical for phenols. Standard curves for authentic samples were analytical grade purchased reagents (Sigma Chemical Co.). Samples were obtained by dissolving 0.30 mg of dry extract in 0.5 mL HPLC grade methanol [7]. The final results represent the mean of three to five measurements.

Results and Discussion

The total content of polyphenols and flavonoids are included in the table below.

Table I

The content of the active ingredients of the *Pelargonium hispidum* and *P. zonale*

Samples	Polyphenols content (mg% dry extract)	Flavonoid content (mg% dry extract)
P. hispidum (P1)	2475.92	788.8
P. zonale (P2)	5694.12	770.1

The polyphenols are known as natural antioxidants with important biologic activities [2]. Their structure and concentration usually differs from one plant product to another. Therefore, the chemical analysis by HPLC is of great importance to obtain information about the polyphenol structures found in raw material extracts, although there are limitations in terms of the used detector. Nevertheless, a general spectrum of compounds is more than enough to explain certain bioactivities of the vegetal extracts.

Overall, concentration of *P. zonale* extract was richer in polyphenols, so it is to be expected that the antioxidant potential is higher than *P. hispidum* sample. However, literature data indicates that not only the quantity is relevant for the strength of the activity, but also the type of the structures and their proportion might induce modifications to the biological proprieties of a plant extract [4].

Out of 14 standards used for comparison, we were able to identify: catechin, epicatechin, cyanidol, quercetin-3-arabinoside, quercetol, luteolin, kaempferol and cinnamic acid derivatives. The quantities varied from one sample to another, as indicated in the Table II.

Table II

Compounds found in *Pelargonium hyspidum* and *P. zonale* extracts

Sample	Compound (mg / g dry extract)							
	Catechin	Epicatechin	Cyanidol	Cinnamic deriv.	Quercetin-3-arabinoside	Quercetol	Luteolin	Kaemferol
P. hyspidum	8.7166	1.4218	-	0.0519	3.9962	1.0770	0.4069	0.4199
P. zonale	5.4694	1.0727	2.1378	0.0175	8.0002	4.4602	0.7008	0.1334

Our results showed that *P. zonale* was richer in cyanidol, flavonols and its derivatives, whereas *P. hispidum* contains more catechins. However, apigenin was not detected in any investigated sample, similar to previous research studies [15]. A DAD detector cannot provide all data regarding the compounds in our samples, but in the future we plan to use a much more sensitive detector. The chromatograms samples are presented in Figure 1.

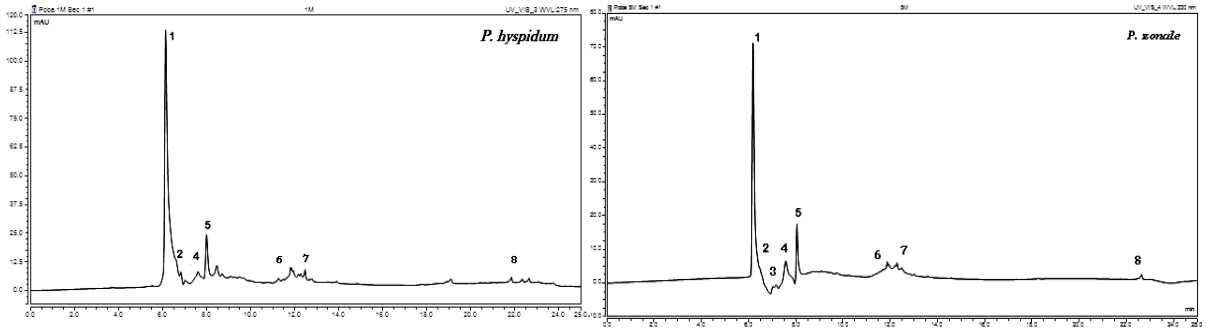

Figure 1.

HPLC chromatograms for the investigated samples

Legend: Catechin (1); Cinnamic deriv.(2); Cyanidol(3); Quercetin-3-arabinoside(4); Epi-catechin(5); Luteolin(6); Quercetol(7); Kaemferol(8).

Our data was in accordance with the literature related to the types of compounds usually found in *Pelargonium species*, but unlike other authors we found only relatively small amounts of tannins in both samples (less than 0.5 mg / g).

The antiradical activity of each methanolic extract was determined using the DPPH and ABTS methods, whereas the reducing potential was quantified by the reducing power assay.

The antioxidant activity of the vegetal extracts was first evaluated as the scavenging capacity towards the DPPH radical [12]. When a strong scavenger reacts with DPPH violet solution at 517 nm, a change to yellow diphenyl-picrylhydrazine compound indicates the existence of the activity. Moreover, the capacity of detaching electrons or hydrogen is another way of expressing compounds' antioxidant ability [9].

Therefore, ABTS method was used to complete the antioxidant profile.

Analysing the results, we can observe that the *P. zonale* sample has a higher activity comparing to *P. hispidum* (Table III). This can be explained by the type and the total amount of polyphenols determined in the samples (Tables I and II). The scavenging activity was expressed as the quantity of the sample that has the capacity to reduce 50% (EC_{50}) of the radicals found in the test tube. On the other hand, a lower value of EC_{50} indicates a higher antioxidant activity (Table III).

Table I

Results in the antioxidants assays: EC_{50} values

Samples	**DPPH assay** EC_{50} (mg / mL)	**ABTS assay** EC_{50} (mg / mL)	**Red. power assay** EC_{50} (mg / mL)
P. hispidum	14.11 ± 0.09	176.99 ± 1.71	448.63 ± 0.57
P. zonale	4.69 ± 0.05	99.51 ± 1.75	124.64 ± 0.67
Gallic acid	4.48 ± 0.03	79.23 ± 0.94	103.21 ± 0.21

In the reducing power assay, the presence of antioxidants in the sample would result in the transformation of Fe^{3+} to Fe^{2+} by donating an electron. The reducing power assay correlated with the results obtained by the DPPH and ABTS assays (Figure 2), showed that the *P. zonale* extract had a stronger activity than *P. hispidum* sample. These activities are due to the presence of phenolic equivalents.

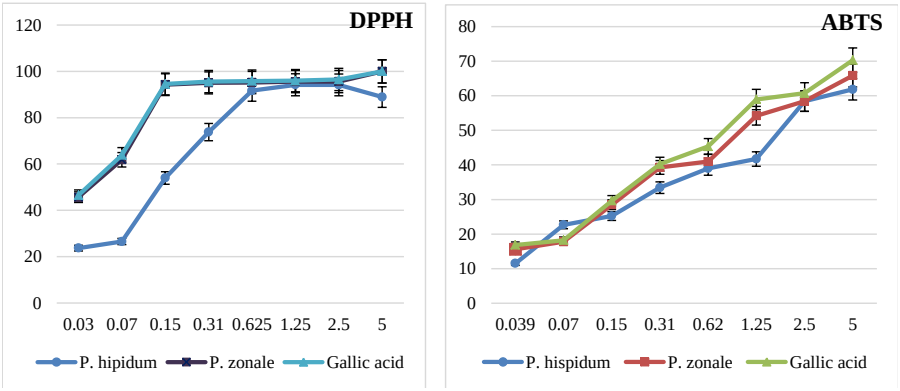

Figure 2.
Graphic representation of inhibition in DPPH and ABTS assays

The reducing capacity of compounds may serve as a significant indicator of its potential. A stronger absorbance indicates increased reducing power, whereas low values signify that the sample has a poor effect of converting free radicals to more stable products [1]. In our study, the reducing power of methanolic extract increased with the dose (Figure 3).

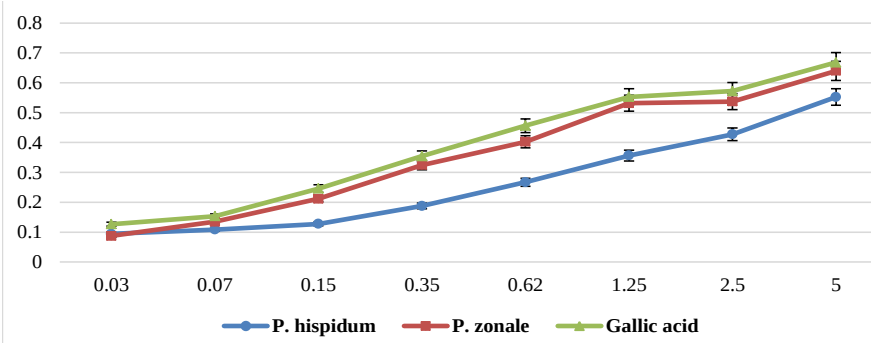

Figure 3.
Reducing power assay absorbance chart

All the results presented above are directly correlated to the total polyphenols content [3]. This was also confirmed by the trend line curve (R^2 values > 0.9). However, the values registered for R^2 related to flavonoid concentration in the samples showed inconsistency in regards to the biological potential.

The membrane stabilization assay on RBCs was used to offer information about the possible anti-inflammatory effect of the studied compounds. The current test is based on increasing the stability of cell membranes by inhibition of proinflammatory compounds released from the cells when using the investigated samples [2]. The results for the stability of cell membranes test are presented in Figure 4. For all tested compounds, we observed that the extracts have a protective activity on the erythrocyte membrane as compared to the standard anti-inflammatory (diclofenac).

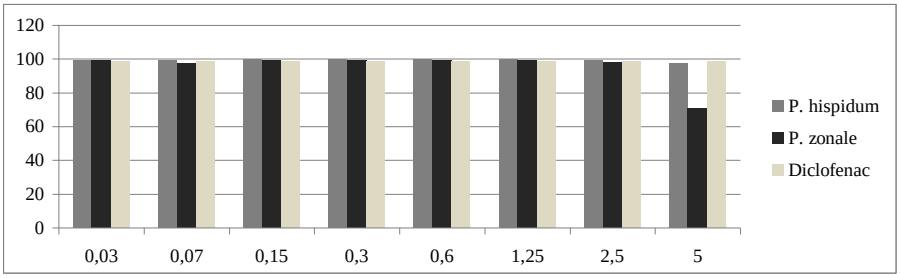

Figure 4.
Stability of the human blood cells assay

Therefore, once again, both *Pelargonium* extracts proved to be good sources of secondary metabolites that act as protectors for the cell membranes. Such results are not uncommon; literature shows that many plant polyphenols with good antioxidant activity can protect the cells against external damage such as toxic compounds or UV rays. We noticed low membrane stability percentage for high concentrations of *P. zonale* extract and we correlated it with the possibility of an interaction between the composition of the extract and the compounds in the cell membrane, but this will be studied in the future.
A noticeable observation is that correlating all quantitative, qualitative and biological test results, *P. zonale* extract is more important regarding the terms of concentration of active compounds (three times higher than the other sample) and in regards to biological potential (at least twice as stronger than *P. hispidum* extract). Even though, both samples have similar potency on the RBCs stability at the same concentrations, the extra value of *P. zonale* extract is given by the previous test results.

Conclusions

Correlating the phenolic contents with antioxidant and protective activities our samples represent natural resources for potential therapeutic preparations, although extracts may give different conclusions depending on the choice of assay, due to different structure-activity relationships between polyphenol substituents. According to HPLC analysis we were able to show the differences between species, but also to prove that the extracts have a significant biologic activity.
Although the compounds are found almost in the same quantity, *P. zonale* extract has cyanidol and quercetol derivatives in its composition, all known as strong cell protective agents. Significant correlations between the presence of proanthocyanidins and the efficient antioxidant capacity sustain the use of *Pelargonium species* in futuer studies.

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