https://doi.org/10.31925/farmacia.2023.2.11 **ORIGINAL ARTICLE**

ANTIOXIDANT AND ANTICANCER EFFECT OF SOME *PELARGONIUM SPECIES* EXTRACTS

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Abstract

Pelargonium species are medicinal plant species containing many polyphenolic compounds, namely flavonoids and phenolic acids. The aim of the present study was to investigate the antioxidant potential of two methanolic extracts of *Pelargonium radens* and *Pelargonium zonale* as scavenger of reactive oxygen species (superoxide radical, hydroxyl radical), and chelating the ferrous iron. Superoxide radical was generated by the reduced form of nicotinamid-adenindinucleotide-ferozinmetosulphate that reduces the tetrazolium nitrate (NBT) to a blue compound measured at 560 nm. Hydroxyl radical was generated by the Fe^{3+}- EDTA/ascorbate Fenton system, and assayed by evaluating deoxyribose degradation using the thiobarbituric acid method. Fe^{2+} combined with ferrozine forms a red complex measured with maximum absorbance at 562 nm that is reduced in the presence of the vegetal extracts. The yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)] is reduced to a violet compound in the presence of a living cells. The absorbance was measured at 550 nm and the cell line used for MTT test was human melanoma cells A375. In the presence of the vegetal extracts the cells viability decreased depending on the concentration. The scratch assay showed the capacity of the extracts to stimulate or reduce the rebound of the cells that were destroyed through a mechanical technique.

Rezumat

Reprezentanții genului *Pelargonium* sunt surse bogate de polifenoli, și anume flavonoide și acizi fenolici. Scopul acestui studiu a fost de a investiga potențialul antioxidant a două extracte metanolice de *Pelargonium radens* și *Pelargonium zonale* ca *scavenger* al speciilor reactive de oxigen (radical superoxid, radical hidroxil) și de chelare a ionului feros. Radicalul superoxid a fost generat de forma redusă de nicotinamid-adenindinucleotidă-ferozinmetosulfat care reduce nitratul de tetrazoliu (NBT) la un compus albastru măsurat la 560 nm. Radicalul hidroxil a fost generat de sistemul Fe^{3+}-EDTA/ascorbat Fenton și a fost testat prin evaluarea degradării dezoxiribozei folosind metoda acidului tiobarbituric. Fe^{2+} combinat cu ferozina formează un complex roșu cu absorbanță maximă la 562 nm care se reduce în prezența extractelor vegetale. Sarea galbenă de tetrazolium [bromură de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazoliu (MTT)] este redusă la un compus violet în prezența celulelor vii. Absorbanța a fost măsurată la 550 nm, iar linia celulară utilizată pentru testul MTT a fost reprezentată de celule de melanom uman A375. În prezența extractelor vegetale viabilitatea celulelor tumorale a scăzut în funcție de concentrație. Testul Scratch a arătat capacitatea extractelor de a stimula sau reduce *rebound*-ul celulelor care au fost distruse printr-o tehnică mecanică.

Keywords: antioxidant, anticancer, human melanoma, *Pelargonium* extracts

Introduction

The bacterial resistance to different classes of chemotherapy is well known; this translates into the impossibility of getting favourable results with certain treatments for infectious diseases [13]. Although there have been periods when different synthetic drugs were considered irreplaceable and eclipsed plant sources, once with the world development and the appearance of many illnesses of the modern civilization, the scientific world once again turned its attention to herbs, hoping to find valuable solutions for modern therapy [31].

Thus, there are several premises that require research regarding the exploitation of natural resources with obvious therapeutic value. Therefore, our attention is focused on easily accessible species on which literature scarce in information from a chemical and

biological point of view, and we started from the information available in the traditional medicine.

Pelargonium species can be found in the history of modern medicine from the time of British Major Stevens, who, in 1897, launched Stevens' Consumption Cure drug, which treated tuberculosis [49]. The experience he had gained in South Africa was the basis for the introduction of umckaloabo preparations (decoction of the roots of *Pelargonium sidoides*), although the first clinical trial was conducted in 1920 on 800 patients [49].

In 1930, it was established the first antituberculostatic treatment before synthetic chemotherapeutic agents start to be used. In this sense, the purpose of this paper is to highlight new sources related to species of *Pelargonium* which would constitute important therapeutic resources [49].

One of the most common source of the oxidative stress is the hydroxyl radical that is generated by the transitional metals. This radical is known as the most aggressive one for the biological structures. However, this radical is neutralised in the presence of reductive compounds such as vitamins or polyphenols compounds. Many studies has shown that, by modulating the high concentrations of iron and copper, decreases tissue aggression induced by reactive oxygen species [17, 39, 40, 50].

Elevated reactive oxygen species (ROS) concentration induces lipid peroxidation, protein oxidation, DNA destruction, as well as mitochondrial dysfunctions. Specific signals are triggered at the cellular level such as the inability to respond to the action of some proteins or the transcription of factors such as p53/ p73, proteins that will eventually lead to apoptosis. In order to avoid them, it is attempted to identify molecules that exhibit cytoprotective effects. According to the literature, flavonoids are substances that exhibit neuroprotective and cardioprotective effects precisely because of the ability to reduce the activity of ROS and the negative effects induced by them. The vegetal extracts are rich in polyphenolic compounds that have also an antioxidant capacity [17, 25, 27, 55].

Pelargonium radens and *Pelargonium zonale* are species belonging to the *Geraniaceae* family, whose essential oil is widely used in cosmetics, aromatherapy and as a food flavouring [17]. The genus *Pelargonium* contains more than 250 species widely distributed in North Africa, and has been widely used in folk medicine to treat various diseases and many studies have reported its chemical composition. According to the literature the pharmacological properties reported for *Pelargonium species*, in general, are probably due to the diversity of chemicals found in the extracts. A wide variety of flavonoids, mainly glycosylated, are also found in the plant. The aglycone unit most present in these compounds are quercetin and kaempferol and some other complex compounds derived of gallic acid are reported. This variety of compounds also justifies the antioxidant activity of the *Pelargonium* extracts, as reported previously by many researchers [19, 48].

The current study is presenting results that we have obtained in the antioxidant and the anticancer assays on the A375 human melanoma cell line.

Materials and Methods

Plant material

We used for this research two *Pelargonium* species: *radens* (PrM) and *zonale* (PzM). Specimens were obtained from the "Anastasie Fătu" Botanical Garden, Iași, Romania. The plants were kept in similar growth conditions to provide minimum environmental impact.

Preparation of the alcoholic extract

The vegetal extracts were obtained from dried leaves which were grounded prior to extraction. 2 g of each sample were extracted three times with methanol at 85°C, on a thermostatic water bath, and were brought to a level of 100 mL in a volumetric flask. The vegetal extracts were dried at 40°C. We used the dried extract for the assays described below [9].

Iron (FeII) chelating activity assay

This test used the method of Dinis *et al*. [16] with minor changes. Briefly, several dilutions (0.0390625 - 5 mg/mL) in DMSO were prepared from the dried extracts. Fe^{2+} in the presence of ferrozine forms a pink complex with absorbance at 562 nm. The presence of a chelating agent in the reaction medium reduces the absorbance of the complex formed. All following steps were as described by Vicaș *et al*. [52].

Superoxide anion radical scavenging capacity

The superoxide anion radical ($O2\cdot$-), generated by the nicotinamide–reduced adenine dinucleotide – ferrozine methosulfate system (NADH-PMS), reduces tetrazole nitrate (NBT) to a blue mixture with absorbance at 560 nm.

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 [33].

Hydroxyl radical assay

The deoxyribose method for determining the scavenging effect of the vegetal extracts on hydroxyl radicals was performed based on the reaction between ascorbic acid, $FeCl_3$, EDTA, H_2O_2, deoxyribose and vegetal extract. Trichloroacetic acid and thiobarbituric acid (TBA) were added afterwards. The absorbance of the resulting solution was measured at 532 nm [12, 50].

All determinations for the antioxidant tests were performed in triplicate, with results expressed as the mean of three determinations ± standard deviation. The IC_{50} (inhibitory concentration 50) value was calculated considering (for each sample, as well as for the control) the first lower value and, respectively, the first value higher than 50%, obtaining by linear

interpolation the chelating concentration that corresponds to an activity of 50%. Well-known antioxidant compound such as gallic acid was used as standard.

Cell viability assay

The evaluation of cell viability by MTT assay is based on the reducing capacity of the vegetal extracts on the MTT reagent. The yellow solution of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) was reduced to violet formazan in the presence of living cells. The absorbance of the solution after solubilization in DMSO was determined spectrophotometrically at 550 nm. The cell line was replicated and incubated for 48 h along with the investigated extracts. The procedure consisted in the technique described by Gaiddonn *et al*. [21]. The presented data is the average of four consecutive determinations.

The concentrations of vegetal extracts (expressed in mg/mL) used in these tests varied from 0.0195; 0.3125; 0.625 to 1.25.

The scratch assay

The evaluation of cell division capacity by using the scratch technique is based on *in vitro* assessment of the ability of plant extracts to stimulate the process of restoring intercellular bonds that were destroyed mechanically. For this test we used human melanoma cell line (A375) that were cultivated in 12 wells plates and then treated with DMSO (control), 0.0195 mg/mL and 1.25 mg/mL methanolic extract of each sample. For quantification purposes the stimulated cells were photographed (10X magnification) at certain times (initially, at 3 h and 24 h, respectively) with DP74 camera built into Olympus IX73 microscope. Further data was calculated with CellSense Dimension soft-ware. [15, 55, 56].

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).

Statistical analysis

Results were presented as mean ± standard error of the mean. Data and biochemical assessment were analysed by one-way ANOVA followed by Tuckey's post hoc multiple comparison test, considering treatment as a factor. For significant results comparison was set at $p < 0.05$.

Results and Discussion

In the previous photodiode array-liquid chromatography (PDA-HPLC) studies, we were able to identify: catechin, epicatechin, cyanidol, quercetin-3-arabinoside, quercetin, luteolin, kaempferol and cinnamic acid derivatives. The quantities varied from one species to another. Our results showed that the methanolic extract of *Pelargonium zonale* was richer in cyanidol, epicatechin, catechin, cinnamic derivatives, quercetin and lutein, whereas the methanolic extract of *Pelargonium radens*, contains more epicatechins [2].

Antioxidant activity evaluation

Natural antioxidants can increase the rate of cell survival by scavenging free radicals, which is not always possible due to the short half-life of radicals such as the hydroxyl radical (about 1 ns) [18]. Under pathological conditions, iron and superoxide metabolism interact to exacerbate each other's toxicity resulting in extensive cellular destruction effects [40].

The components of an oxidative process are the substrate, the oxidizing compound and the initiator of the process, their knowledge allows the identification of the intermediate and final products in order to determine the antioxidant capacity. In the assessment of antioxidant capacity, the sources of reactive oxygen species and the target substrate are the most targeted. Such antioxidant compounds protect lipids from oxidation, but can accelerate the destruction of other biological molecules [22].

The most common procedures that are used to evaluate the antioxidant activity of the molecules are based on the oxidative acceleration method using an oxidative process initiator to manipulate several variables in the oxidative reactions [42]. These initiators were oxygen, temperature and pressure, concurrently with environmental exposure to a metal accelerator, but also exposure to ultraviolet rays to favour singlet oxygen photosensitized oxidation [10].

Among the most common reaction activators are copper and iron. These ions have the properties of enzyme cofactors, so that the enzymes involved in oxidative processes are activated and increase the reaction speed [11].

For the *in vitro* determination of oxygen free radical species, the methods were adapted to allow the quantification of some primary products of the redox reactions.

Iron (FeII) chelating activity assay

Iron is an indispensable element for the human body considering its presence in the structure of compounds with biological activity such as haemoglobin, myoglobin, cytochromes and heminic enzymes. In the structure of the respective compounds, being found in the form of ferric or ferrous ions, the ions that are in a free state can become promoters of oxidation reactions with negative consequences for biological structures [2, 24]. Controlling the amount of free Fe^{2+} or Fe^{3+} ions at the cellular or plasma level represents an indirect way of controlling oxidative processes [8, 20]. Chelation of the ferrous ion confers protection against oxidative stress by reducing its concentration in the reaction medium, which leads to the decrease of hydroxyl radicals normally generated following Fenton-type reactions [24]. In this context, the present test evaluates the ability of *Pelargonium* extracts to compete with ferrozine to chelate ferrous ions present in the reaction medium. The results obtained high-

lighted the remarkable binding capacity of iron ions, thus suggesting that the investigated extracts can block peroxidation based on chelating mechanisms.

Table I

Inhibition percentage and the IC_{50} values of the methanolic extracts on the iron (FeII) chelating activity assay

Conc. (mg/mL)	Samples – inhibition % ± standard deviation		
	PrM	PzM	Gallic acid
0.0390625	12.26 ± 0.02	13.81 ± 0.84	13.42 ± 0.41
0.078125	14.43 ± 0.29	15.32 ± 0.98	14.32 ± 0.36
0.15625	15.02 ± 1.12	19.47 ± 1.06	18.08 ± 0.13
0.3125	16.93 ± 1.32	22.47 ± 1.34	20.69 ± 0.68
0.625	21.07 ± 0.97	29.98 ± 0.64	25.97 ± 0.64
1.25	22.03 ± 0.46	57.20 ± 0.25	44.35 ± 0.94
2.5	41.41 ± 0.39	69.67 ± 0.71	52.22 ± 0.31
5	71.50 ± 0.37	70.35 ± 0.59	67.52 ± 0.31
IC_{50} (µg/mL)	30.46 ± 0.51	77.06 ± 0.34	19.14 ± 0.09

These data support what is already known from previous research published worldwide with reference to the special chelating capacity of flavonoid compounds present in plant extracts [20]. Due to the structural conformation, flavonoids can bind transition metals in the form of complexes, increasing their hydrophilic character and thereby the elimination rate from the body. In this way, the complexes formed are extremely stable blocking the induction of pro-oxidant compounds.

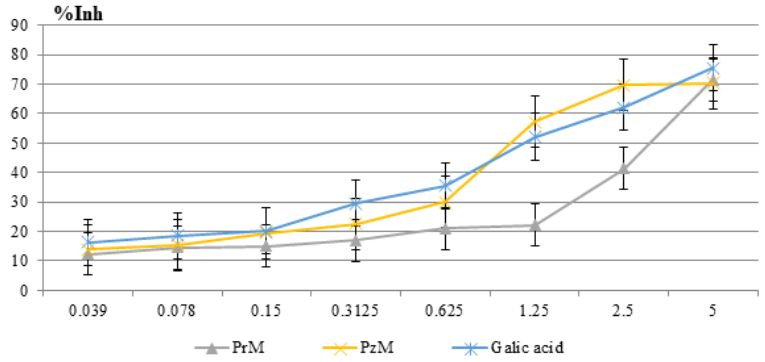

Figure 1.

Graphic representation of the inhibition percentage of the methanolic extracts on the iron (FeII) chelating activity assay

Also, natural metabolites containing two or more of the following functional groups (OH, SH, COOH, PO_3H_2, C=O, NR_2, S-, O-) favourably conformationally positioned, exhibit marked chelating potential. Such compounds are frequently found in phytocomplexes isolated from vegetal products and are quantified under the generic term of total polyphenols or reducing compounds [22, 25]. The methanolic extracts that we investigated are rich in such compounds so it was possible to correlate the concentration in total polyphenols with the IC_{50} values obtained in the ferrous ion chelation test. In Figure 1 it is illustrated that the methanolic extract of *Pelargonium zonale* has a higher chelating potential than the methanolic extract of *Pelargonium radens*.

Superoxide anion radical scavenging capacity

The superoxide radical anion is produced in the human body in both physiological and pathological conditions, it is also involved in the appearance of some pathological situations and in the acceleration of the body's aging process [26, 37].

From a physiological point of view, the synthesis of the superoxide radical anion is quantitatively limited, thus it is considered that a maximum of 4% of the amount of oxygen that reaches the mitochondrial level can be transformed into the superoxide radical anion (at the level of complexes I and III in the respiratory chain) which will be transformed by the mitochondrial superoxide dismutase [7].

Another physiological pathway of superoxide radical anion synthesis is catalysed by xanthine oxidase, the most important enzyme used for uric acid synthesis. On the other hand, the superoxide radical anion is produced by phagocytes in the immune system and is used to destroy microorganisms that have entered the human body.

The imbalance between the production of superoxide radical anion and the ability of superoxide dismutase to transform it determines the intensification of oxidative

processes at the cellular level with damage to lipids, especially unsaturated ones, proteins, and DNA [27, 33]. Under these conditions, it is necessary to support the endogenous antioxidant systems by administering antioxidant compounds capable of neutralizing the superoxide radical anion, a category in which compounds of plant origin such as polyphenols fall [25].

Plants are recognized as sources of protective active principles, they synthesize such substances to survive in environmental conditions [47, 48, 50].

Thus, plant extracts have a high content in compounds with polyphenolic structures and more, which have the ability to control oxidation processes in the body and reduce the risk of developing various pathologies resulting from them [27]. It has been shown that a series of antioxidant compounds can be isolated from various plant products, of which the most frequently encountered are catechin, quercetin/rutoside, chlorogenic acid or caffeic acid [41].

For the scavenger capacity of the methanolic extracts PrM and PzM on the superoxide anion radical, the inhibitory concentration 50 (IC_{50}) was calculated. The results we obtained at this assay are showed in the Table II.

The lowest intensity of action was clearly observed for PrM, which ranks last, the inhibition intensity suffering visible inflections compared to the upward trend of the other sample.

Table II

Inhibition percentage and the IC_{50} values of the methanolic extracts of the superoxide anion radical scavenging capacity

Conc. (mg/mL)	Samples – inhibition % ± standard deviation		
	PrM	PzM	Galic acid
0.0390625	7.07 ± 1.27	18.12 ± 0.69	22.82 ± 0.08
0.078125	21.25 ± 0.88	32.23 ± 0.68	29.44 ± 0.57
0.15625	23.07 ± 1.62	47.08 ± 0.96	36.26 ± 0.31
0.3125	38.56 ± 0.64	59.67 ± 0.58	45.29 ± 0.97
0.625	47.49 ± 0.93	70.77 ± 1.34	56.82 ± 0.12
1.25	51.56 ± 1.13	82.60 ± 1.09	67.45 ± 0.34
2.5	71.08 ± 1.07	90.93 ± 0.64	78.30 ± 0.01
5	75.19 ± 0.66	99.64 ± 0.58	95.56 ± 0.34
IC_{50} (µg/mL)	95.83 ± 0.67	18.34 ± 0.44	41.46 ± 0.09

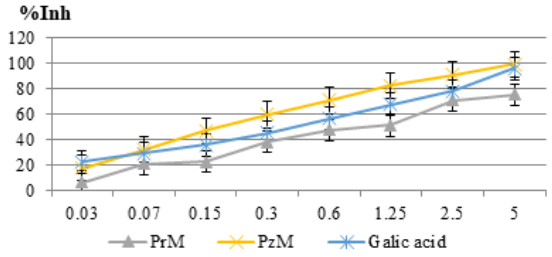

Figure 2.

Graphic representation of the inhibition percentage of the methanolic extracts of the superoxide anion radical scavenging capacity

Hydroxyl radical assay

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl (OH), peroxyl, and alkoxyl radicals may attack biological macromolecules giving rise to oxidative stress originated diseases. Since OH is very short lived, secondary products resulting from OH action on various sample are measured [5]. The hydroxyl radical (OH) is the most reactive product of ROS formed by successive 1-electron reductions of molecular oxygen (O_2) in cell metabolism, and is primarily responsible for the cytotoxic effects observed in aerobic organisms extending from bacteria to plants and animals. It is generally assumed that OH is generated in biological systems from H_2O_2 by the Fenton reaction [53]. Oxidative attack of hydroxyl radicals generated from such a Fenton reaction on deoxyribose produces malondialdehyde (MDA) and similar substances that are colourimetrically reactive toward thiobarbituric acid, forming the essence of OH detection [3]. The inhibition percentage and the IC_{50} results of the PrM and PzM are presented in Table III.

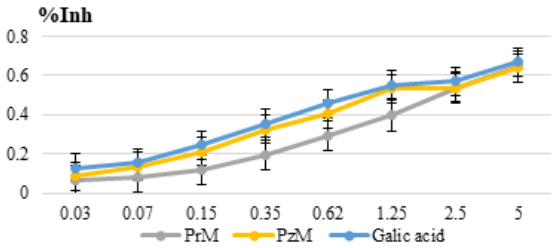

Figure 3.

Graphic representation of the inhibition percentage of the methanolic extracts on the hydroxyl radical assay

Hydroxyl radicals generated by iron-EDTA + H_2O_2 in the presence of ascorbic acid oxidize deoxyribose to a pink chromogen with maximum absorbance at 532 nm. Hydroxyl radical scavengers added to the medium compete with the deoxyribose and diminish

chromogen formation, enabling the calculation of second order rate constants of OH scavenging [39].

According to the results, the methanolic extract of PzM has a higher capacity of scavenging the hydroxyl radical. This might be linked to the higher concentration of polyphenolic compounds in this extract. These results are also showed in the graphic representation in Figure 3. Correlating both, the plant source and the type of extract used in the study, for this test it was revealed that both variables influence the intensity of action. Also, the most important determining factor remains the concentration of active principles of the vegetal sample, since in methanol we could extract a different number of polyphenolic compounds.

Table III

Inhibition percentage and the IC_{50} values of the methanolic extracts on the hydroxyl radical assay

Conc. (mg/mL)	Samples – inhibition % ± standard deviation		
	PrM	PzM	Galic acid
0.0390625	0.0649 ± 0.94	0.0872 ± 0.64	0.1286 ± 0.06
0.078125	0.0781 ± 0.74	0.1348 ± 0.58	0.1534 ± 0.52
0.15625	0.1183 ± 0.34	0.2118 ± 0.28	0.2461 ± 0.25
0.3125	0.1920 ± 0.61	0.3246 ± 0.57	0.3542 ± 0.28
0.625	0.2943 ± 0.66	0.4024 ± 0.37	0.4563 ± 0.12
1.25	0.3955 ± 1.06	0.5317 ± 0.84	0.5527 ± 0.01
2.5	0.5386 ± 0.37	0.5371 ± 0.74	0.5724 ± 0.07
5	0.6449 ± 0.61	0.6402 ± 1.09	0.6682 ± 0.22
IC_{50} (µg/mL)	24.563 ± 0.59	12.464 ± 0.67	17.943 ± 0.58

Cytoprotective activity and scratch assay

Considering the cytoprotective and antioxidant properties of the polyphenols and their presence in the extracts, we proposed to investigate these effects for the methanolic extracts from the *Pelargonium species*. The methanolic extracts have a much higher flavonoid content compared to the ethanolic ones as showed in the literature, but also are rich in other phenolic derivatives (caffeic acid, gallic acid, cyanidol derivatives and catechin compounds) with remarkable antioxidant properties [34]. The cell line we focused on is A375 – cells isolated from human melanoma. The studies on such tumour cells aim both at the identification of molecules with an antitumor effect, but also at the apoptotic or necrotic mechanisms that can take place in the tumour cells. *In vitro* studies on A375 cells have shown that high NO production attenuates tumour cell proliferation by inhibiting DNA synthesis [51, 54]. The cytostatic effect of NO is probably attributed to a series of biochemical reactions at the cellular level such as inhibition of mitochondrial activity, blocking of DNA synthesis or interference with sulphide proteins [1].

According to literature data, compounds that promote NO release are intensively studied for their antitumour and cytostatic effect. Analysing the results of the studies related to the antitumor properties of vegetal compounds, it is observed that gallic acid derivatives favour the release of NO [30].

Starting from these data and from the results obtained from the phytochemical analysis that revealed the fact that the analysed extracts are rich in gallic acid derivatives, we performed the cell viability test on the A375 line to complete the biological profile of the methanolic extracts of *P. radens* and *P. zonale*. The test also sought to establish a correlation between the polyphenol's concentration in the extracts and the effect of these extracts of different concentrations on the division of human melanoma cells [38].

Currently, there are various techniques that can be used to determine the influence of substances or plant extracts on cell viability [55]. However, each type of test presents advantages and disadvantages, some being faster, more sensitive, presenting a less laborious technique, being equally much more expensive. The tests used in the present research, however, have the advantage of being more accessible and allow obtaining conclusive results. Obtaining the most complete information regarding the biological activity of some plant extracts requires performing tests that are relevant regarding the mechanisms of action and the molecular targets of the compounds present in these extracts. That is why cell viability tests are an important indicator in guiding studies regarding the action of the compounds present in the extracts on general metabolism or enzyme activity as a predictor of cell viability.

The test is carried out by incubating the substrate with live cells when a signal is generated proportional to the number of cells that are present and have retained their viability [23]. Through their apoptosis, a certain amount of substrate remains unchanged, which leads to the appearance of spectrophotometrically detectable colour differences. Tetrazolium reduction assays were the first used to demonstrate cell viability in the format known today (96-well).

MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazole bromide) is a positively charged molecule that can cross the membrane of nucleated cells without external intervention [5]. That is why the MTT technique is widely used for the purpose of preliminary *in vitro* testing of vegetal extracts. At the cellular level MTT

is converted by reduction into a violet-coloured formazan derivative that has a maximum absorbance at 570 nm [21].

The installation of apoptosis no longer allows the transformation of MTT, so the coloured compound (formazan) can be considered a marker of cell viability. It has been postulated that the electron transfer reduction reaction of MTT involves the participation of NADH or compounds with similar potential. On the other hand, various researchers believe that this transformation is catalysed by mitochondrial enzymes [35].

MTT reduction is a reaction that gives information on the percentage of cells that retain their viability, which is not the same as detecting cell proliferation [45]. The viability of A375 cells in the presence of methanolic extracts of the *Pelargonium* species studied are presented in Figure 4.

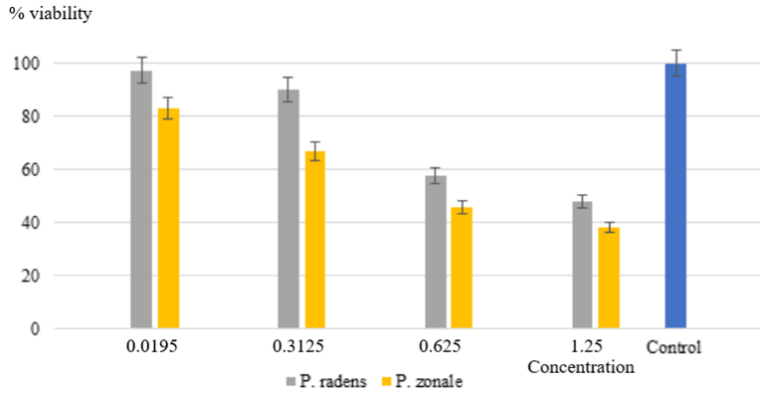

Figure 4.

Graphic representation of the cell viability of the A 375 cell line in the presence of the methanolic extracts

The results obtained for the concentrations of the extracts of 0.625 and 1.25 mg/mL are statistically significant ($p < 0.01$).

A375 are cells isolated from human melanoma and have attracted the interest of many researchers because they are useful in studies to identify compounds with cytostatic effects.

As can be seen by analysing the graphic, the percentage of viability in the presence of methanolic extracts at the highest concentration is below 50% for PrM extract, and below 40% in the case of PzM extract. The strongest inhibitory action in the case of PzM extract could be correlated with the content of active principles, thus PzM contains the highest amount of polyphenols (5694.12 mg%), as we presented in a previous study that we published [29].

In order to highlight a clearer correlation between these compounds and their cytoprotective/cytotoxic effect, much more in-depth further studies are needed.

The compounds directly responsible for this biological activity are of polyphenolic type and are generally those that show antioxidant potential through their reducing properties.

Assessment of cell division capacity by using the Scratch technique

Medicinal plants are frequently used in the treatment of skin diseases and especially skin lesions [6]. Since ancient times plants and herbal pharmaceuticals have been used very frequently in the treatment of wounds [46, 56]. The process of restoring the damaged tissue is very complex and includes a long series of local biochemical reactions and at the level of the whole organism. This process is initiated by inflammation and continues with the formation and remodelling of new tissue [36].

The scratch test involves a series of steps, namely the creation of a line called a scratch at the confluence area between cells, followed by the migration of cells to the area of the newly created line in order to restore that area, and finally the re-establishment of new connections between cells [32]. For this purpose, a scratch is made, then the microscopic image is photographed at the beginning of the experiment and at regular time intervals to determine the proliferation and movement of the cells, and finally the images are compared to establish the influence of the test compound on the cells used in the test [54]. This technique is an *in vitro* wound healing method and is used to determine the effect of test substances on the ability of cells to migrate.

The A375 cell line is a cell line isolated from human melanoma, a tumour developed at the level of melanocytes and characterized by a very high invasive capacity and risk of metastasis. The most common forms of melanoma are those of ectodermal origin and those of cutaneous nature [14].

The therapeutic approach to human melanoma is difficult due to the lack of cellular sensitivity to radiotherapy, so the tumour develops rapidly and requires the administration of immunosuppressants or alkylating agents [44]. Added to these are the severe side effects and cellular non-selectivity of chemotherapeutic compounds. All this led to the continuous attempt of scientists to identify and isolate

from different plant extracts, compounds with significant biological activity on different types of malignant cell lines [4]. Considering the results obtained in the previous tests, both antioxidant tests and cell viability tests, we performed Scratch test on the methanolic extracts using two concentrations 0.0195 mg/mL and 1.25 mg/mL and we photographed the plates with camera built into the microscope. The results that we obtained are presented in Figure 5.

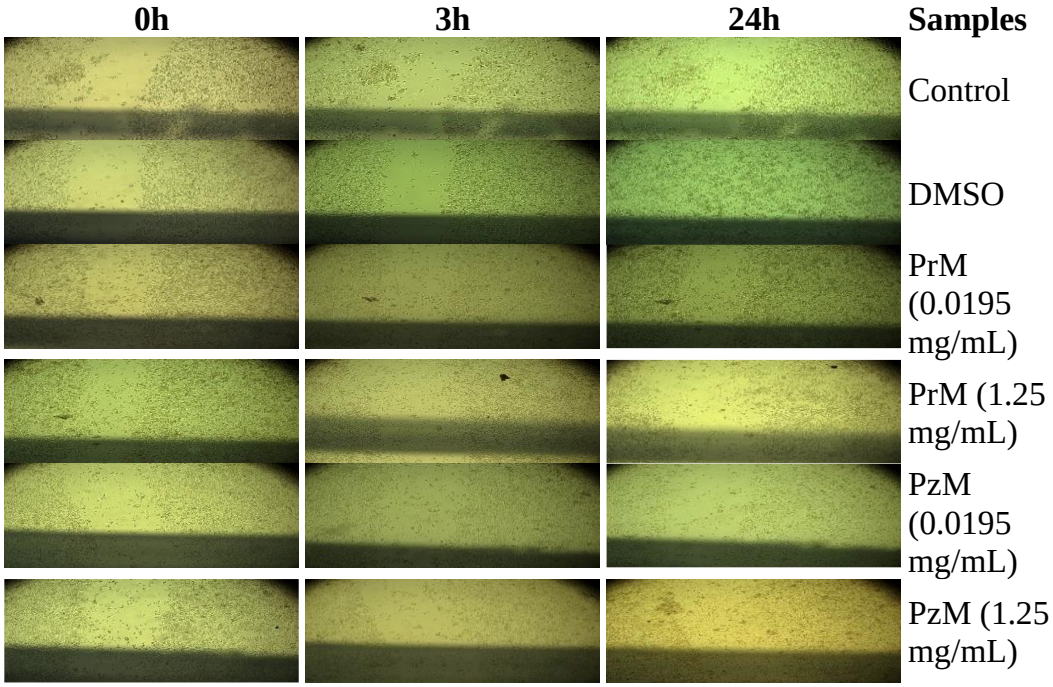

Figure 5.
The antimigratory effect of the methanolic extracts on the A375 cell line

According to the images in Figure 5, the methanolic extracts of *Pelaronium* species have an antimigratory effect on A375 cells, the most conclusive results can be seen after 24 h. As expected, at the concentration of 1.25 mg/mL the effect is much more obvious compared to 0.0195 mg/mL.

In the case of the plates treated with methanolic extract of *Pelargonium radens* (PrM) and *Pelargonium zonale* (PzM) it can be observed much more clearly at the concentration of 1.25 mg/mL that the cells showed a decrease in density and a reduction in the aggregation capacity, the effect being more intense at PzM. According to another study that aimed to test different types of vegetal extracts, the methanolic extracts helps in reducing the cancer cells number as they become round and displayed the apoptotic like features [43].

Conclusions

Due to the numerous mechanisms involved in oxidation, plant extracts and natural secondary metabolites represent the best option as antioxidants. As proven by the obtained results, the investigated *Pelargonium* extracts possess good antioxidant effects based on their multiple targets and capabilities to interact with different oxidizing agents, thus blocking the conversion into reactive species.

In order to evaluate metabolic markers and to determine the viability of cells exposed to the action of different plant extracts, a wide range of biological tests can be used, each presenting advantages and disadvantages.

The results obtained after performing these tests demonstrated that the degree of cytotoxicity of the extracts is closely related to their chemical composition.

The MTT test is based on an oxidation-reduction reaction, but which also involves the activity of mitochondrial enzymes from the studied cells. It should be noted that this test does not offer the possibility of accurately establishing the mechanisms that take place at the cellular level, especially in the case of cancer cells that have a much more alert development rate and in which the metabolic processes take place according to less strict rules. The test provides indicative information on the cytotoxic/cyto-protective effect of the plant extracts and it is necessary to carry out other tests at the same time to evaluate the intervention at the cellular metabolism level of the compounds of plant origin.

Conflict of interest

The authors declare no conflict of interest.

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