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Antibacterial and antitumor activity of the species *Prunella vulgaris L.*

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Abstract

***Background**: Prunella vulgaris L., known as self-healing herb, is a widely spread species in the spontaneous flora with beneficial effects on human health, a fact proven in particular by Asian researchers. The aim of this study was to evaluate the antitumor activity and the antibacterial effect on different bacterial strains, including multidrug-resistant ones, depending on the type of solvent used (aqueous, hydroalcoholic), the plant product taken into consideration (spike inflorescence, leaf), its quantity and the concentration of active principles. **Material and method**: For screening of antimicrobial susceptibility, both minimum inhibitory concentration and minimum bactericidal concentration were determined on Escherichia coli, Klebsiella pneumoniae, Providencia stuartii, Pseu-*

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domonas aeruginosai, and methicillin-resistant Staphylococcus aureus species, including reference strains and hospital strains. Leaves and flower extracts (aqueous and 70% methanolic) were first assessed, and the one with the best antibacterial potential was further tested as a concentrated extract. The antitumor activity was determined on MDA-MB-231 breast adenocarcinoma cells and on a non-tumor cell line, MCF-10A breast epithelial cells by means of Alamar blue technique and Scratch assay. ***Results****: Inflorescence extracts showed better bacteriostatic effects than leaf extracts on most bacteria, in both aqueous and hydroalcoholic extracts. The concentrated extract of spike inflorescence showed measurable activity with good effects on Gram-positive bacteria, but also on multidrug-resistant Gram-negative ones. The 70% methanolic extract of the species Prunella vulgaris L. (spike inflorescence) demonstrated a concentration-dependent cytotoxic and anti-migratory activity on MDA-MB-231 breast cancer cells, while affecting the non-tumor cell line less.* ***Conclusions****: The results suggest that Prunella vulgaris extracts present antibacterial potential in the complementary treatment of multidrug-resistant infections. The extract from the spike inflorescence of Prunella vulgaris L. produced a dose and time-dependent reduction in cell viability and migration, eliciting a stronger effect on the breast adenocarcinoma cell line.*

Keywords: *Prunella vulgaris L., antibacterial, antitumor*

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Introduction

The increased interest for the species *Prunella vulgaris* L., commonly used in traditional medicine in some countries in Europe, Asia, America, and Australia, has contributed to the intensification of phytochemical and pharmacological studies in order to identify bioactive compounds, justify the use in traditional medicine and develop standardized extracts with constant, reproducible effects (1,2). *Prunella vulgaris* L. grows in the spontaneous flora of Romania in humid places, fields, meadows, grasslands, unpopulated areas, and unpaved lawns, both in the sun and in the shade (3). The species under consideration presents an important source of active principles with numerous pharmacological actions. *Prunella vulgaris* L. has multiple therapeutic benefits including antimicrobial and antitumor action (4-6).

Studies on the action of aqueous, alcoholic and hydroalcoholic extract have highlighted the antimicrobial, antifungal, and antiviral properties. The aqueous and hydroalcoholic extract of the species *Prunella vulgaris* L. revealed the antibacterial effect on *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris*, and on different *Staphylococcus* species (7-9). The methanolic extract of *Prunella vulgaris* L. (root) has demonstrated *in vitro* antiproliferative activity on different breast cancer cell lines: MCF-7, MDA-MB-231BO. The study conducted by Roh et al. on MCF-7 cell line demonstrated that cell growth was stopped, the cell apoptosis was induced and the pathway PI3K/ AKT was modulated, which is an important target for anticancer agents as it is an activated pathway in cancer cells, especially in ovarian, breast, and urothelial cancers. The results of the aqueous extract of the spiciform inflorescences (10% V/ V) indicated an increased action on skin, maintaining the skin integrity by inhibiting 11ß- hydroxysteroid dehydrogenase 1 (11ß - HSD1) activation, an enzyme that helps skin cells synthesize cortisol) (4,10,11). Considering the favorable biological effects, these natural extracts could be alternative sources of compounds that can be used in infection control. Currently there are reported studies conducted mainly with the herbal drugs collected from China, Czech Republic, South Korea, etc. However,

to our knowledge, no studies were conducted with the herbal drug collected in Romania. As it is known, climatic factors and geographical conditions can exert a high influence upon the chemical composition of plants. Considering that differences between the same species from different regions of the globe are to be expected, the present research aimed to investigate the phytochemical profile and pharmacological actions of *Prunella vulgaris* L. from Romania and to compare the outcome with results from other studies conducted on the same species harvested from various world regions.

Materials and Methods

HPLC/MS analysis

Sample preparation

Two grams of crushed spike inflorescence were mixed with either 100 mL of 70% methanolic solution or with 100 mL ultrapure water. The sample was placed in an ultrasonic bath at 40°C for 60 minutes. After cooling, the extracts were filtered and stored at -50°C until analysis.

Chromatographic and mass spectrometry conditions

Rosmarinic acid was identified and quantified using a validated LC-MS analytical method described by Vlase et al (12,13). The equipment that was used was an Agilent 1100 HPLC Series system (Agilent, Santa Clara, CA, USA) equipped with a binary pump, auto sampler, column thermostat (set at 48°C), and UV detector. The mass spectrometer was an Agilent Ion Trap 1100 SL (LC/MSD Ion Trap VL, Agilent, Santa Clara, CA, USA) equipped with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The injection volume was 3 µL and the flow rate was 1 mL/ min. Compound separation was performed on a reverse-phase analytical column (Zorbax SB-C18 100 X 3.0 mm i.d., 3.5 µm particle). The elution solvents were 1 mM ammonium acetate (A) and acetonitrile (B) in water. Samples were eluted according to a linear gradient: 0–3.3 min, 5–25% B; 3.3–3.4 min, 25–90% B; 3.4–3.8 min, 90% isocratic B; 3.8–3.9 min, 90-5% B. The MS with ESI source operated in negative ionization mode and was set for fragmentation and isolation of deprotonated rosmarinic acid molecule with *m/z* = 359. The quantification of this bioactive compound was assessed based on the deprotonated molecule.

The calibration curve of rosmarinic acid was performed in the concentration range of 0.095-1.520 µg / ml. All solutions were prepared in ultrapure water.

Antibacterial effect

The inhibitory (MIC) and bactericidal (MBC) activity of the extracts were assessed on seven bacterial strains which are representative for Gram-positive (*Staphylococcus aureus* ATCC 43300 - MRSA, *Staphylococcus aureus* ATCC 25923 - MSSA) and Gram-negative (*Klebsiella pneumoniae* ATCC13883, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, multidrug resistant *Klebsiella pneumoniae*, multidrug resistant *Providencia stuartii*) bacteria, including reference strains and multidrug-resistant clinical isolates (Table 1).

The plant extracts were sterilized by filtration using 0.45µ Whatman 25mm syringe filters (GE Healthcare).

The MIC was assessed by microdilution method, based on CLSI 2018 standard 11th edition, document M07 (14). Shortly, in a sterile 96-well plate, each well of the first column was filled with 200 µl of each plant extract. From each extract, 100µl were subsequently diluted with 100µl sterile water in the remaining wells from each row. Each well of the plate was then filled with a bacterial inoculum (approximately 10^4 CFU/ml) in 2x concentrated Muller-Hinton broth. One plate was prepared for each bacterial strain. Pos-

Table 1. Characteristics of the clinical isolates

Strain	Source	Susceptible	Resistant
Klebsiella pneumoniae	Respiratory tract secretion	Tigecycline, Trimethoprim	Ticarcillin/clavulanic acid, Piperacillin, Cefuroxime, Cefixime, Ceftriaxone, Cefepime, Aztreonam, Meropenem, Levofloxacin, Moxifloxacin, Minocycline
Providencia stuartii	Respiratory tract secretion	Aztreonam	Ticarcillin/clavulanic acid, Piperacillin, Ceftazidime, Cefepime, Aztreonam, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Minocycline, Trimethoprim/ sulfamethoxazole

itive and negative growth controls were used for all bacteria. The plates were incubated at 35°C overnight. The MICs were interpreted by visual reading in the last well that did not show bacterial growth.

The MBCs were assessed by inoculating 1 µl from the last wells that did not show bacterial growth in the MIC plate in the wells of a new plate that contained 100 µl Muller-Hinton broth/ well. The MBCs were interpreted similarly as for MIC, by visual reading, in the last well that did not show bacterial growth.

In vitro antitumoral effects of Prunella vulgaris extract

Cell culture

MDA-MB-231 – human breast adenocarcinoma cell line (ATCC® HTB-26™) and MCF-10A - non-tumorigenic breast epithelial cell line (ATCC® CRL-10317 ™) were purchased from the American Type Culture Collection (ATCC). MDA-MB-231 cells were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich), supplemented with 10% fetal bovine serum (FBS; Gibco, ThermoFisher Scientific) and 1% Penicillin/ Streptomycin mixture (Pen/Strep, 10,000 IU/ml; Sigma-Aldrich). MCF-10A cells were cultured in 1:1 mixture DMEM:F-12 medium (ATCC) supplemented with 20ng/mL epidermal growth factor (EGF; Gibco, ThermoFisher Scientific), 500 ng/mL hydrocortisone (Sigma-Aldrich), 0.01 ng/mL insulin (Sigma-Aldrich), 500 ng/mL hydrocortisone (Sigma-Aldrich), 5% FBS and 1% Pen/Strep mixture. The culture plates were kept in standard conditions, 37°C in a humidified atmosphere containing 5% CO_2 and passaged three times a week.

Alamar blue assay

Alamar blue is a technique used for cell viability assessment. The principle of the assay consists of the ability of metabolically active cells (viable cells) to reduce resazurin (the dark blue compound) to resorufin (the pink compound with an intense fluorescence). The assay was conducted as previously described (15). Briefly, a number of 1×10^4cells/ well were plated in 96-well plates and allowed to adhere overnight. Afterwards, the cells were stimulated with different concentrations (1, 10, 50, 100, 250, 500 and 750 µg/ mL) of *Prunella vulgaris* extract (spike inflorescence) and incubated for 24, 48, and 72h. Control cells were stimulated with the same amount of dimethyl sulfoxide (DMSO), the solvent used for the preparation of the stock solution. After the incubation period, Alamar blue (20 *µL*/well - 10% of the volume of cell culture medium) was added and the cells were incubated at 37°C for 3h. After that, the absorbance was measured spectrophotometrically at 570 and 600 nm with a microplate reader (xMark Microplate Spectrophotometer, Bio-Rad).

Scratch assay

This *in vitro* method evaluates the anti-migratory potential of *Prunella vulgaris* extract (spike inflorescence) on the two cell lines. The assay was conducted as previously described (16). Briefly, a number of 2×10^5 cells/ well were cultured in 12-well plates until 90% confluence was reached. A sterile pipette tip was used to draw scratches on well-defined zones of the cells monolayer and the detached cells were removed by washing with phosphate-buffered saline (PBS) prior to stimulation. Cells were stimulated with different concentrations (1, 10, 50, 100 and 250 µg/mL) of *Prunella vulgaris* extract (spike inflorescence). Images of the cells in culture were taken at the starting point of the experiment (0h) and after 24h and were compared to Control cells (cells stimulated with the solvent DMSO) using Olympus IX73 inverted microscope provided with DP74 camera (Olympus, Tokyo, Japan). Cell growth analysis was evaluated with cell Sense Dimension software. The scratch closure rate was calculated according to the formula (15):

$$\text{Scratch closure rate} = \left[\frac{A_{t0} - A_t}{A_{t0}}\right] * 100$$

where: A_{t0} is the scratch area at time 0; A_t is the scratch area at 24h.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). One-way ANOVA test followed by Dunnett's multiple comparison test was used for comparison among the groups for the *in vitro* antitumor effects. A p value of ≤ 0.05 was considered to be of statistical significance. For the statistical analysis GraphPad Prism 5 was used.

Results

HPLC/MS analysis

An HPLC-MS method was used for the quantification of rosmarinic acid in the extracts from *P. vulgaris* spike.

The recorded full-scan mass spectrum of a rosmarinic acid solution is shown in figure 1. The expected ion, according to the molecular mass of the rosmarinic acid (M = 360.2) and depending on the ionization mode (negative) would be the ion with *m/z* 359, corresponding to the deprotonated molecule.

In order to increase the selectivity of the LC/MS method, fragmentation of the characteristic rosmarinic acid ion (*m/z* 359) was performed and the MS spectrum was recorded (Figure 2).

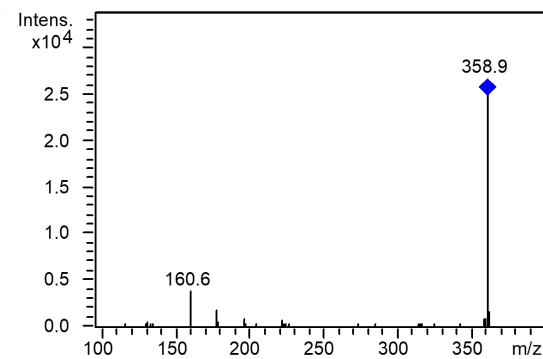

Fig. 1. Full-scan spectrum of rosmarinic acid in mobile phase

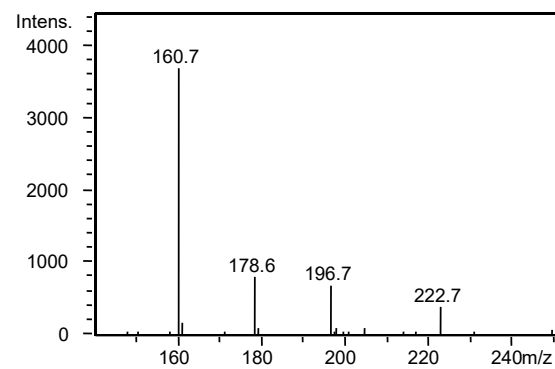

Fig. 2. MS/MS spectrum of rosmarinic acid in mobile phase

By fragmentation, the rosmarinic acid is disintegrated into four main fragments, with m / z 160.7, 178.6, 196.7 respectively 222.7.
Following the HPLC-MS determination, 27.69 µg rosmarinic acid / mL (1.38 mg rosmarinic acid/g dry material plant) was obtained in the aqueous extract of the crushed spike, and 97.19 µg rosmarinic acid / mL (4.85 mg rosmarinic acid/g dry material plant) in the 70% methanolic extract.

Antibacterial effect

We tested the antibacterial activity of two different *Prunella* extracts: aqueous solution, methanolic extract and concentrated aqueous solution after initial extraction with methanol. Leaves and flower extracts were first assessed and the one with the best antibacterial potential was further used as concentrated extract. No significant differences were found between leaf and flower aqueous extracts, both types presenting low antibacterial effect, with inhibitory concentrations at values ranging from 25 to more than 50 mg/ml. Both Gram-positive and Gram-negative bacteria presented a quasi-equal behavior to the tested substances. The methanolic extracts presented the best antibacterial effect, with MICs as low as 6.25 or 12.5 mg/ml, but these values were also observed for 70% alcohol. Of the Gram-negative bacteria, the most susceptible were the non-glucose-fermenter *Pseudomonas aeruginosa* and the clinical isolate of *Klebsiella pneumoniae* with MICs of 6.25 and 12.5 mg/ml respectively. Due to the low concentration of active substance in aqueous and methanolic extracts, their real MICs could not be determined, most values being of more than 50 mg/ml. Instead, the concentrated extract presented quantifiable activity, with good effect on Gram-positive bacteria. Moreover, in concentrated extract, the bactericidal effect was found to be at the same concentration as the inhibitory effect (MBC=MIC). Of the Gram-negative bacteria, *Klebsiella pneumoniae* was the least susceptible. It is known that *Klebsiella* species present a protective mucopolysaccharidic capsule, which makes this bacterial species resistant to several environmental factors, including antibacterial agents (17,18).
Details of the antibacterial activity of the tested extracts can be found in Table 2.
If reported to the antimicrobial effect of 70% methanol, the results show a lower effect for leaf extracts than for flower extracts. Nevertheless, the overall effects were equal with that of the 70% methanol, with a single exception: against MSSA, where both the leaf and flower extracts were more efficient. A sub-unitary index obtained by dividing the MIC/MBC of the extract to the MIC/MBC of methanol equals a better effect of the extract than the methanol alone (Table 3).
The flower extracts presented better bacteriostatic effect than the leaf extracts on most bacteria, both the aqueous and methanolic extracts. A sub-unitary index obtained by dividing the MIC/MBC of the flower extract to the MIC/MBC of the leaf extract equals a better effect of the first one (Table 4).

In vitro cytotoxic and anti-migratory effects of Prunella vulgaris extract

Cells viability assessment

Prunella vulgaris extract (spike inflorescence) was tested for its cytotoxic effects on MDA-MB-231 - breast adenocarcinoma cells and on healthy MCF-10A breast epithelial cells at different incubation periods (24h, 48h, 72h). A Control group - cells stimulated with the solvent DMSO was also included. Figure 3 depicts the effect of the extract on the non-tumorigenic cell line. Results have shown that stimulation with *Prunella vulgaris* extract induced a dose and time dependent decrease in MCF-10A cells viability, at the highest tested concentrations, namely 500 and 750 µg/mL. At doses ranging between 1-250 µg/mL, the extract did not affect significant MCF-

Table 2. Bacterial minimum inhibitory (MIC) and bactericidal (MBC) concentrations (mg/ml) of the extracts.

Strain		Aqueous extracts				Methanolic extracts				Methanol		Concentrated extract
		Leaves		Flowers		Leaves		Flowers				Flowers
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC=MBC
Gram negative	*Klebsiella pneumoniae* MDR	>50	>50	50	>50	25	50	12.5	25	12.5	25	333.5
	Klebsiella pneumoniae ATCC13883	>50	>50	50	>50	25	50	25	25	12.5	25	333.5
	Providencia stuartii MDR	>50	>50	50	>50	50	50	25	25	12.5	25	83.4
	Escherichia coli ATCC25922	>50	>50	>50	>50	50	50	25	25	12.5	25	83.4
	Pseudomonas aeruginosa ATCC27853	>50	>50	25	>50	12.5	50	6.25	25	6.25	25	166.8
Gram positive	MRSA ATCC 43300	>50	>50	>50	>50	25	25	25	25	25	25	41.7
	MSSA ATCC 25923	>50	>50	50	>50	25	25	25	25	25	50	41.7

Table 3. Normalized indexes of MIC/MBC against alcohol

Strain		MICs for methanolic extracts		MBCs for methanolic extracts	
		Leaf	Flower	Leaf	Flower
Gram negative	*Klebsiella pneumoniae* MDR	2	1	2	1
	Klebsiella pneumoniae ATCC 13883	2	2	2	1
	Providencia stuartii MDR	4	2	2	1
	Escherichia coli ATCC 25922	4	2	2	1
	Pseudomonas aeruginosa ATCC 27853	2	1	2	1
Gram positive	MRSA ATCC 43300	1	1	1	1
	MSSA ATCC 25923	1	1	0.5	0.5

Index <1 – low MIC/MBC (better effect than methanol);
Index 1 – MIC/MBC equal with alcohol;
Index >1 – high MIC/MBC (lower effect than methanol);

Table 4. Indexes of MIC/MBC for flower extracts reported to leaf extracts

	Strain	MIC		MBC	
		Flower/Leaf Aqueous extract	Flower/Leaf Methanolic extract	Flower/Leaf Aqueous extract	Flower/Leaf Methanolic extract
Gram negative	*Klebsiella pneumoniae* MDR	>1	0.5	>1	0.5
	Klebsiella pneumoniae ATCC 13883	>1	1	>1	0.5
	Providencia stuartii MDR	>1	0.5	>1	0.5
	Escherichia coli ATCC 25922	>1	0.5	>1	0.5
	Pseudomonas aeruginosa ATCC 27853	>1	0.5	>1	0.5
Gram positive	MRSA ATCC 43300	>1	1	>1	1
	MSSA ATCC 25923	>1	1	>1	1

Index <1 – low MIC/MBC for flower (better effect than leaf); Index 1 – equal MIC/MBC for flower and leaf; Index >1 – high MIC/MBC for flower (lower effect than leaf);

10A breast cells viability regardless of the incubation period.

Figure 4 describes the effect of *Prunella vulgaris* extract (spike inflorescence) on the selected breast adenocarcinoma cell line (MDA-MB-231). One can observe that at low concentrations (1 and 10 µg/mL), the extract did not affect cell viability. By increasing the concentration, a significant dose and time dependent decrease in tumor cell viability was noticed, with the most potent effect obtained at 72h post-stimulation. Thus, it can be concluded that starting from the concentration of 250 µg/mL *Prunella vulgaris* extract presents selectivity against the cancer cell lines.

Evaluation of the anti-migratory potential

In order to evaluate the anti-migratory effect of *Prunella vulgaris* extract (spike inflorescence) the scratch assay technique was used. Breast cancer cells were stimulated with different concentrations of the extract and compared to Control cells (cells stimulated with the solvent DMSO). The two highest concentrations of the extract (500 and 750 µg/mL) were not tested due to the fact that the percent of viable cells was very low. At the lowest doses tested (1 and 10 µg/mL), the extract stimulated cell migration (scratch closure rate 100%), but by increasing the concentration (50, 100 and 250 µg/mL) a significant reduction in tumor cell migration was obtained; scratch closure rates were 73.9% (50 µg/mL), 0.1% (100 µg/mL) and -1.9% (250 µg/mL). Moreover, at 24h after stimulation, at the highest tested doses (100 and 250 µg/mL) changes in tumor cell morphology can be observed – cells display round shape and some of them are detached from the plate, indicating that *Prunella vulgaris* extract affects breast adenocarcinoma cells.

Discussions

According to the scientific literature, extracts obtained from the spike inflorescences of *Prunella vulgaris* have antitumor activity.

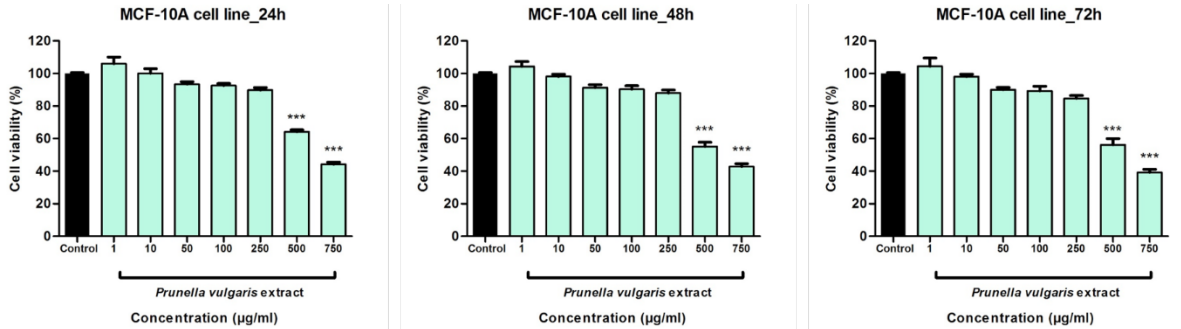

Fig. 3. *In vitro* effects of *Prunella vulgaris* extract (1, 10, 50, 100, 250, 500 and 750 µg/mL) on MCF-10A - non-tumorigenic breast epithelial cell line at 24, 48 and 72h post-stimulation determined by Alamar blue technique. (Results are presented as mean ± SD. Experiments were performed in triplicate) (* p <0.05; ** p <0.01; *** p <0.001 vs control calculated by One-way ANOVA followed by Dunnett's post-test).

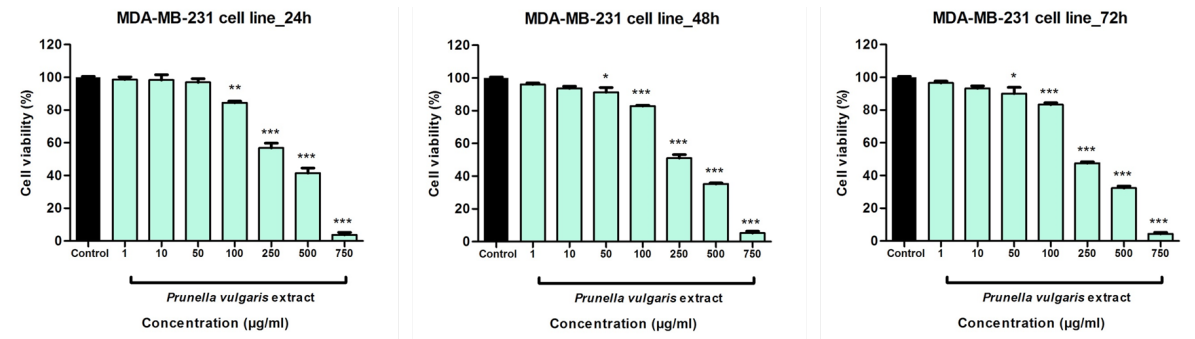

Fig. 4. *In vitro* effects of *Prunella vulgaris* extract (1, 10, 50, 100, 250, 500 and 750 µg/mL) on MDA-MB-231—human breast adenocarcinoma cell line at 24, 48 and 72h post-stimulation determined by Alamar blue technique. (Results are presented as mean ± SD. Experiments were performed in triplicate) (* p <0.05; ** p <0.01; *** p <0.001 vs control calculated by One-way ANOVA followed by Dunnett's post-test).

These effects were found in the *in vitro* study, however, a higher concentration of active principles (rosmarinic acid) is needed to highlight the antitumor action of spike inflorescence, but efficient concentrations are improbable *in vivo*. Due to the high concentration of phenolic compounds, the spike inflorescence of *Prunella vulgaris* L. exhibits antioxidant action. The active compounds can directly inhibit the growth and proliferation of cancer cells (19-24). The present study has demonstrated that the extract (concentrated aqueous solution after initial extraction with methanol) from the spike inflorescence of *Prunella vulgaris* L. (collected from Romania) presents cytotoxic and anti-migratory potential in a dose and time dependent manner on the tested breast adenocarcinoma cell line, namely MDA-MB-231 while affecting less the non-tumorigenic breast epithelial cells.

Phenolic compounds (from the spike inflorescence), namely flavonoids, anthocyanins and phenolcarboxylic acids have antioxidant activities (25,26). Phenolic compounds, as secondary metabolites of plants that can be found in abundance in plant sources, have pharmacological profiles of interest. In addition to the antioxidant action, their antibacterial activity can be exploited therapeutically with a minimum of side effects. This effect is described for all phenolic phytocompounds, but is intensively studied

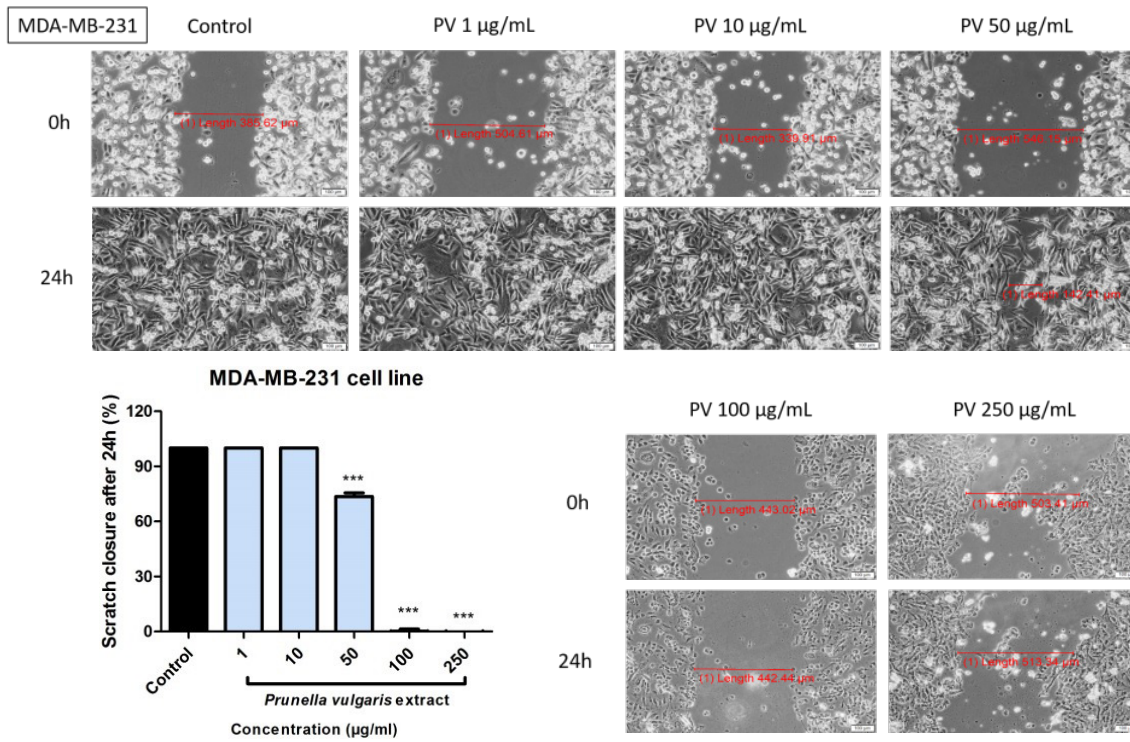

Fig. 5. Anti-migratory effect of *Prunella vulgaris* extract (1, 10, 50, 100 and 250 µg/mL) on MDA-MB-231—human breast adenocarcinoma cell line. Images were taken by light microscopy at 10X magnification at 0h and 24h post-stimulation. The bar graph represents the percentage of scratch closure after 24h compared to the starting point of the experiment (0h). One-way ANOVA test followed by Dunnett's multiple comparison test was used for comparison among the groups (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. Control-cells stimulated with the solvent DMSO).

for phenol-carboxylic acids, such as rosmarinic acid, which is present in the studied extracts (7). According to the literature, the methanolic extract of *Prunella vulgaris* L. has been shown to have significant antibacterial action. *In vitro* experiments have indicated that it has inhibitory activity on Gram-positive bacteria (8). However, our experiments show an antibacterial action of almost equal intensity on Gram-negative bacteria, both on standard strains and on clinical isolates (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Providencia stuartii*). Aside already published data this makes us conclude that in methanol extracts, the antibacterial effect is achieved mostly because of the methanol itself and to a lesser extent by the extracted active compounds (27).

The antimicrobial action is influenced by the plant part taken into the study, the concentration of active principles and the type of solvent used. The most intense activity was presented by the more concentrated extract of spike inflorescence. This is due to variations in the composition of bioactive phenolic compounds, such as rosmarinic acid, ursolic acid and oleanolic acid, with the highest concentrations in full-flowering stage (28). Its antimicrobial action is dose and extraction type dependent. This was also found

in other studies, methanolic extracts presenting more potent antimicrobial activity (29, 30). Our results showed that Gram negative bacteria were less susceptible to *Prunella vulgaris* L. extracts, but without significant differences among the representative species. Mahboubi et al. suggested that antibacterial activity is not directly related to the phenolic acid content (29), but rather to the flavonoid content (31). Nevertheless, phenolic acids such as rosmarinic acid enhance the activity of antibiotics against bacteria and provide a better time kill kinetics (32). Overall, our results support the positive biological activity potential of *Prunella vulgaris* L.

Conclusions

The methanolic extract of the species *Prunella vulgaris* L. (spike inflorescence) demonstrated a dose-dependent antitumor and antibacterial activity, including multidrug resistant strains. *In vitro* studies show a biological activity that may be the basis for the selection of compounds that are present in the extract to be subsequently tested *in vivo*, in order to determine the pharmacokinetic and pharmacodynamic properties in various animal models. *In vitro* screening may be the first step in identifying potential antitumoral and antibacterial compounds to be evaluated for appropriate test batteries for their biological activity.

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Author Contributions

Conceptualization: A.G., C.E.V., R.S. and L.D.M.; Data curation, C.E.V., C.D., I.Z.P., Cr.D. and A.M.; Formal analysis: A.G. and R.E.D.; Methodology: A.G., R.S., R.E.D., I.Z.P., C.D., Cr.D., A.M. and L.V.; Supervision: L.D.M.; Validation: L.V. and L.D.M.; Writing – original draft: A.G., R.S. and A.M.; Writing – review & editing: C.E.V., A.M., L.V. and L.D.M. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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