

Phytochemical profile, antioxidant capacity and wound healing potential of *Viscum album* L. growing on *Robinia pseudoacacia* L.

Simona I. VICAS^{1,2,a}, Adriana R. MEMETE^{1,b}, Eva KLESZKEN²,
Florina MIERE (GROZA)^{3,c}, Mariana GANEA^{3*}, Simona CAVALU³,
Angela ANTONESCU³, Tudor E. FERTIG^{4,5}, Daciana S. MARTA^{4,6}

¹University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru st., Oradea 410048, Romania; svicas@uoradea.ro; adrianamemete@yahoo.com

²University of Oradea, Doctoral School of Biomedical Science, 1 Universitatii st., Oradea, 410087, Romania; eva.kleszken@gmail.com

³University of Oradea, Faculty of Medicine and Pharmacy, 10 Piața 1 Decembrie st., Oradea, 410068, Romania; florinamiere@uoradea.ro; mganea@uoradea.ro (*corresponding author); simona.cavalu@gmail.com; antonescu.angela@yahoo.com

⁴Victor Babeș National Institute of Pathology, Ultrastructural Pathology and Bioimaging Lab., Bucharest, Romania; emanuel.fertig@ivb.ro; daciana.marta@ivb.ro

⁵“Carol Davila” University of Medicine and Pharmacy, 8 Bulevardul Eroii Sanitari, Bucharest, 050474 Romania

⁶“Titu Maiorescu” University, Faculty of Medicine, 67/A Gheorghe Petrașcu st., sect. 3, Bucharest, Romania

^{a,b,c} These authors contributed equally to this work.

Abstract

Mistletoe (*Viscum album* L.), a semi-parasitic medicinal plant, continues to be of interest, due to its phytochemical composition. The leaves of mistletoe contain phenols, which have a variety of biological effects. The main goal was to characterize the mistletoe that parasitized *Robinia pseudoacacia* L., (called VAR) in terms of phenolic compounds and to assess the wound healing potential *in vitro* using the scratch method. Furthermore, the antioxidant capacity was evaluated both by spectrophotometric techniques (DPPH, FRAP, TEAC), as well as by the ability of the mistletoe extract to synthesize green selenium nanoparticles. Among the phenolic acids, dihydroxybenzoic acid is in high level (2.86 ± 0.03 mg/g dw), whereas isorhamnetin-glucuronides dominate the flavonol class (0.593 ± 0.03 mg/g dw). The presence of phenolic compounds in the VAR leaves provides antioxidant capacity. The reducing capacity of VAR extract was demonstrated for the first time by the biosynthesis of nanoselenium particles (NSePs) with a regular, spherical shape and a diameter of around 130 nm. The VAR concentrations of 25-200 $\mu\text{g/mL}$ showed no toxic effect on normal human dermal fibroblasts (NHDF), and the concentration of 100 $\mu\text{g/mL}$ exhibited the best percentage of wound surface closure *in vitro* (94.08%). The results show that mistletoe is a promising plant because of its phytochemical composition and antioxidant capacity, which can modulate the wound repair process *in vitro*.

Keywords: antioxidant capacity; normal dermal fibroblast; phenols; selenium nanoparticles; scratch assay; *Viscum album*; wound healing

Received: 28 Nov 2023. Received in revised form: 12 Mar 2024. Accepted: 20 Mar 2024. Published online: 27 Mar 2024.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Mistletoe (*Viscum album* L.) is a semi-parasitic, evergreen plant that grows on a variety of host trees, both deciduous and coniferous, and is dependent on the host's nutrients and water, but can produce its own carbohydrates by photosynthesis (Szurpnicka *et al.*, 2019; Kleszken *et al.*, 2022b). It is part of the genus *Viscum* and includes several species that are distributed mainly in Europe, Africa, Asia, America and Australia (Kleszken *et al.*, 2022b). Mistletoe is hosted by poplar, apple, black locust, fir, plum trees, to mention only a few (Barbu, 2012; Kleszken *et al.*, 2022b, 2022a).

Several chemical and pharmacological investigations have provided evidence that *Viscum* extracts and their diverse preparations contain a wide range of compounds including lectins, viscotoxins, lignans, amines, amino acids, alkaloids, polyphenols, flavoflavonoids, and polysaccharides (Vergara-Barberán *et al.*, 2017; Peñaloza *et al.*, 2020). These extracts are widely used as complementary and alternative medicines for the treatment of various neurological disorders. Additionally, they are recognized for their antiviral, antibacterial, anti-inflammatory, antiepileptic, and immunostimulatory properties (Szurpnicka *et al.*, 2019; Kwon *et al.*, 2021; Pietrzak and Nowak, 2021; Jones, 2022). Flavonoids and phenolic acids, which occur naturally as antioxidants, have been identified as compounds present in the mistletoe plant. These compounds play a crucial role in the biological activity of the plant and have been utilized in disease prevention, particularly in the context of oxidative stress-induced ailments such as cancer, which are attributed to the presence of free radicals within the body (Bonamin *et al.*, 2017; Pietrzak and Nowak, 2021; Kleszken *et al.*, 2022a). The application of mistletoe plants in cancer therapy offers significant importance (Fritz *et al.*, 2004; Wode *et al.*, 2020; Pietrzak and Nowak, 2021). Previous studies have revealed additional bioactive properties of mistletoe, such as its ability to promote wound healing and provide protective effects on the skin (Kuonen *et al.*, 2013; Choi *et al.*, 2022).

Using herbal products to heal skin wounds is a therapeutic challenge for traditional medicine, while the conventional therapy involves various synthetic drugs (antibiotics and antifungals) which may cause tolerance, and consequently, increased dose applied in order to diminish the resistance of microorganisms involved in possible infections (Miere *et al.*, 2021; Choi *et al.*, 2022; Burlou-Nagy *et al.*, 2023; Memete *et al.*, 2023a). In this context, a search for new therapeutic options based on plants extract and their metabolites is required, as a great source of novel and unexplored biomolecules, while wound healing comprises a cascade of biochemical and cellular events that include tissue repair and renewal. Particularly, topical antiseptics and antimicrobial therapy are crucial in order to control microbial colonization.

On the other hand, within the nanotechnological approach, plants extracts may be used to synthesize nanoparticles in a way that is both ecologically friendly and safe to use, producing nanoparticles with well-defined features such as size and form, being employed for different bio-medical or environmental applications. It has been demonstrated that secondary metabolites such as flavonoids and alkaloids possess an important role in metal salt reduction, capping and stabilizing the nanoparticles generated by green synthesis (Chopra *et al.*, 2022).

Hence, the biological methods used to synthesize nanoparticles are preferred over chemical or physical methods. In this context, bio-synthesis of selenium nanoparticles was previously reported in several papers (Cavalu *et al.*, 2018b, 2018a; Vicas *et al.*, 2021a, 2021b; Laslo *et al.*, 2022) being used as for bioremediation purpose or biomedical applications. Selenium nan-forms have been reported to have a higher antimicrobial and antioxidant activity than other forms, either organic or inorganic (Rayman, 2000; Tran and Webster, 2011; Khiralla and El-Deeb, 2015; Huang *et al.*, 2016). Furthermore, selenium, an essential micronutrient, is necessary for the control and enhancement of various physiological processes in organisms. It serves as an essential element of glutathione peroxidase, one of the primary antioxidant enzymes. When comparing selenomethionine and nano-selenium, it can be noticed that nano-selenium exhibits reduced toxicity while maintaining comparable effectiveness in enhancing the activities of selenoenzymes (Cavalu *et al.*, 2017).

The main goal of our study was to identify the main phytochemical compounds and to investigate the wound healing potential of *Viscum album* L. extract, harvested from black locust (*Robinia pseudoacacia* L.) by *in vitro* assay, using normal human dermal fibroblasts (NHDF) and the “scratch” technique. Another goal was to assess the antioxidant capacity of mistletoe extract by DPPH, FRAP and TEAC assay. Finally, we aimed to synthesize nanoselenium particles (NSePs) employing mistletoe extract, along with their physico-chemical characterization by DLS and TEM techniques.

Materials and Methods

Plant materials

The plant material used in our study is represented by mistletoe leaves (*V. album* L. subsp. *album*) that were collected from the black locust (*Robinia pseudoacacia* L.) near the town of Mișca, in the spring of 2022. The town of Mișca is located in Bihor County, in the North-East of Romania, at an altitude of 198 m above the sea level, having the following coordinates: 47° 26' North and 22° 26' East, in the North - East of Bihor County. A specimen of the mistletoe (*V. album* L. subsp. *album*) was kept in the Herbarium of the Faculty of Medicine and Pharmacy Oradea, Romania, registered in NYBG Steere Herbarium, PUO 05361 code.

Chemicals

The HPLC reference standards of gallic acid, chlorogenic acid, rutin and solvents (acetonitrile, acetic acid) were purchased from Sigma Aldrich (St. Louis, MO, USA). The water for the HPLC analysis was purified using a Milli-Q system (Merck Millipore). For spectrophotometric methods, Folin & Ciocalteu's phenol-Aldrich, Quercetin, 2,2-Diphenyl-1-picryl-hydrazyl-hydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, were purchased from Sigma Aldrich (St. Louis, MO, USA), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma Life Science), potassium persulfate, 2,4,6-Tris(2-pyridyl)-s-triazine, aluminium chloride were purchased from Fluka (Charlotte, North Carolina, US). Methanol was of analytical grade.

Determination of phenol compounds from mistletoe leaves by HPLC-DAD-MS-ESI⁺

The mistletoe leaves harvested from black locust host tree were air-dried in the dark, at room temperature, and powdered. The dry powder leaves (0.5 g) was mixed with 5 mL ethanol 70 %, vortex for 1 minute followed by 30 minutes ultrasonic treatment. The sample was kept macerated for 24 hours in the cold (4 °C) after which it was centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through a nylon filter (Chromafil Xtra nylon) with a pore size of 0.45 μm and a volume of 20 μl was injected into the HPLC system. Chromatographic measurements were performed using an Agilent 1200 HPLC system equipped with a quaternary pump, solvent degasser, autosampler, photodiode UV-VIS detector (DAD) coupled to an electrospray (ESI⁺) mass detector (MS-6110) with a single quadrupole (Agilent-Technologies, CA, USA). The separation of the compounds was carried out on a Kinetex XB-C18 column, with the following size: 4.6 x 150 nm, with 5 μm particles (Phenomenex, USA), for 30 minutes, at a temperature of 25 °C, and a flow rate of 0.5 mL/min. The mobile phases are solvent A containing of 0.1 % acetic acid in ultrapure water and solvent B containing of 0.1 % acetic acid in acetonitrile. Ultrapure water was purified by Direct-Q UV system, Millipore (USA), and HPLC grade acetonitrile being purchased from Merck (Germany). A gradient elution of the mobile phase, of the two solvents, was used, according to Table 1.

Table 1. Mobile phase gradient required for analysis of bioactive compounds in mistletoe extracts from three host trees

Time (min)	Solvent (A)	Solvent (B)
0	95%	5%
2	95%	5%
2-18	95-60%	5-40%
18-20	60-10%	40-90%
20-24	10%	90%
24-25	10-95%	5%
25-30	95%	5%

Fragmentations were applied, in the range of 50-100 V. Mass spectrophotometric detection of positively charged ions was performed using scan mode under the following operating conditions: capillary voltage 3000 V, temperature 350 °C, nitrogen flow 7 L/min, nebulizer pressure 35 psi, fragmentor 100 V and m/z 120-1500.

The chromatograms were recorded at the following wavelengths: $\lambda = 280$ nm, and $\lambda = 340$ nm for all peaks. The wavelength $\lambda = 280$ nm is specific to hydroxybenzoic acids, and the wavelength $\lambda = 340$ nm is specific to hydroxycinnamic acids and flavonols (Kleszken *et al.*, 2022a).

Spectrophotometric determination of total phenols and flavonoids content

Sample preparation

The collected plant material was washed, dried completely at room temperature and ground to form a powder. The powder obtained from mistletoe leaves was macerated in 70% ethanol in a ratio of 1:10 (w/v). After 24 hours and periodic shaking in the dark, the mixture was centrifuged (NÜVE NF 200 BENCH TOP CENTRIFUGE, Turkey) at 5000 rpm for 20 minutes. The collected supernatant was concentrated, by evaporation of ethanol, in a vacuum rotary evaporator (Heidolph rotary evaporator, Laborota 4000 rotavapor, Schwabach, Germany) at 40 °C. The mistletoe leaf extract obtained was freeze-drying using Christ Alpha 1-2 LDplus Lyophilizer (Osterode am Harz, Germany) and stored at -20 °C until further use. For the evaluation of bioactive compounds, such as total polyphenols, total flavonoids and antioxidant capacity determined by FRAP, DPPH and TEAC assays and for cell culture analysis, the powder was freshly prepared by dissolving in distilled water to obtain a stock solution (1 mg/mL) that was further diluted to various concentrations.

Total phenols content

The total phenolic content of mistletoe (*V. album* L.) leaves grown on black locust (*Robinia pseudoacacia* L) was determined. The total content of polyphenols was determined by the Folin-Ciocalteu method, with small modifications (Singleton *et al.*, 1999; Vicas *et al.*, 2011). Mistletoe extracts (100 μ L) were mixed with 1700 μ L of distilled water, 200 μ L of Folin-Ciocalteu reagent (freshly prepared), dilution 1:10 (v/v) and Na₂CO₃ solution 7.5%. The mixtures were kept at room temperature in the dark for 2 h. Absorbance was measured at 765 nm using Shimadzu mini-UV-VIS spectrophotometer, and the results were expressed as mg gallic acid equivalent (GAE) / g dw, using gallic acid as standard ($y = 27.637x + 0.0069$, $R^2 = 0.9994$).

Total flavonoids content

The total flavonoid content of mistletoe leaves was determined by the AlCl₃ colorimetric method as described by Miere Groza *et al.* (2021). Briefly, a volume of 1 mL of ethanolic mistletoe leaf extract was carefully transferred into a 10.0 mL volumetric flask, which already contained 4 mL of distilled water. Subsequently, a quantity of 300 μ L of a 5% NaNO₂ solution was introduced into the flask. After a period of interval of 5 minutes, a volume of 300 μ L of a 10% AlCl₃ solution was introduced, and then, after a time interval of 6 minutes, a volume of 2 mL of a 1 M NaOH solution was added. The flask was filled with 10 mL of distilled

water. The measurement of absorbance was conducted at a wavelength of 510 nm with respect to the blank sample. In order to quantify the total flavonoids, the compound quercetin was used as a reference standard. The results were reported in milligrams of quercetin equivalent (QE)/g dw, following a linear regression equation of $y = 0.8475x + 0.0065$, with an R-squared value of 0.9976.

The antioxidant capacity

DPPH assay

The antioxidant capacity of ethanolic extracts of mistletoe leaves to scavenge the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined according to the method of Brand-Williams *et al.* (1995) with minor modifications (Brand-Williams *et al.*, 1995; Memete *et al.*, 2022). A volume of 100 μ L of mistletoe extract was mixed with 2800 μ L of 80 μ M DPPH solution and stored at room temperature in the dark. After 30 minutes, the absorbance of the samples was measured using a Shimadzu mini-UV-VIS spectrophotometer at a wavelength of 517 nm. The results were expressed as mmol Trolox equivalent (TE)/g dw.

The radical scavenging activity was calculated using Equation 1.

$$\text{Radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where, A₀ – Absorbance of DPPH in methanol; A₁ – Sample absorbance;

FRAP assay

The antioxidant capacity of mistletoe leaves extract was determined by the FRAP test, according to Benzie and Strain (1996) with minor modification to Bandici *et al.* (2021). This method is based on the reduction of the ferric tripyridyltriazine complex (Fe(III)-TPTZ) to the ferrous tripyridyltriazine complex (Fe(II)-TPTZ) by a reductant at acidic pH. A volume of 100 μ L extract was mixed with 2000 μ L distilled water and 500 μ L FRAP solution, freshly prepared consisting of 300 mM acetate buffer, pH=3.6, 20mM FeCl₃ · 6H₂O and 10 mM sol TPTZ, in a ratio of 10:1:1 (v/v/v). This mixture was kept at room temperature in the dark, for 60 min. Absorbance was measured at 595 nm and results were expressed as mmol Trolox equivalent (TE) /g dw.

TEAC assay

TEAC spectrophotometric method was used to evaluate the antioxidant capacity of mistletoe harvested from black locust (*Robinia pseudoacacia* L.) according to the method of (Memete *et al.*, 2023b). This method measures the ability of compounds to scavenge the ABTS cationic radical. The blue/green ABTS⁺ cation is the reaction product of 7 mM ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) solution with 2.45 mM potassium persulfate solution. The mixture was stored in the dark at room temperature for 12 - 16 hours and then was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 100 μ L of mistletoe extract to 2.9 mL of diluted ABTS stock solution, the antioxidant capacity was monitored at 734 nm, after exactly 30 seconds. The results were expressed in mmolTE/ g dw. The results were expressed as mM Trolox equivalent/g fresh matter. The ABTS⁺ radical changes color from blue/green to colourless depending on the antioxidant capacity of the mistletoe extracts.

The reduction of sodium selenite (Se⁴⁺) to NSePs by mistletoe extract

Fresh mistletoe leaves harvested from black locust were washed and freeze-dried (Freeze dryer Alpha 1-2 Christ - Martin Christ, Germany). The obtained powder was homogenized (Heidolf homogenizer) with distilled water in the ratio 1:10 (w/v). The extract was sterilized using a 0.22 μ m sterile filter (ISOLAB, Laborgeräte GmbH, Germany). The mistletoe extract was mixed with sterilized 25 mM of sodium selenite (Na₂SeO₃) solution in a ratio 1:10 v/v and allowed to stay at room temperature, in the dark for few days. When the solution color turns red, it was centrifuged at 15000 rpm for 15 minutes, and the NSePs were washed with

sterilized water for 3 times. The characterization of NSePs was performed by DLS, while morphological details were observed by TEM (Negative-Stain). DLS measurements were performed by using ZEN 3690 (Malvern Instruments, Cambridge, United Kingdom) in order to determine the average particle size, size distribution and zeta potential of NSePs.

Negative stain transmission electron microscopy

Carbon-coated copper grids (300 mesh, S160-3, Agar Scientific, UK) were glow discharged to reduce surface hydrophobicity, then incubated with 3 μ L of sample, at room temperature. After removing excess sample by blotting, the grids were stained with three successive drops of 2% uranyl acetate. Image acquisition was done on a 200 kV Talos F200C TEM equipped with a 4 \times 4k Ceta camera (Thermo Fisher Scientific, Waltham, MA, USA).

In vitro determination of the wound healing effects of the V. album L. extract using the scratch method

Cell culture and treatments

The study utilized normal human dermal fibroblasts (NHDF) obtained from Lonza Pharma & Biotech (Basel, Switzerland). The specific culture kit employed for the experiment was the Fibro-blast Growth Medium-2 BulletKit. The culture medium utilized in this study was Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (FBS), gentamicin at a concentration of 50 mg/mL, amphotericin at a concentration of 50 mg/mL, and recombinant fibroblast growth factor hFG (CC-4065) at a concentration of 1 mg/mL.

A stock solution of 1 mg/mL of the lyophilized mistletoe leaves extract (VAR) was prepared and filtered by a sterile filter with a pore size of 0.22 μ m. Different dilutions of stock solution were made with the cell culture medium resulting the final concentrations of 25, 50, 100 and 200 μ g/mL (VAR_25, VAR_50, VAR_100 and VAR_200, respectively) for determination of the viability, proliferation assay and performing of the scratch assay.

Cell viability and cell proliferation (MTS) assays

The NHDF cells were seeded in 24-well plates at 1×10^4 cells/well and incubated at 37 °C with 5% CO₂ for 24 h. Then the cells were treated with different concentrations of VAR extracts (25, 50, 100 and 200 μ g/mL), and with 50 μ g/mL of Allantoin (ALA_50) which represents the positive control. As a control sample, cells without treatment were used (CTRL). After 12, 24 and 36 hours of incubation, cells were trypsinized (trypsin/EDTA solution (0.25 mg/mL), LONZA), neutralized (TNS-Trypsin Neutralization Solution, LONZA) and centrifuged (1000 rpm/5 min). The resulting pellets were suspended in culture medium and cell viability was performed by applying the trypan blue test, using the EVE Automated Cell Counter (NanoEnTek Inc., Seoul, Republic of Korea) device (Antonescu *et al.*, 2021).

The calculation of cell viability percentage was performed using equation (2), and the resulting values were expressed as the percentage of cell viability in the treated cells compared to the control group (untreated cells - CTRL).

$$\text{Cell viability (\%)} = \frac{\text{number of living cells}}{\text{number of total cells}} \times 100 \quad (2)$$

The MTS assay utilizes the reduction of the tetrazolium compound MTS by viable cells (in particular, NHDF cells) to generate a soluble dye known as formazan within the cell culture medium. The proliferation assay was conducted by seeding cells in sterile 98-well plates at a density of 2×10^4 /mL. The treatments with mistletoe extract of different concentrations, CTRL and ALA_50 were applied. The reduction of the tetrazolium was monitored for 12, 24 and 36 h.

In each well of plate, at the suitable time, 10 μL of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H tetrazolium) solution containing PMS (phenazine) was added and incubated for 2 h at 37 °C. The colored, soluble formazan produced was recorded at 492 nm with a reference wavelength of 630 nm using a microplate reader (Stat Fax 2100, Palm City, USA). Cell proliferation was determined at the initial time, 12 h, 24 h and 36 h (Malich *et al.*, 1997; Kabakov *et al.*, 2011). Results were expressed as percentage of cell viability compared to control at initial time. Tests were performed in triplicate.

Scratch assay

Dermal cells were seeded at a concentration of 5×10^4 cells/well in 6-well plates and incubated under a 5% CO_2 atmosphere at 37 °C for 72 h until confluence was reached. Confluent NHDF cells were “scratched” vertically using sterile 50 μL pipette tip. The desquamated cells were removed by washing the well with a PBS solution. The VAE samples were applied on NHDF cells to obtain final concentrations of VAE_25 $\mu\text{g}/\text{mL}$, VAE_50 $\mu\text{g}/\text{mL}$, VAE_100 $\mu\text{g}/\text{mL}$, VAE_200 $\mu\text{g}/\text{mL}$. Allantoin 50 $\mu\text{g}/\text{mL}$ (ALA_50) was used as a positive control and fibroblast without treatment represent the control (CTRL). Using the CytoSMART Lux3BR® device, the samples were monitored in real-time for 36 h. The evaluation of wound closure per area (%) was analysis using the equation (3) for each sample at 12, 24 and 36 h (Memete *et al.*, 2023a).

$$\text{Wd}_A (\%) = \frac{\text{Average area of wound_sample } (\mu\text{m}^2) \text{ at time } t}{\text{Average area of wound_sample } (\mu\text{m}^2) \text{ at time } t = T_0} \times 100 \quad (3)$$

Statistical analysis

All values were represented as mean \pm SD (standard deviation) and all experiments were performed in triplicate (n = 3). Data were statistically processed using GraphPadPrism (GraphPad Software, Inc., La Jolla, CA, USA) and one-way analysis of variance, followed by Tukey’s multiple comparison test at $p < 0.05$, was used to indicate a statistically significant difference.

Results and Discussion

Mistletoe (*Viscum album* L) belongs to the Santalaceae family, genus *Viscum* (Büssing 2000). Mistletoe is more often found on the branches of woody species, and less often on their trunk, but on black locust (*Robinia pseudoacacia* L) it often grows on the trunk as well (**Figure 1a**). It penetrates through the bark of trees, passes through the fibrous tissue, and reaches the cambium, a plant tissue located between the woody and fibrous tissue that ensures the secondary growth of the stem, from where it takes water and mineral salts (Schulze *et al.*, 1984; Glatzel and Geils, 2009) with the help of haustoria and cortical threads that ensure the lateral spread of mistletoe on the host tree (Büssing, 2000). Mistletoe is an evergreen plant, contains photosynthetic pigments, the leaves are arranged in pairs, and are yellow-green to dark green in color (Figure 1b). The female flowers are small, 2 - 3 mm, yellow-green in color, have 4 petals, a pistil with an ovary, a simple central style and a stigma. The male flowers are 7 mm, have 4 tepals, filaments with about 50 pollen compartments, no anthers (Figure 1 c,d). The fruits are small, spherical, white, translucent, of about 8 mm in diameter (Figure 1e).

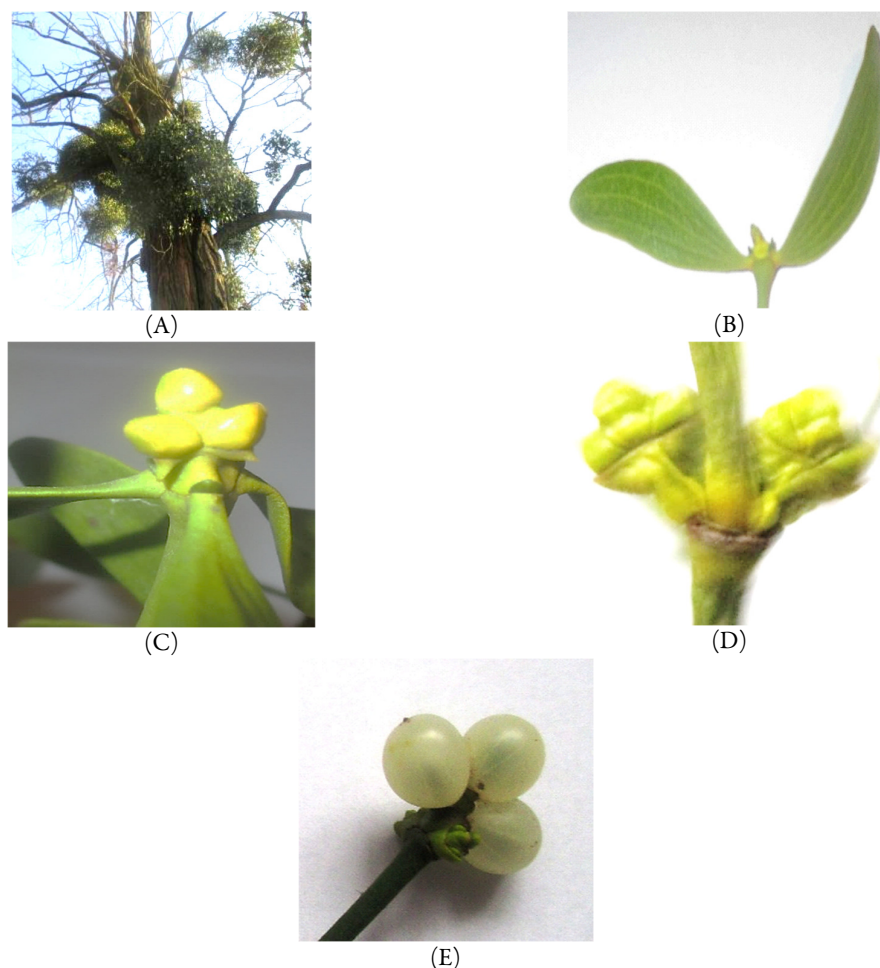


Figure 1. Morphology of mistletoe (*Viscum album* L.); (A) mistletoe on black locust (*Robinia pseudoacacia* L.); (B) mistletoe leaves; (C) mistletoe female flower; (D) mistletoe male flower; (E) mistletoe fruits

Phytochemical characterization of V. album L. and its antioxidant capacity

Mistletoe (*V. album* L.) is a medicinal plant much studied by researchers, because it has a complex phytochemical composition, contains phenolic acids, flavonoids, lectins, viscotoxins, polysaccharides, organic acids (Luczkiewicz *et al.*, 2001; Vicas *et al.*, 2011; Marvibaigi *et al.*, 2014; Szurpnicka *et al.*, 2019; Peñaloza *et al.*, 2020; Kleszken *et al.*, 2022a).

High performance chromatography method coupled with mass spectroscopy (HPLC-DAD-ESI⁺ / MS) identified 16 bioactive compounds that were separated and tentatively assigned based on retention times, mass/charge ratio (m/z) and their main fragments. Table 2 contains the retention time (R_t), the maximum wavelength (λ_{max}), the specific m/z [M + H]⁺ values, the tentative identification of the bioactive compounds, their inclusion in the phenolic subclasses, and the mean values of the phenolic compounds (mg/g dw) from mistletoe ethanolic extracts harvested from black locust (*Robinia pseudoacacia* L.).

Table 2. HPLC-DAD-MS (ESI⁺) tentative phenolic compounds identified and quantified (mg/g dw) in the *V. album* L. subs. *album* harvested from the *Robinia pseudoacacia* L. (VAR)

R _t (min)	UV λ _{max} (nm)	[M+H] ⁺ (m/z)	Compound	Subclass	VAR (mg/g dw)
3.33	265	155	Dihydroxybenzoic acid	Hydroxybenzoic acid	2.864 ± 0.03
10.85	323	355,163	3-Caffeoylquinic acid (Neochlorogenic acid)	Hydroxycinnamic acids	0.104 ± 0.01
12.47	323	355,163	5-Caffeoylquinic acid (Chlorogenic acid)		0.292 ± 0.01
13.21	330	387,223	Sinapic acid-glucoside		0.338 ± 0.02
14.28	330	399,223	3-Sinapoylquinic acid		0.336 ± 0.02
14.84	330	399,223	5-Sinapoylquinic acid		0.259 ± 0.01
16.18	255, 360	465,303	Quercetin-glucoside		0.534 ± 0.03
17.01	255, 360	609,303	Quercetin-O-[hydroxymethylglutaryl] hexoside (Quercetin derivative)	Flavonol	0.254 ± 0.01
17.69	240, 350	479,317	Isorhamnetin-glucoside		0.291 ± 0.01
18.11	240, 350	623,317	Isorhamnetin -O- [hydroxymethylglutaryl] hexoside (Isorhamnetin derivative)		0.416 ± 0.01
18.77	240, 350	493,317	Isorhamnetin-glucuronide		0.593 ± 0.03
19.51	240, 350	755,317	Isorhamnetin-(dirhamnosyl)- rhamnoside		0.554 ± 0.03
20.35	245, 350	493,331	Rhamnazin-glucoside		0.442 ± 0.01
20.71	245, 350	639,331	Rhamnazin-rutinoside		0.347 ± 0.02
21.64	255, 360	303	Quercetin		0.328 ± 0.02
24.03	240, 350	317	Isorhamnetin	0.281 ± 0.03	

16 phenolic compounds, namely, one hydroxybenzoic acid (compound 1), five hydroxycinnamic acids (compounds 2 - 6) and ten flavonols (compounds 7 - 16) were identified in the ethanolic extract of mistletoe harvested from black locust (VAR). It was noticed that the VAR extract is richer in dihydroxybenzoic acid (compound 1) having a concentration of 2.864 ± 0.03 mg/g dw, than in hydroxycinnamic acids or flavonols. The main chlorogenic acid isomers detected in mistletoe extracts include 3-Caffeoylquinic acid (compound 2 (m/z 355, and 163)) and 5-Caffeoylquinic acid (compound 3 m/z 355,163). The presence of chlorogenic acid isomers was previously reported in *Viscum album* grown on pine (*Pinus silvestris*) (Stefanucci *et al.*, 2020). Compound 3 (chlorogenic acid) was identified in *Viscum album* L extracts by Vicas *et al.* (2011). Compound 4 showed fragment ions at m/z 387,223 and was identified as sinapic acid-glucoside. Compounds 5 and 6 showed sinapic acid in their fragment molecules (m/z 399,223 [M + H]⁺) are characterized as cinnamate esters: 3-Sinapoylquinic acid and 5-Sinapoylquinic acid. Compound 5 (3-Sinapoylquinic acid) is found in a concentration of 0.336 ± 0.02 mg/g dw, and compound 6 (5-Sinapoylquinic acid) has a concentration of 0.259 ± 0.01 mg/g dw.

Flavonols are another group of compounds found in mistletoe extracts (Table 2). Mistletoe leaves (*Viscum album* L.) present a complex mixture of ten flavonols predominantly in the form of glucosides, with a low amount (0.281 ± 0.03 mg/g dw) of isorhamnetin (compound 16). The predominant flavonol found in mistletoe leaves was Isorhamnetin-glucuronide (m/z 493,317) (compound 11). Isorhamnetin glucoside (compound 9) shows fragmentation ions m/z 479,317. Flavonols such as Isorhamnetin -O-[hydroxymethylglutaryl] hexoside (Isorhamnetin derivatives) (compound 10) and Isorhamnetin-(dirhamnosyl)-rhamnoside (compound 12) are derivatives of Isorhamnetin, which are in a concentration of 0.554 ± 0.03 mg/g dw (compound 12), respectively 0.416 ± 0.01 (compound 10). Quercetin-glucoside (compound 7) with fragmentation ions at m/z 465 and 303[M + H]⁺, and Quercetin-O-

[hydroxymethylglutaryl] hexoside (Quercetin derivative) (compound 8) with fragmentation ions at m/z 609, 303 $[M + H]^+$ were identified. Two O-methylated flavonols were also identified: Rhamnazine glucoside (compound 18, m/z 493, and 331 $[M + H]^+$) and Rhamnazine rutinoside (compound 19, m/z 639 and 331 $[M + H]^+$). Rhamnazine is also found in other medicinal plants and has been shown in the literature to have antioxidant, antitumor, antiviral, anti-inflammatory and other biological functions (Yu *et al.*, 2018). Quercetin (compound 15) showed fragment ions at m/z 303 and is found in a small percentage of 3.97 % compared to the other phenolic compounds. The HPLC chromatogram of the mistletoe extract harvested from black locust (*Robinia pseudoacacia* L.) was recorded at $\lambda = 340$ nm and 280 nm.

Mistletoe (*Viscum album* L.) harvested from black locust (VAR) was evaluated for its total phenols and flavonoids content as well as its antioxidant capacity (Table 3).

Table 3. Total phenols, flavonoids and antioxidant capacity determined by the DPPH, FRAP and TEAC assay of *V. album* L. subs. *album* harvested from the *Robinia pseudoacacia* L. (VAR)

Sample	TPh (mgGAE/g dw)	TFLAV (mgQE/g dw)	DPPH ($\mu\text{molTE/g dw}$)	FRAP ($\mu\text{molTE/g dw}$)	TEAC ($\mu\text{molTE/g dw}$)
VAR	14.86 \pm 0.31	6.31 \pm 0.85	647.27 \pm 7.75	57.53 \pm 0.43	0.32 \pm 0.02

VAR –mistletoe harvested from the *Robinia pseudoacacia* L.; TPh-total phenols content; TFLAV-total flavonoid content; DPPH- 2,2-diphenyl-1-picrylhydrazyl; FRAP- Ferric Reducing Antioxidant Power, TEAC- Trolox equivalent antioxidant capacity

The results of this study showed that the extract of mistletoe (*V. album* L.) harvested from black locust (VAR) possesses an amount of phenols and flavonoids of 14.86 \pm 0.31 mg GAE/ g dw and 6.31 \pm 0.85 mg QE/g dw, respectively, comparable to values reported in other studies. Analysing total phenolics from mistletoe extracts harvested from black locust, Vicas *et al.* (2011), reported a value of 7.58 \pm 0.0 mg GAE/ g dw. Yadang *et al.* (2021), analysing mistletoe ethanolic extracts, showed a flavonoid content between 13.61 \pm 0.01 and 15.94 \pm 0.01 respectively (mg rutin equivalent/g dw) (Yadang *et al.*, 2022). Condrat *et al.* (2005), investigated 9 phanerogamous plants, including mistletoe, and determined the antioxidant capacity of these plant extracts and presented a capacity of 0.20 $\mu\text{mol TE / g dry matter}$, respectively, 0.16 $\mu\text{mol TE / g dry matter}$ (Condrat *et al.*, 2009). Mistletoe hosted on black locust has an ABTS+ cation radical scavenging value of 0.32 \pm 0.02 $\mu\text{mol TE/g dw}$. Vicas *et al.* (2011), recorded a scavenging level of ABTS+ cationic radicals (1.7 \pm 0.04 mM TE/g fresh matter) of mistletoe aqueous extract harvested from black locust (*Robinia pseudoacacia* L.).

Our data are in accordance with the results of other research which highlighted the fact that the host tree can influence the chemical composition of mistletoe extracts and implicitly their antioxidant capacity (Luczkiewicz *et al.*, 2001; Vicas *et al.*, 2011; Pietrzak and Nowak, 2021). The total amount of phenolics, flavonoids and antioxidant capacity determined from mistletoe leaves depends on a variety of factors, such as the extraction method, the host tree, the season in which they were harvested and their stage of maturity, as well as climatic conditions. This fact was confirmed in another study, in which the content of total polyphenols, total flavonoids and antioxidant capacity was determined in mistletoe leaves harvested from host trees such as *Malus domestica*, *Prunus domestica* and *Populus alba*, and the phytochemical composition varied according to the tree host that the mistletoe parasitizes (Kleszken *et al.*, 2022a). Furthermore, it has been shown that there is a close correlation between total polyphenol content and antioxidant capacity (Cavalu and Damian, 2003; Önay-Uçar *et al.*, 2006; Vicas *et al.*, 2011; Pietrzak and Nowak, 2021).

Pietrzak and Nowak, (2021) emphasized the correlation between climatic conditions and the presence of phenolic compounds and antioxidant activity in mistletoe leaves. Specifically, they found that a higher concentration of these compounds is associated with autumn-winter seasons and reduced sunlight, which promotes the accumulation of secondary metabolites (Pietrzak and Nowak, 2021). Also, Vicas *et al.* (2011),

investigated the phytochemical composition of *Viscum album* L. that parasitized different host trees, such as *Acer campestre*, *Fraxinus excelsior*, *Populus nigra*, *Malus domestica* and *Robinia pseudoacacia*, and the antioxidant capacity of mistletoe extract from *Robinia pseudoacacia* recorded higher values in the case of the DPPH method ($76.60 \pm 0.02\%$ for the ethanolic extracts harvested in December) (Vicas *et al.*, 2011).

Önay-Uçar *et al.* (2006) studied the *in vitro* antioxidant capacity of crude methanolic extracts of *V. album ssp. album* grown on different host trees by three widely used methods, namely, DPPH, thiocyanate methods ferric and thiobarbituric acid and to compare the results, depending on the host tree (*Tilia argentea*, *Acer campestre* L., *Robinia pseudoacacia* L.), harvested in two different seasons (February and July). Regarding mistletoe leaves developed on *Acer campestre* L. and *Robinia pseudoacacia* L. trees, the reported results revealed a higher antioxidant capacity, in mistletoe leaves harvested in February compared to July, and the highest value obtained was registered in the mistletoe leaves developed on the *Robinia pseudoacacia* L. tree ($91.36 \pm 2.61\%$ inhibition of DPPH), compared to the other samples studied (89.60 ± 0.47 and 88.97 ± 1.50 respectively % inhibition of DPPH). A different model was recorded in the mistletoe leaves developed on the *Tilia argentea* tree, where the highest antioxidant capacity was reported in July, ($95.12 \pm 2.37\%$ inhibition of DPPH), also representing the highest reported value, compared to the other samples studied in both seasons. Because there is no standardized method for determining the content of phenols, flavonoids, or antioxidant capacity, sometimes, comparing results can be difficult.

Biomolecules in plant extracts, including phenolic compounds, are known to be potential selenium reducing agents. In addition, due to their phytochemical composition, plant extracts also have nanoparticle-stabilizing properties. The evidence of NSePs formation using mistletoe extract was demonstrated by the color change: the extract's typical light brown colour changed to an orange-red colour characteristic to NSePs. Using the DLS method, the size distribution and zeta potential of NSePs were investigated and shown in Figure 2 a, b.

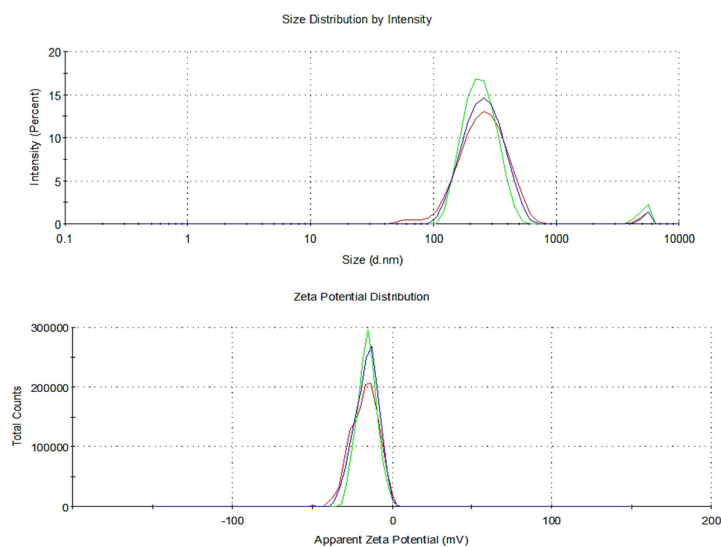


Figure 2. (A) Size distribution of NSePs recorded by DLS measurement; (B) Zeta potential measurement of NSePs

The DLS technique highlighted a maximum size of 159 nm with a gaussian distribution, while the apparent zeta potential presented a negative value of -24.5 mV, indicating a good stability, without a tendency of aggregation. The morphology of NSePs, as evidenced by TEM, indicated regular, spherical shape, with diameter around 130 nm (Figure 3).

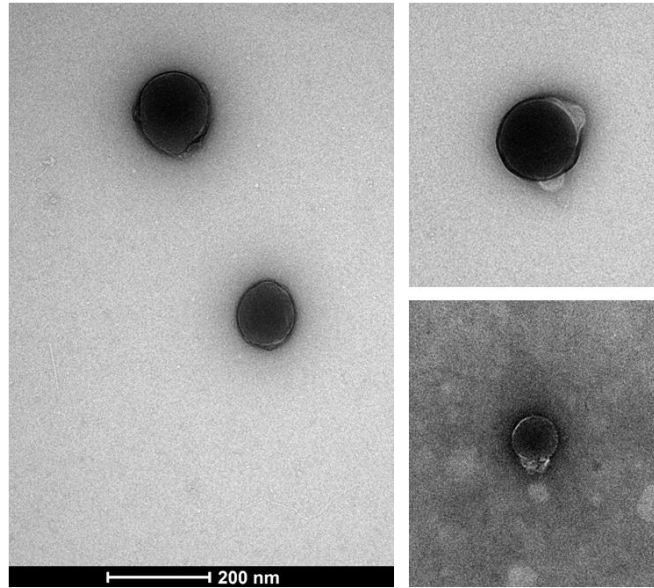


Figure 3. Representative negative stain TEM images of NSePs particles, biosynthesized by mistletoe extract. Scale bar applies to all three images

Similar results were reported by Cui and colleagues (2018), synthesizing NPSe using a hawthorn fruit extract (*Crataegus hupehensis* Sarg.), with a median size of 113 nm (Cui *et al.*, 2018). Other studies reported spherical NPSe with size around 68.12 nm in diameter produced using leaf extract from *Azadirachta indica* (Abdel-Gaber *et al.*, 2023), while parsley leaves extract (*Petroselinum crispum*) was reported to form spherical selenium nanoparticles with diameter of up to 300 nm (Fritea *et al.*, 2017). According to Miere *et al.* (2022) findings, the plant *Stellaria media* (L.) Vill. can synthesize NPSe with a wide range of diameters from 24 to 342 nm.

Cell culture and treatment

Cell viability and proliferation assay

Determining cell viability is one of the most important elements in the formation of cell culture, thus, for the application of the scratch method, a cell viability and confluence degree of at least 80% is required (Memete *et al.*, 2023 a). Thus, in Table 4, the cell viability of the samples is presented.

Table 4. Cell viability effect of VAR in NHDF cells line

Samples*	% of cell viability
CTRL	95.22 ± 3.21
ALA_50	95.67 ± 2.52
VAR_25	95.17 ± 2.21
VAR_50	96.53 ± 3.79
VAR_100	95.64 ± 4.11
VAR_200	95.86 ± 2.26

*CTRL-control (NHDF without treatment); ALA_50 – positive control (NHDF treated with 50 µg/mL allantoin); VAR – *V. album* L. growth on black locust (*Robinia pseudoacacia* L.); VAR_25, VAR_50, VAR_100 and VAR_200 – NHDF treated with different concentrations of mistletoe: 25 µg/mL (VAR_25), 50 µg/mL (VAR_50), 100 µg/mL (VAR_100) and 200 µg/mL (VAR_200)

To determine the number of viable cells, the trypan blue dye exclusion test was used. The principle underlying this method is that living cells possess intact cell membranes, which determines the exclusion of the

trypan blue dye, while dead cells do not (Stoddart, 2011; Strober, 2015). Based on our results, no significant difference was observed between the four concentrations of mistletoe studied (VAR_25, VAR_50, VAR_100, VAR_200 $\mu\text{g}/\text{mL}$) and ALA_50 compared to CTRL.

Cell proliferation was performed using the MTS assay, on different concentrations of mistletoe extract (varied between 25 - 200 $\mu\text{g}/\text{mL}$), compared to 50 $\mu\text{g}/\text{mL}$ allantoin (ALA_50) which represents the positive control, as well as with CTRL (Figure 4). There was no inhibition of NHDF cell proliferation in the samples tested. Based on the results obtained (Figure 4), mistletoe showed no significantly increases of cell viability percentage compared to CTRL, during the first 12 hours. After 24 or 36 hours, respectively, the percentage of cell viability shows an increase in all mistletoe samples, more significant for VAR at 25 $\mu\text{g}/\text{mL}$ and 50 $\mu\text{g}/\text{mL}$. Furthermore, the VAR concentrations tested (ranging from 25 to 200 $\mu\text{g}/\text{mL}$) had no cytotoxic effect on NHDF 36 hours after treatment, enabling the extracts obtained from mistletoe leaves to be tested on the cell proliferation process and the cicatrizing effect.

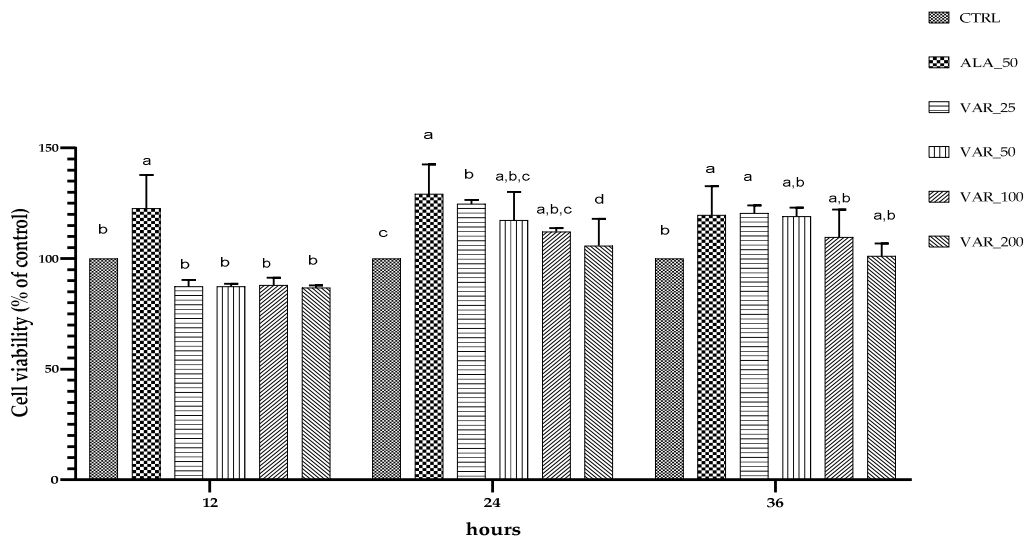


Figure 4. The effect of different concentrations of *V. album* harvested from black locust (25, 50, 100 and 200 $\mu\text{g}/\text{mL}$) on cell viability of NHDF after 12-, 24- and 36-hours using MTS assay CTRL-untreated cells; ALA_50-allantoin 50 $\mu\text{g}/\text{mL}$; VAR_25-mistletoe extract 25 $\mu\text{g}/\text{mL}$; VAR_50-mistletoe extract 50 $\mu\text{g}/\text{mL}$; VAR_100-mistletoe extract 100 $\mu\text{g}/\text{mL}$; VAR_200-mistletoe extract 200 $\mu\text{g}/\text{mL}$. Different lowercase letters indicate significant differences between samples ($p < 0.05$) for each time analyzed (12, 24 and 36 hours, respectively).

It is known that, at certain concentrations, mistletoe has a cytotoxic effect, that can influence the results of the functional wound healing test (Kuonen *et al.*, 2013; Choi *et al.*, 2022; Wright *et al.*, 2022). Kuonen *et al.* (2013) investigated the effects of mistletoe lipophilic extract and oleanolic acid on the migration of NIH/3T3 fibroblasts as well as HaCat keratinocytes using a wound healing assay, and the results showed that concentrations of mistletoe under 100 $\mu\text{g}/\text{mL}$ and oleanolic acid under 12.5 $\mu\text{g}/\text{mL}$ were non-toxic to NIH/3T3 fibroblast. After 48 hours of treatment, concentrations of mistletoe less than 200 $\mu\text{g}/\text{mL}$ and oleanolic acid less than 10 $\mu\text{g}/\text{mL}$ were non-toxic to HaCat keratinocytes, with cell viability around 90%. On wound closure, mistletoe concentrations ranging from 500 to 2000 $\mu\text{g}/\text{mL}$ and oleanolic acid of 50 $\mu\text{g}/\text{mL}$ inhibited NIH/3T3 fibroblasts significantly (Kuonen *et al.*, 2013).

In vitro wound healing potential of *V. album* L. (VAR) on NHDF

The scratch method is an *in vitro* technique, which provides valuable information about the biological properties of different extracts or compounds present in plants, widely used to evaluate the potential of cell proliferation for wound healing (Salehi *et al.*, 2020; Antonescu (Mintaş) *et al.*, 2021; Memete *et al.*, 2023a). Wound healing is a complex process, represented by the proliferation and migration activity of fibroblasts, characterized by a well-coordinated and progressive sequence of events, structured both in time and space (Eming *et al.*, 2007; Guo and DiPietro, 2010; Junker *et al.*, 2013).

In the present study, normal human dermal fibroblasts (NHDFs) were subjected to varying concentrations of mistletoe extract (25, 50, 100, and 200 µg/mL) in order to evaluate cell migration over different time intervals (0, 12, 24, and 36 h) (Figure 5).

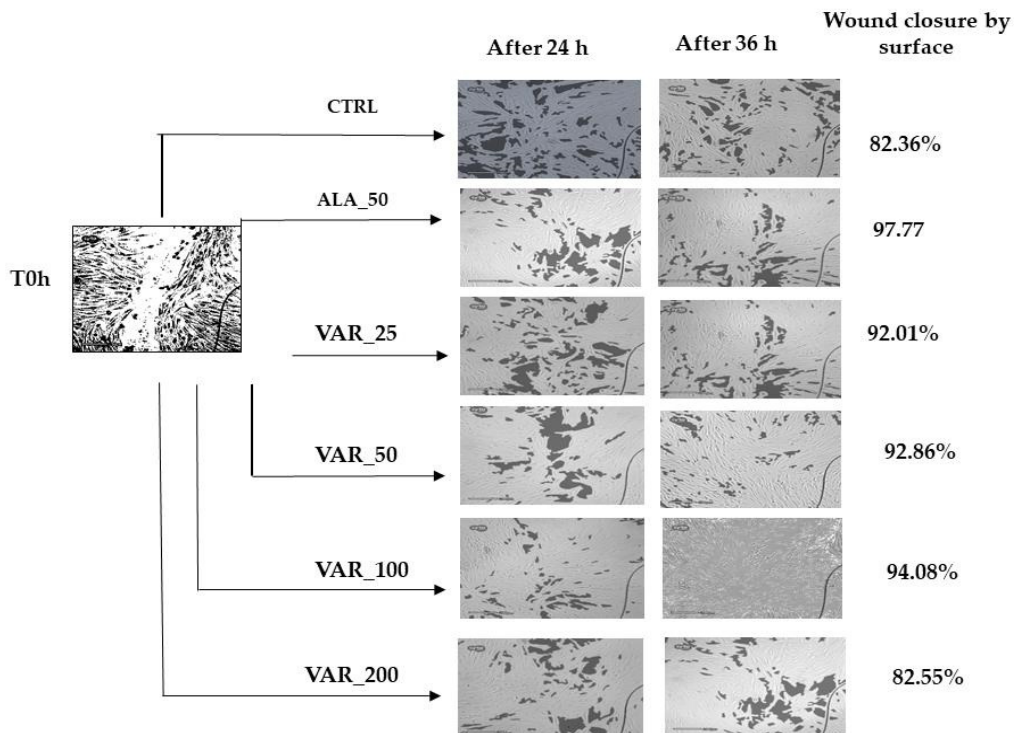


Figure 5. The evolution over time of the migration of normal dermal fibroblasts (NHDFs) for healing the wound formed *in vitro* by the scratch method according on the different concentrations of VAR extract (VAR_25-mistletoe extract 25 µg/mL; VAR_50-mistletoe extract 50 µg/mL; VAR_100-mistletoe extract 100 µg/mL; VAR_200-mistletoe extract 200 µg/mL). CTRL-untreated cells; ALA_50-allantoin 50 µg/mL

The images from Figure 5 were obtained using the CytoSMART Lux3BR® device. The wound closure by surface (W_{nd_A}) (%) were calculated using CytoSMART Lux3BR® and ImageJ software and the results are presented in Table 5.

Figure 5 shows the evolution over time of the closure of the “scratch” induced *in vitro* based on the treatment used. The results showed that the most effective was the treatment with VAR extract in a concentration of 100 µg/mL (VAR_100), the *in vitro* scratch coverage area was 94.08% in 36 h, a value close to ALA_50 (97.77%), used as positive control. According to the data in Table 5, it can be seen that, in the case of sample VAR_100 µg/mL, a statistically significant increase ($p < 0.05$) in the percentage of wound closure is obtained starting from the 24-36-hour time, compared to the 12-24-hour time. Also, significant differences

were noted between the concentrations of VAR_25, 50, 100 µg/mL and the concentration of VAR_200 µg/mL, which also showed the lowest percentage of closure.

Table 5. Wound closure according to the surface (%), following the application of different treatments at 12, 24 and 36 h

Samples Hours	CTRL	ALA_50	VAR_25	VAR_50	VAR_100	VAR_200
Wound closure by surface (Wd_A) (%)						
12	35.66 ± 1.32 ^b	47.13 ± 2.11 ^a	37.61 ± 1.16 ^b	34.72 ± 1.89 ^{b,c}	34.14 ± 1.86 ^{b,c}	31.63 ± 1.15 ^c
24	51.42 ± 1.27 ^c	64.25 ± 1.62 ^a	57.84 ± 1.08 ^b	53.89 ± 1.18 ^c	45.58 ± 1.23 ^d	45.58 ± 1.11 ^d
36	82.36 ± 1.57 ^c	97.77 ± 1.56 ^a	92.01 ± 1.12 ^b	92.86 ± 1.57 ^b	94.08 ± 1.11 ^b	82.55 ± 1.21 ^c

CTRL-untreated cells; ALA_50-allantoin 50 µg/mL; VAR_25-mistletoe extract 25 µg/mL; VAR_50-mistletoe extract 50 µg/mL; VAR_100-mistletoe extract 100 µg/mL; VAR_200-mistletoe extract 200 µg/mL. Different superscripts letters indicate significant differences (p < 0.05) on the line (between samples at the same time (hours)).

The wound healing process is a highly complex process and involves a number of factors acting in three stages (Kuonen *et al.*, 2013). The first stage is represented by vasoconstriction, which stops bleeding and takes care of self-cleaning of the wound; Also here, at the level of the wound, the transformation of prothrombin into thrombin and fibrinogen into fibrin takes place, then a crust of platelets is formed. The second stage is the pro-inflammatory stage and is generated by the immune system, where vasodilation occurs and phagocytic cells are focused on cleaning the wound, acting as antimicrobial elements (Antonescu (Mintaş) *et al.*, 2021; Miere *et al.*, 2021; Memete *et al.*, 2023a). VAR extract is a rich source of primary active ingredients consisting of glycoproteins know as mistletoe lectins (ML) and viscotoxins, as well as secondary ingredients such as alkaloids, phenolic acids, flavonoids, phytocompounds that have been shown to have antimicrobial activity and can be considered a stimulator of wound scar formation (Eming *et al.*, 2007; Metelmann *et al.*, 2012; Kleszken *et al.*, 2022b, 2022a; Wright *et al.*, 2022). The third phase is based on cell proliferation and wound remodeling. This stage begins with the phenomenon of migration and proliferation of fibroblasts and then the cell matrix and re-epithelialization are formed (Kuonen *et al.*, 2013; Wright *et al.*, 2022; Memete *et al.*, 2023a).

There are few studies that have tested the effect of topical mistletoe extract treatment on wound healing (Kunz *et al.*, 2011; Kuonen *et al.*, 2013; Wright *et al.*, 2022). For example, Kunz *et al.* (2011) used an ointment containing lipophilic mistletoe extract for the topical treatment of basal cell carcinoma. Clinical findings included the promotion of wound healing as well as antitumor properties of mistletoe extract, and a primary hemostatic effect of subsequent treatment was reported, with the aim of wound closure and partial or complete remission of basal cell carcinoma (Kunz *et al.* 2011). The authors Im *et al.* (2022) and Choi *et al.* (2022), demonstrated that a high content of phenolic compounds, respectively antioxidant compounds and vitamins, has beneficial effects on skin eruptions and helps in the stimulation and proliferation of dermal fibroblasts.

Phytocompounds, such as phenolic acids, flavonoids, from extracts can be considered as stimulators of dermal cell proliferation and migration, followed by re-epithelialization (Wilkinson and Hardman, 2020). Based on data from the literature (Wilkinson and Hardman, 2020), there is a direct relationship between chemical compounds, such as phenolic acids (ferulic, caffeic, vanillic), kaempferol (Özay *et al.*, 2019), Quercetin derivatives (Beken *et al.*, 2020), compounds that are also present in mistletoe extracts, and the process of regeneration and wound healing.

Conclusions

Mistletoe leaves that parasitize black locust were examined in this study. Spectrophotometric and chromatographic analyses have revealed that mistletoe leaves contain significant amounts of phenolic compounds, which contribute to mistletoe's antioxidant capacity. The ability of mistletoe leaves to reduce the inorganic selenium salt in NSePs with a size of approximately 130 nm was highlighted for the first time in the literature. It was also stated that the mistletoe aqueous extract is not toxic to NHDF at the tested concentrations and has the ability to repair the artificially induced wound *in vitro*. More research is needed to reveal the mechanisms underlying wound healing and to identify the compounds responsible for this process.

Authors' Contributions

Conceptualization, S.I.V., S.C. and M.G.; Formal analysis, S.I.V.; Funding acquisition, M.G.; Investigation, A.R.M., F.M., T.E.F., D.S.M.; Methodology, A.R.M., F.M., E.K.; Project administration, MG. and A.A.; Resources, S.I.V. and M.G.; Supervision, S.I.V.; Validation, T.E.F., A.A. and A.R.M.; writing original draft—S.I.V., A.R.M., F.M. and S.C.; writing—review and editing, S.I.V. and A.R.M. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the University of Oradea through the grant competition “Excellent scientific research related to the priority fields with the goal of technology transfer: INO-TRANSFER-UO” (grant number 309/21.12.2021).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abdel-Gaber R, Hawsah MA, Al-Shaebi EM, Al-Otaibi T, Thagfan FA, ... Dkhil MA (2023). Effect of biosynthesized nanoselenium using *Azadirachta indica* (Meliaceae) leaf extracts against *Eimeria papillata* infection. *Microscopy Research and Technique* 86:714-724. <http://doi.org/10.1002/jemt.24331>
- Antonescu (Mintaş) IA, Antonescu A, Miere (Groza) F, Fritea L, Teuşdea AC, Vicaş L, ... Cavalu S (2021). Evaluation of wound healing potential of novel hydrogel based on *Ocimum basilicum* and *Trifolium pratense* extracts. *Processes* 9:2096. <https://doi.org/10.3390/pr9112096>
- Bandici L, Teuşdea A, Milian OP, Memete AR, Vicas SI, Timar AV, Bandici GE (2021). The use of microwaves in the process of reducing the browning of apple slices. In: 2021 16th International Conference on Engineering of

- Modern Electric Systems (EMES), 1–4. Oradea, Romania: IEEE.
<https://doi.org/10.1109/EMES52337.2021.9484152>
- Barbu CO (2012). Impact of white mistletoe (*Viscum album* ssp. *abietis*) infection on needles and crown morphology of silver fir (*Abies alba* Mill.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 40:152-158.
<https://doi.org/10.15835/nbha4027906>
- Beken B, Serttas R, Yazicioglu M, Turkekul K, Erdogan S (2020). Quercetin improves inflammation, oxidative stress, and impaired wound healing in atopic dermatitis model of human keratinocytes. *Pediatric Allergy, Immunology, and Pulmonology* 33:69-79. <https://doi.org/10.1089/ped.2019.1137>
- Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of “Antioxidant Power”: The FRAP assay. *Analytical Biochemistry* 239:70-76. <https://doi.org/10.1006/abio.1996.0292>
- Bonamin LV, de Carvalho AC, Waisse S (2017). *Viscum album* (L.) in experimental animal tumors: A meta-analysis. *Experimental and Therapeutic Medicine* 13:2723-2740. <https://doi.org/10.3892/etm.2017.4372>
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* 28:25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).
- Burlou-Nagy C, Bănică F, Negrean RA, Jurca T, Vicaș LG, Marian E, ... Pallag A (2023). Determination of the bioactive compounds from *Echinacea purpurea* (L.) Moench leaves extracts in correlation with the antimicrobial activity and the *in vitro* wound healing potential. *Molecules* 28:5711. <https://doi.org/10.3390/molecules28155711>
- Büssing Arndt (2000). Mistletoe [Elektronisk resurs] the genus *Viscum*. Harwood Academic, Amsterdam.
- Cavalu S, Antoniac IV, Fritea L, Mates IM, Milea C, Laslo V, Vicas S, Mohan A (2018a). Surface modifications of the titanium mesh for cranioplasty using selenium nanoparticles coating. *Journal of Adhesion Science and Technology* 32:2509-2522. <https://doi.org/10.1080/01694243.2018.1490067>
- Cavalu S, Damian G (2003). Rotational correlation times of 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-yloxy spin label with respect to heme and nonheme proteins. *Biomacromolecules* 4:1630-1635.
<https://doi.org/10.1021/bm034093z>
- Cavalu S, Kamel E, Laslo V, Fritea L, Costea T, Antoniac IV, ... Vicas S (2018b). Eco-friendly, facile and rapid way for synthesis of selenium nanoparticles production, structural and morphological characterization. *Revista de Chimie* 68:2963-2966.
- Cavalu S, Prokisch J, Laslo V, Vicas S (2017). Preparation, structural characterization and release study of novel hybrid microspheres entrapping nanoselenium, produced by green synthesis. *IET Nanobiotechnology* 11:426-432.
<https://doi.org/10.1049/iet-nbt.2016.0107>
- Choi HJ, Park S-J, Choi YN, Kim S-D, Kwag E-B, Song S-Y, ... Yoo H-S (2022). Selective Immune Modulating Activities of *Viscum album* and Its Components; A Possibility of Therapeutics on Skin Rash Induced by EGFR Inhibitors. *Integrative Cancer Therapy* 21:15347354221118332. <https://doi.org/10.1177/15347354221118332>
- Chopra H, Bibi S, Singh I, Hasan MM, Khan MS, Yousafi Q, Baig AA, Rahman MdM, Islam F, Emran TB, Cavalu S (2022). Green metallic nanoparticles: biosynthesis to applications. *Frontiers in Bioengineering and Biotechnology* 10:874742. <https://doi.org/10.3389/fbioe.2022.874742>
- Condrat D, Szabo M-R, Crișan F, Lupea A-X (2009). Antioxidant activity of some phanerogam plant extracts. *FSTR* 15:95-98. <https://doi.org/10.3136/fstr.15.95>
- Cui D, Liang T, Sun L, Meng L, Yang C, Wang L, Liang T, Li Q (2018). Green synthesis of selenium nanoparticles with extract of hawthorn fruit induced HepG2 cells apoptosis. *Pharmaceutical Biology* 56:528-534.
<https://doi.org/10.1080/13880209.2018.1510974>
- Eming SA, Krieg T, Davidson JM (2007). Inflammation in wound repair: molecular and cellular mechanisms. *Journal of Investigative Dermatology* 127:514-525.
- Fritea L, Vasile L, Cavalu S, Costea T, Vicaș S (2017). Green biosynthesis of selenium nanoparticles using parsley (*Petroselinum crispum*) leaves extract. *Studia Universitatis Vasile Goldis Arad, Seria Stiintele Vietii* 27:203-208.
- Fritz P, Dippon J, Kierschke T, Siegle I, Möhring A, Moisa A, Mürdter TE (2004). Impact of mistletoe lectin binding in breast cancer. *Anticancer Research* 24:1187-1192.
- Glatzel G, Geils BW (2009). Mistletoe ecophysiology: host–parasite interactions. This review is one of a collection of papers based on a presentation from the Stem and Shoot Fungal Pathogens and Parasitic Plants: the Values of Biological Diversity session of the XXII International Union of Forestry Research Organization World Congress meeting held in Brisbane, Queensland, Australia, in 2005. *Botany* 87:10-15. <https://doi.org/10.1139/B08-096>

- Guo S, DiPietro LA (2010). Factors affecting wound healing. *Journal of Dental Research* 89:219-229. <https://doi.org/10.1177/0022034509359125>
- Huang X, Chen X, Chen Q, Yu Q, Sun D, Liu J (2016). Investigation of functional selenium nanoparticles as potent antimicrobial agents against superbugs. *Acta Biomaterialia* 30:397-407. <https://doi.org/10.1016/j.actbio.2015.10.041>
- Im J, Hyun J, Kim S-W, Bhang SH (2022). Enhancing the angiogenic and proliferative capacity of dermal fibroblasts with mulberry (*Morus alba*. L) root extract. *Tissue Engineering and Regenerative Medicine* 19:49-57. <https://doi.org/10.1007/s13770-021-00404-6>
- Jones P (2022). The Tenbury Mistletoe Festival & National Mistletoe Day. <https://holidappy.com/holidays/The-Tenbury-Mistletoe-Festival>
- Junker JPE, Caterson EJ, Eriksson E (2013). The microenvironment of wound healing. *Journal of Craniofacial Surgery* 24:12-16. <https://doi.org/10.1097/SCS.0b013e31827104fb>
- Kabakov AE, Kudryavtsev VA, Gabai VL (2011). Determination of Cell Survival or Death. In: Calderwood S.K., Prince T.L. (eds) *Molecular Chaperones*. Humana Press, Totowa, NJ, pp 231–244. https://doi.org/10.1007/978-1-61779-295-3_17
- Khiralla GM, El-Deeb BA (2015). Antimicrobial and antibiofilm effects of selenium nanoparticles on some foodborne pathogens. *LWT - Food Science and Technology* 63:1001-1007. <https://doi.org/10.1016/j.lwt.2015.03.086>
- Kleszken E, Purcarea C, Pallag A, Ranga F, Memete AR, Miere (Groza) F, Vicas SI (2022a). Phytochemical profile and antioxidant capacity of *Viscum album* L. Subsp. album and effects on its host trees. *Plants* 11:3021. <https://doi.org/10.3390/plants11223021>
- Kleszken E, Timar AV, Memete AR, Miere F, Vicas SI (2022b). On overview of bioactive compounds, biological and pharmacological effects of mistletoe (*Viscum album* L). *Pharmacophore* 13:10-26. <https://doi.org/10.51847/Tmo2sXGQRs>
- Kunz C, Heiligttag H, Hintze A, Urech K (2011). Treatment of basal cell carcinoma with *Viscum album* lipophilic extract—a case series study. *Phytomedicine: International Journal of Phytotherapy & Phytopharmacology* 18:S25-S25.
- Kuonen R, Weissenstein U, Urech K, Kunz M, Hostanska K, Estko M, Heusser P, Baumgartner S (2013). Effects of lipophilic extract of *Viscum album* L. and oleanolic acid on migratory activity of NIH/3T3 fibroblasts and on HaCat keratinocytes. *Evidence-Based Complementary and Alternative Medicine* 2013:e718105. <https://doi.org/10.1155/2013/718105>
- Kwon Y-S, Chun S-Y, Kim M-K, Nan H-Y, Lee C, Kim S (2021). Mistletoe extract targets the STAT3-FOXO1 pathway to induce apoptosis and inhibits metastasis in breast cancer cells. *The American Journal of Chinese Medicine* 49:487-504. <https://doi.org/10.1142/S0192415X21500221>
- Laslo V, Pinzaru SC, Zagula G, Kluz M, Vicas SI, Cavalu S (2022). Synergic effect of selenium nanoparticles and lactic acid bacteria in reduction cadmium toxicity. *Journal of Molecular Structure* 1247:131325. <https://doi.org/10.1016/j.molstruc.2021.131325>
- Luczkiewicz M, Cisowski W, Kaiser P, Ochocka R, Piotrowski A (2001). Comparative analysis of phenolic acids in mistletoe plants from various hosts. *Acta Polonica Pharma* 58:373-379.
- Malich G, Markovic B, Winder C (1997). The sensitivity and specificity of the MTS tetrazolium assay for detecting the *in vitro* cytotoxicity of 20 chemicals using human cell lines. *Toxicology* 124:179-192. [https://doi.org/10.1016/S0300-483X\(97\)00151-0](https://doi.org/10.1016/S0300-483X(97)00151-0)
- Marvibaigi M, Supriyanto E, Amini N, Abdul Majid FA, Jaganathan SK (2014). Preclinical and clinical effects of mistletoe against breast cancer. *Biomed Research International* 2014:785479. <https://doi.org/10.1155/2014/785479>
- Memete AR, Miere (Groza) F, Laslo V, Purcarea C, Vicas L, Ganea M, Antonescu A, Vicas SI (2023a). An *in vitro* study of the healing potential of black mulberry (*Morus nigra* L.) extract in a liposomal formulation. *Applied Sciences* 13:1041. <https://doi.org/10.3390/app13021041>
- Memete AR, Sărac I, Teusdea AC, Budău R, Bei M, Vicas SI (2023b). Bioactive compounds and antioxidant capacity of several blackberry (*Rubus* spp.) fruits cultivars grown in Romania. *Horticulturae* 9:556. <https://doi.org/10.3390/horticulturae9050556>

- Memete AR, Teusdea AC, Timar AV, Vuscan AN, Mintaş OS, Cavalu S, Vicas SI (2022). Effects of different edible coatings on the shelf life of fresh black mulberry fruits (*Morus nigra* L.). Agriculture 12:1068. <https://doi.org/10.3390/agriculture12071068>
- Metelmann HR, Brandner J, Schumann H, Bross F, Hoffmann M, Podmelle F (2012). Accelerating the aesthetic benefit of wound healing by triterpene. Journal of Cranio-Maxillofacial Surgery 40:e150-e154.
- Miere F, Teuşdea A, Vasile L, Cavalu S, Luminita F, Dobjanschi L, ... Vicaş S (2021). Evaluation of *in vitro* wound-healing potential, antioxidant capacity, and antimicrobial activity of *Stellaria media* (L.) Vill. Applied Sciences 11:11526. <https://doi.org/10.3390/app112311526>
- Miere FG, Ganea M, Teodorescu AG, Fritea L, Lestyan M, Horvath T, ... Vicaş SI (2022). The green synthesis of silver and selenium nanoparticles using the plant *Stellaria media* (L.) Vill. Pharmacophore 13:88-95. <https://doi.org/10.51847/rzjBEulH9c>
- Önay-Uçar E, Karagöz A, Arda N (2006). Antioxidant activity of *Viscum album* ssp. album. Fitoterapia 77:556-560. <https://doi.org/10.1016/j.fitote.2006.08.001>
- Özay Y, Güzel S, Yumrutaş Ö, Pehlivanoglu B, Erdoğan İH, Yildirim Z, Türk BA, Darcan S (2019). Wound healing effect of kaempferol in diabetic and nondiabetic rats. Journal of Surgical Research 233:284-296. <https://doi.org/10.1016/j.jss.2018.08.009>
- Peñaloza E, Holandino C, Scherr C, Araujo PIP de, Borges RM, Urech K, Baumgartner S, Garrett R (2020). Comprehensive metabolome analysis of fermented aqueous extracts of *Viscum album* L. by liquid chromatography-high resolution tandem mass spectrometry. Molecules 25:E4006. <https://doi.org/10.3390/molecules25174006>
- Pietrzak W, Nowak R (2021). Impact of harvest conditions and host tree species on chemical composition and antioxidant activity of extracts from *Viscum album* L. Molecules 26:3741. <https://doi.org/10.3390/molecules26123741>
- Rayman MP (2000). The importance of selenium to human health. The Lancet 356:233-241. [https://doi.org/10.1016/S0140-6736\(00\)02490-9](https://doi.org/10.1016/S0140-6736(00)02490-9)
- Salehi B, Butnariu M, Corneanu M, Sarac I, Vlavisavljevic S, Kitic D, ... Martins N (2020). Chronic pelvic pain syndrome: Highlighting medicinal plants toward biomolecules discovery for upcoming drugs formulation. Phytotherapy Research 34:769-787. <https://doi.org/10.1002/ptr.6576>
- Schulze E-D, Turner NC, Glatzel G (1984). Carbon, water and nutrient relations of two mistletoes and their hosts: A hypothesis*. Plant, Cell & Environment 7:293-299. <https://doi.org/10.1111/1365-3040.ep11589756>
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. Elsevier, pp 152-178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Stefanucci A, Zengin G, Llorent-Martinez EJ, Dimmito MP, Della Valle A, Pieretti S, Ak G, Sinan KI, Mollica A (2020). *Viscum album* L. homogenizer-assisted and ultrasound-assisted extracts as potential sources of bioactive compounds. Journal of Food Biochemistry 44. <https://doi.org/10.1111/jfbc.13377>
- Stoddart MJ (2011). Cell viability assays: introduction. In: Stoddart MJ (Eds). Mammalian Cell Viability. Humana Press, Totowa, NJ, pp 1-6. https://doi.org/10.1007/978-1-61779-108-6_1
- Strober W (2015). Trypan blue exclusion test of cell viability. Current Protocols in Immunology 111. <https://doi.org/10.1002/0471142735.ima03bs111>
- Szurpnicka A, Zjawiony JK, Szterk A (2019). Therapeutic potential of mistletoe in CNS-related neurological disorders and the chemical composition of *Viscum* species. Journal of Ethnopharmacology 231:241-252. <https://doi.org/10.1016/j.jep.2018.11.025>
- Tran PA, Webster TJ (2011). Selenium nanoparticles inhibit *Staphylococcus aureus* growth. International Journal Nanomedicine 6:1553-1558. <https://doi.org/10.2147/IJN.S21729>
- Vergara-Barberán M, Lerma-García MJ, Nicoletti M, Simó-Alfonso EF, Herrero-Martínez JM, Fasoli E, Righetti PG (2017). Proteomic fingerprinting of mistletoe (*Viscum album* L.) via combinatorial peptide ligand libraries and mass spectrometry analysis. Journal of Proteomics 164:52-58. <https://doi.org/10.1016/j.jpro.2017.05.025>
- Vicas SI, Laslo V, Timar AV, Balta C, Herman H, Ciceu A, ... Hermenean A (2021a). Nano selenium—enriched probiotics as functional food products against cadmium liver toxicity. Materials 14:2257. <https://doi.org/10.3390/ma14092257>

- Vicas SI, Laslo V, Timar AV, Balta C, Herman H, Ciceu A, ... Hermenean A (2021b). Functional food product based on nanoselenium-enriched *Lactobacillus casei* against cadmium kidney toxicity. Applied Sciences 11:4220. <https://doi.org/10.3390/app11094220>
- Vicas SI, Rugin D, Socaciu C (2011). Comparative study about antioxidant activities of *Viscum album* from different host trees, harvested in different seasons. JMPR 5:2237-2244. <https://doi.org/10.5897/JMPR.9000380>
- Wilkinson HN, Hardman MJ (2020). Wound healing: cellular mechanisms and pathological outcomes. Open Biology 10:200223. <https://doi.org/10.1098/rsob.200223>
- Wode K, Hök Nordberg J, Kienle GS, Elander NO, Bernhardson B-M, Sunde B, Sharp L, Henriksson R, Fransson P (2020). Efficacy of mistletoe extract as a complement to standard treatment in advanced pancreatic cancer: study protocol for a multicentre, parallel group, double-blind, randomised, placebo-controlled clinical trial (MISTRAL). Trials 21:783. <https://doi.org/10.1186/s13063-020-04581-y>
- Wright A, Watanabe R, Koehler JW (2022). European mistletoe (*Viscum album*) extract is cytotoxic to canine high-grade astrocytoma cells *in vitro* and has additive effects with mebendazole. Veterinary Sciences 9:31. <https://doi.org/10.3390/vetsci9010031>.
- Yadang F, Kom C, Ngezeye Y, Mamat A, Diboue Betote PH, Agbor G (2022). *In vitro* anti-oxidative and anti-inflammatory properties of *Viscum album*. Academia Journal of Medicinal Plants 10:061-071. <https://doi.org/10.15413/ajmp.2021.0138>
- Yu Y, Zhou X-Z, Ye L, Yuan Q, Freeberg S, Shi C, Zhu P-W, Bao J, Jiang N, Shao Y (2018). Rhamnazin attenuates inflammation and inhibits alkali burn-induced corneal neovascularization in rats. RSC Advances 8:26696-26706. <https://doi.org/10.1039/c8ra03159b>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.
© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.