”BABEȘ-BOLYAI” UNIVERSITY OF CLUJ-NAPOCA

Faculty of Biology and Geology

Integrative Biology Doctoral School

Characterization and biotechnological potential of plant species containing berberine

Ph.D. Thesis

Summary

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Key words:

Plant extracts; morphology and ultrastructure; phytochemical determination; anti-inflammatory activity; antioxidant activity; cytotoxicity; SANS; SAXS

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Introduction

This doctoral thesis presents the results of research carried out on *Berberis vulgaris* L., *Mahonia aquifolium* (Pursh) Nutt., and *Phellodendron amurense* Rupr., as well as the plant extracts obtained from these species. The comparative study of these plant species, with important biological activities, focused on: the morphology, anatomy, and ultrastructure of the leaf, stem, and stem bark; the phytochemical composition; and the biological potential of alcoholic extracts, regarding: in vitro cytotoxicity on tumor cell lines, in vitro anti-inflammatory and antioxidant activities, the ability to reduce $AgNO_3$ for the purpose of silver nanoparticle formation, and the incorporation of plant extracts into lipid complexes.

This doctoral thesis is structured into four major chapters, and the present abstract summarizes the main ideas. The first chapter includes **Literature study** concerning the importance of plant use over time and their contribution to the progress of scientific research. The second chapter describes the **Materials and Methods** used in the research activities, the third chapter presents the main **Results and Discussions**, and the fourth chapter groups the **Conclusions**.

Research scope and objectives

The study investigates the phytochemical and functional profile of extracts from ***Berberis vulgaris, Mahonia aquifolium*** and ***Phellodendron amurense***, correlating chemical composition with effects on oxidative stress, chronic inflammation, and cytotoxicity, while also addressing the relationship between cellular structures and the biosynthesis of active compounds, as well as possible biotechnological applications through the development of nanoparticles and liposomes loaded with extracts.

The specific objectives of the research were:

1. To highlight the plant tissue structures of *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense* species, involved in the biosynthesis and accumulation of bioactive compounds with phytotherapeutic relevance;
2. To perform the phytochemical characterization of stem bark extracts from *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense* using biochemical and physical techniques, in order to evaluate the variability of chemical composition (alkaloids, polyphenols, etc.) depending on the type of extract (tincture) and to correlate it with possible biological effects;
3. To evaluate the cytotoxic activity of extracts from *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense* in in vitro models, on human tumor cell lines A375 and A549;

4. To evaluate the in vivo anti-inflammatory and antioxidant activity of extracts from *Phellodendron amurense*;
5. To use *Berberis vulgaris* extracts for the synthesis of silver (Ag) nanoparticles through green synthesis;
6. To incorporate extracts from *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense* into lipid complexes and to characterize them using physical methods.

1. Literature study

The species *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense*, belonging to the families Berberidaceae and Rutaceae, are distinguished by a phytochemical profile rich in alkaloids, flavonoids, and phenolic compounds, responsible for a wide range of biological effects. *B. vulgaris* has been associated with hepatoprotective, hypolipidemic, and antioxidant effects, mainly attributed to alkaloids such as berberine and berbamine, substances with anti-inflammatory and antimicrobial roles (Mohammadi et al., 2014; Song et al., 2015; Laamech et al., 2017; Aafi et al., 2022; Shakeri, 2024). *M. aquifolium*, native to North America, is valued both ornamentally and ecologically, as well as for its complex phytochemical composition, correlated with antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities (Manosalva et al., 2016; Tuzimski et al., 2023). *P. amurense*, also known as the Amur cork tree and traditionally used under the name "Huang Bai," presents a high concentration of alkaloids – berberine, palmatine, jatrorrhizine, and phellodendrine – responsible for significant antimicrobial, anti-inflammatory, and metabolic effects (Zarmouh et al., 2016; Sun et al., 2016; Akihisa et al., 2017; Sun et al., 2019).

Thus, this doctoral thesis focuses on the comparative study of the phytochemical composition and biological potential of plant extracts from *B. vulgaris*, *M. aquifolium*, and *P. amurense*.

2. Materials and methods

To achieve the objectives of this doctoral thesis, several studies were conducted:

a. Morphological and ultrastructural analysis of the leaf, stem and bark of *B. vurgaris*, *M. aquifolium* and *P. amurense*, by transmission microscopy (S/TEM);
b. Comparative phytochemical analysis of plant extracts from *B. vulgaris*, *M. aquifolium*, and *P. amurense* with regard to their biological potential (*in vitro* cytotoxic activities, *in vivo* anti-inflammatory and antioxidant activities);

c. Biotechnological exploitation of plant extracts from *B. vulgaris*, *M. aquifolium*, and *P. amurense* (green synthesis of Ag nanoparticles and incorporation of plant extracts into lipid complexes).

3. Results and discussions

a. Morphology and ultrastructure of the leaf, stem and bark of *B. vulgaris*, *M. aquifolium* and *P. Amurense*

The leaves, stems, and bark of *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense* were investigated by scanning electron microscopy (SEM) (Figures 1, 2, and 3). The leaves are the site of natural compound synthesis, while the bark is used for storage. The morphological appearance of the three organs provides information regarding the general health status of the plant (Ciorîță et al., 2024). SEM characterization highlighted the presence of stomata on the stem surface (Figure 2), while transverse and longitudinal sections showed a normal distribution of cells without microbial infections (Figure 3).

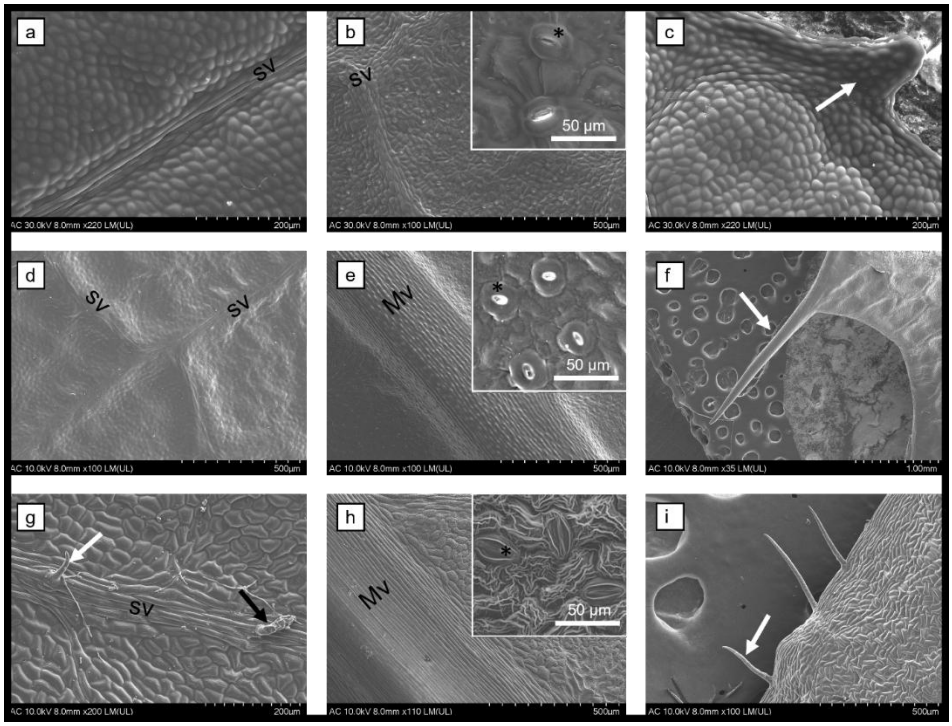

Figure 1. Scanning electron microscopy images of *B. vulgaris* (**a–c**), *M. aquifolium* (**d–f**) and *P. amurense* (**g–i**) **leaf** showing the upper epidermis (**a,d,g**), lower epidermis (**b,e,h**) and margins (**c,f,i**); Mv = midvein; sv = secondary vein; white arrow = protuberance/tector trichome; black arrow = secretory trichome; * = stomata (Ciorîță et al., 2024).

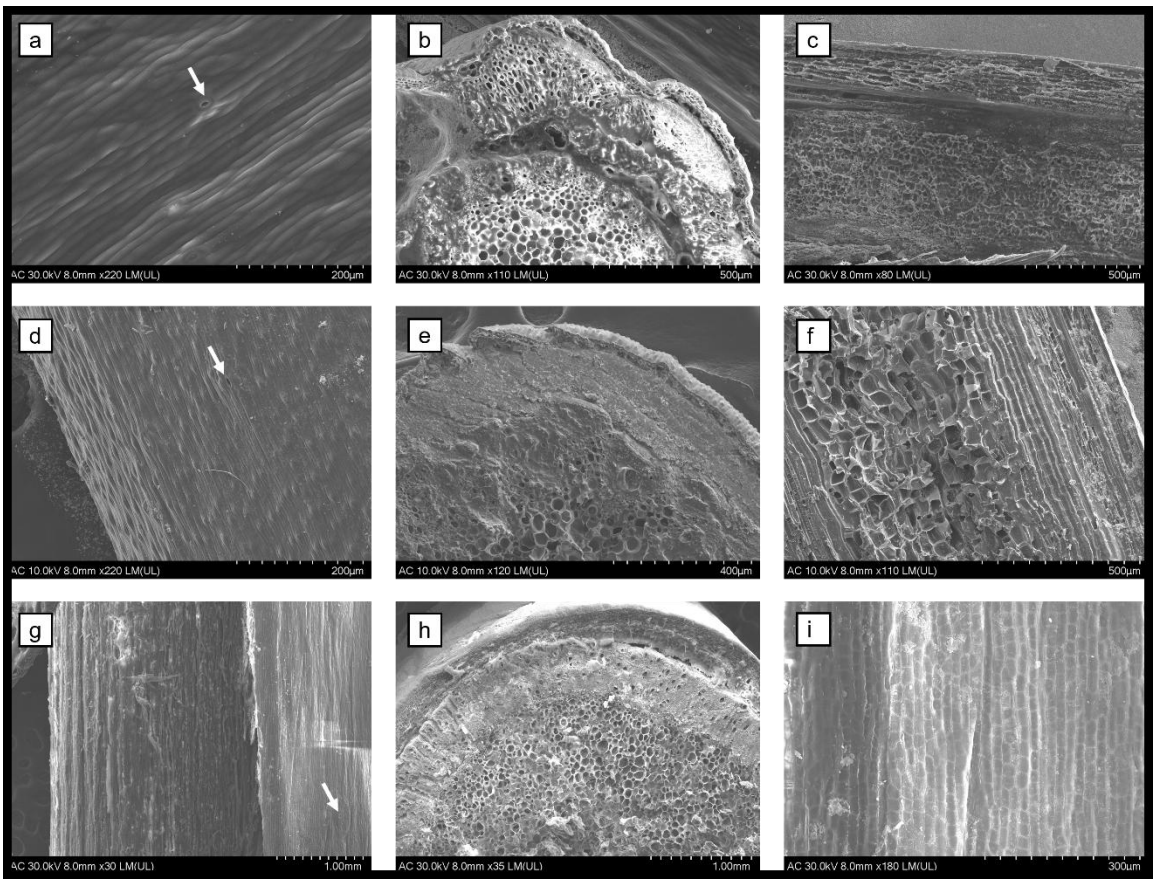

Figure 2. Scanning electron microscopy images of *B. vulgaris* (**a-c**), *M. aquifolium* (**d-f**) and *P. amurense* (**g-i**) **stems** showing the surfaces (**a,d,g**), cross-sections (**b,e,h**) and longitudinal sections (**c,f,i**); of the stems with randomly distributed stomata (white arrow) and normal appearance of the vascular bundles (Ciorîță et al., 2024).

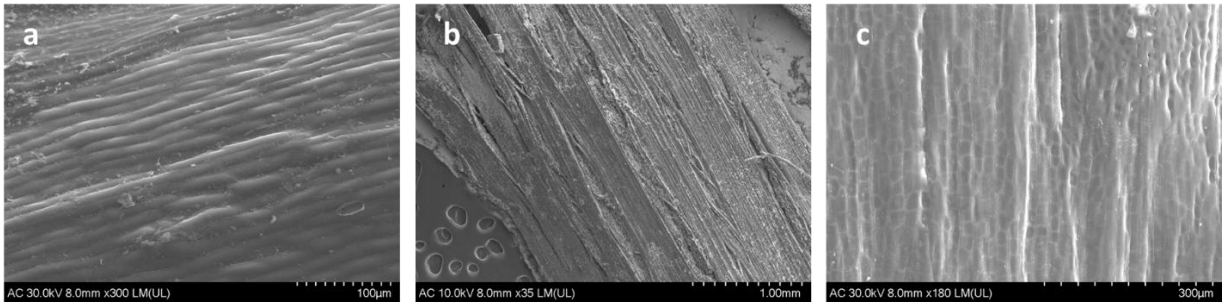

Figure 3. Scanning electron microscopy images of *B. vulgaris* (**a**), *M. aquifolium* (**b**) and *P. amurense* (**c**) **bark** that showed a normal appearance.

Analiza morfologică și ultrastructurală a frunzei (Figura 4 a-f), tulpinii (Figura 5 a-f) și scoarței tulpinii (Figura 6 a-i) celor trei plante de interes, au fost investigate prin microscopie electronică cu transmisie (TEM). Cu ajutorul acestui tip de investigare s-au putut evidenția veziculele bogate în fitoconstituenți, întâlnite la nivel celular, în cazul celor trei tipuri de plante investigate, dar și structura celulelor și a pereților celulari. De asemenea, cu ajutorul TEM s-a putut evidenția ultrastructura normală a celor trei plante de *B. vulvaris, M. aqifolium* și *P. amurense.*

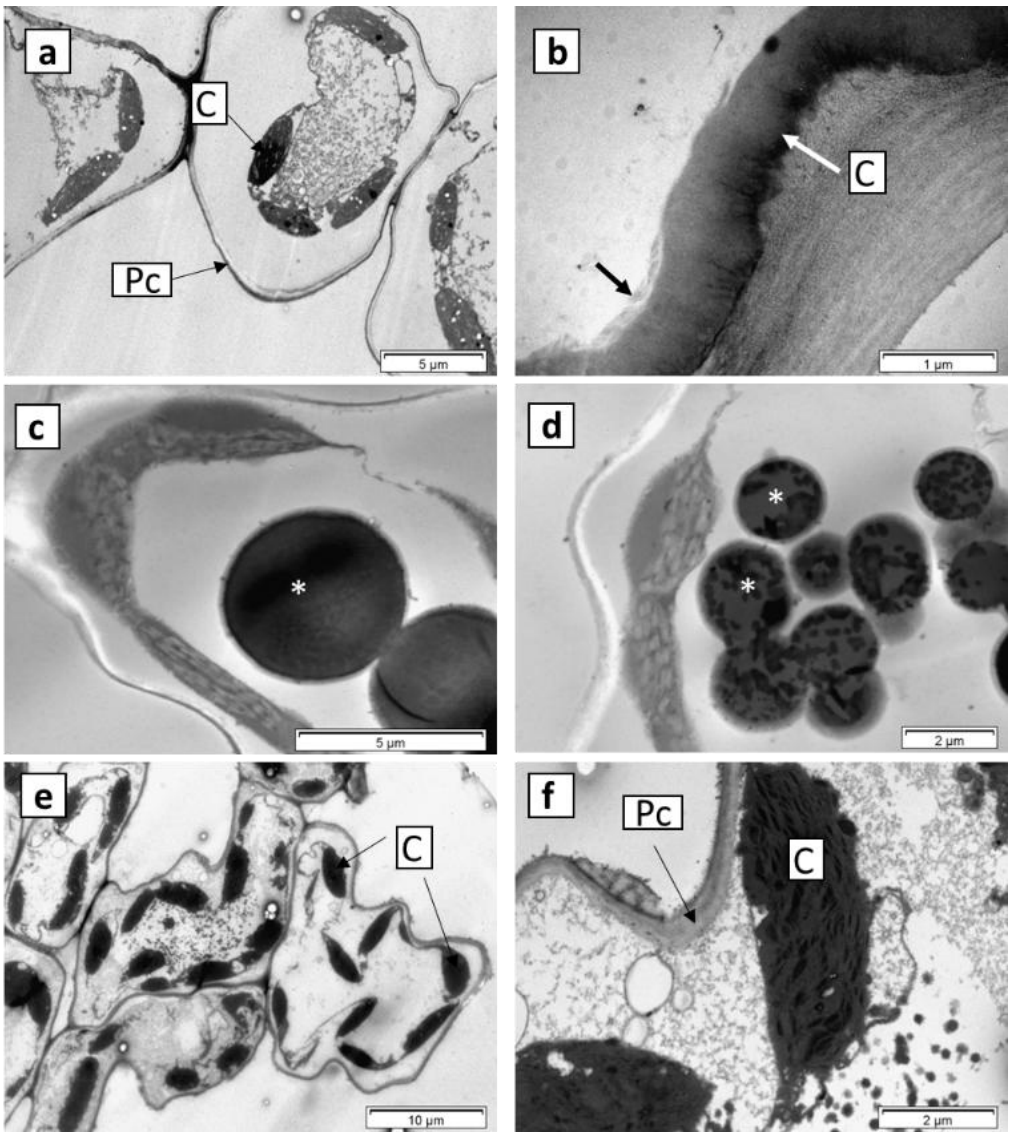

Figure 4. Transmission electron microscopy images of ultrathin sections through leaves of *Berberis vulgaris* (a–b), *Mahonia aquifolium* (c–d), and *Phellodendron amurense* (e–f). Chloroplasts (C), cell wall structure (Pc), and vesicles with phytoconstituents marked with an asterisk (*) can be observed. Arrows indicate areas of cell wall differentiation and the ultrastructural organization of chloroplasts. Scale bars: 1–10 µm.

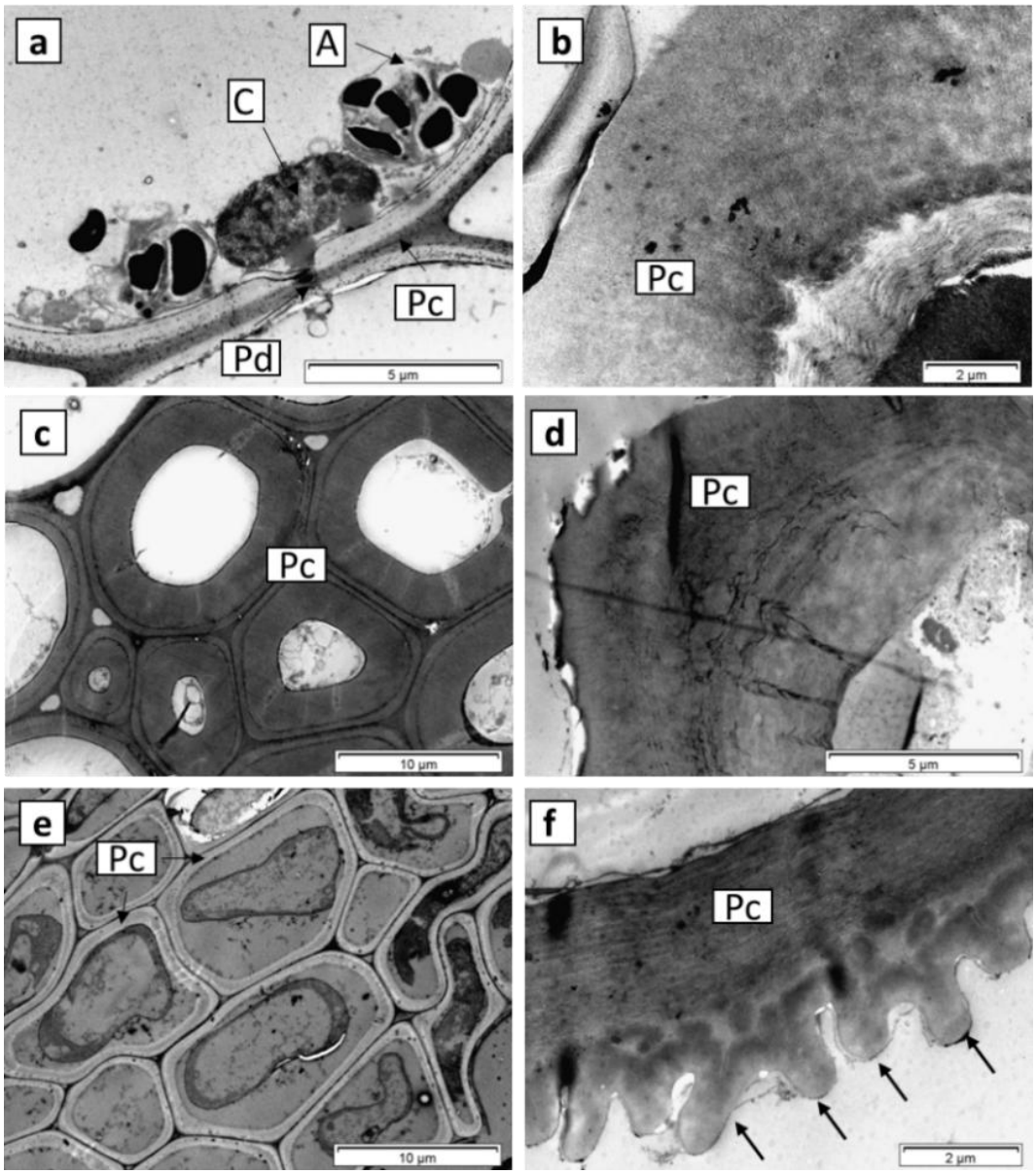

Figure 5. Transmission electron microscopy images of ultrathin transverse sections through the stem of *Berberis vulgaris* (a–b), *Mahonia aquifolium* (c–d), and *Phellodendron amurense* (e–f). The cell wall (Pc), chloroplasts (C), amyloplasts (A), and plasmodesmata (Pd) can be observed. Arrows indicate structural features of the cell wall and areas of intercellular connection.

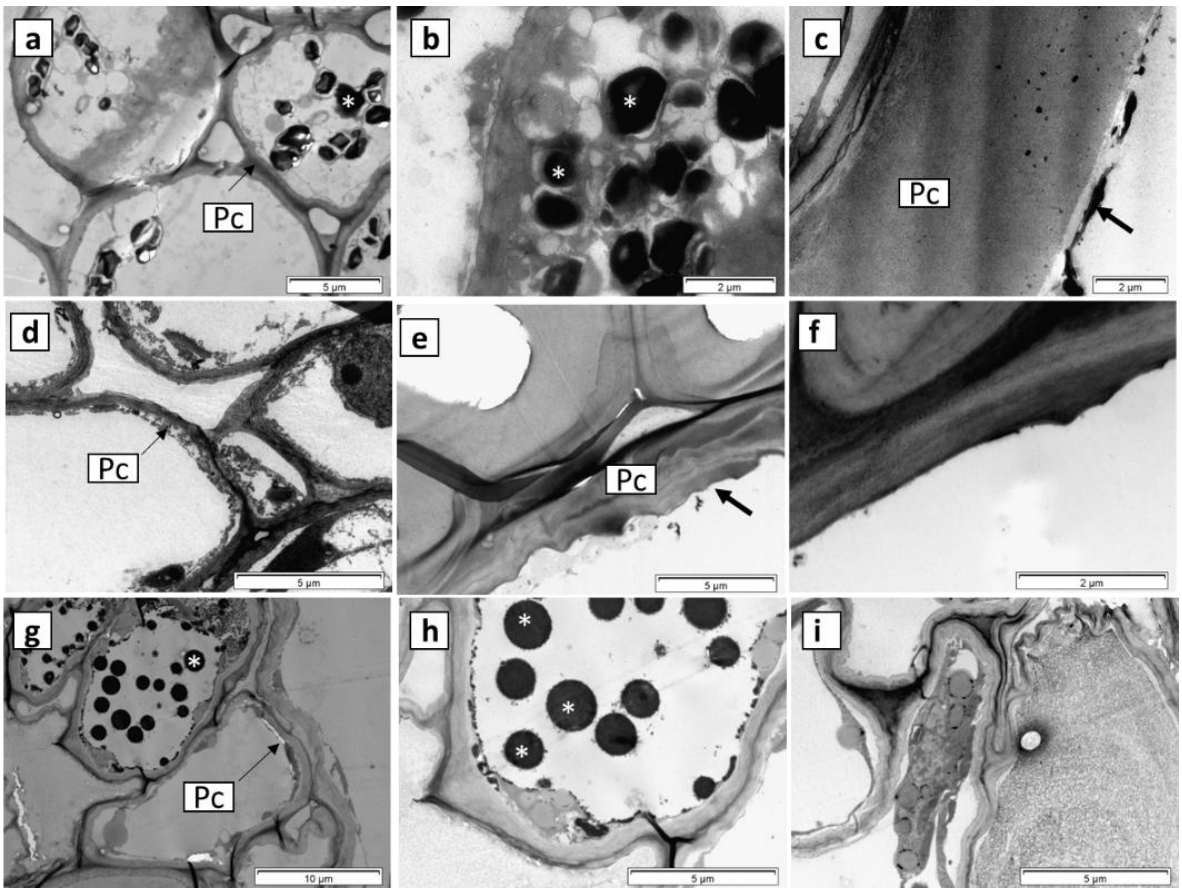

Figure 6. Transmission electron microscopy images of transverse sections through the inner bark of *Berberis vulgaris* (a–c), *Mahonia aquifolium* (d–f), and *Phellodendron amurense* (g–i), highlighting the cell wall structure (Pc) and the presence of vesicles with phytoconstituents (*). Arrows indicate areas of thickening and differentiation of the cell wall.

b. Phytochemical composition and pharmacological potential of extracts of *B. vulgaris*, *M. aquifolium* and *P. amurense*

The determination of the phytochemical composition showed that berberine, the compound of interest, was identified in each of the three plant species in different concentrations (*B. vulgaris* – 10.2±1.1 mg/g; *M. aquifolium* – 2.84±0.23 mg/g; *P. amurense* – 2.63±0.22) (Figure 1 and Table 1). The cytotoxic activity of plant extracts from *B. vulgaris*, *M. aquifolium*, and *P. amurense* is dependent on species and phytochemical composition. The cytotoxic activity of the three investigated plant extracts was tested on two tumor cell lines: A375 (melanoma) and A549 (lung adenocarcinoma) (Figures 2, 3, and 4) (Ciorîță et al., 2024). The results showed that *B. vulgaris* has the strongest inhibitory capacity against both cell lines, while *M. aquifolium* has a better potential against the A375 cell line, and *P. amurense* was the least toxic among the three tested plant extracts.

Table 1. Quantitative determination of chemical compounds from extracts of *Berberis vulgaris*, *Mahonia aquifolium* and *Phellodendron amurense* plants (Ciorîță et al., 2024).

No.	Compounds	Elution Time (min)	*B. vulgaris* (mg/g)	*M. aquifolium* (mg/g)	*P. amurense* (mg/g)
1	**Gallic acid**	**3.32**	0.08±0.02	<LOD	0.10±0.01
2	**4-hydroxybenzoic acid**	**9.98**	0.26±0.03	<LOD	0.38±0.03
3	**Caffeic acid**	**12.25**	<LOD	<LOD	<LOD
4	**P-coumaric acid**	**15.79**	<LOD	<LOD	<LOD
5	**Ferulic acid**	**17.24**	<LOD	<LOD	<LOD
6	**Berbamine**	**21.80**	1.32±0.11	1.09±0.12	<LOD
7	**Jatrorrhizine**	**24.70**	5.27±0.43	12.7±1.0	0.37±0.03
8	**Palmatine**	**29.70**	0.15±0.02	2.02±0.17	0.09±0.01
9	**Berberine**	**31.59**	10.2±1.1	2.84±0.23	2.63±0.22
LOD = limit of detection					

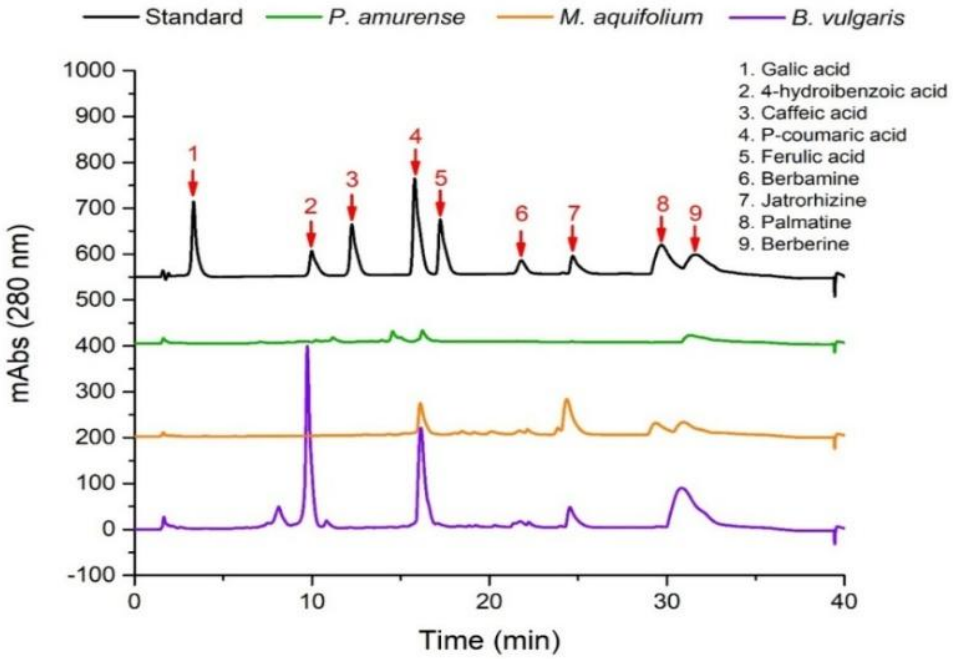

Figure 1. HPLC chromatograms of extracts from *B. vulgaris*, *M. aquifolium*, and *P. amurense* monitored at 280 nm; quantitative data are presented in Table 1.

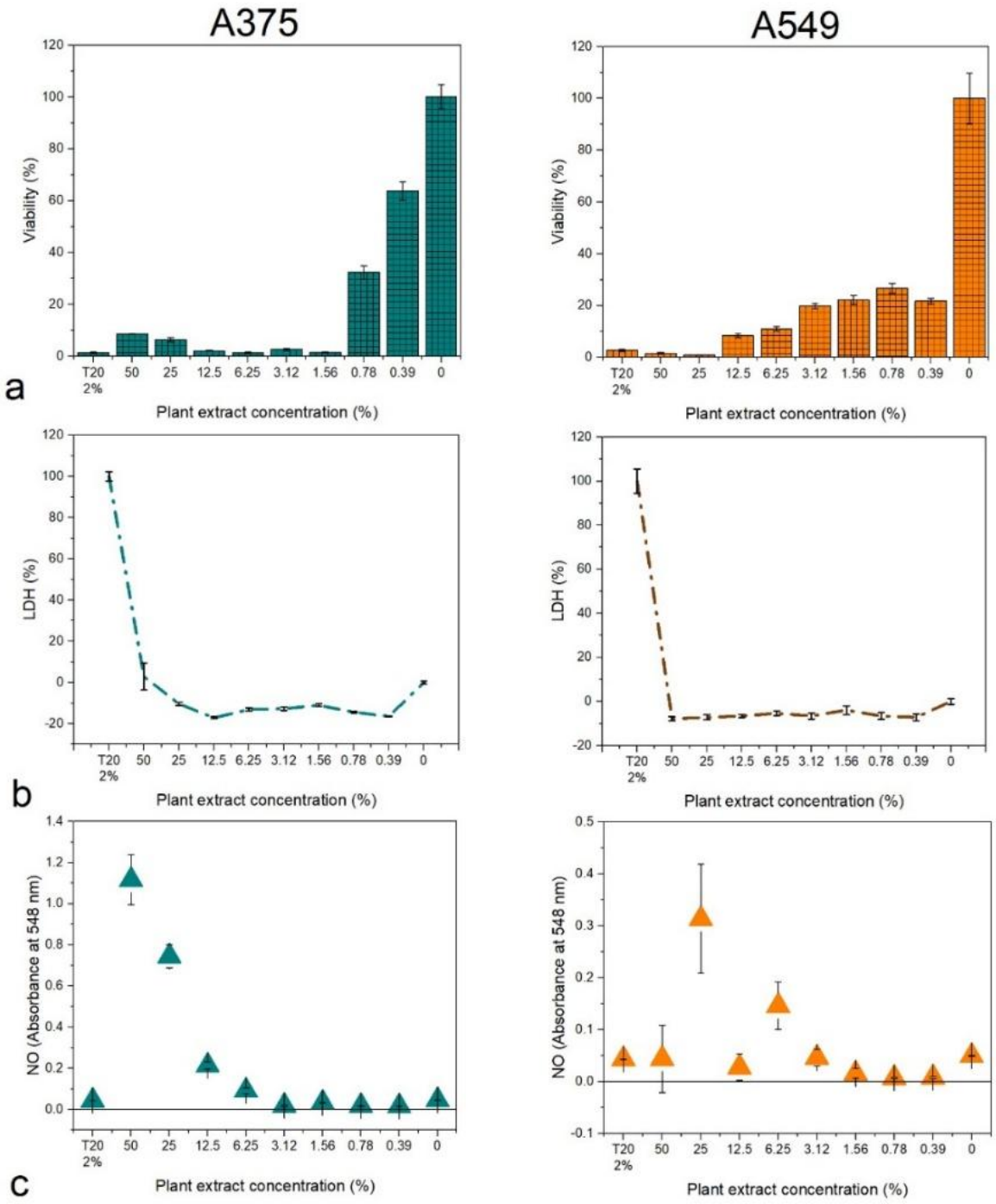

Figure 2. Cytotoxicity of *B. vulgaris* extract on human melanoma A375 cells and lung adenocarcinoma A549 cells; a) MTT viability assay, b) LDH membrane integrity assay, c) NO Griess assay. T20 = negative control Tween 20 (Ciorîță et al., 2024).

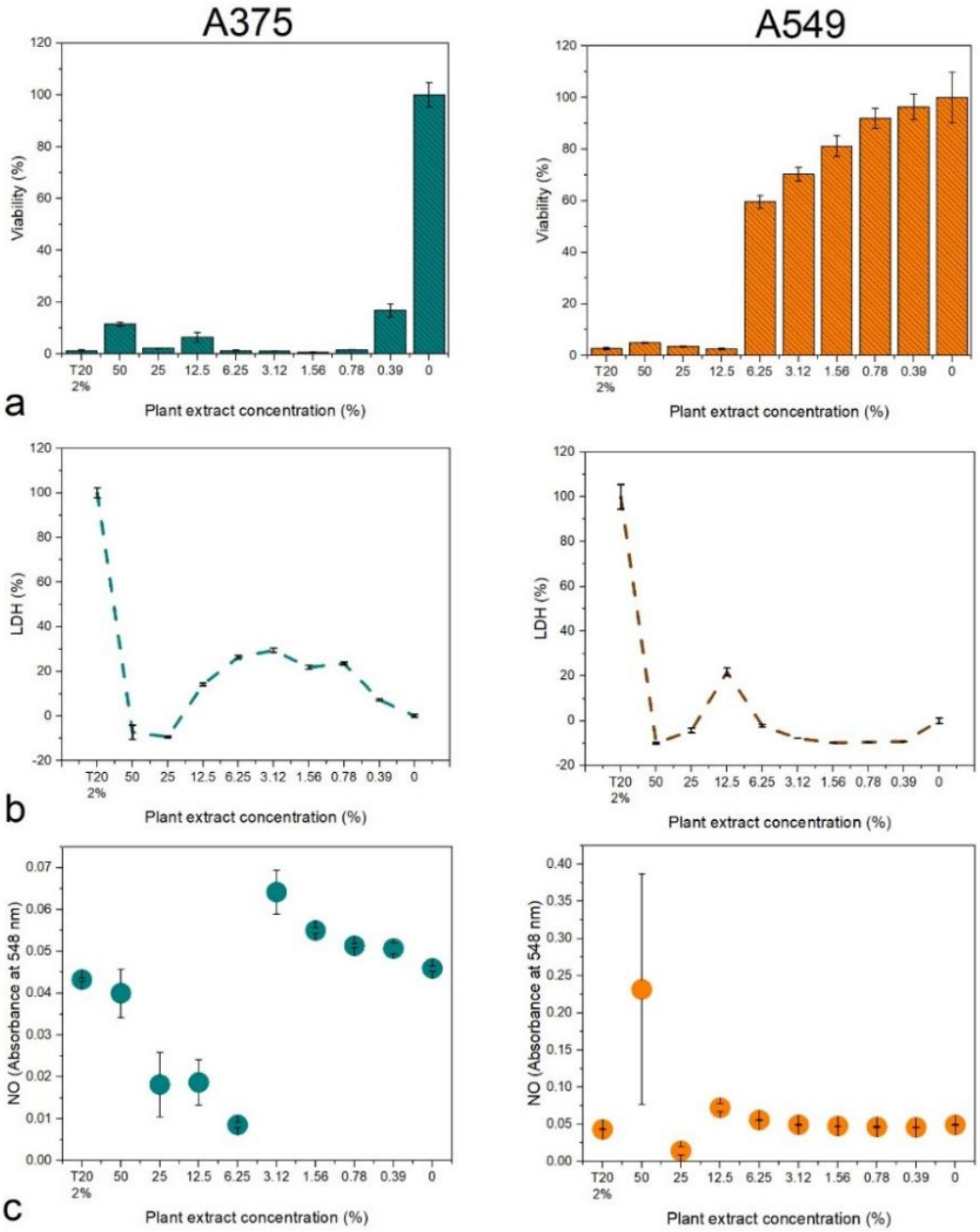

Figure 3. Cytotoxicity of *M. aquifolium* extract on human melanoma A375 cells and lung adenocarcinoma A549 cells; a) MTT viability assay, b) LDH membrane integrity assay, c) NO Griess assay. T20 = negative control Tween 20 (Ciorîță et al., 2024).

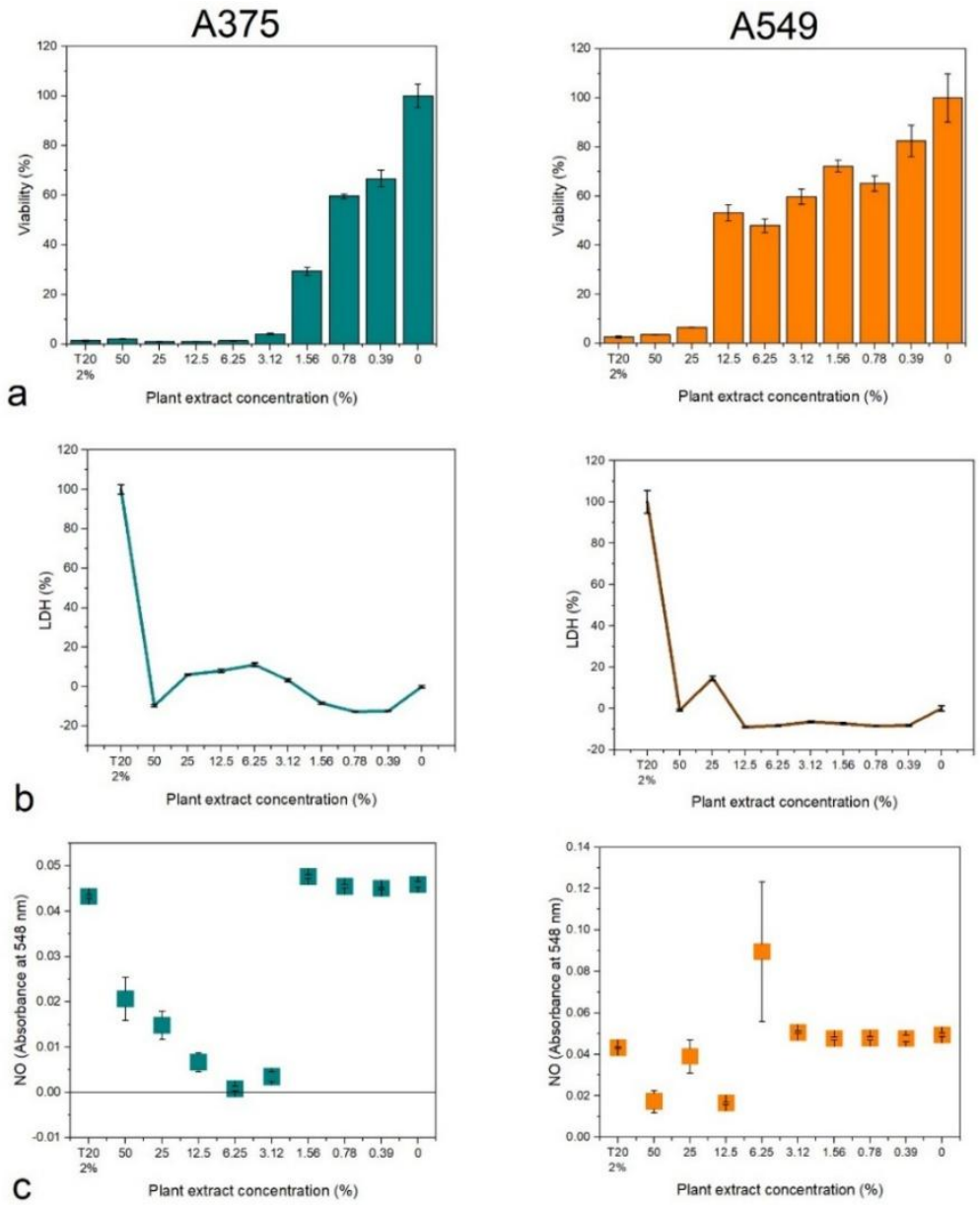

Figure 4. Cytotoxicity of *P. amurense* extract on human melanoma A375 cells and lung adenocarcinoma A549 cells; a) MTT viability assay, b) LDH membrane integrity assay, c) NO Griess assay. T20 = negative control Tween 20 (Ciorîță et al., 2024).

The extract of *P. amurense* proved to have anti-inflammatory activity, through the reduction of NF-κB, NOx, and 3NT, more prominent when administered as therapeutic treatment, and antioxidant activity, through the reduction of oxidative stress biomarkers MDA, TOS, and OSI, more prominent in the case of prophylactic treatment in turpentine-induced inflammation, in an in vivo experimental model on rats (Figure 5) (Erhan et al., 2023).

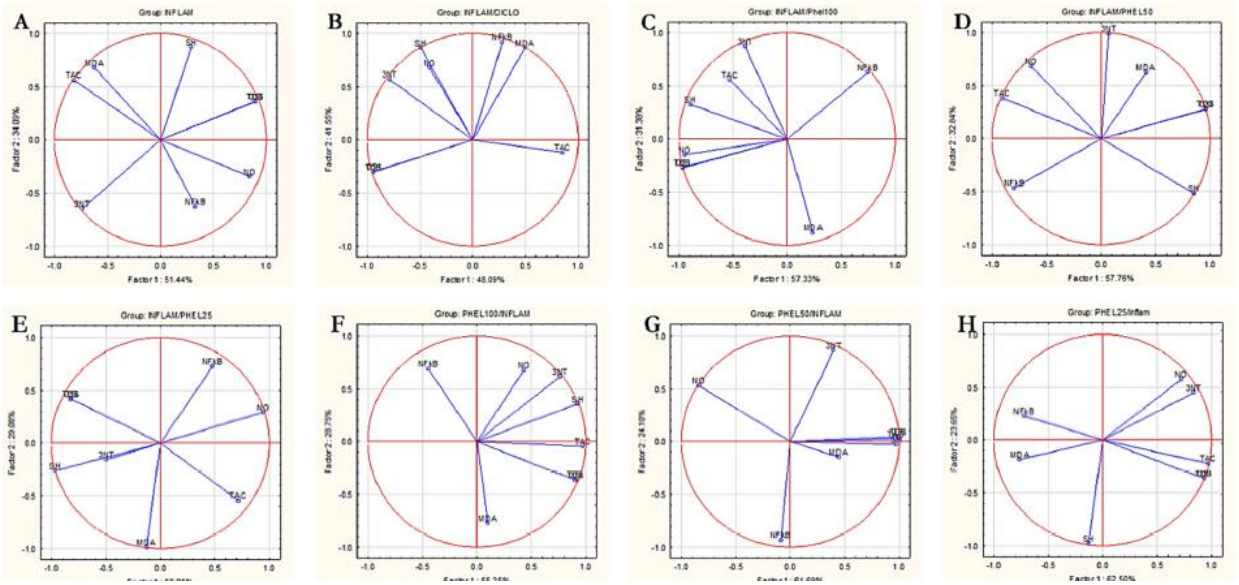

Figure 13. Anti-inflammatory and oxidative stress tests PCA correlation monoplots of rats with turpentine oil-induced acute inflammation and *P. amurense* bark extract treatments. (A) Inflam - inflammation group; B) Diclo - inflammation group treated with diclofenac; C) Inflam/Phel100 - inflammation group with *P. amurense* extract 100% treatment; D) Inflam/Phel50 - inflammation group with *P. amurense* extract 50% treatment; E) Inflam/Phel25 - inflammation group with *P. amurense* extract 25% treatment; F) group with Phel100/Inflam - *P. amurense* extract extract 100% prophylaxy followed by inflammation; G) group with Phel50/Inflam - *P. amurense* extract extract 50% prophylaxy followed by inflammation; H) group with Phel25/Inflam - *P. amurense* extract extract 25% prophylaxy followed by inflammation (Erhan și colab., 2023)

c. Biotechnological exploitation of plant extracts from *B. vulgaris*, *M. aquifolium*, and *P. amurense* (green synthesis of Ag nanoparticles and incorporation of plant extracts into lipid complexes).

The ability of *B. vulgaris* extract to reduce silver nitrate was investigated, focusing on reaction times of 5 and 18 hours of continuous stirring (Figure 5). The aim of the method was to highlight the possibility of obtaining NPs through green synthesis, targeting the production of small and uniform nanoparticles. This approach, which uses plant extracts, is becoming increasingly popular due to its ecological character and potential for accelerated production.

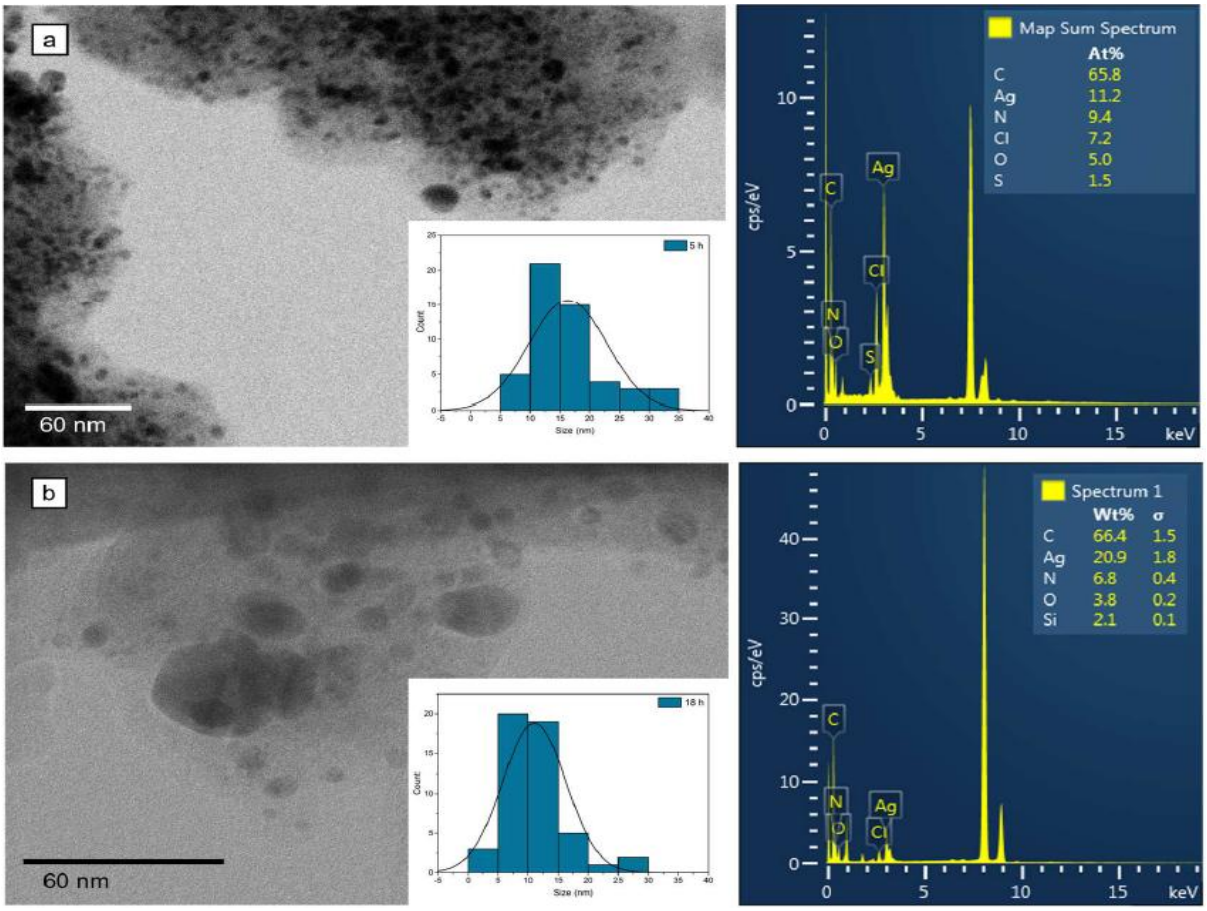

Figure 6. Transmission electron microscopy images and EDX spectra of Ag nanoparticles synthesized with *B. vulgaris* extract for 5 hours (a) and 18 hours (b). It can be observed that nanoparticles can form after 5 hours of incubation with the plant extract, with a broader size distribution (insets) compared to 18 hours of incubation. EDX confirmed the presence of silver in the samples, together with organic elements such as C, N, O, S, and Cl, which can be attributed to compounds from the plant extract, suggesting a core–shell structure with a metallic Ag core and an organic coating layer that provides stability to the nanoparticles (Ciorîță et al., 2024).

Considering that berberine, the main alkaloid common to *B. vulgaris*, *M. aquifolium*, and *P. amurense*, is unstable (Aljiin et al., 2017; Calvo et al., 2020; Duong et al., 2021), the incorporation of these plant extracts into three different types of lipid complexes was chosen: DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), and POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine). To investigate their stability under different temperature conditions (Figure 17), as well as the influence of plant extracts on the structure of the lipid complexes (Figures 18–23), two structural investigation methods were employed: small-angle neutron scattering and small-angle X-ray scattering (SANS and SAXS), methods that bring novelty to this doctoral thesis.

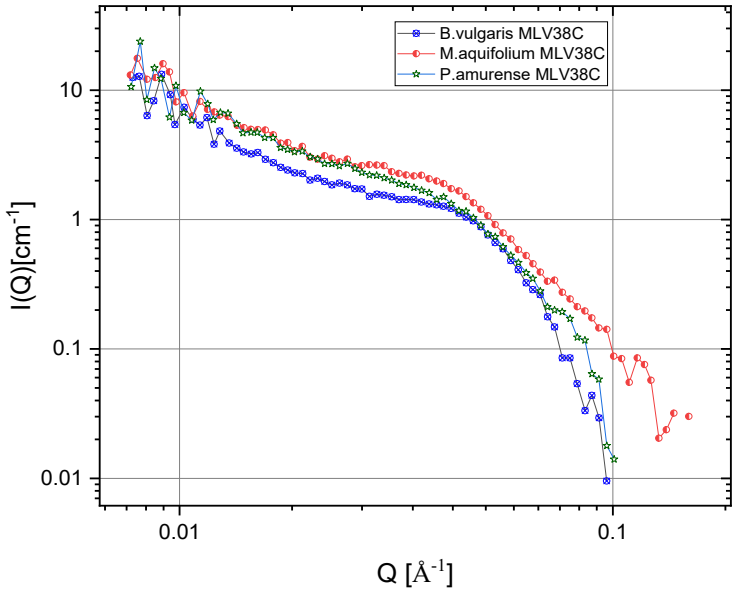

Figure 7. SANS scattering spectra showing the evolution of structural stability for multilamellar lipid complexes loaded with plant extracts of *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense*: DPPC MLV + *B. vulgaris*, DPPC MLV + *M. aquifolium*, DPPC MLV + *P. amurense*, tested at the same temperature of 38 °C (I(Q) [cm^{-1}] – scattering intensity; Q [$Å^{-1}$] – scattering vector; *B. vulgaris* MLV 38 °C; *M. aquifolium* MLV 38 °C; *P. amurense* MLV 38 °C).

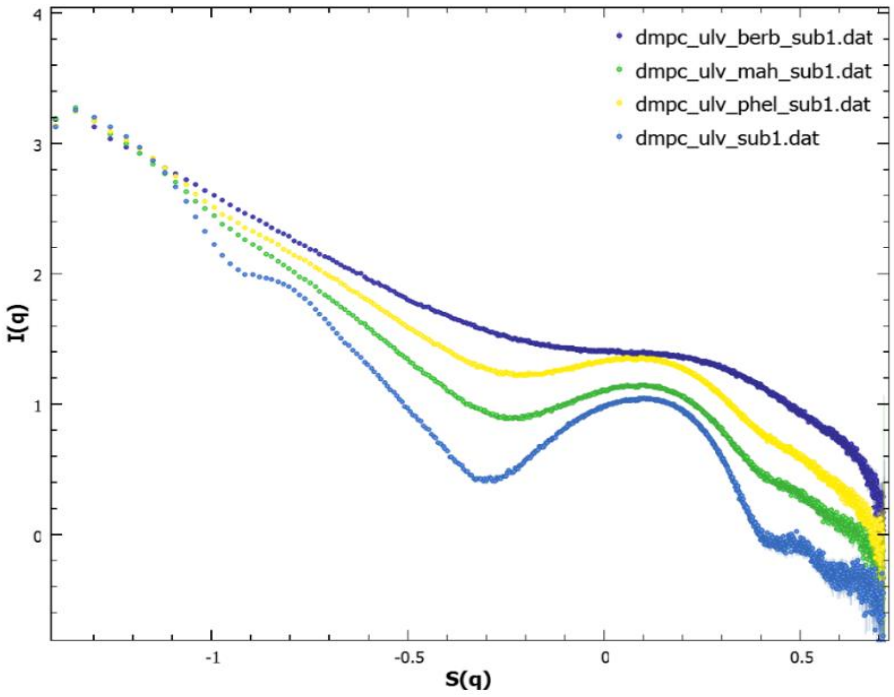

Figure 8. SAXS scattering spectra for lipid complexes DMPC ULV + *B. vulgaris* (indigo – dmpc_ulv_berb_sub1.dat), DMPC ULV + *M. aquifolium* (green – dmpc_ulv_mah_sub1.dat), DMPC ULV + *P. amurense* (yellow – dmpc_ulv_phel_sub1.dat), and DMPC + Milli-Q ultrapure water (blue – dmpc_ulv_sub1.dat) (I(q) [cm^{-1}] – scattering intensity; S(q) [$Å^{-1}$] – scattering vector).

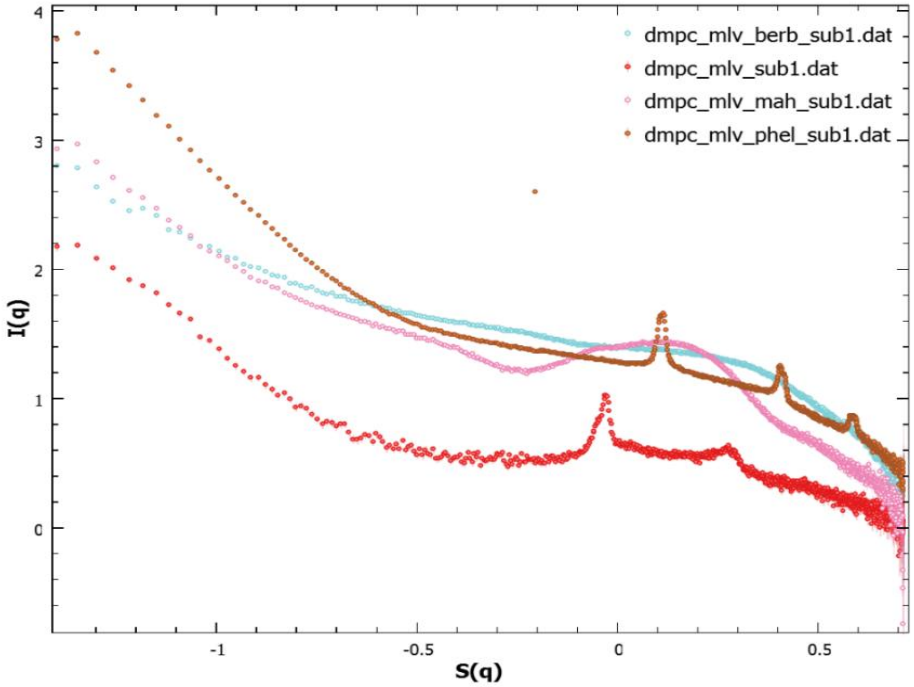

Figure 9. SAXS scattering spectra for lipid complexes DMPC MLV + *B. vulgaris* (turquoise – dmpc_mlv_berb_sub1.dat), DMPC MLV + *M. aquifolium* (pink – dmpc_mlv_mah_sub1.dat), DMPC MLV + *P. amurense* (brown – dmpc_mlv_phel_sub1.dat), and DMPC + Milli-Q ultrapure water (red – dmpc_mlv_sub1.dat) (I(q) [cm^{-1}] – scattering intensity; S(q) [$Å^{-1}$] – scattering vector).

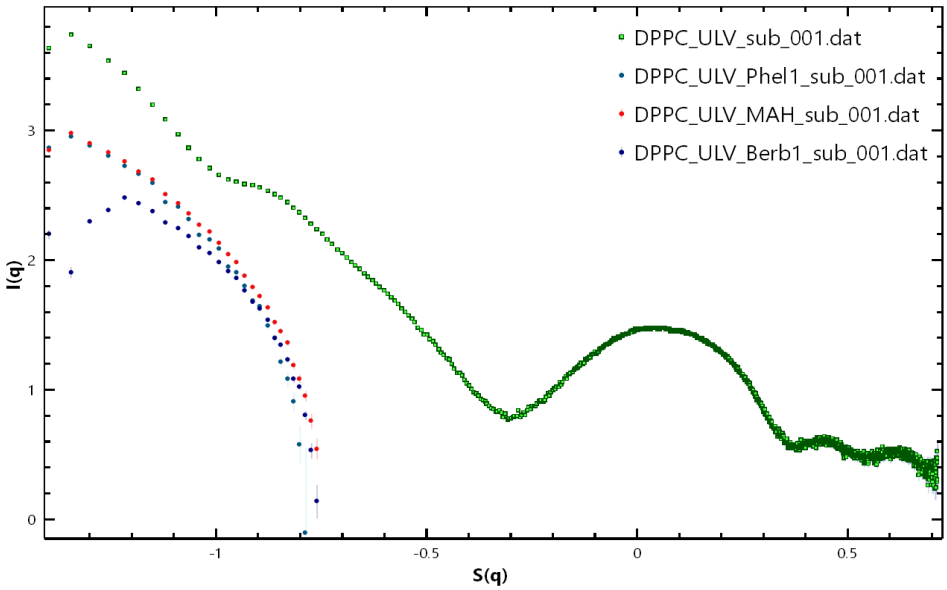

Figure 10. SAXS scattering spectra for lipid complexes DPPC ULV + *B. vulgaris* (Berb1 – indigo – DPPC_ULV_Berb1_sub_001.dat), DPPC ULV + *M. aquifolium* (MAH – red – DPPC_ULV_MAH_sub_001.dat), DPPC ULV + *P. amurense* (Phel1 – blue – DPPC_ULV_Phel1_sub_001.dat), and DPPC ULV + Milli-Q ultrapure water (green – DPPC_ULV_sub001.dat) (I(q) [cm^{-1}] – scattering intensity; S(q) [$Å^{-1}$] – scattering vector).

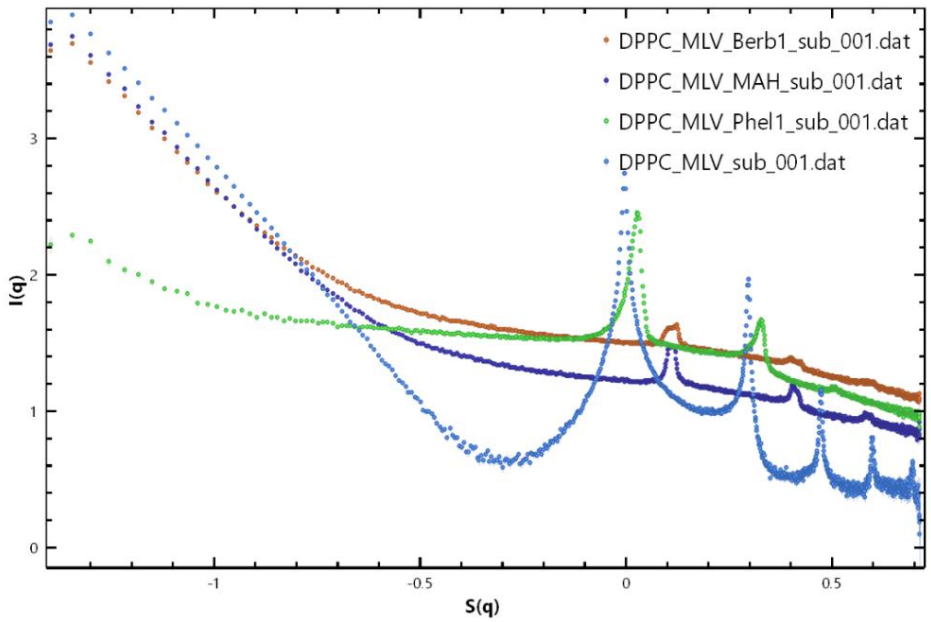

Figure 11. SAXS scattering spectra for lipid complexes DPPC MLV + *B. vulgaris* (Berb1 – orange – DPPC_MLV_Berb1_sub_001.dat), DPPC MLV + *M. aquifolium* (MAH – indigo – DPPC_MLV_MAH_sub_001.dat), DPPC MLV + *P. amurense* (Phel1 – green – DPPC_MLV_Phel1_sub_001.dat), and DPPC MLV + Milli-Q ultrapure water (blue – DPPC_MLV_sub001.dat) (I(q) [cm^{-1}] – scattering intensity; S(q) [$Å^{-1}$] – scattering vector).

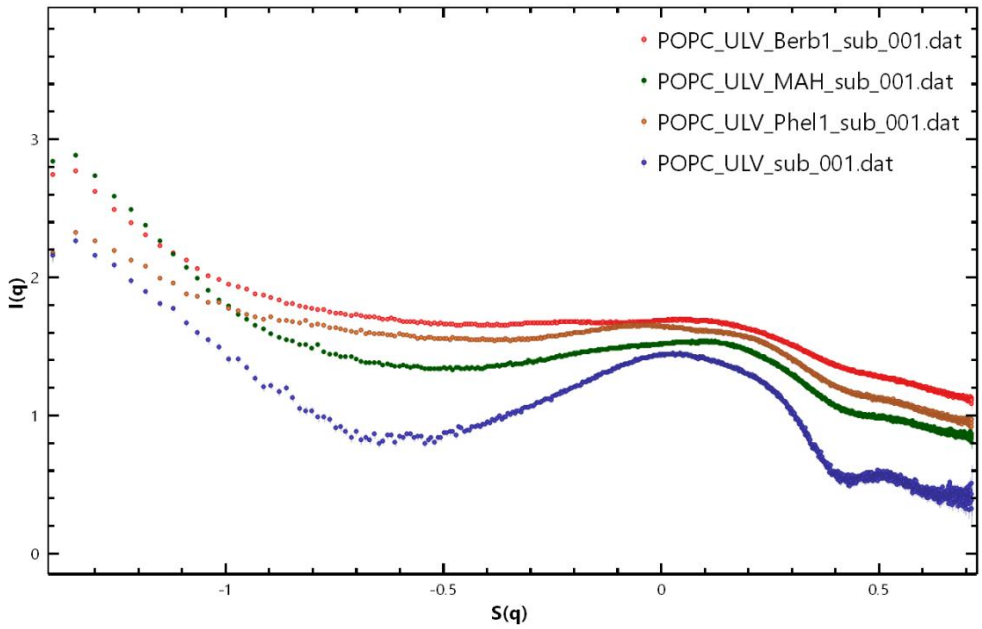

Figure 12. SAXS scattering spectra for lipid complexes POPC ULV + *B. vulgaris* (Berb1 – red – POPC_ULV_Berb1_sub_001.dat), POPC ULV + *M. aquifolium* (MAH – green – POPC_ULV_MAH_sub_001.dat), POPC ULV + *P. amurense* (Phel1 – brown – POPC_ULV_Phel1_sub_001.dat), and POPC ULV + Milli-Q ultrapure water (indigo – POPC_ULV_sub_001.dat) (I(q) [cm^{-1}] – scattering intensity; S(q) [$Å^{-1}$] – scattering vector).

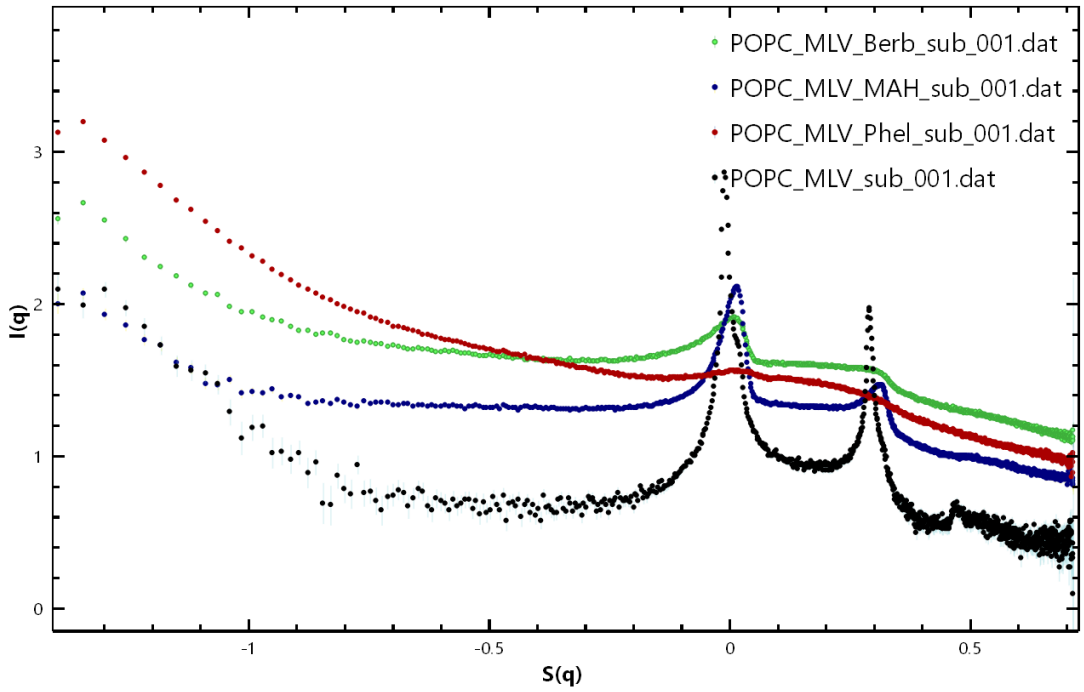

Figure 13. SAXS scattering spectra for lipid complexes POPC MLV + *B. vulgaris* (Berb1 – green – POPC_MLV_Berb1_sub_001.dat), POPC MLV + *M. aquifolium* (MAH – indigo – POPC_MLV_MAH_sub_001.dat), POPC MLV + *P. amurense* (Phel – burgundy – POPC_MLV_Phel1_sub_001.dat), and POPC MLV + Milli-Q ultrapure water (black – POPC_MLV_sub_001.dat) (I(q) [cm^{-1}] – scattering intensity; S(q) [$Å^{-1}$] – scattering vector).

4. Conclusions

The research carried out within this thesis provided relevant data regarding the phytochemical characterization, biological activity, and bionanotechnological applicability of extracts from *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense*, highlighting their bioactive potential and integration into innovative systems with modern biotechnological applications.

- Demonstration of the cytotoxic activity of extracts from *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense* on human lung adenocarcinoma and melanoma cells;
- Highlighting the anti-inflammatory and antioxidant activities of the extract of *Phellodendron amurense*t;
- Obtaining and functionalizing Ag nanoparticles with extract of *Berberis vulgaris*;
- Preparation and characterization of lipid complexes loaded with extract of *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense*.

This doctoral thesis comparatively describes the biological potential of plant extracts from *Berberis vulgaris*, *Mahonia aquifolium*, and *Phellodendron amurense*. The morphological

aspects of the leaf, stem, and stem bark, the chemical composition of the plant extracts, their biological effects, and their influence on the formulation of lipid complexes were investigated by different research methods in several research laboratories.

5. Dissemination

Articles in ISI indexed journals, as main author (* from the thesis topic)

***Erhan**, S.E., Pârvu, A.E., Ciorîță, A., Putri, A.A., Villagrana Molina, A.J., Pârvu, M., Moț, A.C., (2023), Chemical composition and anti-inflammatory effect of *Phellodendron amurense* Rupr. stem bark extract, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, Volume 51, Issue 3, Article number 13306, DOI:10.15835/nbha51313306 **FI:** 1.4; **AIS:** 0.215, Q4, 2023

*Ciorîță, A., **Erhan**, S.E., Soran, M.L., Lung, I., Moț, A.C., Macavei, S.G., Pârvu, M. (2024) Pharmacological Potential of Three Berberine-Containing Plant Extracts Obtained from *Berberis vulgaris* L., *Mahonia aquifolium* (Pursh) Nutt., and *Phellodendron amurense* Rupr, Biomedicine, Volume 12, Article ID 1339, https://doi.org/10.3390/biomedicines12061339 **FI:** 3.9; **AIS:** 0.808, Q2, 2024

Conference participations (* from the thesis topic)

A. National

***Pojar**, S.E, Ciorîță, A., Tripon, S., Soran, L., Lung, I., Macavei, S., Pârvu, M., *Berberis vulgaris* L. Stem Extract used for the Green Synthesis of Copper Nanoparticles with potential applications in phytotherapy, 8th Edition of the BioTA Symposium: Biodiversity-Traditions and Current Affairs, Online, 2021, Cluj-Napoca, România (https://biogeo.ubbcluj.ro/biota-2021/)

***Pojar**, S.E., Ciorîță, A., Pârvu, M., Moț, A.C., The effect of *Berberis vulgaris*, *Mahonia aquifolium* and *Phellodendron amurense* plant extracts over cell viability in different types of tumor cell lines, 9th Edition of the BioTA Symposium: Biodiversity-Traditions and Current Affairs, 2023, Cluj-Napoca, România (https://biogeo.ubbcluj.ro/biota-2023/)

B. International (*from the thesis topic)

***Erhan**, S.E, Heinz Maier-Leibritz Zentrum (MLZ) Conference: Neutrons for Life Science, Online, 2021, Germany, https://indico.frm2.tum.de/event/230/overview

***Erhan**, S.E., Moț, A.C., Pârvu, M., Erhan, R.V., Berberine loaded liposome system structure investigated by SANS and SAXS, Heinz Maier-Leibritz Zentrum (MLZ) Conference: Neutrons for Biomaterials, Poster: Berberine loaded liposome system structure investigated by SANS and SAXS, 2023, Munich, Germany, https://indico.frm2.tum.de/event/391/

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