„BABEŞ-BOLYAI" UNIVERSITY OF CLUJ-NAPOCA

Faculty of Biology and Geology

Integrative Biology Doctoral School

Vinca and *Catharanthus* plant extracts and their therapeutic potential applications

Ph.D. Thesis

Summary

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Key words

plant extracts; morphology and ultrastructure; antioxidant activity; antibacterial activity; cytotoxicity; metal nanoparticles; green synthesis

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Introduction

This doctoral thesis presents the results obtained during the research conducted on *Catharanthus roseus* (L.) G. Don and *Vinca* L. (periwinkle) plant species: *Vinca minor* L., *Vinca major* L., *Vinca herbacea* Waldst. & Kit., *Vinca major* L. var. *variegata* Louden culture variety. The comparative study was aimed to characterize the: morphological and anatomical traits of the leaf; the chemical composition and pharmacological potential of the obtained leaf extracts: antioxidant and antibacterial activities, *in vitro* cytotoxicity against normal and cancerous human cell lines, and the capacity to form metal nanoparticles.

This work is divided in four main chapters and the main ideas are presented in the following summary. Chapter I (**Literature study**) emphasizes on the importance of medicinal plants and the impact that nanotechnology and nanomaterials had on modern medicine. Chapter II describes the **Materials and methods** used to achieve the **Results and discussions** (Chapter III), while the main **Conclusions** are mentioned in Chapter IV.

Research scope and objectives

In the doctoral thesis entitled ***Vinca* and *Catharanthus* plant extracts and their therapeutic potential applications** the comparative study of *Vinca minor* L. (**lesser periwinkle**), *Vinca major* L. (**bigleaf periwinkle**), *Vinca major* L. var. *variegata* Loudon (**greater periwinkle with white margins**), *Vinca herbacea* Waldst. & Kit. (**herbaceous periwinkle**) and *Catharanthus roseus* (L.) G. Don (**Madagascar periwinkle**) and their pharmacological potential was assessed.

The research activities were conducted in the following laboratories:

- „Constantin Crăciun" Electron Microscopy Centre – BBU Cluj-Napoca;
- Natural Extracts from Faculty of Biology and Geology, BBU;
- The Chemistry Department – Faculty of Chemistry and Chemical Engineering, BBU;
- Integrated Electron Microscopy Laboratory, Research Center of Advanced Technologies for Alternative Energies, Integrated Culture Cells and Microbiology Laboratory, Molecular and Biomolecular of the Physics of Nanostructured Systems Department, all from National Institute for Research and Development of Isotopic and Molecular Technologies – INCDTIM, Cluj-Napoca.

1. Literature study

Medicinal plants are versatile regarding the pharmacological potential of the resulted extracts. Such therapeutical effects are the antibacterial, antitumoral, and cytotoxic activities. The antibiotic-resistant bacteria were acknowledged along with the discovery of antibiotics, a breakthrough for modern medicine (Bhagwat și colab., 2014; Jhanji și colab., 2019; Newman, 2008). Moreover, cancer patients

were found to develop resistance to cytostatic drugs as well, a natural process that could lead to dramatic consequences (D'Costa și colab., 2011; Mahmoudi și colab., 2016).

Therefore, the pharmacological potential of: *V. minor* L., *V. major* L., *V. herbacea* Waldst. & Kit.; *V. major* L. var. *variegata* Louden; and *C. roseus* (L.) G. Don cv. Pacifica, was determined comparatively to try and bring novelty to the field. The *Vinca* and *Catharanthus roseus* plant species (**Apocynaceae** family) are rich in alkaloids, natural compounds with demonstrated pharmacological potential (O'Connor, 2008) such as: antioxidant activity (Bahadori și colab., 2012), antibacterial activity (Grujić și colab., 2015), or antitumoral and cytotoxic activities (Garcia-Lazaro și colab., 2020). More and more resources are directed towards the green synthesis of metal nanoparticles using plant extracts, with interdisciplinary applications and in bio-nano-medicine (Suciu și colab., 2020), a research topic conducted in this work as well.

2. Materials and methods

Three main research topics were conducted to meet the established objectives:

a. Morphological and ultrastructural analysis of the *Vinca* and *C. roseus* leaf using photonic microscopy, scanning and transmission electron microscopy (S/TEM) techniques;
b. Comparative phytochemical composition analysis of *Vinca* and *C. roseus* hydroalcoholic leaf extracts regarding the pharmacological potential (antioxidant, antibacterial, and cytotoxic activities);
c. Green nanoparticle synthesis and characterization (Ag-MnO_2 nanoparticles) using *Vinca minor* and/or *Chelidonium majus* plant extracts.

3. Results and discussions

a. *Vinca* and *Catharanthus roseus* leaf morphological and ultrastructural analysis

Several morphological and ultrastructural characteristics of the *Vinca* and *C. roseus* leaves were described using microscopy techniques (Figure 1). Except for *V. minor* all species had stomata on both sides of the leaf, however, they are considered hypostomatic due to the large discrepancy of the stomatal index (SI) between the adaxial and abaxial sides. The highest SI was of *V. minor*, while the lowest was of *V. major*. The trichome distribution on the adaxial side was species dependent and *C. roseus* was the only leaf with trichomes on both sides of the leaf. The mesophyll had pallisadic and spongy parenchyma and for *V. minor* and *V. herbacea* the limitation between the two was more visible. In addition, the mesophyll airspace was for the first time determined in these species through a computational method. The cuticle and epicuticular waxes were described through TEM and *V. minor* and *V. herbacea* had the thickest

epidermises. These characteristics were correlated with the phytochemical composition as well (Ciorîță și colab., 2021a).

b. Phytochemical composition and pharmacological potential of *Vinca* and *C. roseus* plant extracts

The antioxidant, antibacterial, and cytotoxic effects of *Vinca* and *C. roseus* plant extracts are dependent on species and chemical composition. Vincamine was found in four out of five extracts (except *V. herbacea*), while only *C. roseus* had traces of vinblastine (Table 1). *V. minor* had the richest alkaloid content, followed by *C. roseus* and *V. herbacea*, which also had high levels of rutin. This generated the high antioxidant potential of *V. herbacea*, followed by *V. major* var. *variegata*. The greatest antibacterial effect tested against *Staphylococcus aureus* and *Escherichia coli* was of *V. minor*. The *Vinca* extracts acted in a dose-dependent manner against A375 human skin melanoma and HaCaT normal keratinocytes, while *C. roseus* negatively affected the cells at all concentrations. These results proved the pharmacological potential of *Vinca* și *C. roseus* plant extracts (Ciorîță și colab., 2021b).

c. Green synthesis of Ag-MnO_2 nanoparticles using *V. minor* and *Chelidonium majus* plant extracts

Green chemistry implies the synthesis of metal nanoparticles (NPs) using plant extracts. Based on the results obtained after the analysis of the plant extracts (described at point b), the *V. minor* plant extract was chosen to obtain Ag-MnO_2 NPs (Figure 2). Because *Chelidonium majus* was shown to successfully form metal nanoparticles as indicated in other studies, this plant extract was chosen as well, for comparison. Thus, three types of nanoparticles were obtained and characterized: VmNPs (with *V. minor*), CmNPs (with *C. majus*), and MNPs (1:1 extract mix). The NPs were characterized through S/TEM, X-Ray Powder Diffraction, EDX elemental analysis, Fourier Transformed Infra-Red Spectroscopy, while the therapeutic potential was assessed through microbiological and cytotoxic analyses. The NPs had dimensions between 9.3 nm and 32.4 nm, with the best distribution for VmNPs. The NPs were core-shells with Mn core, Ag shell and a layer of organic compound, most probably formed by the plant extracts. The most efficient therapeutic effects were those of VmNPs, followed by MNPs, and CmNPs (Figure 3). These results showed that *V. minor* and *C. majus* are suited for NP synthesis and the formed nanoparticles gave a great pharmacological potential (Ciorîță și colab., 2020).

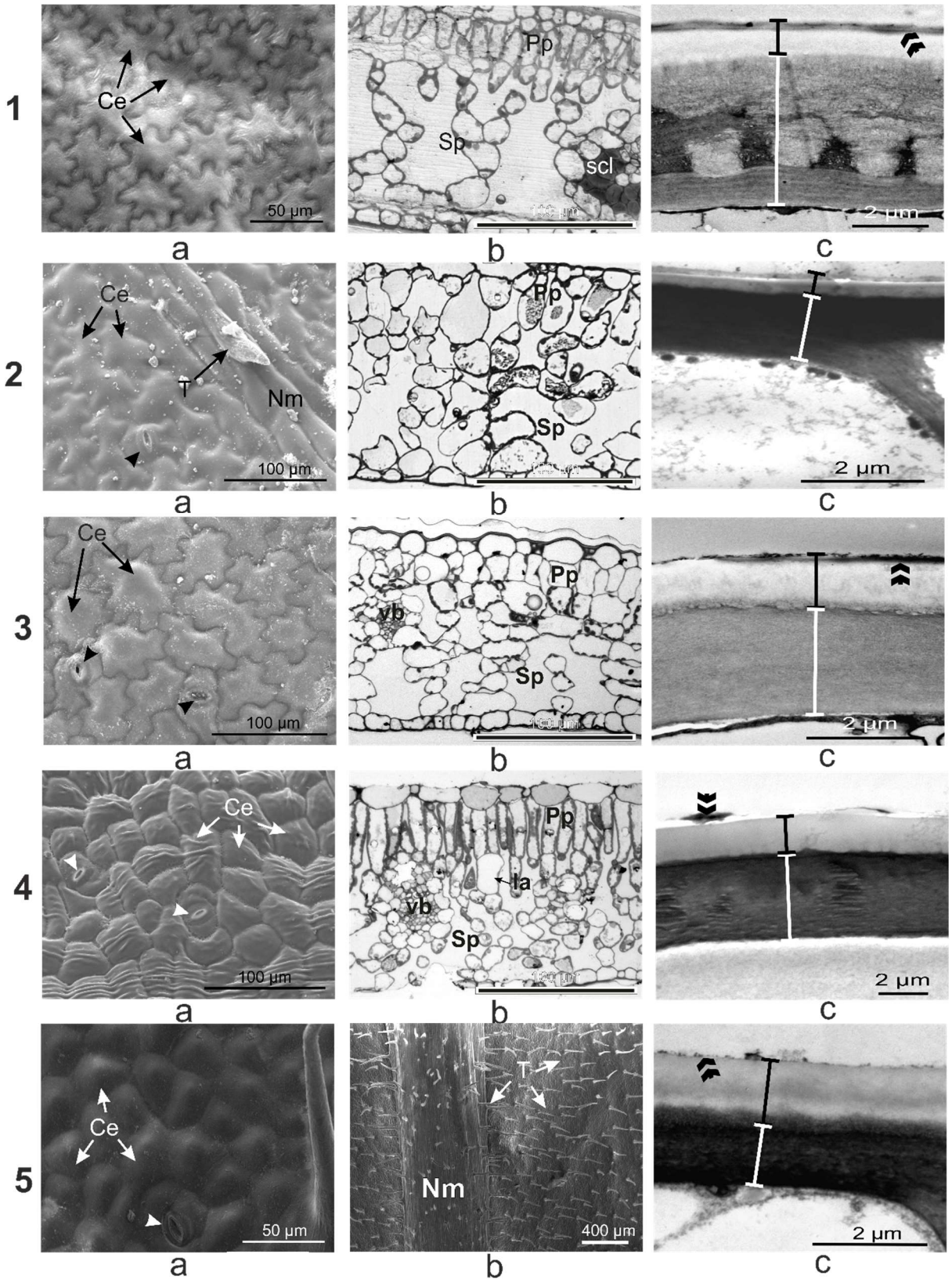

Figure 1. Light microscopy, SEM, and TEM images of *V. minor* (1), *V. major* (2), *V. major* var. *variegata* (3), *V. herbacea* (4) and *C. roseus* cv. Pacifica (5) leaves. Ce = epiderma cell, Nm = Midvein, T = trichome, Ia = idioblast, Pp = pallisadic parenchyma, Sp = spongy parenchyma, scl = sclerenchyma, vb = vascular bundle, simple arrowhead = stomata, double arrowhead = cutin

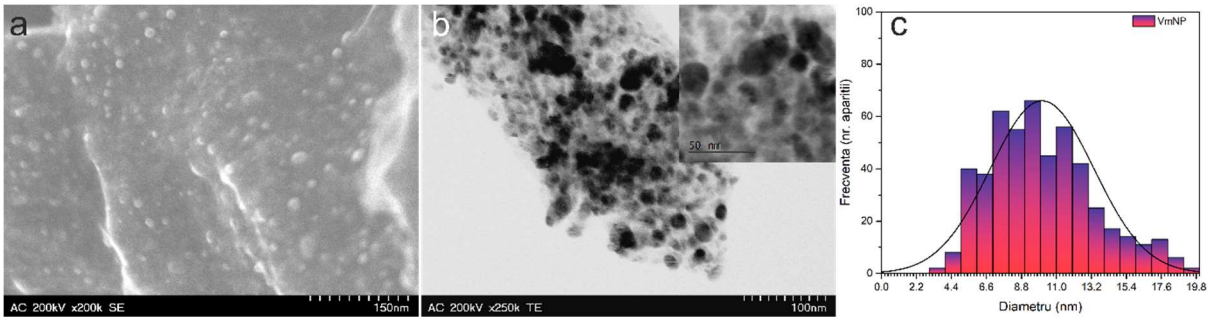

Figure 2. SEM (a), TEM (b), and size distribution (c) green-synthesized Ag-MnO_2 NPs using de *V. minor* extract

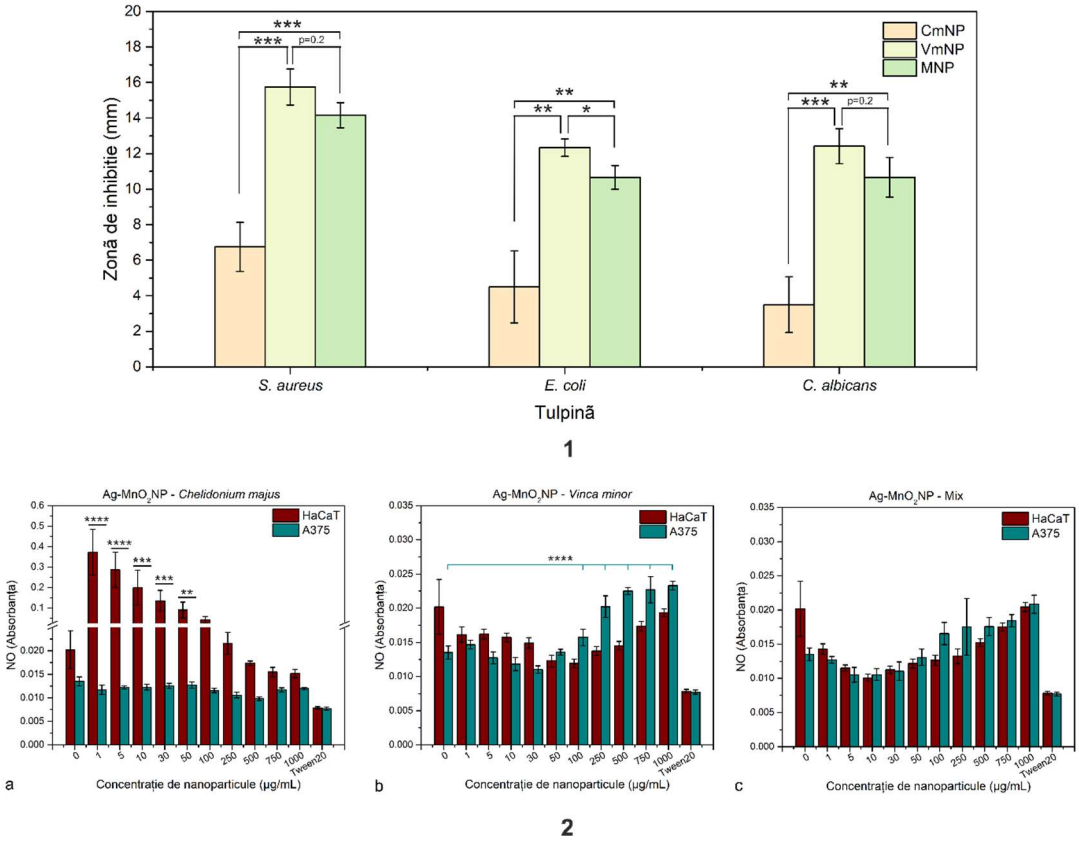

Figure 3. Antimicrobial (1) and cytotoxic (2) effects of Ag-MnO_2

Table 1. The main phytoconstituents determined in *Vinca* and *C. roseus* plant extracts

Compound	Compound concentration (µg/g)				
	Vm	VM	VMv	Vh	Crp
Chlorogenic acid	4112±13	675±160	932±260	1538±200	2959±240
Caffeic acid	229±2	13±2	182±23	13±1	13±1
Rutin	73±1	11±1	94±10	2528±160	44±2
Isoquercitrin	12±1	12±1	38±4	87±4	82±7
Quercitrin	52±4	11±1	45±8	109±10	86±6
Vincamine	65±1	42±3	31±2	n.d.	4±1
Quercetin	21±2	14±2	28±2	20±3	21±2
Vinblastine	n.d.	n.d.	n.d.	n.d.	6.8±2

*Vm = *V. minor*, VM = *V. major*, VMv = *V. major* var. *variegata*, Vh = *V. herbacea*, Crp = *C. roseus* cv. 'Pacifica'; n.d. – not detected.

4. Conclusions

The current Ph.D. thesis describes the comparative pharmacological effects of hydroalcoholic leaf extracts of *Vinca* species and *Catharanthus roseus*. The leaf morphological and anatomical traits, chemical composition of the plant extracts and their pharmacological potential, were determined through various methods in several laboratories. Moreover, the *V. minor* extract was chosen based on the obtained results, to form Ag-MnO_2 nanoparticles, along with *Chelidonium majus* extract. These nanoparticles were then tested for their therapeutical potential against bacteria, fungi, and human cell lines.

This study brings additional information to the field with the help of the published articles. The research was documented based on 352 references, represented by scientific articles from national and international publications. Various research methods were conducted for the analysis of *Vinca* and *Catharanthus* plants and this creates further research opportunities.

5. Dissemination

Articles published in international ISI journals, as first author (* from the subject of the Ph.D. thesis):

1. * **Ciorîță, A.,** Zăgrean-Tuza, C., Moț, A. C., Carpa, R., Pârvu, M., The phytochemical analysis of *Vinca* L. species leaf extracts is correlated with the antioxidant, antibacterial, and antitumor effects. (2021). The phytochemical analysis of *Vinca* L. species leaf extracts is correlated with the antioxidant, antibacterial, and antitumor effects. Molecules, *26*(10), 3040. https://doi.org/10.3390/molecules26103040; **IF:** 3.267; **AIS:** 0.6.
2. * **Ciorîță, A.,** Tripon, S.-C., Mircea, I.-G., Podar, D., Barbu-Tudoran, L., Mircea, C., Pârvu, M. (2021). The morphological and anatomical traits of the leaf in representative *Vinca* species observed on indoor-and outdoor-grown plants. Plants-Basel, *10*(4), 622. https://doi.org/10.3390/plants10040622; **IF:** 2.762.
3. * **Ciorîță, A.,** Suciu, M., Macavei, S., Kacso, I., Lung, I., Soran, M.-L., Pârvu, M. (2020). Green synthesis of Ag-MnO_2 nanoparticles using *Chelidonium majus* and *Vinca minor* extracts and their *in vitro* cytotoxicity; Molecules, *25*(4), 819. https://doi.org/10.3390/molecules25040819; **IF:** 3.267; **AIS**: 0.6.

International conferences

1. **Ciorîță, A.**, Surducan, V., Surducan, E. 12th International Conference „Processes in Isotopes and Molecules''; Poster: *Automated photographic device for real-time monitoring of in vitro biological samples*, 2019, România, http://pim.itim-cj.ro/2019/.
2. **Ciorîță, A.**, Tripon, S.-C., Podar, D., Barbu-Tudoran, L., Pârvu, M. 18th International Balkan Workshop on Applied Physics and Materials Science; Poster: *Light and electron microscopy analysis of the leaves of Vinca species*, 2018, România, http://ibwap.ro/.
3. **Ciorîță, A.,** Bugiel, M., Schaeffer, E., Jannasch, A. Microtubules: From Atoms to Complex Systems – Virtual; Poster: *Single kinesin-8, Kip3, stabilizes microtubules*, 2020, Germania, https://www.embo-embl-symposia.org/symposia/2020/EES20-05/index.html.

Patent application:

1. **Ciorîță, A.,** Surducan, V., Surducan, E. (2019). Device for the real-time observation of the evolution of macroscopic biological material *in vitro*. Patent application no. RO133721A0·2019-11-29.

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4. Ciorîță, A., Tripon, S. C., Mircea, I. G., Podar, D., Barbu-Tudoran, L., Mircea, C., Pârvu, M. (2021a). The Morphological and Anatomical Traits of the Leaf in Representative *Vinca* Species Observed on Indoor- and Outdoor-Grown Plants. *Plants, 10*(4), 622;
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