BABEŞ-BOLYAI UNIVERSITY
FACULTY OF BIOLOGY AND GEOLOGY
DOCTORAL SCHOOL OF INTEGRATIVE BIOLOGY

DOCTORAL THESIS SUMMARY

Characterization of six species of the genus *Allium* and exploration of the properties of plant extracts

Scientific supervisor:
Prof. Dr. Anca-Livia BUTIUC

PhD student:
Ioana Andreea BARBU

Cluj-Napoca,
2025

TABLE OF CONTENTS

SUMMARY

Plants in the *Allium* genus are perennial, herbaceous members of the Liliaceae family. These species have been utilized for both culinary and medicinal purposes since prehistoric times (Harris et al., 2001).

Increasing antibiotic resistance, along with the demand for innovative therapies, has driven researchers to explore plant-based alternatives. Recent studies have demonstrated that *Allium* species and their bioactive compounds - such as polyphenols, flavonoids, and thiosulfinates (particularly allicin) - possess notable antioxidant (El-Saber Batiha et al., 2020; Barbu et al., 2024), antibacterial (Gabriel et al., 2022; Barbu et al., 2023), antifungal (Diba and Alizadeh, 2018), anti-inflammatory (Marefati et al., 2021), and antitumor (Iwar et al., 2024) activities.

The variability of compounds identified in Allium species is determined by a range of factors, including the method employed for tincture preparation, the solvent used in extraction, the biosynthesis of metabolites within the leaf mesophyll, as well as the morphological and structural characteristics of the leaves and prevailing environmental conditions. In Allium species, the synthesis of bioactive compounds occurs predominantly within the foliage, which exhibits interspecific differences in shape, size, developmental stage (phenophase), and leaf number per plant. Although the structural attributes of Allium species have been extensively documented, a definitive correlation between these characteristics and the biosynthetic activity of their bioactive constituents has yet to be established, so this study aims to investigate this.

The promising results obtained using *Allium* extracts led to the expansion of research toward formulating vegetable extracts in systems that can be more easily, efficiently, and stably applied in therapy, such as metallic nanoparticles (Coman et al., 2013; Gabriel et al., 2022; Baran et al., 2023) or hydrogels (Widiyanti and Priskawati, 2023), which have shown significant impact in the medical field.

The aim of this thesis was to characterize six *Allium* extracts and explore their bioactive properties. The study was carried out by combining microbiology, phytochemistry, as well as optical and electron microscopy approaches, in order to characterize the structures involved in syntesis of fitochemical compounds and the biological properties of the extracts. To achieve this goal, the following objectives were accomplished:

1. Structural analysis of the leaves of *A. ursinum, A. cepa, A. sativum, A. senescens* subsp. *montanum, A. fistulosum* and *A. obliquum*.
2. Chemical characterization of *Allium* extracts and testing of antimicrobial and antioxidant activity
3. Green biosynthesis of gold (Au) nanoparticles with *Allium* extract, their characterization and testing of antimicrobial activity.

This thesis is organized into four chapters: the first three outline the activities undertaken to achieve the proposed objectives, while the fourth presents the overall conclusions of the study.

Chapter I presents the structural characterization of the leaves of six *Allium* species and explores their potential correlation with the synthesis of phytochemical compounds and the chemical composition of their extracts, employing light microscopy (LM) and scanning–transmission electron microscopy (S/TEM). SEM analysis revealed substantial differences in leaf morphology. LM observations allowed detailed examination of the upper and lower epidermis as well as the mesophyll. The leaves exhibit an epidermis characterized by thick cell walls, cuticular layers, and epicuticular waxes.

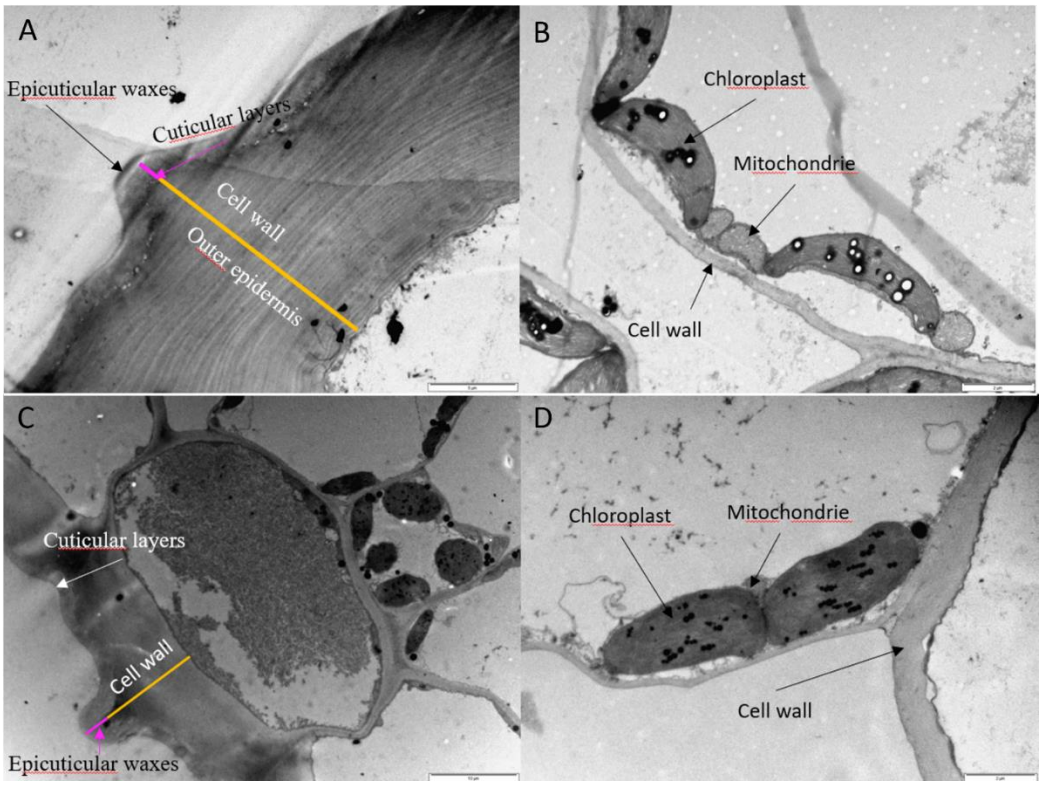

Figure 1. Transmission electron microscopy (TEM) of the leaves. (A, B) *A. fistulosum*; (C, D) *A. obliquum*.

In contrast to the other species examined, *A. fistulosum* and *A. cepa* lack a lower epidermis, with chloroplasts occurring exclusively beneath the outer epidermis.

Ultrastructurally (Figure 1), these two species display similar epidermal cell walls and chloroplast distributions. The presence of both epidermal layers in *A. obliquum*, *A. sativum*, *A. ursinum*, and *A. senescens* may account for their higher allicin content. However, further studies are required to substantiate these findings.

Chapter II is divided into two sub-chapters and aims to describe the obtaining of the extracts and to characterize the antimicrobial and antioxidant effects produced by them.

In the first sub-chapter the hydroalcoholic extracts of six *Allium* species obtained by cold percolation were analyzed regarding their phytochemical compounds and antimicrobial activity. Among the six extracts, differences were observed between the content of polyphenols and flavonoids, while *Allium sativum* and *Allium ursinum* have similar contents of thiosulfinates. The HPLC-DAD method was used to detail the phytochemical composition of species rich in thiosulfinates, *A. sativum* proving to be the richest in allicin. The antimicrobial activity (Figure 2) of *A. sativum* and *A. ursinum* extracts against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Candida parapsilosis* can be correlated with the presence of large amounts of thiosulfinates.

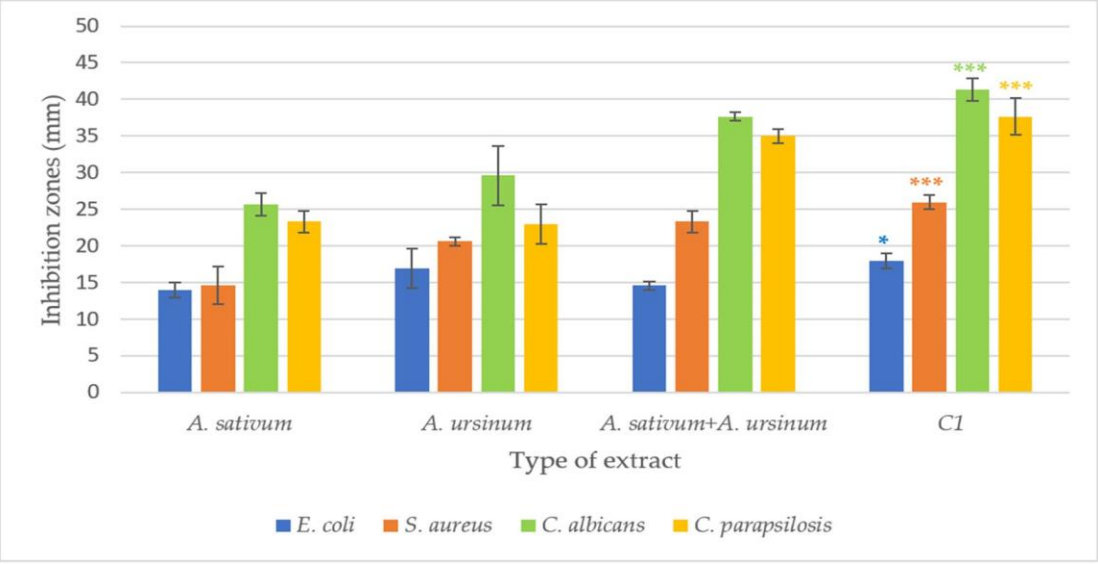

Figure II.1.3. The antimicrobial effect of *Allium sativum*, *A. ursinum*, *A. sativum* and *A. ursinum* 1:1 on *S. aureus, E. coli*, *C. albicans* and *C. parapsilosis*. The values represent the mean of three measurements ± standard deviation; *** $p < 0.0005$, * $p < 0.05$, according to one-way ANOVA.

Both extracts have shown results against *Candida* species and against Gram-positive bacteria, *Staphylococcus aureus*. These results demonstrate the antimicrobial effect of the extracts and suggest their use as an adjuvant treatment for microbial infections. This chapter was published as Barbu, I.A., Ciorîță, A., Carpa, R., Moț, A.C., Butiuc-Keul, A., Pârvu, M. (2023) Phytochemical Characterization and Antimicrobial Activity of Several *Allium* Extracts. *Molecules 28*, 3980. 10.3390/molecules28103980

The second sub-chapter outlines the antioxidant effects of six *Allium* extracts, evaluated through various spectrophotometric methods to demonstrate their antioxidant potential. The extracts tested included *Allium fistulosum*, *Allium ursinum*, Arieș red cultivar of *A. cepa,* and white variety of *A. cepa*, *Allium sativum*, and *Allium senescens* subsp. *montanum*. Their chemical composition was determined using HPLC–MS analysis. Among these, Arieș red cultivar of *A. cepa* exhibited the strongest antioxidant activity, likely attributable to its high levels of polyphenols and alliin. Overall, the findings confirm that *Allium* extracts possess significant antioxidant and free radical scavenging properties. Moreover, their interactions with cytochrome c and hemoglobin suggest promising avenues for future research in developing therapies for oxidative stress-related disorders. This chapter was published as Barbu, I.A., Toma, V.A., Moț, A.C., Vlase, A.-M., Butiuc-Keul, A., Pârvu, M. (2024) Chemical Composition and Antioxidant Activity of Six *Allium* Extracts Using Protein-Based Biomimetic Methods. *Antioxidants 13*, 1182. https://doi.org/10.3390/antiox13101182

Chapter III aimed to examine the antimicrobial properties of AuNPs produced with *Allium sativum* and *Allium ursinum* extracts. These extracts, rich in sulfur compounds (allicin), flavonoids, and polyphenols, not only promoted nanoparticle formation but also enhanced their antimicrobial effectiveness. The resulting nanoparticles were characterized by UV-Vis spectroscopy and subsequently evaluated for antimicrobial activity. In vitro microbiological assays (Figure 3) revealed that the nanoparticles were active against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and fungi (*Candida albicans* and *Candida parapsilosis*). The findings suggest that gold nanoparticles functionalized with *Allium* extracts represent a promising natural alternative for the development of antimicrobial agents, with potential applications in medicine, the food industry, and pharmaceuticals. This chapter was published as Barbu, I.A., Biro, O.M., Carpa, R., Butiuc-Keul, A., Pârvu, M. (2025)

Antimicrobial effects produced by gold nanoparticles obtained with extracts of *Allium sativum* and *Allium ursinum*. *Studia Universitas Biologia*, *70*(1). https://doi.org/10.24193/subbbiol.2025.1.15

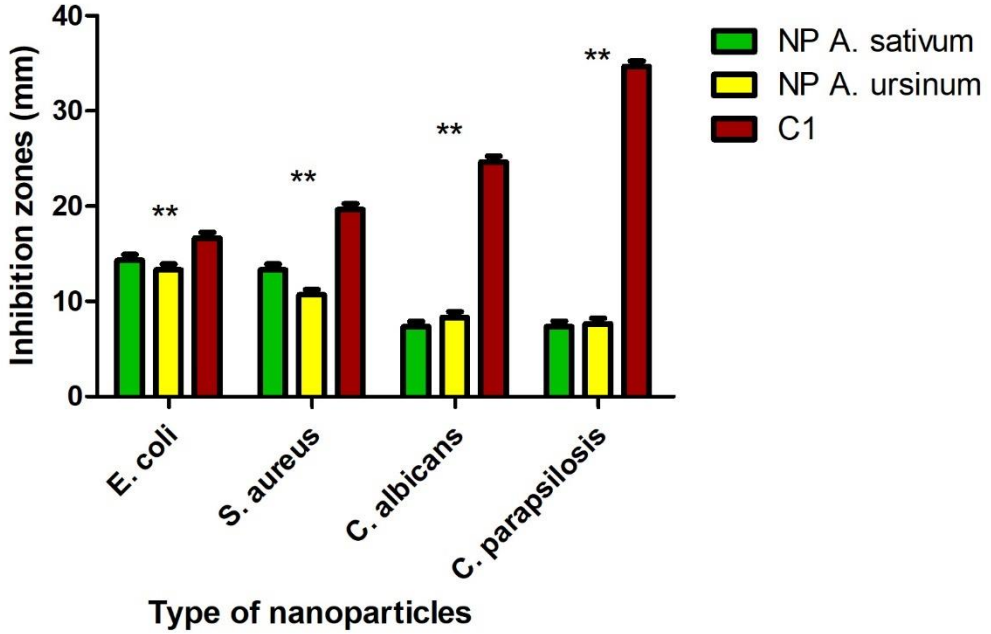

Figure 3. The antimicrobial effect of AuNPs obtained with *Allium sativum* and *A. ursinum* extracts on *S. aureus* (C1 – sulfamethoxazole), *E. coli* (C1 – cefuroxime), *C. albicans* and *C. parapsilosis* (C1 – voriconazole). The values represent the mean of three measurements ± standard deviation; ** $p < 0.005$, according to one-way ANOVA.

Chapter IV presents the main findings and conclusions of this thesis, offering a comprehensive synthesis of the research outcomes while also highlighting potential directions for future studies.

The thesis highlights how leaf structure in *Allium* species affects the biosynthesis of active compounds. Species with both upper and lower epidermal layers (e.g., *A. sativum*, *A. ursinum*) show higher allicin levels than those with a single epidermal layer. *Allium* extracts, especially from *A. sativum* and *A. ursinum*, demonstrate strong antimicrobial effects linked to thiosulfinate content. Antioxidant activity varies across species, with the Arieș red cultivar of *A. cepa* exhibiting the highest due to its polyphenol and alliin content. Gold nanoparticles synthesized from Allium extracts enhance antimicrobial action, offering potential in medicine, pharmaceuticals, and food industries.

Overall, this thesis explores the therapeutic potential of plants from the genus *Allium* by integrating various analytical approaches. Structural analysis of the leaves, phytochemical profiling of the extracts, along with antimicrobial and antioxidant assessments, have provided results that open important perspectives for advancing research in medicinal plants developing nutritional supplements or using extracts as adjuvants in the treatment of various diseases.

LIST OF PUBLICATIONS INCLUDED IN THE THESIS

1. Barbu, I.A., Ciorîță, A., Carpa, R., Moț, A.C., Butiuc-Keul, A. and Pârvu, M. (2023) Phytochemical characterization and antimicrobial activity of several *Allium* extracts, *Molecules*, *28*, 3980. https://doi.org/10.3390/molecules28103980
2. Barbu, I.A.; Toma, V.A.; Moț, A.C.; Vlase, A.-M.; Butiuc-Keul, A.; Pârvu, M. Chemical Composition and Antioxidant Activity of Six *Allium* Extracts Using Protein-Based Biomimetic Methods. *Antioxidants* **2024**, *13*, 1182. https://doi.org/10.3390/antiox13101182
3. Barbu, I.A., Biro, O.M., Carpa, R., Butiuc-Keul, A. and Pârvu, M. (2025) Antimicrobial effects produced by gold nanoparticles obtained with extracts of *Allium sativum* and *Allium ursinum*, *Studia Universitatis Babeș-Bolyai, Biologia 70*(1). https://doi.org/10.24193/subbbiol.2025.1.15

LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS

1. Racz, L.Z., Racz, C.P., Pop, L.-C., Tomoaia, G., Mocanu, A., Barbu, I., Sárközi, M., Roman, I., Avram, A., Tomoaia-Cotisel, M. *et al.* (2022) Strategies for improving bioavailability, bioactivity, and physical-chemical behavior of *curcumin*, *Molecules 27*, 6854. https://doi.org/10.3390/molecules27206854

ATTENDANCES AT NATIONAL AND INTERNATIONAL CONFERENCES

Barbu I.A., Carpa R., Ciorîță A.,Butiuc-Keul A., Pârvu M. (2022) Antimicrobial activity of *Allium sativum* and *Allium ursinum* extracts.

- Poster at the International Conference and XXXIX Scientific Session of the Romanian Society for Cell Biology (RSCB), October 21-23, 2022, Cluj-Napoca, Romania.

Barbu I.A., Carpa R., Moț A.C., Ciorîță A.,Butiuc-Keul A., Pârvu M. (2023) *Allium sativum* and *Allium ursinum* extrects: antimicrobial activity and phytochemical characterization.

- Poster at the Annual International Conference of the *Romanian Society of Biochemistry and Molecular Biology*, September 13-15, 2023, Cluj-Napoca, Romania. *Studia Universitatis Babeș-Bolyai, Biology, 68(2), RSBMB Book of abstracts pp. 93,* Abstract-book-RSBMB-2023_varianta-finala.pdf.

Barbu I.A., Carpa R., Moț A.C., Ciorîță A.,Butiuc-Keul A., Barbu-Tudoran L., Mircea C., Pârvu M. (2023) Structural characterization of two *Allium* species dependent on their phytochemical profile.

- Poster at BIOTA - Biodiversitate, Tradiții și Actualitate, May 5-6, 2023, Cluj-Napoca, România, Book of abstracts pp. 22, https://biogeo.ubbcluj.ro/biota-2023/

References

1. Baran, M.F., Keskin, C,. Baran, A., et al. (2023) Green Synthesis of Silver Nanoparticles from *Allium cepa* L. Peel Extract, Their Antioxidant, Antipathogenic, and Anticholinesterase Activit. *Molecules 28* (5):5. https://doi.org/10.3390/molecules28052310
2. Barbu, I.A., Ciorîță, A., Carpa, R., Moț, A.C., Butiuc-Keul, A., Pârvu, M. (2023) Phytochemical Characterization and Antimicrobial Activity of Several Allium Extracts. *Molecules 28* (10):10. https://doi.org/10.3390/molecules28103980
3. Barbu, I.A., Toma, V.A., Moț, A.C., Vlase A-M., Butiuc-Keul, A., Pârvu, M. (2024) Chemical Composition and Antioxidant Activity of Six *Allium* Extracts Using Protein-Based Biomimetic Methods. *Antioxidants 13*(10): 1182. https://doi.org/10.3390/antiox13101182
4. Coman, C., Leopold, L. F., Rugină, O. D., Barbu-Tudoran, L., Leopold, N., Tofană, M., Socaciu, C. (2013) Green Synthesis of Gold Nanoparticles by *Allium sativum* Extract and Their Assessment as SERS Substrate. *Journal of Nanoparticle Research 16*(1): 2158. https://doi.org/10.1007/s11051-013-2158-4
5. Diba, A. and Alizadeh, F. (2018) In vitro and in vivo Antifungal Activity of *Allium hirtifolium* and *Allium sativum*. *Avicenna Journal of Phytomedicine 8*(5): 465–74. PMID: 30345234
6. El-Saber Batiha, G., Magdy Beshbishy, A., G. Wasef, L., Elewa, Y.H.A., A. Al-Sagan, A., Abd El-Hack, M.E., Taha, A.E., M. Abd-Elhakim, Y., Prasad Devkota, H. (2020) Chemical Constituents and Pharmacological Activities of Garlic (*Allium sativum* L.): a Review. *Nutrients 12*(3): 872. https://doi.org/10.3390/nu12030872
7. Gabriel, T., Vestine, A., Kim, K.D., Kwon, S.J., Sivanesan, I., Chun, S.C. (2022) Antibacterial Activity of Nanoparticles of Garlic (*Allium sativum*) Extract Against Different Bacteria such as *Streptococcus mutans* and *Poryphormonas gingivalis. Applied Sciences 12*(7): 3491. https://doi.org/10.3390/app12073491
8. Harris, J. C., Cottrell, S. L., Plummer, S., Lloyd, D. (2001) Antimicrobial Properties of *Allium sativum* (garlic). *Appl. Microbiol. Biotechnol. 57*(3): 282–286. https://doi.org/10.1007/s002530100722

9. Iwar, K., Ochar, K., Seo, Y.A., Ha, B.-K., Kim, S.-H. (2024) *Alliums* as Potential Antioxidants and Anticancer Agents. *International Journal of Molecular Sciences 25*(15): 8079. https://doi.org/10.3390/ijms25158079
10. Marefati, N., Ghorani, V., Shakeri, F. (2021) A Review of Anti-Inflammatory, Antioxidant, and Immunomodulatory Effects of *Allium Cepa* and Its Main Constituents. *Pharmaceutical Biology 59*(1): 285–300. https://doi.org/10.1080/13880209.2021.1874028
11. Widiyanti, P. and Ayu Priskawati, Y.C. (2023) Synthesis and Characterization of Hydrogel-Based Hyaluronic Acid-Chitosan-*Allium sativum* Extract for Intraperitoneal Antiadhesion Application. *International Journal of Biomaterials* (1): 5172391. https://doi.org/10.1155/2023/5172391