UNIVERSITY OF MEDICINE AND PHARMACY „CAROL DAVILA”, BUCHAREST

DOCTORAL SCHOOL

THE DOMAIN OF PHARMACY

ENHANCED UTILIZATION OF INDIGENOUS PLANT RESOURCES IN BIOMEDICAL APPLICATIONS

Summary of the PhD Thesis

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Ioana-Cătălina Fierăscu

2023

Cuprins

List of published scientific papers

Articles in ISI-listed journals

a. Articles published in the red zone (Q1)

- **Fierăscu I.C.**, Fierăscu I., Fierăscu R.C., Velescu B.Ș., Dinu-Pârvu C.E., *Phytosynthesis of Silver Nanoparticles using Leonurus Cardiaca L. extracts,* Materials, 2023, *16*(9), 3472; https://doi.org/10.3390/ma16093472 (Q1, according to AIS published by UEFISCDI - https://uefiscdi.gov.ro/resource-866361-cuartile.ais.jcr.19.oct.2022.zone.pdf) – *Primary Author;*
- **Fierăscu I.C.**, Fierăscu I., Baroi A.M., Ungureanu C., Ortan A., Avramescu S.M., Somoghi R., Fierăscu R.C., Dinu-Pârvu C.E., *Phytosynthesis of biological active nanoparticles using Echinacea purpurea L. extracts,* Materials, 2022, *15*(20), 7327; https://doi.org/10.3390/ma15207327 (Q1, according to AIS published by UEFISCDI - https://uefiscdi.gov.ro/resource-866361-cuartile.ais.jcr.19.oct.2022.zone.pdf) – *Primary Author; 4 citations (SCOPUS).*
- Fierăscu I., **Fierăscu I.C.**, Brazdis R., Baroi A., Fistos T., Fierăscu R., *Phytosynthesized Metallic Nanoparticles- between Nanomedicine and Toxicology. A Brief Review of 2019's Findings,* Materials, 2020, *13*(3), 574; https://doi.org/10.3390/ma13030574 (Q1, according to IF - https://uefiscdi.gov.ro/resource-820921-if2021.pdf and AIS - https://uefiscdi.gov.ro/resource-820923-ais2021.pdf, lists published by UEFISCDI); *30 citations (SCOPUS).*
- Fierăscu R., Orțan A., **Fierăscu I.C.,** Fierăscu I., *In vitro and in vivo evaluation of antioxidant properties of wild-growing plants. A short review,* Current Opinion in Food Science, 2018, Volume 24, Pages 1-8, https://doi.org/10.1016/j.cofs.2018.08.006 (Q1, according to IF - https://uefiscdi.gov.ro/resource-822843 and AIS - https://uefiscdi.gov.ro/resource-822841, lists published by UEFISCDI); *37 citations (SCOPUS).*

b. Articles published in the yellow zone(Q2)

- Fierăscu R., Fierăscu I., Orțan A., Fierăscu I.C., Anuța V., Velescu B., Pituru S., Dinu-Pirvu C., *Leonurus cardiaca L. as a Source of Bioactive Compounds: An update of the European Medicines Agency Assessment Report,* BioMed Research International, 2019, https://doi.org/10.1155/2019/4303215 (Q2), according to AIS -

https://uefiscdi.gov.ro/resource-821878-clasament2020.ais.pdf published by UEFISCDI) – *Equal contribution author (according to the Acknowldgement section); 6 citations (SCOPUS).*

- Fierăscu I., **Fierăscu I.C.**, Dinu-Pârvu C., Fierăscu R., Anuța V., Velescu B., Jinga M., Jinga V., *A Short Overview of Recent Developments on Antimicrobial Coatings Based on Phytosynthesized Metal Nanoparticles,* Coatings, 2019, *9*(12), 787; https://doi.org/10.3390/coatings9120787 (Q2), according to AIS - https://uefiscdi.gov.ro/resource-821878-clasament2020.ais.pdf, published by UEFISCDI); *21 citations (SCOPUS).*

c. Articles published in the gray zone(Q3 & Q4)

- Fierăscu R., Fierăscu I.C., Dinu-Pârvu C., Fierăscu I., Paunescu A., *The application of essential oils as a next-generation of pesticides: recent developments and future perspectives*, Zeitschrift fur Naturforschung C, 2019, Jul 28;75(7-8):183-204, https://doi.org/10.1515/znc-2019-0160; *33 Citations (SCOPUS)*

Acknowledgment

I would like to begin this ackownledgment page by expressing my deep gratitude and appreciation to all those who have contributed to the success of this PhD thesis. The process of researching and writing this paper has been long and challenging, and without the support and guidance of these individuals, this achievement would not have been possible.

First of all, I would like to thank Prof. Dr. Cristina Elena Dinu-Pîrvu, for being an invaluable source of knwoledge, wisdom and guidance throughout my PhD program. With patience and dedication, you have guided every step of my research and ecouraged me to push my intellectual boundaries. I am deeply grateful for the trust you have placed in me and for being an inspirational example.

I would also like to thank Prof. Dr. Lăcrămioara Popa, Prof. Dr. Mihaela Ghica si Conf. Dr. Andreea Arsene, who, as members of the guidance committee, provided me with careful evaluation of my work and constructive feedback. Your opinions were essential in improving the quality of my thesis.

In addition, I would like to thank the collaborators Radu-Claudiu Fierăscu, Irina Fierăscu, Alina Orțan, Camelia Ungureanu, Sorin Marius Avramescu, Anda-Maria Baroi, Raluca Somoghi, Simona Spinu, Simona-Georgiana Burlacu and Daniela Ionescu, for the resources made available and for an environment conducive to research.

I would not have been able to complete this project without the support of my family and friends. In particular, I would like to thank my brother, Radu, for his encouragement throughout this academic journey. Thank you for your unconditional support and for being there for me in difficult times.

This project was a profound learning experience and helped me develop as an individual and as a researcher. I am deeply grateful to everyone who has contributed to this achievement and for being with me on this academic journey.

Introduction

The last decades have witnessed a high interest from the scientific community in obtaining and characterizing metal nanoparticles through green chemisty methods, with the ultimate goal of eliminating or reducing the use and generation of hazardous substances for human health and the environment [1].

As part of the doctoral study program, research has been conducted on the efficient utilization of indigenous plant resources for the phytosynthesis of silver nanoparticles, in the context of biomedical applications. The main objective of this thesis was to develop a methodology that would enable the use of native plant resources in the field of nanotechnology, with practical applicability in the field of biomedicine. In this study, the capacity of *L. cardiaca* and *E. purpurea* extracts to phytosynthesize silver nanoparticles with relevant biological activities was demonstrated.

Current state of knowledge

1. Nanotechnology

In 1974, Norio Taniguchi from Tokyo Science University defined the term "nanotechnology" as the processing, separation, consolidation and deformation of materials by an atom or molecule. The use of nanotechnology in the treatment, diagnosis and control of diseases is referred to as "nanomedicine" [2].

Among the various methods of nanoparticle synthesis associated with "green chemistry", phytosynthesis is one of the most promising alternatives. This method involves the use of plant extracts and their biocomponents (especially phenolic compounds) as reducing agents in nanoparticle formation reactions, as well as stabilizing agents of the formed nanoparticles.

2. Natural extracts

Throughout history, plants have been exploited in a variety of fields, due to the presence of biologically active substances in their composition. Over the course of human evolution, numerous authors have investigated both the antioxidant and antimicrobial capabilities of plants, advancing the idea of their use in various industries, such as the food or pharmaceutical industries [3].

The physico-chemical and opto-electronic properties of metal nanoparticles are closely dependent on the morphology and size of the particles, being determined by them. In recent decades, nanoparticles with different compositions, shapes, sizes and polydispersity have been developed. [4].

Noble metals, especially Au and Ag, have been extensively tested in phytosynthesis processes to obtain metal nanoparticles with controllable shape and size [5].

3. *Echinacea purpurea* L. and *Leonurus cardiaca* L. – properties and uses

Despite the fact that the isolation and structure of the main compounds of *Echinacea purpurea* L. have been intensively studied, there is no general consensus among researchers regarding the mechanism of action. The main constituents of the plant are considered to be caffeic acid derivatives, alkamides and polysaccharides. Studies have suggested that alkamides play a major role in the immunomodulatory properties of *E. purpurea* extracts, and caffeic acid can be used both for identification and quality control of the extracts [6].

The composition of *Leonurus cardiaca* L.(Motherwort) includes furanic diterpenes, alkaloids, sterols, iridoids, flavonoids, minerals and other compounds [7]. Rusch et al. identified the presence of a major chlorinated iridoid glucoside in the extract of *Leonurus cardiaca* L.[8]. Kuchta et al. quantified using RP-HPLC the presence of ferulic acid, chlorogenic acid, caffeic acid, rutoside, lavandulifolioside, verbacoside and isoquercitrin in *Leonurus cardiaca* L. extract, as well as stachydrin in different parts of the plant [9, 10].

4. Phytosynthesis of nanomaterials – principles and applications

Nanotechnology is the science that deals with obtaining nanoscale particles, devices and systems. These materials and devices can be designed to interact with cells and tissues at the molecular level with a high degree of functional specificity, thus enabling integration between the device and the biological system [11]. The emerging field of nanotechnology is not a single scientific discipline, involving scientists from many different fields, including physicists, chemists, engineers, and biologists. Currently, nanotechnology is gaining importance in biology due to its small size and targeted effects. Nanoparticles can be made from a wide range of materials, such as metals (gold, silver), metal oxides, silicon dioxide, inorganic materials, polymeric materials, and lipids. [12].

Personal contributions

5. Materials and Methods

The drying of the plant material, flowers for Echinacea (*Echinacea purpurea* L.) and respectively the aerial part for Motherwort (*Leonurus cardiaca* L.) was carried out shielded from direct sunlight, drying being considered complete when a constant mass is reached. Once dried, the plant material was grounded and stored in a dark and humidity-controlled environment for future use.

To obtain the natural extracts, two extraction methods were selected, which, according to the literature data, ensure both the extraction of the active principles and the possibility of scaling up, with a view to industrial applications [13].

Classical extraction – the vegetal product, which was previously grounded, was subjected to classical extraction at temperature, using as a solvent a hydroalcoholic mixture, in the ratio ethanol:water = 1:1; the Memmert UN 110 oven was used for extraction, extraction time 3 hours at a temperature of 70ºC. The extract obtained was finally filtered using filter paper.

Microwave-assisted extraction - the grounded vegetal product together with the hydroalcoholic solvent, ethanol:water= 1:1, were heated together using microwave energy, using an Ethos Easy Advanced Microwave Digestion System, and the samples were finally filtered.

After obtaining and filtering the extracts, they were reduced. After removing more than 90% of the solvent, the extract was dried by lyophilization in order to preserve the properties of the extract and to extend its shelf life. The dried extracts were stored in the freezer for further use.

To obtain dispersions of phytosynthesized silver nanoparticles, the previously prepared dried extracts were used, redissolved in purified water at various concentrations (5, 2.5, 2, 1.25, respectively 1 mg/mL) and mixed with the nitrate solution silver, in a 1:1 extract/metallic salt ratio.

Table 5.2. Coding of samples of phytosynthesized silver nanoparticles

Extract (acc. Table 7.1)	**Extract concentration (mg/mL)**	**Codification**
E_e	*5.00*	*EE5*
	2.50	*EE2.5*
	2.00	*EE2*
	1.25	*EE1.25*
	1.00	*EE1*
E_{mw}	*5.00*	*EM5*
	2.50	*EM2.5*
	2.00	*EM2*
	1.25	*EM1.25*
	1.00	*EM1*
L_e	*5.00*	*LE5*
	2.50	*LE2.5*
	2.00	*LE2*
	1.25	*LE1.25*
	1.00	*LE1*
L_{mw}	*5.00*	*LM5*
	2.50	*LM2.5*
	2.00	*LM2*
	1.25	*LM1.25*
	1.00	*LM1*

The gel that served as a vehicle for the incorporation of extracts containing nanoparticles, as well as the control gel, were prepared from Carbopol 940 (Sigma-Aldricht).

6. Characterization of extracts and phytosynthesized silver nanoparticles

The total content of phenolic compounds and flavonoids in the obtained extracts are shown in table 6.1.

Table 6.1. The total content of phenolic compounds and flavonoids in the analyzed extracts. Values represent the mean of five determinations ± SE; values in the same column without a common superscript letter differ statistically ($P<0.05$) as analyzed by one-way ANOVA and the TUKEY test.

Nr. crt.	Extract	**Total content of phenolic compounds (µg GAE/g)**	**Total content of flavonoids (mg RE/g)**
1	E_e	790,23±12,51^{a}	71,54±0.53^{a}
2	E_{mw}	672,25±14,83^{b}	72,82±0.44^{a}
3	L_e	537,75±15,44^{c}	40,80±0.29^{b}
4	L_{mw}	422,13±14,15^{d}	40,91±0.17^{b}

According to the presented results, the classical extraction method (using temperature extraction) represents a more efficient method for the extraction of phenolic compounds, compared to microwave extraction. Echinacea also shows, in both extraction methods, a higher content of phenolic compounds compared to Motherwort.

Regarding the determination of the total content of flavonoids, the absence of statistically significant differences between the two extraction methods can be observed, Echinacea extracts being richer in flavonoids, compared to those of Motherwort; also, the flavonoid content is higher in Echinacea extracts, compared to literature data, and in Motherwort extracts, the total flavonoid content is comparable to the data obtained for purified extracts [14].

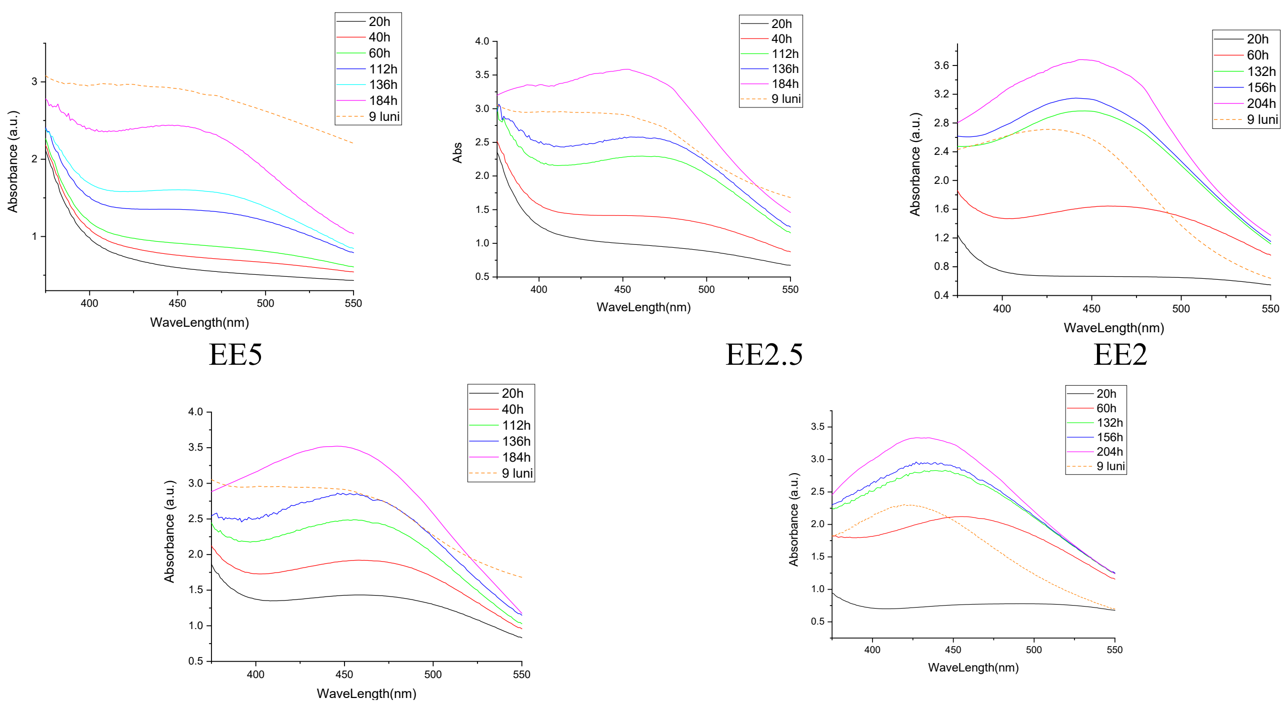


Figure 6.2. UV-Vis spectra for phytosynthesis of silver nanoparticles using Echinacea extracts obtained by the classical extraction method

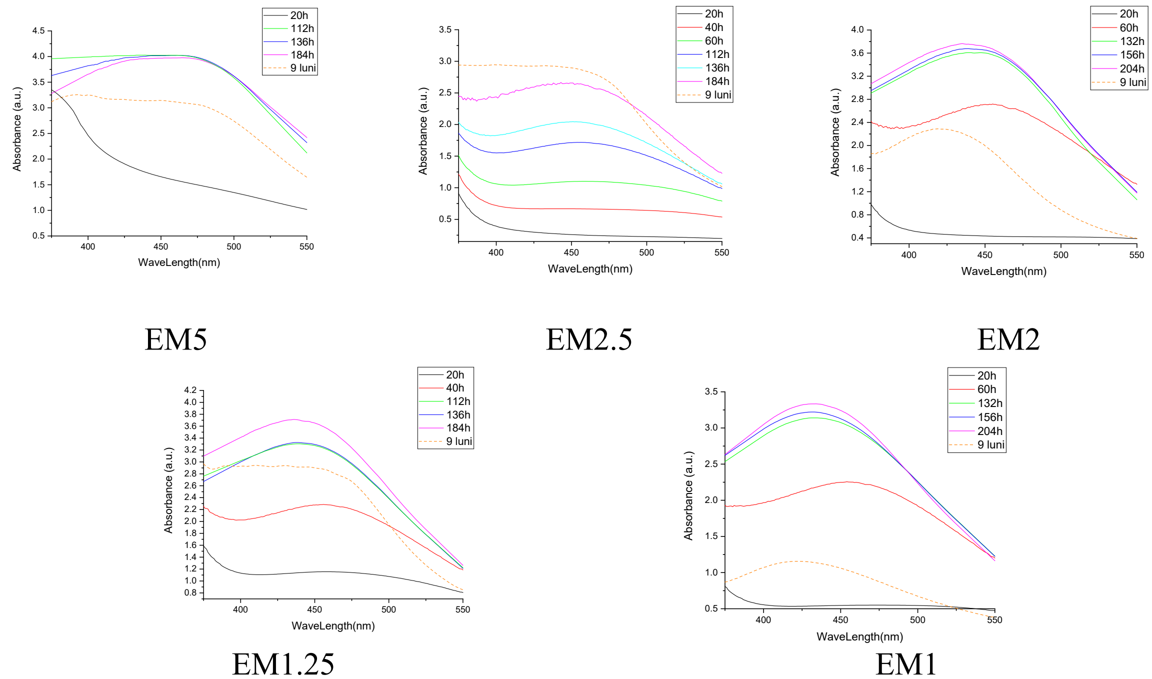


Figure 6.3. UV-Vis spectra for phytosynthesis of silver nanoparticles using Echinacea extracts obtained using the microwave-assisted method

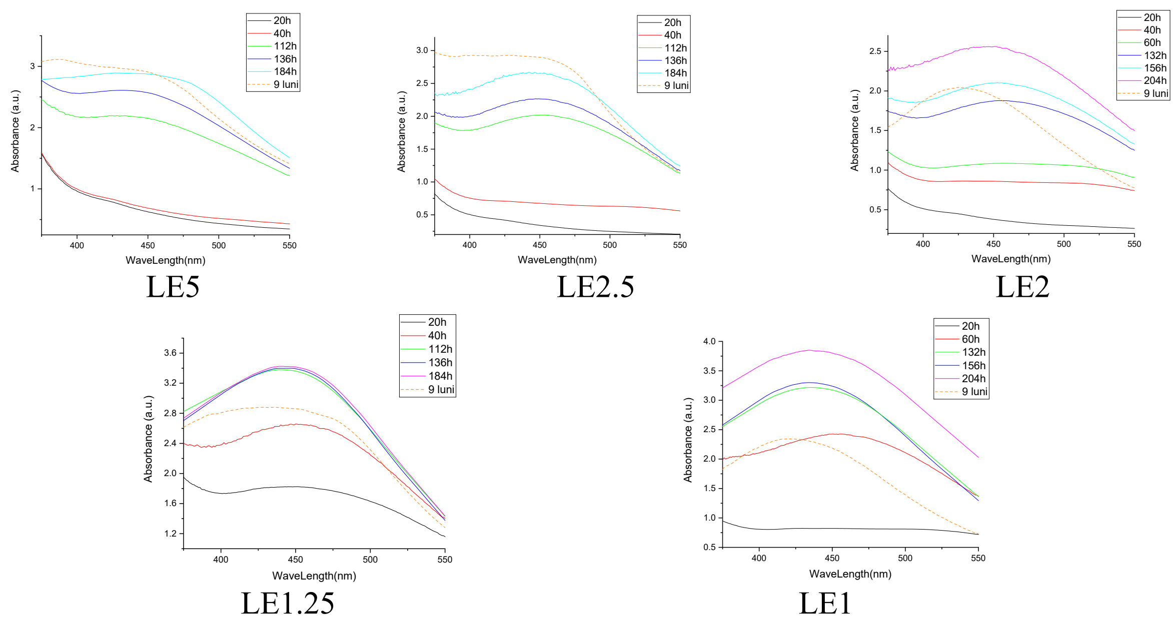
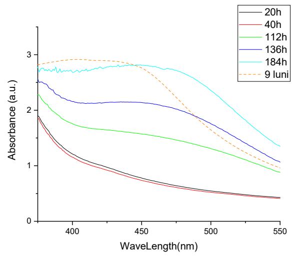
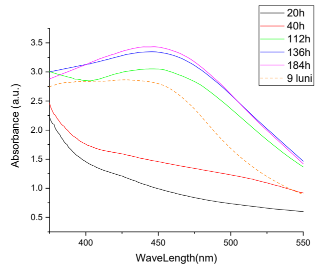


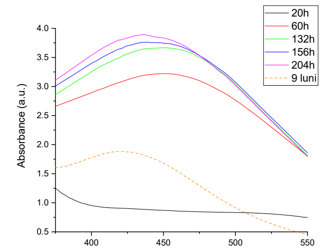
Figure 6.4. UV-Vis spectra for the phytosynthesis of silver nanoparticles using extracts of Motherwort obtained by the classical extraction method



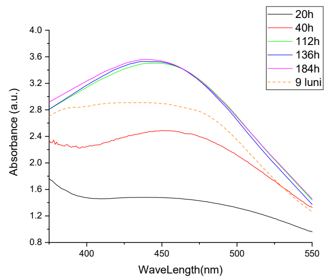
LM5



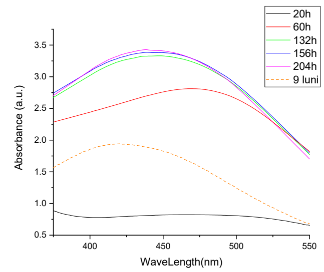
LM2.5



LM2



LM1.25



LM1

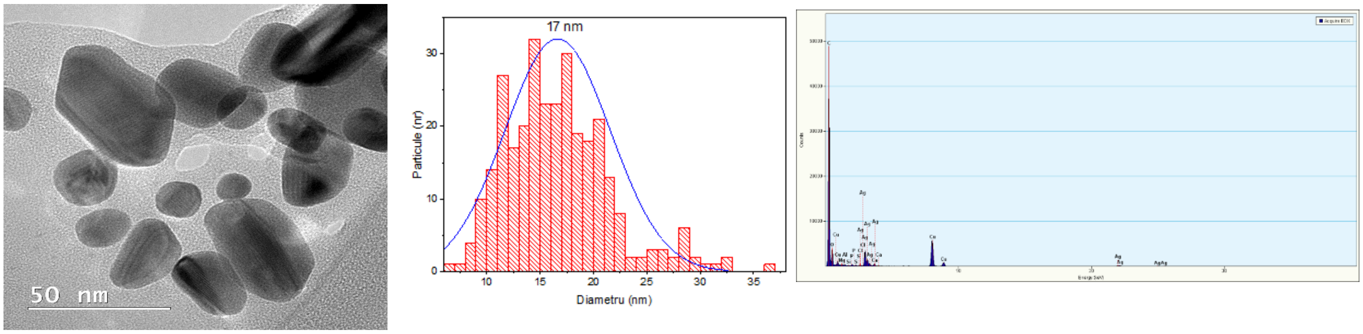
Figure 6.5. UV-Vis spectra for the phytosynthesis of silver nanoparticles using extracts of Motherwort obtained using the microwave-assisted method

This study investigates the synthesis process of silver nanoparticles using extracts from two different plants, Echinacea and Motherwort. The synthesis takes place through various extraction methods and involves varying concentrations of plant extracts. The obtained results show that critical parameters, such as extract concentration and extraction method, have a significant impact on the formation and stability of the resulting nanoparticles.

In general, most of the nanoparticles produced showed good stability, without showing significant aggregation tendencies or major changes in their properties when monitored over time. However, some exceptions to the behavior of the nanoparticles were observed, especially at very low concentrations of extracts or under certain experimental conditions.

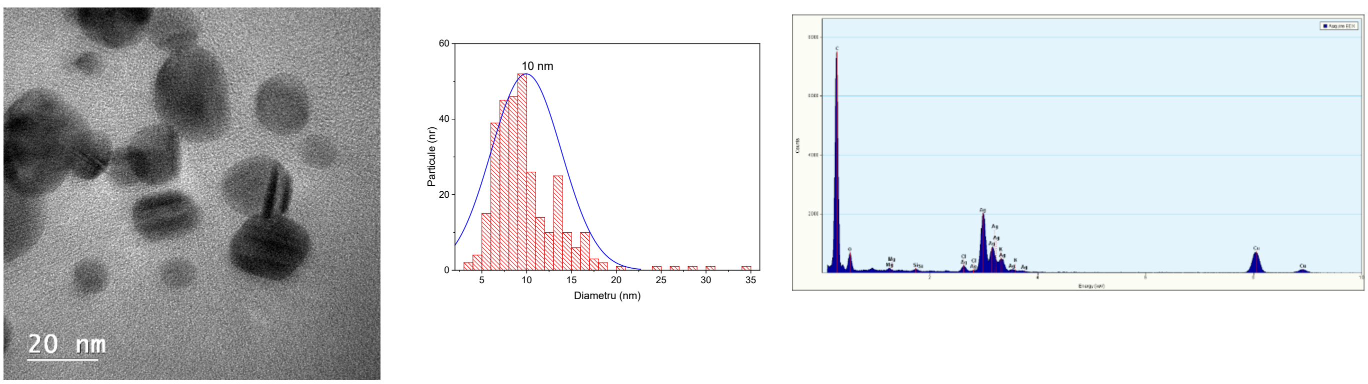
The main conclusion of the study is that the phytosynthesis process of silver nanoparticles can be controlled and optimized by adjusting the concentration of the plant extract and the extraction method according to the type of plant used and the purpose of the synthesis.

Considering all the results obtained, two samples were selected for each set of phytosynthesized nanoparticles (the one with the maximum concentration, respectively an average concentration – 2 mg/mL) for the evaluation of the nanoparticle morphologies. For analysis, a drop of extract containing the nanoparticle dispersion was placed in the center of a copper grid, dried and subjected to microscopic analysis.



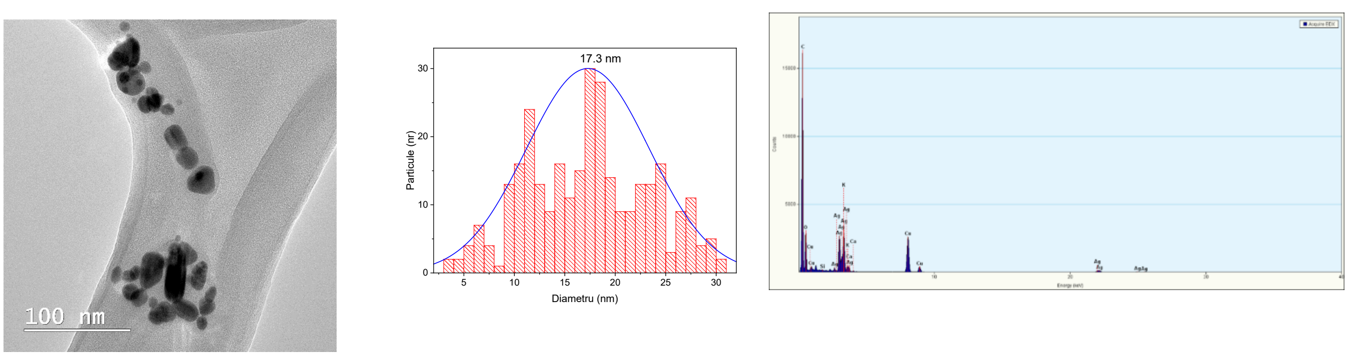
a b c

Figure 6.18 TEM results obtained for the EE5 sample: a) representative TEM image for the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample



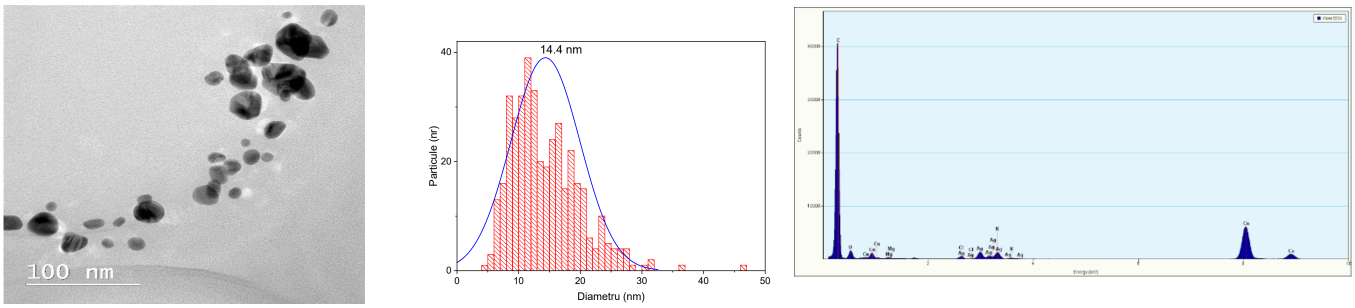
a b c

Figure 6.19. TEM results obtained for the EE2 sample: a) representative TEM image for the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample



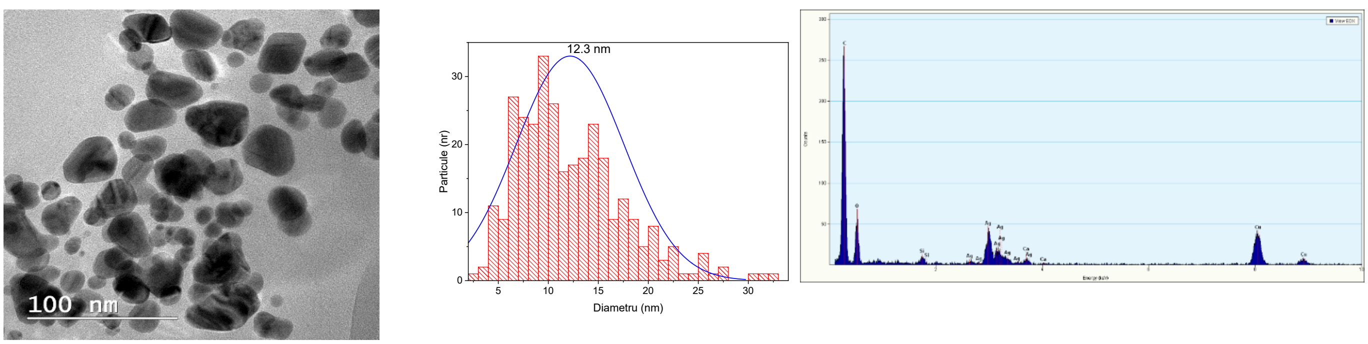
a b c

Figure 6.20. TEM results obtained for the EM5 sample: a) representative TEM image for the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample



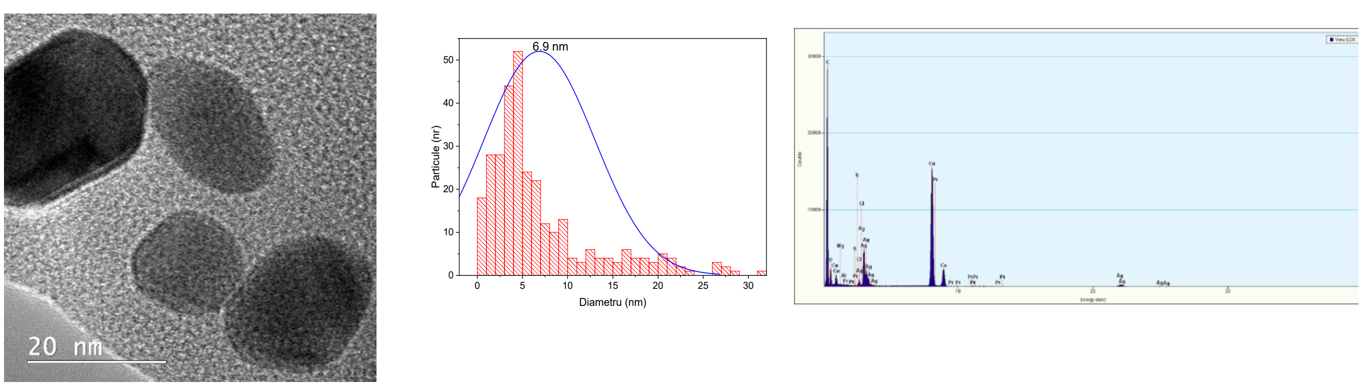
a b c

Figure 6.21. TEM results obtained for the EM2 sample: a) representative TEM image for the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample



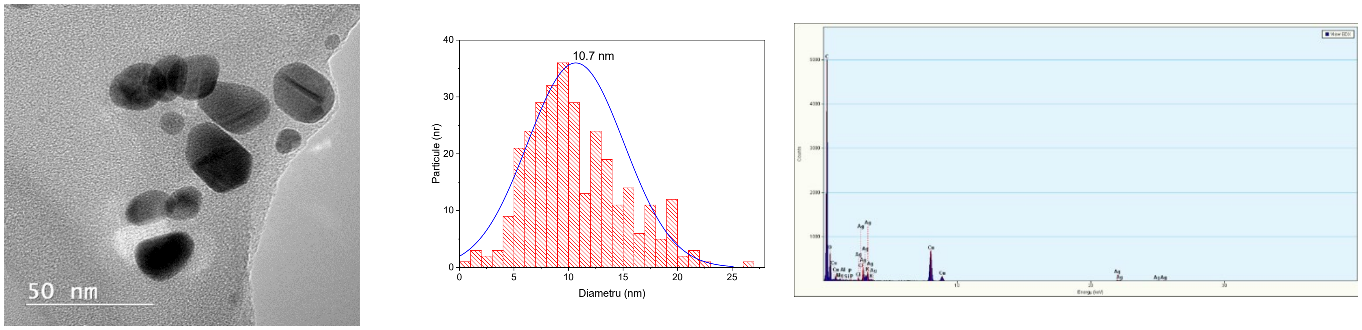
a b c

Figure 6.22. TEM results obtained for the LE5 sample: a) TEM image representative of the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample



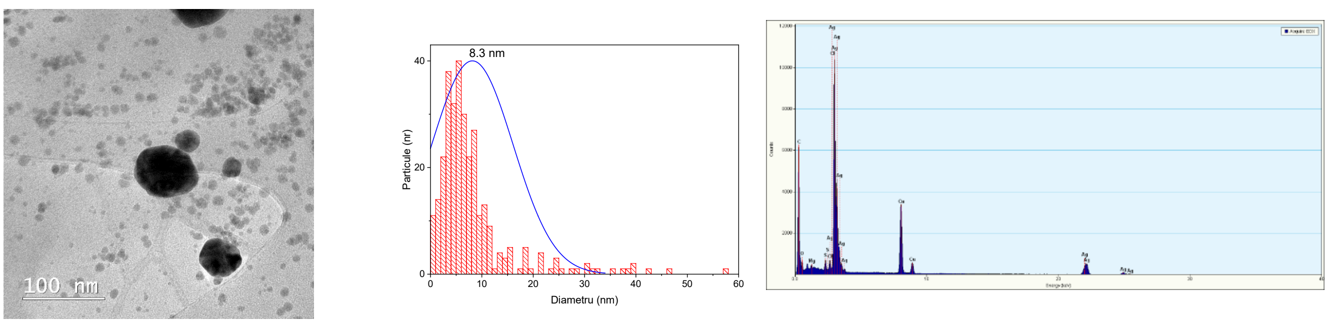
a b c

Figure 6.23. TEM results obtained for the LE2 sample: a) TEM image representative of the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample



a b c

Figure 6.24. TEM results obtained for the LM5 sample: a) TEM image representative of the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample



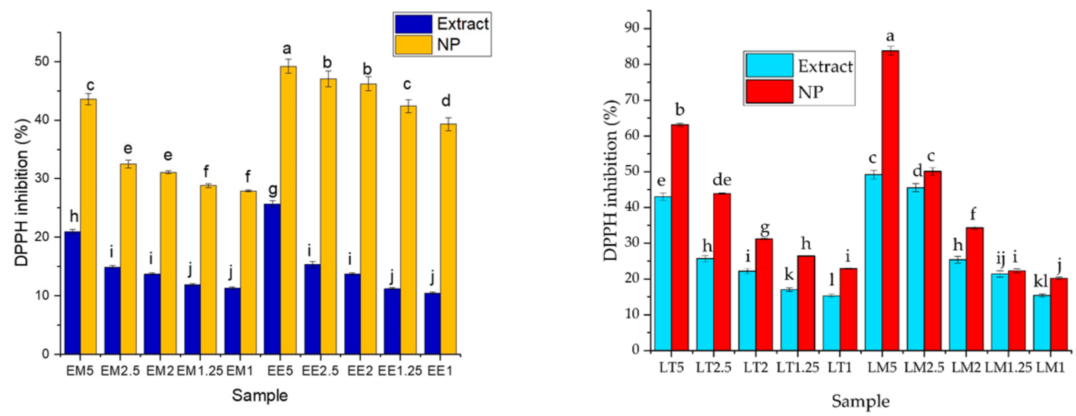
a b c

Figure 6.25. TEM results obtained for the LM2 sample: a) TEM image representative of the morphology of the nanoparticles, b) AgNP size distribution, determined from 150 measurements, c) EDX spectrum of the analyzed sample

TEM analyses confirm the synthesis of nanoparticles with spherical or semi-spherical morphologies, with sizes below 15 nm in general, with a tendency to form nanoparticles with smaller sizes when using lower concentration extract solutions. The EDX spectra confirm in all cases the synthesis of silver nanoparticles, the additional elements present in the spectrum being due to either the TEM analysis grids or the elements present in the phytocomponents.

7. Evaluation of biological properties of phytosynthesized nanoparticles

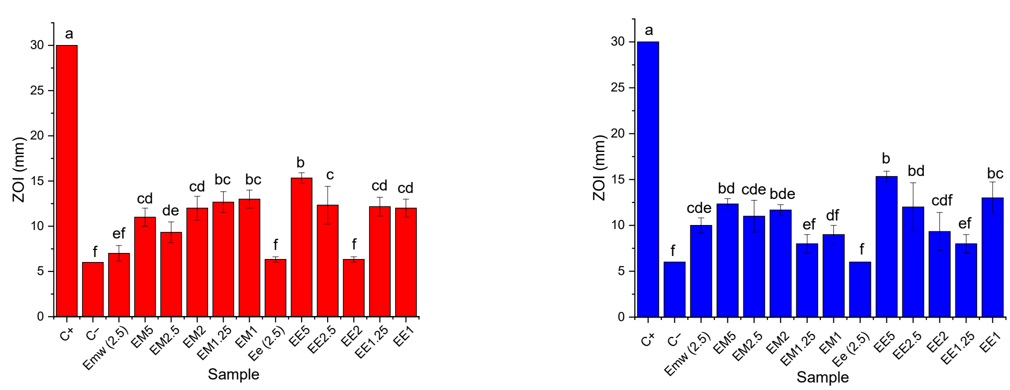
Graphically, the results of the evaluation of the antioxidant activity of the phytosynthesized nanoparticles, compared to the results obtained for the corresponding extracts, are presented in figure 7.1.



a b

Figure 7.1. Antioxidant activity results for phytosynthesized nanoparticle samples using *E. purpurea* (a) and *L.cardiaca* (b) extracts, respectively, compared to the corresponding natural extracts. Results represent the mean of five determinations ± SD; values without a common superscript letter differ statistically (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

In the analyzed study, the antioxidant activity of plant extracts obtained from plants such as Echinacea and Motherwort is investigated, by using different extraction methods. The main results include the increase of antioxidant activity with the concentration of extracts and significant variations in antioxidant activity after phytosynthesis [15, 16]. Interestingly, no clear correlation can be established between antioxidant activity and the content of phenolic compounds or flavonoids. It is also suggested that microwave-assisted extraction may be more effective in obtaining compound-rich extracts.



(a) (b)

Figure 7.3 Antimicrobial activity of phytosynthesized silver nanoparticles using Echinacea extracts, tested against: a) *Candida albicans* ATCC 64548 and b) *Escherichia coli* ATCC 64548; C+ - positive control; C− - negative control (water); Emw (2.5)—microwave-assisted extract at 2.5 mg/mL; Ee (2.5)—classic extract at temperature at 2.5 mg/mL. Values represent mean ± SE, n = 3 per treatment group; values without a common superscript letter differ statistically ($p < 0.05$) as analyzed by one-way ANOVA and the TUKEY test

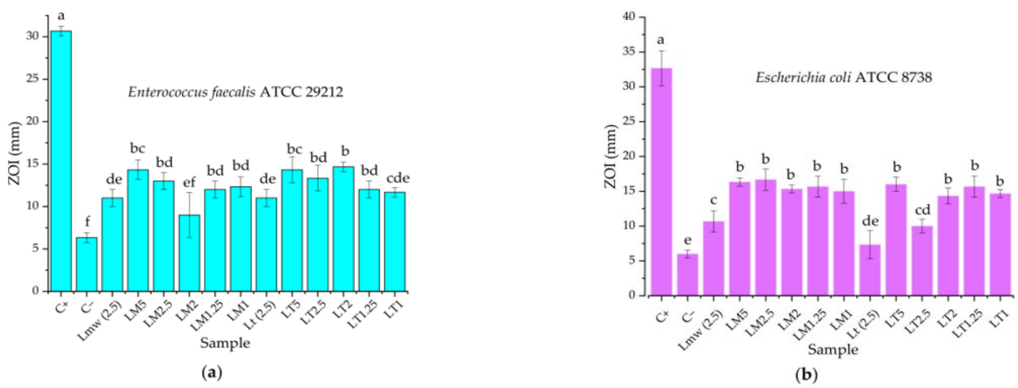


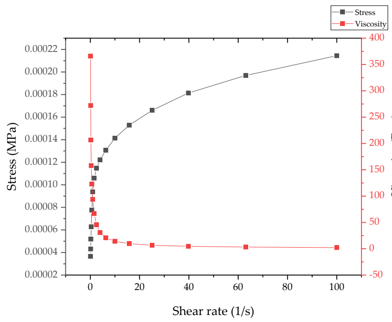
Figure 7.4 Antimicrobial activity of phytosynthesized silver nanoparticles using Motherwort extracts, tested against: a) *Enterococcus faecalis* ATCC 29212 and b) *Escherichia coli* ATCC 64548; C+ - positive control; C− - negative control (water); Emw (2.5)—microwave-assisted extract at 2.5 mg/mL; Ee (2.5)—classic extract at temperature at 2.5 mg/mL. Values represent mean ± SE, n = 3 per treatment group; values without a common superscript letter differ statistically ($p < 0.05$) as analyzed by one-way ANOVA and the TUKEY test

In this study, the antimicrobial properties of phytosynthesized nanoparticles using Echinacea and Motherwort extracts were investigated. For Echinacea extract nanoparticles, the antimicrobial activity was found to increase with extract concentration, reach a plateau, and then increase again, indicating the importance of nanoparticle sizes.

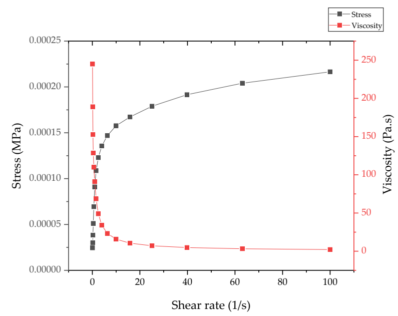
Nanoparticles obtained with *L. cardiaca* extracts showed good antimicrobial activity against Gram-negative and Gram-positive bacteria, with the best results for certain concentrations of the extracts. This study suggests the importance of controlling the size of nanoparticles and that these nanoparticles may be useful for the development of effective antimicrobial agents.

8. Obtaining and characterizing some formulations containing phytosynthesized nanoparticles for topical applications

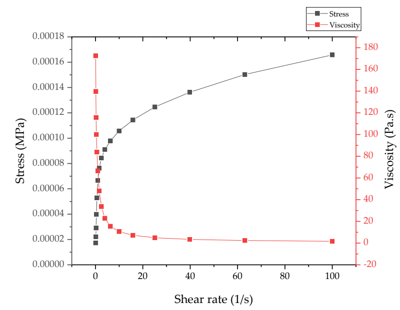
For the development of formulations containing phytosynthesized silver nanoparticles, the nanoparticles obtained using Motherwort extracts were selected.



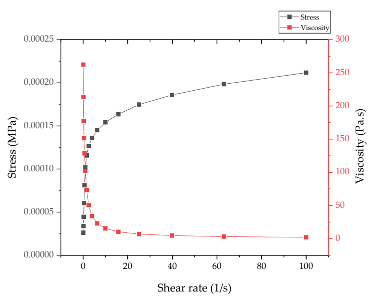
LE1



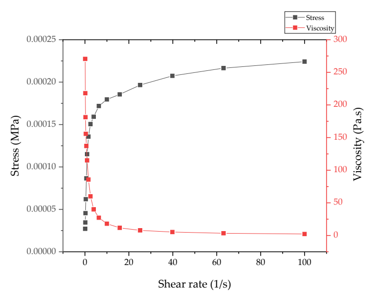
LE1.25



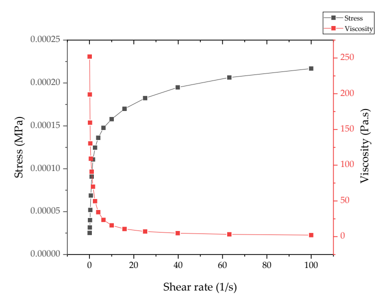
LE2



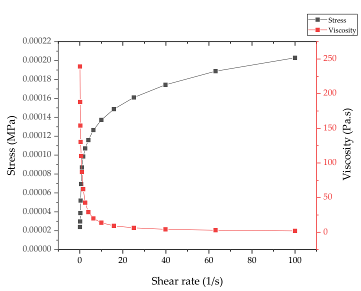
LE2.5



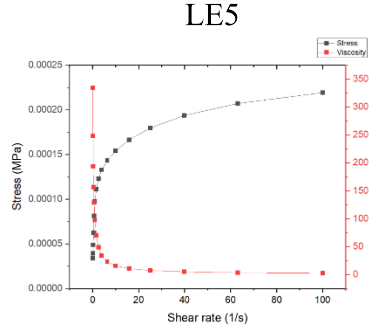
LE5



LM1



LM1.25



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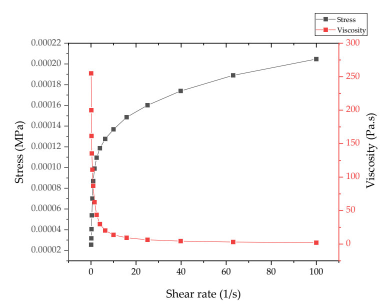

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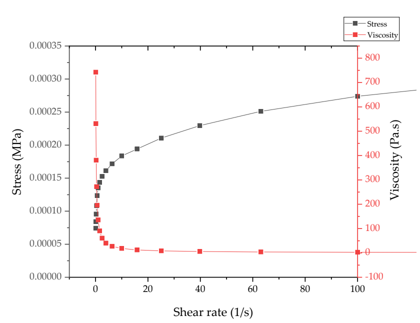


LM5

Figure 8.1 Cumulative rheograms of shear stress as a function of shear rate and viscosity as a function of shear rate for the tested gels

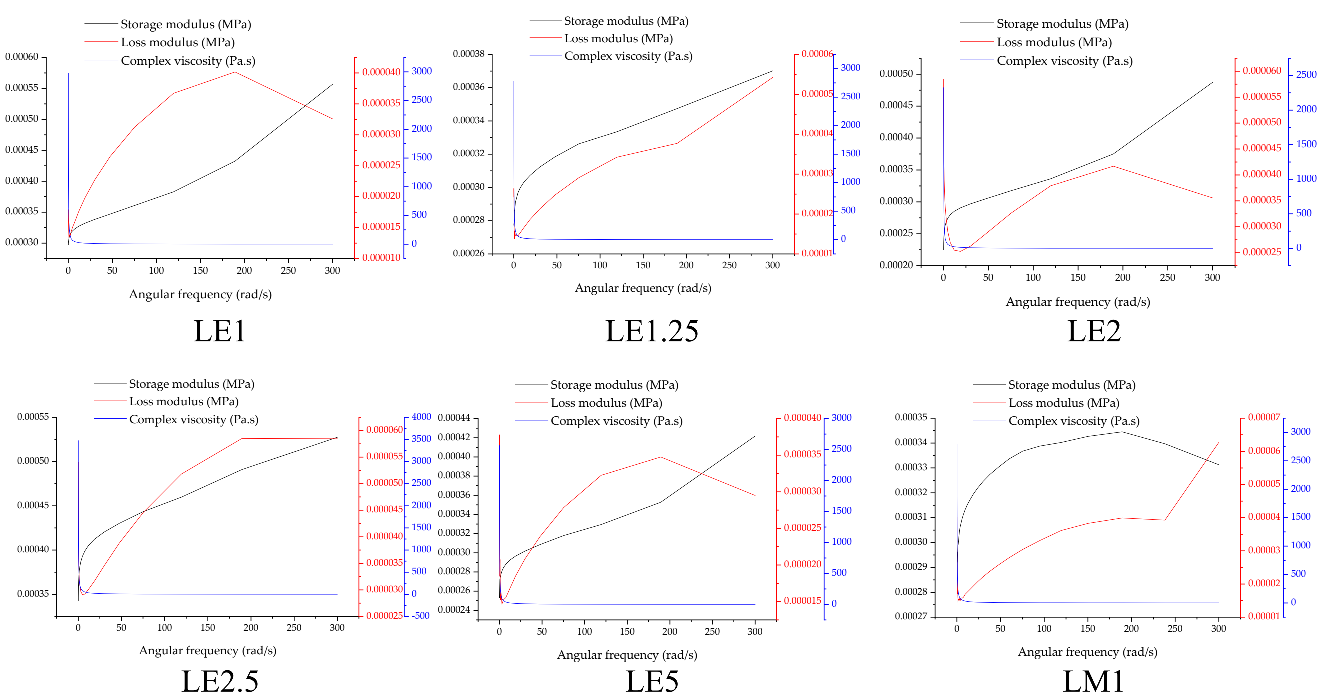
For non-Newtonian systems, the viscosity decreases (pseudoplastic character) or increases (dilatant character) when the shear rate increases, respectively the shear stress.

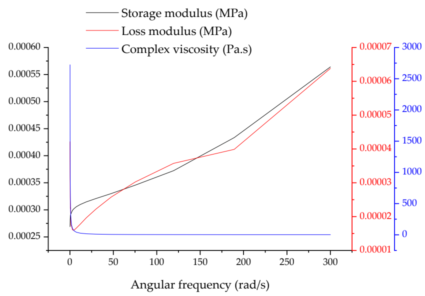
From the rheograms recorded in Figure 8.1, it can be seen that, for all designed formulations, the shear stress increases with increasing shear rate, while the viscosity decreases with shear rate, indicating that the systems exhibit a pseudoplastic character (the system of shear thinning).

Another important aspect discussed is thixotropy, which describes the reversible change in gel properties as a function of shear time and rest periods [17].

In conclusion, silver nanoparticle gels show pseudoplastic reactivity and thixotropy, which has significant implications in their development and use.

To study the eventual thixotropic character of non-Newtonian systems, other analyses, especially oscillatory analyses (Figure 8.2), characteristic of viscoelatic materials, with intermediate properties between liquid and solid, are needed.





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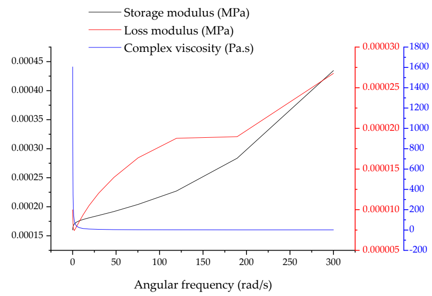

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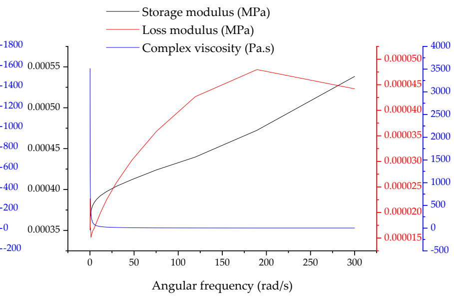

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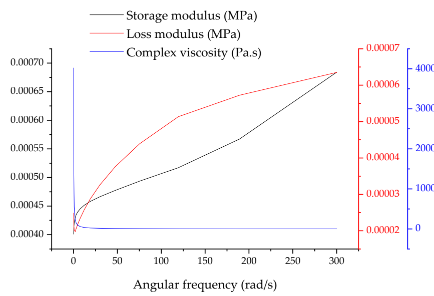

LM5

Figure 8.2 Dependence of the modulus of elasticity (Storage modulus/MPa) and viscosity (Loss modulus/MPa), respectively of the complex viscosity (Complex viscosity/Pa•s) depending on the angular speed (Angular frequency/rad/s) for the tested gels

The evolution of the storage/elastic modulus (G') provides information about the solid (elastic) behavior of the sample, while the loss/viscous modulus (G") provides information about the liquid (viscous) behavior [18].

For samples where the storage modulus was greater than the loss modulus, a greater contribution of the elastic component than the viscous component is suggested (evident in samples LE1.25; LM 2; LM2.5 and LM5).

Rheological measurements indicate an almost frequency-independent G', while G" increased slightly with frequency, as is characteristic of gel-like materials [19].

As for the complex viscosity (η), it is frequency dependent and is determined for a non-Newtonian viscoelastic system by subjecting it to oscillatory shear stress. [20].

The complex viscosity constant (Figure 8.2) suggests interactions between the nanoparticles used and the polymer component in the gel formulations.

9. Conclusions and personal contributions

9.1. Conclusions

Nanotechnology and the development of nanomaterials are a very topical subject, with an impressive number of studies being conducted and published in this field every year. In particular, the phytosynthesis of nanomaterials represents a field in full evolution in recent years.

The aim of the present work was to evaluate the possibilities of phytosynthesis of metal nanoparticles using two plants with proven biomedical applications (*Echinacea purpurea* L. and *Leonurus cardiaca* L.).

Regarding the experimental study on the phytosynthesis of silver nanoparticles using Echinacea extracts, the following conclusions can be formulated:

The obtained experimental results highlight the potential application of Echinacea extracts in the phytosynthesis of metal nanoparticles. The complex characterization of the extracts revealed significantly higher concentrations of total phenolic compounds for the extracts obtained by the classical temperature extraction method, while the microwave-assisted extraction method led to the extraction of significantly higher amounts of flavonoids.

According to the experimental results, the phytosynthesis of nanoparticles with spherical or quasi-spherical morphologies, with sizes below 30 nm in general and with a tendency to form nanoparticles with smaller sizes when lower concentration extract solutions are used, was confirmed.

The antioxidant properties of the extracts could be correlated with the level of total phenolic compounds (TFP). Thus, significantly higher CFT values were recorded for the extract obtained by the classical method at temperature (Ee), the antioxidant activity determined by the DPPH test was also significantly higher for EE5 compared to EM5.

As a general remark, the antimicrobial properties of the phytosynthesized nanoparticles (presented as inhibition zone, in mm) started from high values corresponding to the highest extract concentration and slowly decreased, reaching a plateau (EM2.5 and EE2 for Candida albicans ATCC 64548, respectively EM1.25 and EE1.25 for Escherichia coli ATCC 8738), after which a sudden increase was observed, associated with the increase in the influence of particle sizes on the antimicrobial effect.

By comparing the results of phytochemical analyzes with literature data, lower values of total phenolic compounds were obtained.

Comparing the two extraction methods, it can be seen that the classical extraction at temperature leads to a higher content of total phenolic compounds compared to the microwave extraction, and regarding the total content of flavonoids, the differences are not statistically significant.

HPLC determinations revealed the presence of some phenolic acids (chlorogenic acid, caffeic acid and coumaric acid), at values similar to literature data. The presence of some anthocyanidins and flavonoids (rutin, hyperoside, catechin, naringin and naringenin) was also identified, some of which have not been previously presented in literature studies.

The results of analytical determinations demonstrated the phytosynthesis of silver nanoparticles using extracts of *L. cardiaca*, the smallest sizes of nanoparticles being obtained with the extract developed using the microwave-assisted method at a concentration of 1 mg/mL.

The phytosynthesized silver nanoparticles demonstrated a statistically significant higher antioxidant potential (according to the DPPH test) compared to the extracts used for the synthesis; all tested experimental variants demonstrated significant antimicrobial properties (against both Gram-negative and Gram-positive lines, demonstrated by studying the effects on *Enterococcus faecalis* and *Escherichia coli*).

Considering the obtained results, phytosynthesized nanoparticles using *L. cardiaca* extracts were selected to obtain semi-solid hydrogel type formulations for topical applications (characterized from the rheological point of view).

9.2. Personal contributions

The present work has a high level of originality, personal contributions to the development of knowledge in the field can be summarized as follows:

- Original synthesis of silver nanoparticles with *L. cardiaca* extracts.
- Development of an innovative methodology for the phytosynthesis of silver nanoparticles with *E. purpurea* extracts.

- Carrying out a complex analytical study, which included the characterization of both the extracts and the nanoparticles obtained.
- Evaluation of the antioxidant potential and antimicrobial properties of nanoparticles, with implications in the field of biomedicine.
- Investigating the influence of the extraction method and the extract concentration on the morphology and properties of the nanoparticles.

These contributions were recognized by publishing the results in prestigious scientific journals (Q1 and Q2), in an international book chapter and by participating in international scientific conferences.

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