POLYTECHNIC UNIVERSITY OF BUCHAREST

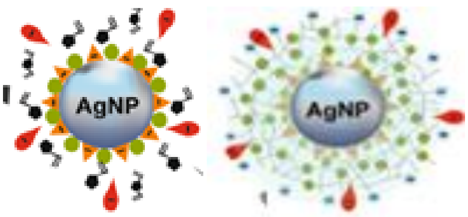
FACULTY OF APPLIED CHEMISTRY AND MATERIALS SCIENCE

PhD THESIS

SUMMARY

DEVELOPMENT OF NEW BIOHYBRIDS BASED ON SILVER NANOPARTICLES AND PLANT EXTRACTS USING GREEN CHEMISTRY

DEZVOLTAREA DE NOI BIOHIBRIZI PE BAZĂ DE NANOPARTICULE DE ARGINT ȘI EXTRACTE DIN PLANTE, UTILIZÂND CHIMIA VERDE



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București, 2022

CONTENT

THANKS

I would like to with great warmth and deep gratitude to my doctoral coordinator, **Professor Emeritus Aurelia Meghea** for the unconditional support and trust given throughout the years of study and during the elaboration of this thesis. Respectful and profound thanks, for the high academic professionalism and outstanding expertise in guiding me the steps of knowledge in the field of scientific research, for the advice and encouraging recommendations offered, with openness and kindness at each stage of further studies and completion of thesis.

I would like to thank to **Dr. Eng. Marcela Elisabeta Pătrașcu-Bărbînța**, associate professor at the Faculty of Physics, Department of Electricity, Solid State Physics and Biophysics, University of Bucharest for permanent support, for the advice full of friendship and professionalism she offered generously to me throughout the experimental and bibliographic research activities. Thank you with gratitude for the example of professional dedication offered throughout the years of study.

I would like to thank the **professor Dr. Eng. Nicoleta Maria Badea** for the example of selflessness and dedication to the scientific research she offered me throughout my years of study, for the professionalism and core approaches, for support, trust and the knowledge provided unconditionally throughout the research activity.

And last but not least, I would like to thank **gratefully Professor Habil. to Dr. eng. Ileana Rău**, director of the Doctoral School for the support, trust and advice offered.

I thank with great respect and gratitude to **Mr. Emil Cătrună**, manager of BIONATURA PLANT S.R.L., the company in which I carried out my daily professional activities, for the understanding and permanent support offered throughout the period of my doctoral studies.

Author

INTRODUCTION

"Let us keep all that was good in the past,
for both past and present
the future is being built. ”
Anatole France (1844 – 1924)

Modern science has known and is experiencing a wide and profound development in all its fields. One of the desideratum of the last two decades is to implement ingenious solutions springing from scientific research. Thus, in the field of bio-nanotechnology there is a live concern regarding the development of new bio-nanotechnologies that propose innovative approaches aimed at improving existing technologies and finding integrative solutions to solve various needs in the fields of health sciences. , of the environment, of different industries.

Traditional phytotherapy, through medicinal and aromatic plants, has proven over time to be an important source in obtaining adequate physiological responses (pain relief, reduction of fever, inflammation, digestive disorders, etc.). Studies and research in the field of pharmacology conducted in recent decades show that phytotherapy is one of the application branches of the Pharmacology of herbal medicines, an aspect mentioned in the book suggestively entitled "Phytotherapy - plants source of health" [1].

Also, the physico-chemical qualities and biological effects of precious and semi-precious metals (Au, Ag, Pl, Cu, Zn, etc.) are known and recognized since antiquity. Of these, silver, in various forms, has a well-defined place throughout history, due to the proven qualities of catalyst, antimicrobial, anti-inflammatory, healing.

Harry Margraf, of Saint Louis University, U.S.A. stated about silver the following: "Silver is the best fighter, in all respects, against the pathogenic germs we have." [5]. The same researcher published a study in 1977 [6] in which he highlighted the qualities of a broad-spectrum germicidal agent and a healing agent of a silver and zinc compound used in the treatment of chronic skin ulcers (leg ulcers, these are known to have, currently, diabetic etiology). It is currently used in medical practice, chronic wound care, colloidal silver dressings. Such examples, which highlight the beneficial effects of colloidal silver used alone or in combination with other preparations may continue.

Modern science, through interdisciplinary studies (biology, chemistry, biophysics, pharmacology) conducted in recent decades and their corroboration with the nano field contributes to obtaining new metal bio-nanohybrids, which depending on the morpho-structural characteristics have physico-chemical, optical quality, electromagnetic, electrical, photovoltaic, biological (eg microbiological) properties, superior to there components used separately.

The present thesis entitled: "Development of new biohybrid based on silver nanoparticles and plant extracts, using green chemistry" presents studies of obtaining biohybrid hybrids based on phytogenic silver nanoparticles, highlighting their physico-chemical and biological properties and new plants are proposed that could be used to develop formulations with antioxidant, protective, regenerating properties to improve quality of life. Thus, in the studies performed and presented in this paper to obtain different types of biohybrids, phyto-nanoparticles of silver were preparated, using extracts from various plants (*Salvia sclarea, Arctium lappa, Artemisia abrotanum, Asparagus officinalis, Cirsium arvense, Harpagophytum procumbens*) biomimetic membranes based on liposomes, and together with vitamin C and pectin extracted from lemon peel, bio-nanohybrids containing phytogenic AgNPs were obtained. All bio-nanosystems obtained were characterized in terms of physico-chemical, morpho-structural properties (UV-Vis, DLS, Zeta Potential, SEM) and biological properties (antioxidant, antimicrobial activity). Highlighting the qualities of nano-biohybrids was performed *in vitro*.

The experiments were performed at the Research Center for Environmental Protection and Ecofriendly Technologies of the Faculty of Applied Chemistry and Materials Science, Polytechnica University of Bucharest, in the laboratories of the Department of Biophysics, Faculty of Physics, University of Bucharest and in the laboratories from the National Institute for Lasers, Plasma and Physical Radiation, Bucharest-Magurele.

This paper is structured in two parts, consisting of 9 chapters, followed by the final conclusions and bibliography.

Part I. The bibliographic research consists of 3 chapters:

❖ **Chapter 1** entitled **Metallic Nanoparticles** presents information on the basic elements related to metallic nanoparticles - types of metallic nanoparticles and methods of their synthesis, methods of nano-phytosynthesis using plant extracts and information on nanoparticle hybrids metallic - types of hybrids, methods of obtaining nanohybrids.

❖ In **Chapter 2** entitled ,,Plant sources with potential to be used for the synthesis of metal nanoparticles" some information are presented on the current situation of metal bio-nanosynthesis through Green Chemistry, the main factors involved in optimizing metal nano-biosynthesis, mechanisms of action antibacterial of metal nanoparticles synthesized through plants, biological activities of biogenerated nanoparticles, methods for characterizing metal nanoparticles, as well as proposals for suitable plant sources that can be used to obtain silver nanoparticles.

❖ **Chapter 3** of this paper, „Applications of metallic nanoparticles and their biohybrids" presents examples of fields of research and application of nanotechnology and some examples of applications of metallic bio-nanostructures in some fields of activity - bio-medical, environment.

❖ **Part II: Original contributions** presents the research objectives, includes 6 chapters, finally presenting the conclusions and bibliography of the thesis.

❖ **Chapter 4** entitled „Methods for the preparation and characterization of biohybrids based on silver nanoparticles (AgNP), obtained by methods of "green chemistry" " presents the materials and experimental methods used for the synthesis of silver nanoparticles and biohybrids, as well as the methods of characterization

❖ In **Chapter 5** is presented the „Optimization of the method of synthesis of silver nanoparticles using the root extract of *Harpagophytum procumbens*".

❖ In the most extensive chapter of this paper, **Chapter 6** „Biosynthesis and characterization of silver nanoparticles obtained by "green" methods" is presented the ecological approach for obtaining silver nanoparticles through four plants (Burdock (*Arctium lappa*), St. John's wort (*Salvia sclarea*), Asparagus (*Asparagus officinalis*), Wood of the Lord (*Artemisia abrotanum*)]), highlighting their qualities in the phytogeneration of silver nanoparticles, evaluation of antioxidant activity through two methods (chemiluminescence and ABTS) and highlighting the antibacterial activity of AgNPs

❖ **Chapter 7** - Phytosynthesized AgNP-based biohybrids from *Artemisia abrotanum* extract and chlorophyll *a*-labeled liposomes is intended to present the way to make bio-nanohybrids based on AgNPs and liposomes and highlight their antioxidant qualities.

❖ **Chapter 8** Biohybrids with antioxidant properties prepared from aqueous Brusture extract (*Arctium lappa*), highlights the antioxidant qualities of bio-nanohybrids obtained from AgNPs synthesized through Arctium lappa extract, biomimetic membranes (liposomes) and vitamin C.

❖ **Chapter 9** Phyto-generated bio-active nanomaterials from field weed, Cirsium arvense presents an innovative system composed of bio-active components - phyto-synthesized AgNPs from aqueous extract from *Cirsium arvense* and lemon pectin and highlights their antioxidant and antibacterial properties that can recommend them to be used in biomedical applications.

The doctoral thesis ends with "Conclusions" in which the results of the research are synthetically punctuated, their originality, followed by the presentation of study perspectives in order to obtain new bio-active systems with new properties or the improvement of those already highlighted.

The Thesis contains 13 tables, 94 figures and 222 bibliographical references.

The results of the studies were presented by publication in 5 ISI-rated journals (cumulative impact factor FI = 3,104).

Keywords: phyto-nanosynthesis, AgNPs, biomimetic membranes, vitamin C, lemon pectin, bio-activites (antioxidant and antibacterial activity).

I. ORIGINAL CONTRIBUTIONS

Chapter 5

OPTIMIZATION OF GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING *Harpagophytum procumbens* ROOT EXTRACT

This chapter presents the optimization study of the method for obtaining ecosynthesized silver nanoparticles (AgNPs) using aqueous extract of *Harpagophytum procumbens* root powder, in order to be used later in various fields of industry, medicine and pharmacy, in different natural / traditional therapies.

H. procumbens, belonging to *Pedaliaceae* family, is a creeper plant growing spontaneously in sandy soils from dry hill and submountain areas of South Africa (Namibia, Botswana), covered by a woody crust endowed with two central prickles and other two rows of smaller prickles (Figure 5.1A). In traditional phytotherapy both root and tubers are used (Figure 5.1B).

A.  **B.** 

Figure 5.1 Photographs of the plant *Harpagophytum procumbens*: A) Flower and thorns [149]; B) Plant tubers [150].

In specialty studies on *Devil's claw* the anti-inflammatory effects [157] of the phyto-compounds contained by this plant are mentioned such as: glicozide iridoides – harpagoside (esther of cinnamic acid), harpagides [157], phenylethanoides (verbacosides and isoverbacosides), phenols and flavonoids [158], and also lipids, fibers, proteins, polyphenols, carbohydrates [159]. Among other properties of this plant are the following activities: antioxidant, antimutagenic, antitumor [160], hepatoprotective, hemodynamic, antimicrobian, antiinflamatory, antiallergenic [157, 160, 161], antiarthritic [21]; it can be used in the therapy of degenerative diseases of bony and muscular systems [162]; anti-obesity [163]; prevents osteoporosis, confers protection against bon losses induced by ovarictemy [162]; stimulates uterotonic spastic activity, being used in inducing/accelerating travail and placenta expulsion after birth [163].

The present work reports on biogeneration of silver nanoparticles by a simple method, by using *Harpagophytum procumbens* root extract. AgNPs thus obtained are spectrally characterized by means of absorption UV-Vis spectroscopy, and their size has been estimated by dynamic light scattering (DLS) measurements. Moreover, the antioxidant activity of AgNPs has been evaluated by chemiluminescence and ABTS techniques.

5.1 Obtaining the plant extract from the root of *Harpagophytum procumbens*

The method of obtaining silver bio-nanoparticles made using *Harpagophytum procumbens* root powder extract is presented in Chapter 4.

Abbreviations of the prepared samples are presented in Table 5.1.

Table 5.1. Abbreviations of the analyzed samples [132]

Sample name	Sample abreviation
Extract from the root of *Harpagophytum procumbens*	HE
AgNP biosynthesized from the root of *Harpagophytum*, ratis extract:$AgNO_{3(aq)}$, de: 1:10 (mL/mL), after 1 h	S1_1h
AgNP biosynthesized from the root of *Harpagophytum*, ratist extract:$AgNO_{3(aq)}$, de: 1:10 (mL/mL), after 2 h	S1_2h
AgNP biosynthesized from the root of *Harpagophytum*, ratis extract: $AgNO_{3(aq)}$, de: 1:10 (mL/mL), after 24 h	S1_24h
AgNP biosynthesized from the root of *Harpagophytum*, ratis extract: $AgNO_{3(aq)}$, de: 1:100 (mL/mL), after 1 h	S2_1h
AgNP biosynthesized from the root of *Harpagophytum*, ratis extract: $AgNO_{3(aq)}$, de: 1:100 (mL/mL), after 2 h	S2_2h
AgNP biosynthesized from the root of *Harpagophytum*, ratis extract: $AgNO_{3(aq)}$, de: 1:100 (mL/mL), after 24 h	S2_24h
Suspension of AgNPs (S2_24) centrifuged for 30 minutes, at 4ºC, 15000 rpm	S2_1
Sample S2_1 ultrasound for 5 minutes	S2_2
Sample S2_2 ultrasound for 5 minutes (total ultrasound time = 10 min.)	S2_3

5.1.1 Monitoring by UV-Vis absorption spectroscopy of nanoparticles obtained by *Harpagophytum procumbens* extract

Highlighting the ability of the aqueous extract of *Harpagophytum procumbens* root to synthesize silver nanoparticles was performed using UV-Vis absorption spectra correlated with dynamic light scattering (DLS) measurements.

Figure 5.2 illustrates the evolution in time of metal nanoparticles suspensions prepared using two volumetric ratios extract: $AgNO_{3(aq)}$ of 1:10 (Figure 5.2A) and 1: 100 (mL/mL) (Figure 5.2B).

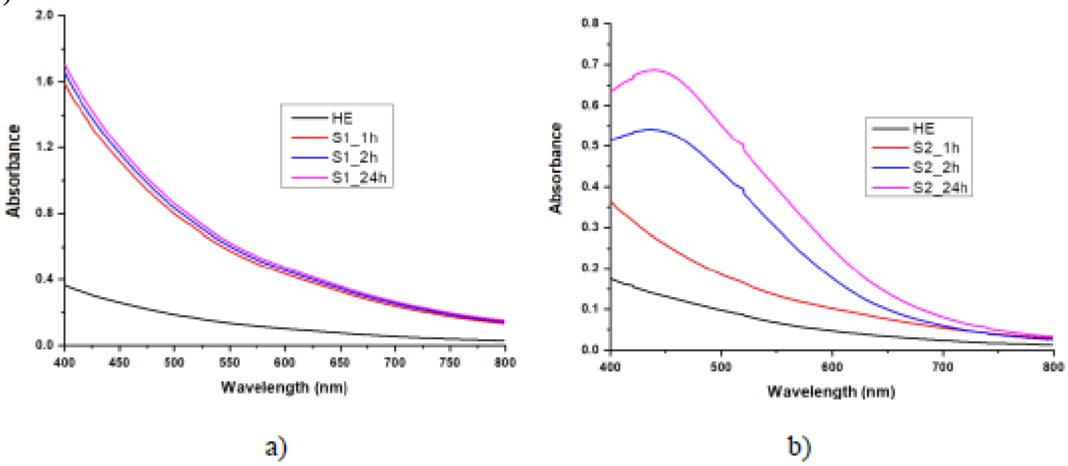

Figure 5.2. Time evolution of metallic nanoparticles suspensions by using two volumetric ratios of extract:$AgNO_{3(aq)}$ de: A) 1:10 (mL/mL) și B) 1:100 (mL/mL) [132].

One can be noticed, in figure 5.2B, that the surface plasmon resonance (SPR) band is well defined for the volumetric ratio 1:100, this being an optimal ratio for AgNPs phytogeneration. These nanoparticles have been further processed and spectrally characterized in order to optimize the protocol for AgNPs obtaining.

Thus, the AgNPs suspension has been centrifuged for 30 minutes, at 4°C, 15000 rpm (SIGMA 12K 15 centrifuge); the resulted sediment (sample S2_1) has been separated and resuspended into distilled water (1mL). Later on the sample has been ultrasonicated by using a sonicator endowed with a titanium probe (Hielser, UP 100 H) for 5 minutes (sample S2_2) and for 10 minutes (sample S2_3).

Figure 5.3 presents dependence of SPR band as function of sonication time. One may observe a strong blue shift of SPR band, from 449 nm towards 441 nm after ultrasound irradiation. These results are explained by the decrease of AgNPs size, as will be later demonstrated by DLS measurements (Figure 5.4).

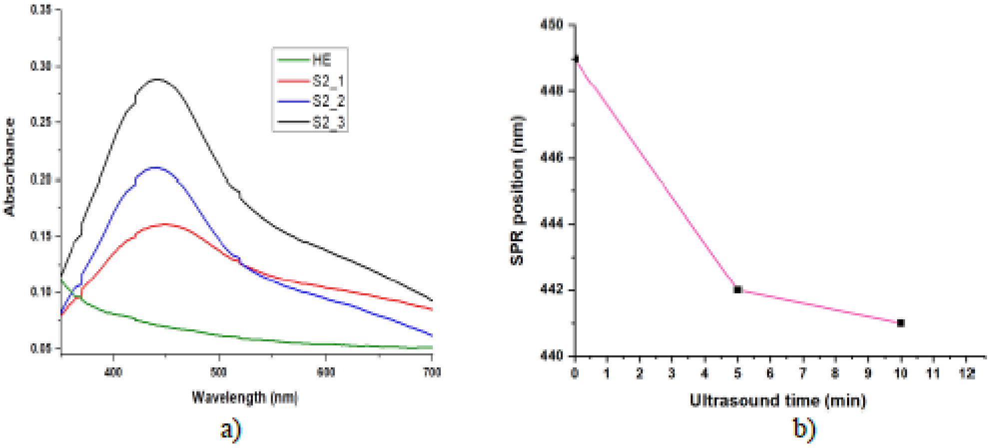

Figura 5.3. Dependence of SPR band function of sonication time: a) UV-Vis absorption spectra of AgNPs samples: S2_1, S2_2 and S2_3. b) The blue shift of SPR band function of sonication time [132]

5.1.2 Evaluation of the size of the obtained nanoparticles

The size of AgNPs is drastically decreasing after 5 minutes of irradiation from 240 nm (sample S2_1) to 79 nm (S2_2). Also, the polydispersity index, PdI, is slightely changed from 0.4 to 0.38. After other 5 minutes of sonication, PdI rises to 0.47, indicating the presence of many nanoparticle populations, while the average size of these NPs increases to 82 nm. One may conclude that the optimal sonication time is of 5 min, a longer time producing insignificant effects.

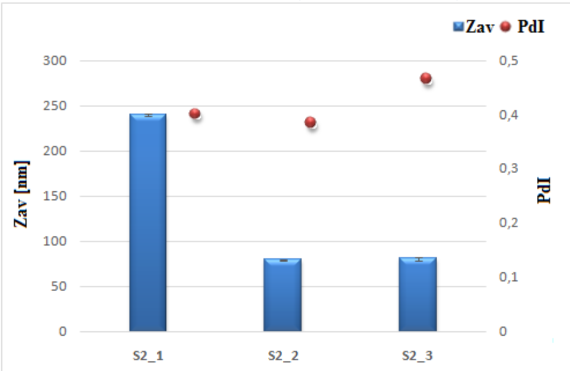

Figura 5.4. Nanoparticles size of samples S2 by DLS measurements [132].

5.2 Estimation of the antioxidant activity of the nanoparticles

The influence of silver nanoparticles size on the antioxidant activity has been comparatively studied by two methods: chemiluminescence and ABTS techniques. While by chemiluminescence the quenching capacity of short-lived free radicals is evaluated (example reactive oxygen species – ROS), by ABTS the capture capacity of long-lived free radical, $ABTS^{\bullet +}$, is estimated.

Samples of AgNPS having particle size between 79 – 220 nm showed a good antioxidant activity of ROS radicals ranging between 68.5 – 75.5 % as compared with *Harpagophytum procumbens* (60%). This amplified antioxidant activity of sample S2_2 manifested against ROS from chemiluminescent system (Figure 5.5) can be associated with the synergistic effect produced by the complex structures of the main bioactive compounds from *Harpagophytum procumbens* extract and the nanosize effect, as these nanoparticles can generate much more reaction centers able to scavenge free radicals [164].

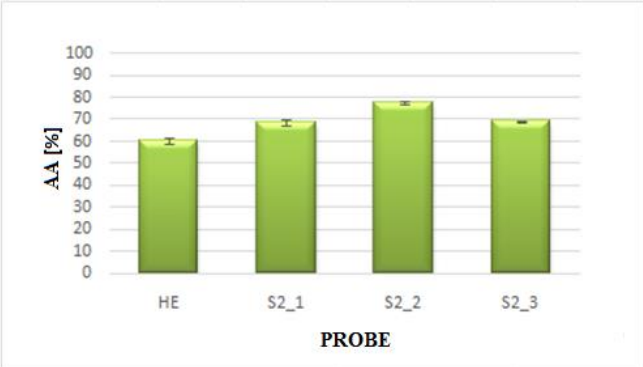

Figura 5.5. Evaluation of antioxidant activity by chemiluminescence [132]

A rather moderate antioxidant activity for $ABTS^{\bullet +}$ cation radical capture has been observed in case of silver nanoparticles synthetized with *Harpagophytum procumbens*, (Inh = 18.3%), but still higher than that of nativ extract (Inh = 12.5%) (Fig. 5.6). Also in this case the nanosize effect is obvious, the best inhibition value being obtained for the smallest nanoparticles of 79 nm (Sample S2_2)

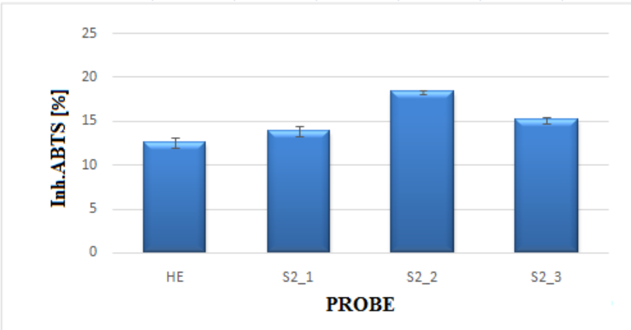

Figura 5.6. Evaluation of antioxidant activity by ABTS method [132]

5.3. Partial conclusions

This study reports on optimization of protocol for obtaining of silver nanoparticles phytosynthetized by means of aqueous extract of *Harpagophytum procumbens* root, for biomedical applications. Among the samples prepared in various proportions of plant extract and $AgNO_3$ de 1 mM, the most efficient was the ratio of 1: 100 (mL/mL).

Evolution in time of AgNPs formation has been monitored by SPR band in visible domain, whose blue shift has been correlated with decreasing of nanoparticle size, as confirmed by DLS measurements.

Silver nanoparticles prepared by this biosynthesis exhibit a good scavenge capacity for short-lived free radicals (73%) and moderate capacity to capture long-lived free radicals (18.3%), thus highlighting that bringing active compounds at nanometric scale results in enhancing their antioxidant activity.

Chapter 6

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES OBTAINED BY "GREEN" METHODS

This chapter presents the original contribution to the development of new antioxidant & antibacterial nanosystems based on AgNPs phyto-generated from aqueous extracts of aerial parts of Burdock (*Arctium lappa)* (Figure 6.1A), Clary sage (*Salvia sclarea*) (Figure 6.1B), Asparagus (*Asparagus officinalis*) (Figure 6.1C) and Southernwood (*Artemisia abrotanum*) (Figure 6.1D) for biomedical purposes.

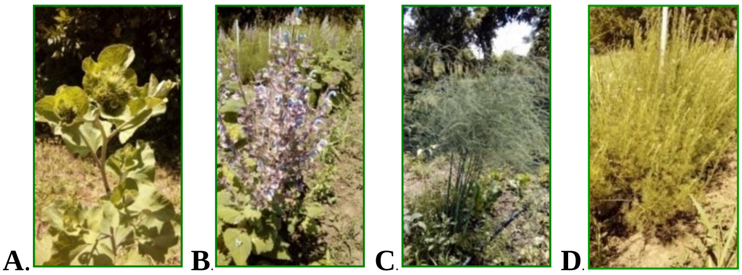

Figure 6.1. Photos of the plants used in this study:
A. Brusture (*Arctium lappa*); B. St. John's Wort (*Salvia sclarea*); C. Asparagus (*Asparagus officinalis*);
D. Lord's Wood (*Artemisia abrotanum*).

The obtained AgNPs were spectrally characterized by UV-Vis absorption spectroscopy, DLS measurements, and their physical stability was estimated by zeta potential determinations. The antioxidant properties were evaluated by two methods: chemiluminescence technique and ABTS$\bullet^{+}$ assay. The antibacterial effectiveness of the developed biogenic AgNPs was tested against the human pathogen *Escherichia coli* ATCC 8738.

6.1. Obtaining plant extracts from Burdock (*Arctium lappa*), St. John's wort (*Salvia sclarea*), Asparagus (*Asparagus officinalis*) and Lord's Wood (*Artemisia abrotanum*)

The "green" biosynthesis of silver nanoparticles (AgNPs) was performed through the aqueous extract obtained from the plants studied: *Arctium lappa, Salvia sclarea, Asparagus officinalis, Artemisia abrotanum.*

The abbreviations of the prepared samples are presented in Table 6.1

Table 6.1. Abbreviations of the analyzed samples [131]

Sample description	Sample abbreviation
Clary sage (*Salvia sclarea*)	ISI
AgNPs "green" synthesized from clary sage	AgNP-ISI
Burdock (*Arctium lappa*)	Br
AgNPs "green" synthesized from burdock	AgNP-Br
Southernwood (*Artemisia abrotanum*)	LD
AgNPs "green" synthesized from southernwood	AgNP-LD
Asparagus (*Asparagus officinalis*)	Asp
AgNPs "green" synthesized from asparagus	AgNP-Asp

6.1.1. Obtaining silver nanoparticles and spectral monitoring by UV-Vis absorption spectroscopy

Through UV-Vis absorption spectra correlated with DLS measurements, the ability of aqueous extracts of *Asparagus officinalis, Artemisia abrotanum, Arctium lappa, Salvia sclarea* to synthesize silver nanoparticles was highlighted.

All four types of plants studied, although of low intensity, showed bands specific to the resonance of surface plasmons (SPR) of silver nanoparticles in the area 450 - 480 nm. Figure 6.3 shows the details of highlighting the synthesis of silver nanoparticles prepared through natural plant extracts: *Salvia sclarea, Arctium lappa, Artemisia abrotanum, respectively Asparagus officinalis*, (Figures 6.3. A, B, C, D). They confirm the formation of silver nanoparticles [174, 175]. The strong SPR band at 452, 438, 467 and 454 nm for AgNPs-ISI, AgNPs-Br, AgNPs-LD and AgNPs-Asp is highlighted.

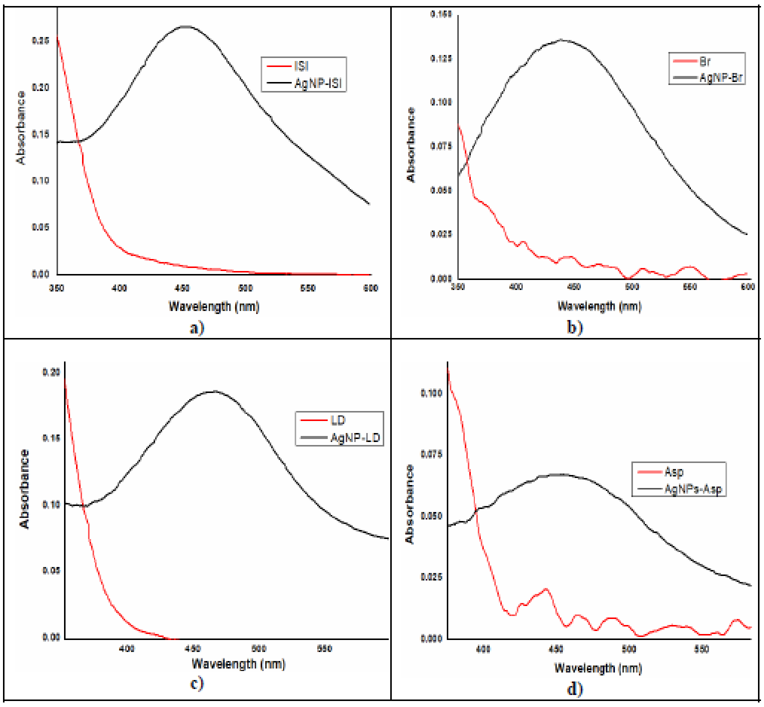

Figura 6.3. SPR band of "green" synthesized AgNPs using the extract from: A. *Salvia sclarea* (AgNPs-ISI); B. *Arctium lappa* (AgNPs-Br); C. *Artemisia abrotanum* (AgNPs-LD); E. *Asparagus officinalis* (AgNPs-Asp) [131].

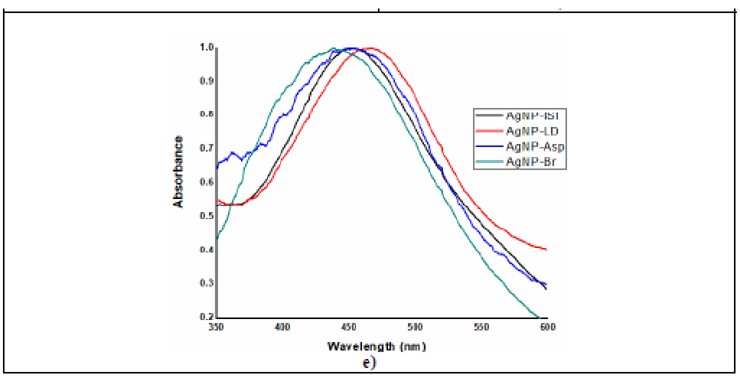

Figura 6.4. Comparison of the SPR bands of the nominated spectra for all samples: (AgNPs-ISI = black band; AgNPs-LD = red band; AgNPs-Asp = blue band; AgNPs-Br = green-turquoise band) [131].

Figure 6.4 displays a comparison of the normalized spectra at SPR peak for all the samples, revealing the band widths which are closely related to particle dimension and to the size distribution.

As observed, AgNP-LD and AgNP-ISI present the narrowest bands, followed by AgNP-Asp and AgNP-Br. It is expected that mean size increases as follows: size(AgNP-LD) ~ size(AgNP-ISI) < size(AgNP-Asp) < size(AgNP-Br).

These assumptions were further confirmed by DLS measurements (Figure. 6.6) showing the nano-scaled dimension of the prepared AgNPs those average diameters presented the following values: 51,05 nm (AgNP-LD), 74,52 nm (AgNP-ISI), 122,3 nm (AgNP-Asp) and 123,6 nm (AgNP-Br), which is in perfect agreement with the order of dimensions provided by the electronic spectra of the SPR band.

6.1.2. Determining the size of AgNPs by DLS technique

Figure 6.6 shows that the size of AgNPs obtained by "green" synthesis varies depending on the plant extract used in biosynthesis. Thus, the silver nanoparticles synthesized in the presence of *Salvia sclarea* extract (AgNPs-ISI) have an average diameter of Z_{av} = 75 nm and a polydispersion coefficient (PdI) of 0.23; for *Arctium lappa* extract (AgNPs-Br), an average diameter of Z_{av} = 125 nm and a polydispersity coefficient of 0.37 are obtained; in the case of *Artemisia abrotanum* extract (AgNPs-LD), the silver nanoparticles have an average diameter of Z_{av} = 51 nm and a polydispersion coefficient of 0.132, and in the presence of *Asparagus officinalis* extract (AgNPs-Asp), nanoparticles are obtained with a diameter average Z_{av} = 121 nm, PdI = 0.37.

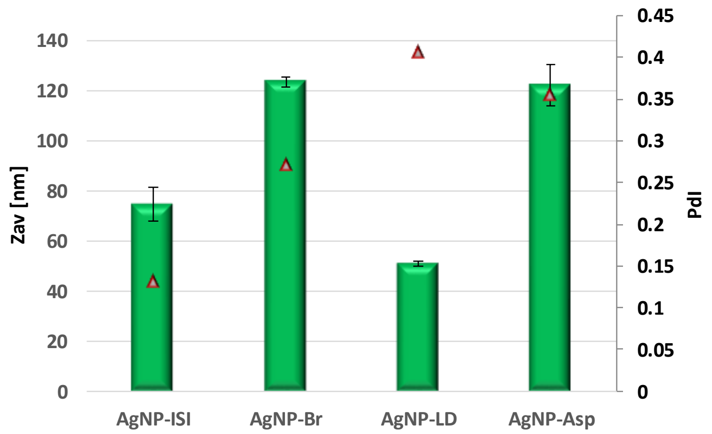

Figura 6.6. Determination of the size of silver nanoparticles obtained based on *Salvia sclarea* (AgNPs-ISI), *Arctium lappa* (AgNPs-Br), *Artemisia abrotanum* (AgNPs-LD), *Asparagus officinalis* (AgNPs-Asp), analyzed with DLS technique [131].

AgNP-ISI showed the smallest polydispersity index (PdI = 0.132), therefore the most uniform population of particles.

6.1.3. Assessment of the physical stability of AgNPs

The physical stability of the phytogenerated silver nanoparticles was evaluated in terms of Zeta Potential (ξ) which is a parameter close by related to the surface charge of particles.

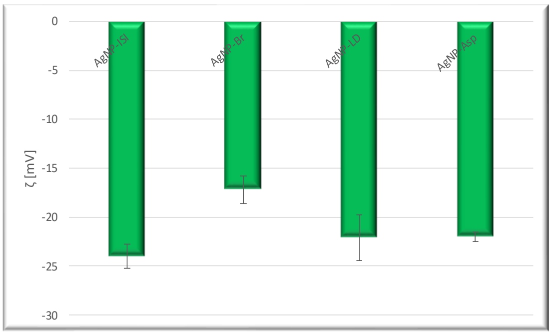

Figura 6.7. Variation in physical stability of biosynthesized AgNPs based on *S. sclarea* (AgNPs-ISI), *A. lappa* (AgNPs-Br), *A. abrotanum* (AgNPs-LD), *A. officinalis* (AgNPs-Asp), evaluated in terms of Zeta potential [mV], [131].

As observed in Figure 6.7, AgNP-Br presented moderate stability (ξ = –17.20 ± 1.42 mV), as compared to the other samples that showed good stability assured by repulsive inter-particle forces: ξ(AgNP-ISI) = –24.00 ± 1.20 mV; ξ(AgNP-LD) = –22.10 ± 2.33 mV; ξ(AgNP-Asp) = –22.00 ± 0.50 mV

6.2. Evaluation of the biological activities of developed phytogenic nanoparticles

The antioxidant potential of the obtained samples was determined *in vitro* by two bio-assays: chemiluminescence technique and ABTS method (as described in the chapter 4).

6.2.1. Antioxidant activity evaluated by the chemiluminescence method

All the prepared specimens presented antioxidant properties. Moreover, the bio-prepared silver nanoparticles exhibited higher capacity of free radicals scavenging, as compared to natural extracts; our previous studies highlighted similar behavior of biogenic AgNPs as compared to their precursors vegetal extracts [174, 176, 177].

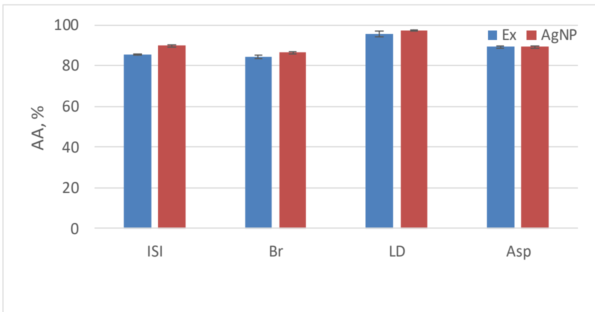

Figura 6.8. The antioxidant activity (evaluated by the chemiluminescence technique) of silver nanoparticles and precursor plant extracts: *Salvia sclarea, Arctium lappa, Artemisia abrotanum, Asparagus officinalis* (Data are presented as mean ± SD), [131].

Chemiluminescence results (Figure 6.8) revealed high capacity for scavenging short-life free radicals, in the following sequence: AgNPs-LD>AgNPs-ISI>AgNPs-Asp>AgNPs-Br.

6.2.2. Antioxidant activity evaluated by cation-radicals $ABTS^{\bullet +}$

$ABTS\bullet^{+}$ method (Figure. 6.9) highlighted that ABTS radical cation inhibition ranged between 3,74 and 11,84% for natural extracts. Phyto-generated AgNPs showed better ability to inhibit $ABTS\bullet^{+}$, with values of $ABTS\bullet^{+}$ capturing ranging between 32,44 and 61,26%.

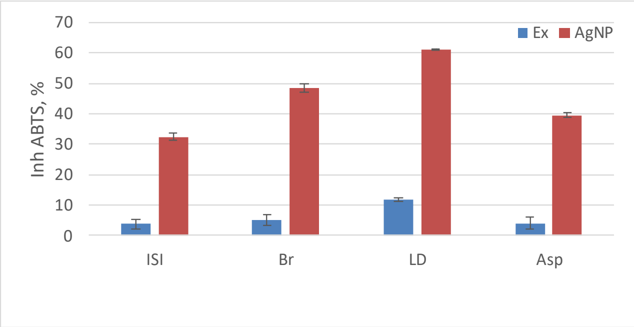

Figura 6.9. Antioxidant activity (evaluated by ABTS method) of phytosynthesized AgNPs from aqueous extracts: *Salvia sclarea* (ISI), *Arctium lappa* (Br), *Artemisia abrotanum* (LD), *Asparagus officinalius* (Asp) (Data are presented as mean ± SD), [131].

Southernwood extract (LD) and AgNPs phyto-synthesized from this extract (AgNP-LD) proved to be the most efficient antioxidant systems, showing remarkable ability to scavenge both short-life (AA = 95,53% for LD, and 97,39% for AgNP-LD) and long-life (11,84% for LD, and 61,26% for AgNP-LD) free radicals. These bio-properties can be attributed to the presence of cocktails of antioxidant phytochemicals in the composition of the vegetal materials, which are rich in phenolic acids, flavonoids, terpenoids, or lignans.

6.3. Determination of the antibacterial activity of ecologically synthesized AgNP

The antibacterial activity of phytosynthesized AgNPs was evaluated against the Gram-negative bacterium *Escherichia coli* (*E. coli*), a common pathogen in the normal microflora of the intestinal tract of humans, often used as an indicator of faecal contamination [28]. Anti-bacterial potency of the obtained silver nanoparticles was estimated in terms of inhibition zone (ZOI). The pictures of Petri dishes showing the halos of these zones are illustrated in Figure 6.10.

Figure 6.10 displays the ZOI diameters (in mm) exhibited by the obtained biogenic silver nanoparticles against *Escherichia coli* ATCC 8738, with ZOI value of 20.67 ± 3.05 mm for AgNP-ISI, 21.67 ± 2.08 mm for AgNP-Br, 23.33 ± 2.09 mm for AgNP-LD, and 25.33 ± 1.52 mm for AgNP-Asp. These results indicated that all the prepared AgNPs have a marked effect on the inhibition of *E. coli*, the most potent being AgNP-Asp.

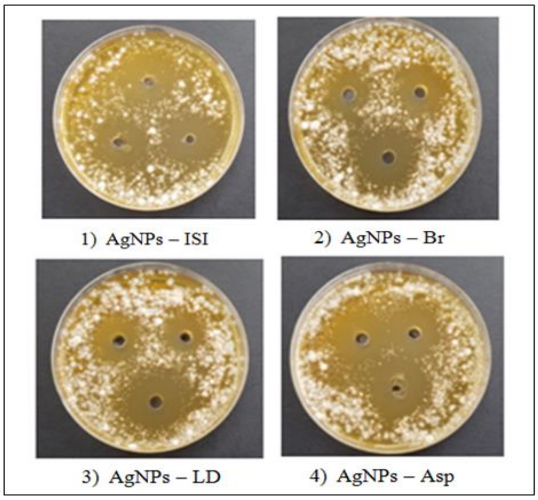

Figura 6.10. Photographs of Petri dishes highlighting the antibacterial activity of AgNPs: *Salvia sclarea* (AgNPs-ISI), *Arctium lappa* (AgNPs-Br), *Artemisia abrotanum* (AgNPS-LD), *Asparagus officinalius* (AgNPs-Asp), [131]

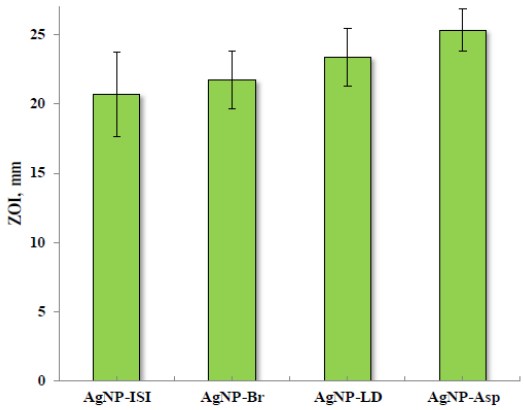

Figure 6.11. Comparison of the antibacterial activity of the studied AgNPs: *Salvia sclarea* (AgNPs-ISI), *Arctium lappa* (AgNPs-Br), *Artemisia abrotanum* (AgNPS-LD), *Asparagus officinalius* (AgNPs-Asp), [131]

The antibacterial action of the obtained biogenic silver nanoparticles could be attributable to a series of events such as:

i) direct physical contact of AgNPs with the bacterial cell surface, leading to the deterioration of cell wall, and then to the cell membrane damaging, resulting in the release of the cell content;

ii) penetration of AgNPs inside the bacterial cell causing the membrane dysfunction and finally the cell death [179].

6.4. Partial conclusions

This study described the phytosynthesis and biophysical characterization of silver nanoparticles generated from natural extracts of Clary sage (*Salvia sclarea*), Burdock (*Arctium lappa*), Southernwood (*Artemisia abrotanum*) and Asparagus (*Asparagus officinalis*). The ability of these plants to bio-reduce the silver ions was monitored by the UV-Vis absorption spectra which highlighted the spectral signatures of all developed AgNPs; strong SPR bands appeared between 438 and 467 nm, confirming the phytosynthesis of silver nanoparticles. The SPR band widths are in close connection with the particle size, fact demonstrated by DLS measurements which proved also the formation of nano-scaled particles with mean diameters ranging from 51 to 123,6 nm.

The biogenic silver nanoparticles developed in this study demonstrated good physical stability, and high capacity to capture short- and long-term life free radicals. The smallest silver nanoparticles, AgNP-LD ("green" synthesized from southernwood), with a mean size of 51,05 nm possessed the highest capacity to scavenge short- life (AA = 97,39%) and also long-life (Inh $_{ABTS\bullet+}$ = 61,26%) free radicals.

It could be mentioned the impressive antibacterial effect of the AgNPs biogenerated from *Asparagus* (AgNPs-Asp), showing a ZOI diameter of 25,33 ± 1,52 mm against *Escherichia coli* bacterium.

Our results are encouraging, the developed phytogenic silver nanoparticles proved to be promising materials to eliminate bacteria and short-life & long-life free radicals arising from oxidative stress. Moreover, this study has a great economic, environmental and medical impact, since herbs & food wastes could be converted into "green" metallic nanoparticles with high biomedical value.

Chapter 7
BIOHYBRIDS BASED ON PHYTOSYNTHETIZED AgNPs FROM *ARTEMISIA ABROTANUM* EXTRACT and CHLOROPHYL *A*-LABELED LIPOSOMES

The original contribution of this research work is the "green" design of antioxidant composites based on biomimetic membranes and phyto-generated silver nanoparticles (AgNPs) [135]. The artificial lipid bilayers were labeled with a natural porphyrin – chlorophyll *a*, which was used as a spectral marker to monitor the formation of biocomposites. This bioporphyrin was successfully used in previous studies [184, 185] as a spectral sensor for monitoring the action of different agents on biomimetic membranes, at molecular level.

In this study, three types of composites have been used containing bio-inspired lipid membranes and different Southernwood-generated AgNPs content. Upon our knowledge, this is the first report on "green" synthesis of AgNPs and of composites based on biomimetic liquid crystals and biogenic silver nanoparticles generated from A. abrotanum L. aqueous extract. The obtained materials were characterized by spectral techniques: UV–Vis absorption and emission spectroscopy, and dynamic light scattering (DLS). The physical stability was estimated by zeta potential measurements. The chemiluminescence method was used to assess the antioxidant properties of the samples

7.1. Obtaining the plant extract of *Artemisia abrotanum*, liposomes and bio-nanohybrids based on AgNPs

AgNP-based bio-nanohybrid hybrids were obtained using *Artemisia abrotanum* plant extract, biomimetic lipid membranes (MLV) and Chlorophyll a (Chl a) according to procedures presented in Chapter 4.

Table 7.1 Shows the abbreviations of the samples obtained.

Table 7.1. Abbreviations of the samples obtained [135]

SAMPLE	CODE
Artemisia abrotanum L. aqueous extract	Ex
AgNPs phytogenerated from Artemisia abrotanum L	AgNPs
Chla-lecithin MLVs (Liposomes)	MLV
Phosphate saline buffer pH 7,4	PBS
MLV: PBS (29: 1, V / V)	MLV1
MLV: PBS (5: 1, V / V)	MLV2
MLV: PBS (1: 1, V / V)	MLV3
PBS: AgNPs (29: 1, V / V)	MG1
PBS: AgNPs (5: 1, V / V)	MG2
PBS: AgNPs (1: 1, V / V)	MG3
MLV: AgNPs (29: 1, V / V) biohybrids	BH1
MLV: AgNPs (5: 1, V / V) biohybrids	BH2
MLV: AgNPs (1: 1, V / V) biohybrids	BH3

7.2. Spectral monitoring of the formation of silver nanoparticles and biohybrids

a. Spectral characterization of the obtained samples

A deep insight into the synthesis of lipo-nanosilver composites biogenerated using *A. abrotanum L.* water extract, was provided by spectral analysis in UV–Vis absorption (figure 7.1), emission spectroscopy (Figure 7.2) and by fluorescence emission spectra (Figure 7.2).

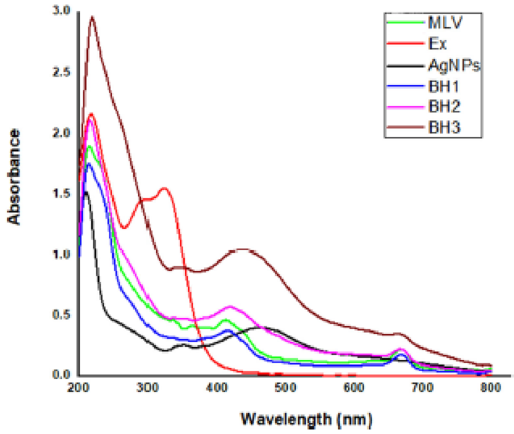

Figura 7.1. Spectral analysis in UV-Vis of the samples [135].

Figure 7.1 displays the UV–Vis absorption spectra of the samples. The UV–Vis absorption spectrum of AgNPs exhibits an intense sharp band at 464 nm, due to surface plasmon resonance of AgNPs, while the spectrum of *Southernwood* extract has no peaks in this region, thus demonstrating the biosynthesis of AgNPs.

UV–Vis absorption spectra of chlorophyll-based samples revealed the spectral signature of the *Chla* fluorophore: an absorption band located at 668 nm in MLV and in BH1, which blue shifted to 667 nm in BH2 and to 663 nm in BH3. The blue shift is more pronounced when the AgNP content is greater, as observed in the case of BH3 biohybrids that have the highest content of AgNPs (53,5 µg/mL). These findings suggest a strong interaction between biomimetic lipid layers and phytogenerated silver nanoparticles in the case of BH3 hybrid systems. The absorption spectrum of BH3 shows the spectral fingerprints of its building blocks: *Chla* and of AgNPs.

7.3. Evaluation of the size and physical stability of biohybrid

7.3.1 Evaluation of the size of the obtained biohybrids

The particle size of the samples was estimated by DLS measurements (Figure 7.3). AgNPs present a mean diameter of 51 nm. The multilamellar vesicles MLVs have a mean diameter of 370 nm.

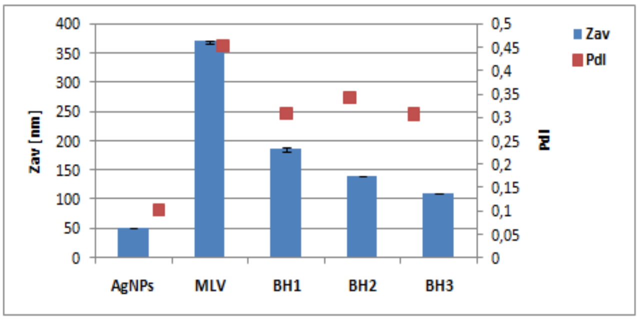

Figura 7.3. Dimensional DLS analysis of AgNPs nanoparticles and biohybrids [135].

The ultrasound-assisted incorporation of AgNPs in MLVs resulted in a reorganization of biomimetic membranes, resulting three nano-scale lipo-nanosilver composites:

- BH1: Z_{av} = 185 nm pentru C_{AgNPs} = 3,57 µg / mL,
- BH2: Z_{av} = 139 nm pentru C_{AgNPs} = 17,83 µg / mL,
- BH3: Z_{av} = 109 nm pentru C_{AgNPs}= 53,5 µg / mL.

This decreasing sequence of nanoparticle size with the increase in the proportion of AgNPs confirms their incorporation into liposomal structures.

7.3.2 Assessment of the physical stability of biohybrid

The physical stability of the composites and their building blocks was evaluated by measuring zeta potential values. ZP is an indicator of the magnitude of the interparticle interactions and its value is directly related to the particle charge. All the samples are negatively charged (Figure 7.5), so the particles tend to repel each other to assure the stability of the colloidal system.

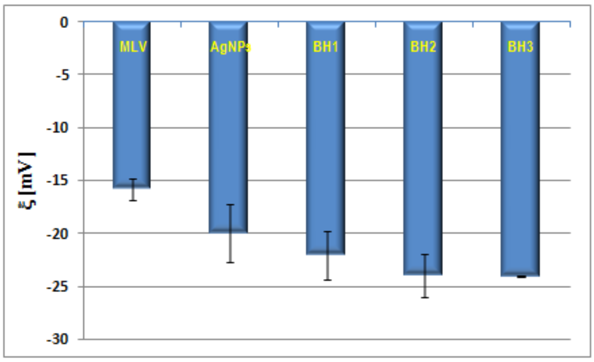

Figura 7.5. Comparative evaluation of the physical stability (potential Zeta, mV) of the obtained biohybrid (BH1, BH2, BH3) *versus* liposomes (MLV) and AgNPs [135]

The physical stability of phytogenerated silver nanoparticles enhanced after incorporation in biomimetic systems, so the ZP value of AgNPs increased from - 20 mV to -22.1 mV in BH1, to -24 mV in BH2, and to -24.1 mV in BH3.

7.4. Evaluation of antioxidant properties

The antioxidant activity of the samples was assessed through chemiluminescence technique (Figure 7.6).

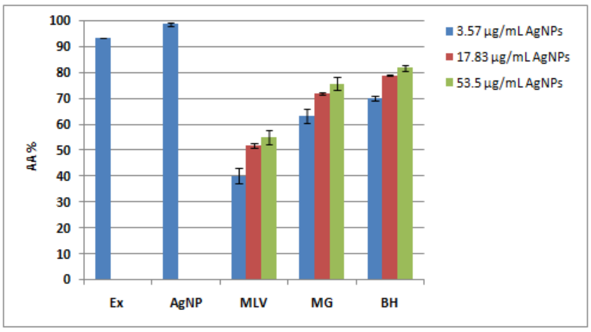

Figura 7.6. The antioxidant activity of the obtained biocomposites and their components [135]

The Southernwood aqueous extract, Ex, proved to be strong antioxidant (AA = 93,24%) due to the high content of polyphenols, flavonols, flavonoids (aglycones and glycosylates), artemisin and hydroxycinnamic derivatives of *A. abrotanum* L. plant [171]. AgNPs phytogenerated from this herb showed also strong antioxidant activity (AA = 98,63%) due to the presence of capping antioxidant phyto-molecules arising from the *A. abrotanum* extract. Liposomes alone, MLV, exhibited low level of antioxidant activity (between 40 and 50%), the diluted suspensions of AgNPs, MG, showed medium to good level of antioxidant activity (between 63.24 and 75.75%), while the obtained biocomposites presented high antioxidant activities (70–82%). It was observed that the antioxidant activity of biohybrids increased with AgNPs content.

7.5. Partial conclusions

1. Three types of lipo-nanosilver composites based on artificial lipid membranes and "green" silver nanoparticles were prepared.

2. The size of the AgNPs obtained was on average 51 nm, the obtained bio-based materials showed good physical stability (ZP_{AgNPs} = -20 mV, ZP_{BH1} = -2,1 mV, ZP_{BH2} = -24 mV și ZP_{BH3} = -24,1 mV), and also exhibited good antioxidant properties, depend- ing on the AgNP content. The biocomposites with the highest AgNPs concentration (53,5 μg/mL) presented high antioxidant activity (AA = 82%) and the smallest size (Z_{av} =109 nm).

3. The biocomposites generated by *A. abrotanum* water extract presented high antioxidant activity (AA = 70 – 82%).

Chapter 8

BIOHYBRIDS WITH ANTIOXIDANT PROPERTIES GENERATED FROM BURDOCK (*Arctium lappa*) AQUEOUS EXTRACT

This chapter of the doctoral dissertation presents a "green" *bottom-up* strategy for obtaining antioxidant release systems based on vitamin C, biomimetic membranes (liposomes) and phyto its generated silver nanoparticles by mens of burdock extract (*Arctium lappa*) (Figure 5.A), is a popular plant widely used in treatment of hepatitis, atherosclerosis, hypertension, geriatric diseases due to their polyphenolic constituents with antioxidant properties [187]. This wild plant was chosen due to its valuable bio-activities and therapeutic effectiveness, and also due to economic reasons, taking into account that burdock is one of the most widespread plant in our country, Romania.

The antioxidant activity of the composites bio-developed in the present study was estimated by chemiluminescence assay - which is a rapid method of testing the ability to capture the short-lived free radicals [195-196]. The novelty of our study is the incorporation of AgNPs into liposomes with vitamin C that increases their bioactivity and stability; the developed biocomposites can serve as „building blocks" for the development of new bio-active materials that have a high potential for applicability in the biomedical field. Vitamin C or ascorbic acid is an antioxidant molecule which prevents and scavenges the formation of reactive oxygen species [179], while its incorporation in liposomes significantly improves their properties and applications [198]. The developed silver-based biocomposites were characterized by UV-Vis absorption spectroscopy and Dynamic Light Scattering measurements; their physical stability was estimated through zeta potential measurements, and the antioxidant properties by chemiluminescence technique. The presence of silver nanoparticles and of vitamin C in biocomposites was demonstrated by UV-Vis absorption spectra.

8.1. Obtaining biohybrids based on AgNPs, liposomes and vitamin C

In this paper we designed for the first time silver-based composites based on liposomes, vitamin C, and AgNPs synthesized from burdock, with two silver:liposomes ratios. The biocomposites were prepared by ultrasonic irradiation (15 minutes with breaks, on ultrasound bath Elmasonic S60H) of a mixture containing burdock-derived AgNPs (nS) and liposomes (L or LC) in a volumetric ratio of 1:1 and 2:1, resulting in 4 types of composites: nSL1, nSL2, nSLC1 și nSLC2 (Table 8.1).

In addition, liposomes confer stability and protection to the components of biocomposites: AgNPs and vitamin C. Marsanasco et al highlighted the protective role of liposomes as vehicles for vitamins E and C [199].

Table 8.1 Abbreviations of the samples obtained [138]

SAMPLE	CODE
Burdock extract	BE
Nano-silver (AgNPs) phytogenerated from burdock extract	nS
Soybean lecithin MLVs	L
Soybean lecithin MLVs – vitamin C	LC
Liposomes/AgNPs biohybrids (1:1, v/v)	nSL1
Liposomes/AgNPs biohybrids (1:2, v/v)	nSL2
Liposomes–vitamin C /AgNPs biohybrids (1:1, v/v)	nSLC1
Liposomes–vitamin C /AgNPs biohybrids (1:2, v/v)	nSLC2

In previous studies reprorted by Bărbînța-Pătrașcu et al., are presented TEM images showing that the silver nanoparticles are coated with liposomes [178], and could be located both in the intervesicular medium and in the vesicular multilamellar structures [200].

8.2. Spectral monitoring of silver-based composites

UV-Vis absorption spectroscopy and Dynamic Light Scattering measurements were used to demonstrate the formation of silver nanoparticles and silver-based composite systems.

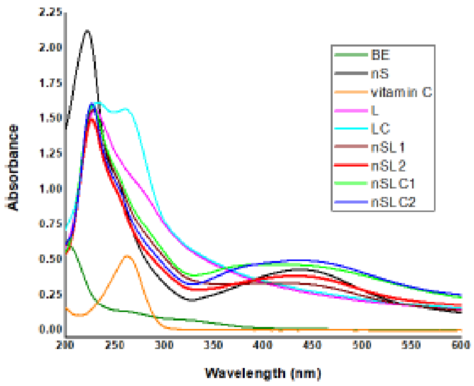

Figura 8.1. Comparison of UV-Vis absorption spectra of the obtained samples [138]

The UV-Vis absorption spectrum of AgNPs "green" synthesized from *Arctium lappa* leaves (sample nS in Figure 8.1) showed a strong SPR peak located at 437 nm wavelength (peak not present in the BE spectrum), occurring after addition of burdock extract (BE) in 1 mM $AgNO_3$ solution, thus demonstrating the biosynthesis of silver nanoparticles.

The characteristic peak of vitamin C located at 263 nm was shifted to 259 nm in absorption spectrum of liposomes LC.

The presence of vitamin C and AgNPs in liposome-based biocomposites was demonstrated by the presence of spectral signatures of AgNPs and vitamin C in the UV-Vis absorption spectra of liposome-based biocomposites, as well as through the "shifts" of these "spectral fingerprints".

The spectral fingerprint of AgNPs in silver-based biocomposites was blue-shifted from 437 nm (for nS) to 409 nm for nSL1, 431 nm for nSL2, 417,5 nm for nSLC1, and 435 nm for nSLC2, confirming the synthesis of biocomposites. These shifts are more pronounced for samples nSL1 and nSLC1, with liposomes:AgNPs ratio of 1:1 (v/v) as compared to the samples with liposomes:AgNPs ratio of 1:2 (v/v). On the other hand, it could be observed that absorption spectra of composites containing ascorbic acid display a slight blue shift. This different behaviour could be explained by a different structural organization of biocomposites loaded with vitamin C.

Mean diameter of nanoparticles estimated by DLS measurements (Figure. 8.2) ranged from 103 to 194,5 nm. Among all developed biocomposites, those containing burdock-nanosilver and bio-inspired membranes loaded with vitamin C (1:1, v/v) presented the smallest size, with a mean diameter of 103 nm ± 4,25 (Figure 8.2).

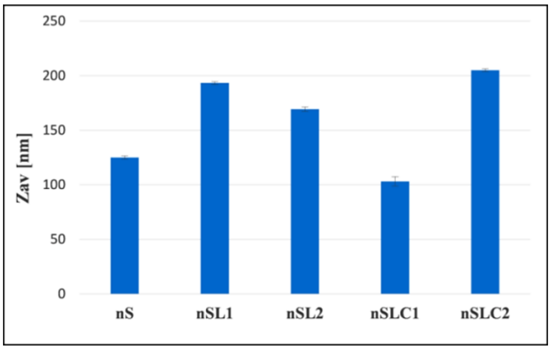

Figura 8. 2. Mean size values of silver-based nano-biocomposites [138]

8.3. Estimation of the physical stability of biohybrid

The physical stability of the bio-based composites was estimated in terms of zeta potential, ZP (Figure 8.3), based on electrophoretic mobility; ZP value is related to the surface particle charge.

All the samples presented a negative surface charge that induces repulsive forces among particles (Figure 8.3), assuring short-term stability for nanosilver nS (ZP (nS) = -17 mV) and liposomes L (ZP (L) = -20.45 mV), and good stability for the other samples as well. Samples loaded with ascorbic acid have more negative ZP values, therefore they are more stable (Figure 8.3).

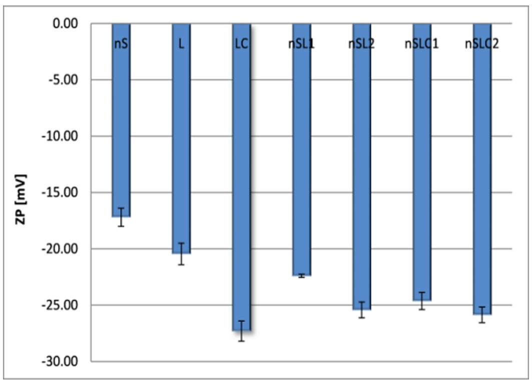

Figura 8.3. Zeta potential values of prepared biocomposites and their components [138]

Liposomal formulation of vitamin C showed good stability as compared to liposomes alone (ZP_{LC}=-27.3 mV; ZP_{L} = -20.45 mV). Thus, addition of vitamin C resulted in enhancing the ZP magnitude, from |-22.4| mV to |-24.63| mV for systems containing AgNPs and liposomes in a ratio of 1:1, v/v, and from |-25.43| mV to |-25.85| mV for systems containing AgNPs and liposomes in a ratio of 2:1, v/v. Combination of biomimetic membranes with burdock-derived silver nanoparticles (nS) assured an enhancement of physical stability of nS. These results are in agreement with our previous works [173, 178].

8.4. Determination of biological properties

8.4.1. Evaluation of antioxidant activity by the chemiluminescence method

The antioxidant properties of the samples were tested through chemiluminescence technique, and the values of AA% (calculated using equation 1) are comparatively displayed in Figure 8.4.

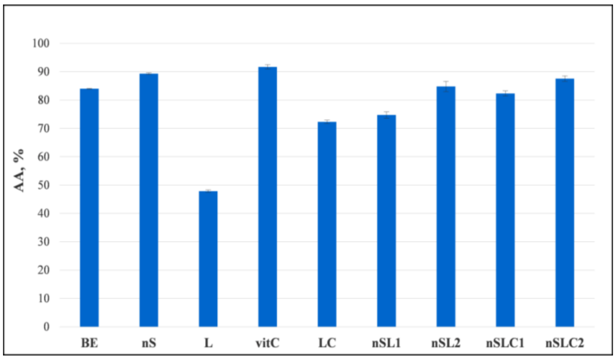

Figure 8.4. Antioxidant activity of silver-based bio-nanosystems obtained from *A. lappa* leaf extract [138]

The "green" synthesized silver nanoparticles, nS, presented high value of antioxidant activity: 89.3%, and the liposomal formulations of vitamin C showed an AA% value of 72.3%, greater than liposomes alone (AA% = 47.8%). The biogenic silver nanoparticles, nS, exhibited an amplified value of antioxidant activity (89.3%) as compared to vegetal extract alone (83%), this amplification being assessed to the nano-scaled size of metallic nanoparticles which offers an increase in total surface area providing many reaction centers for free radical scavenging [173]. This enhancement of AA% value was highlighted also in previous studies [173, 178].

In contrast, liposomal formulations with vitamin C showed a slightly lower AA% value of 72.3%, but higher than that of liposomes alone (AA% = 47.8%).

Moreover, the presence of ascorbic acid gave rise to biosystems with enhanced antioxidant activities (83% and 88% for nSLC1 and nSLC2, respectively) as compared to liposomes free of vitamin C (75% and 85% for nSL1 and nSL2, respectively).

Even in dilution of 1-5 or 2 fold, in lipid vesicles, the silver nanoparticles bio-generated from burdock exhibited high antioxidant activity. The AA% values of silver - based composites containing vitamin C, developed in this study, are higher than for nanosilver/lecithin - biocomposites derived from *Geranium* (72% [194]) and sage (86.5% [175]).

The antioxidative potential of burdock-derived nanoparticles is conferred by the presence of antioxidant biocomponents in the burdock leaf composition: flavone derivatives (such as rutin, quercetin, luteolin, crocin, and cyanine) and polyphenol carboxylic acids (such as chlorogenic and caffeic acids) [187, 201].

8.5. Partial conclusions

Artificial cell membranes and phytogenic silver nanoparticles "green" generated from *Arctium lappa* extract were used to bottom-up design of antioxidant biogenic systems with and without vitamin C. UV-Vis absorption spectra demonstrated the formation of burdock-generated silver nanoparticles and their composites with bio-inspired lipid membranes loaded or not with ascorbic acid. DLS measurements displayed the nano-scaled size of the developed silver - based composite systems.

The shifts of spectral fingerprints of vitamin C and "green" silver nanoparticles in the UV-Vis absorption spectra of liposome-based biocomposites proved the presence of AgNPs and vitamin C in the obtained biocomposites.

The addition of vitamin C in biocomposites improved the properties of the obtained materials. Thus, silver-based systems containing vitamin C are more stable than silver-loaded systems without vitamin C, so bio-based systems containing vitamin C showed enhanced physical stability as compared to systems without vitamin C (the increase was 33.5% for liposomes alone, 10% for biocomposites with liposomes:AgNPs ratio of 1:1, v/v, and 1.7% for biocomposites with liposomes:AgNPs ratio of 1:2, v/v). In addition, the insertion of vitamin C in the burdock-derived silver-based composites significantly improved the antioxidant efficiency of such composites.

The liposomal components of biocomposites developed in our study, assured an enhancement of "green" nanosilver stability.

These developed materials could be applied in dermatologic biomedical field as free radical scavenger, and as adjuvants in treatment of oxidative stress – related diseases.

Chapter 9
PHYTO-GENERATED BIO-ACTIVE NANOMATERIALS FROM FIELD WEED, THISTLE (*CIRSIUM ARVENSE*)

The need to "green" *design* novel strategies for biomedical and (bio)photonic applications is an intensive trend in scientific research area, over last few decades [189, 192, 202, 203, 204].

This study reports an innovative design of bio-active materials containing lemon pectin and silver nanoparticles (AgNPs) phyto-synthesized from natural aqueous extracts of thistle (*Cirsium arvense*) leaves, motivation starting from their recognition in the treatment of many diseases, especially in the era of antibiotic resistance, when many commercially available synthetic antibiotics are becoming less efficient against several human diseases [205].

Cirsium arvense (Figure 9.1) is a medicinal herbaceous plant, a perennial species from the family *Asteraceae*, often found as noxious weed in grasslands and riparian [206], widespread in Europe and parts of North Africa and Asia [207].


Figure 9.1. Photographs of the plant *Cirsium arvense* [208].

In popular medicine the medicinal applications include the treatment of peptic ulcer, metrorrhagia, syphilis, leukaemia, diabetes, eye infections, skin sores, gonorrhoea, some mouth diseases and tuberculosis [206].

Various therapeutic uses attributed to *Cirsium* species stimulate researchers to screen extracts and compounds (bioactive secondary metabolites and essential oils) of these plants. Thus, the significant therapeutic potential of *Cirsium arvense*, is based on the high content of flavonoid compounds. High content of linarin (5,7-dihydroxy-4- methoxyflavone, 7-0-[0-α-L-rhamnopyranosyl-β-D-glu- copyranoside) from leaves, apigenin, luteolin, 3-O-methyl kaempferol, cosmosiin, and acacetin 7-β-D- glucopyranosid uronic acid from flowers have been detected [202].

Pectin is a heterogeneous polysaccharide with different biological activities. Utilization and valorization of *Citrus* peels has been a subject of various researches as a potential source of natural antioxidants and phenolic compounds. It has been reported that pectin decreased blood lipid level and peroxidative status, and showed antioxidant activities in kidney toxicity induced by octylphenol [213]. The special *in vitro* antibacterial properties of pectin were first reported by Russian researchers in the late 1990s nevertheless the findings remained almost ignored until the second decade of the 2000s, when a few studies started to report new pectin-based substances with high bactericidal activity [214].

9.1. Obtaining phyto-generated bioactive nanomaterials from thistle, (C*irsium arvense*)

The main steps for bio-*design* of thistle-based materials are the following:

i) Preparation of thistle extract.

ii) Phyto-mediated preparation of silver-based materials.

The detailed presentation of the two steps for obtaining silver phyto-nanoparticles based on thistle leaves is an presented in Chapter 4.

Table 9.1 contains a summary of the abbreviated codes of the samples developed in this study.

Table 9.1. Abbreviations of the samples developed in this study [133]

Samplas developed in this study	Code
Thistle leaf extract	TST
Phyto-generated silver nanoparticles from thistle extract	TST-AgNPs
Pectin isolated from lemon peel	LP
Lemon pectin and thistle AgNPs based on hybrids	TST-AgNPs-LP

9.2. Optical monitoring of bio-nanoparticles obtained based on *Cirsium arvense*

A comparative presentation of UV-Vis absorption spectra of all samples (Figure 9.2) displayed the following absorption peaks: between 206-219 nm - assigned to the carbohydrates and/or peptide bonds in proteins; between 256-282 nm - attributed to the aromatic aminoacid residues of proteins and also to the carbohydrates [191, 215]; between 315-327 nm - assigned to the flavonoids [216].

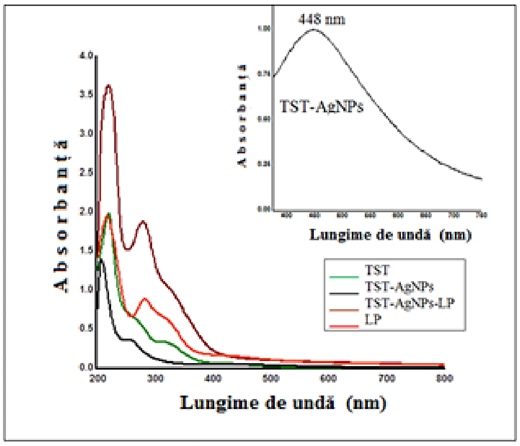

Figure 9. 2. Absorption spectra of the obtained samples; Insertion: SPR band of AgNPs obtained from *Cirsium arvense* [133**]**

Cirsium – mediated synthesis of AgNPs was firstly demonstrated by the apparition of a single SPR band located at 448 nm, as shown in Figure 9.2, Inset. This band is characteristic for spherical shaped silver nanoparticles [217, 219].

9.3. Assessment of the stability of pectin-coated materials

The silver-based particles have negative surface charge, quantified by zeta potential measurements (Table 9.2).

Table 9.2. Zeta potential values, average size and polydispersity index of materials containing AgNPs obtained from Cirsium arvense [133]

Probe	ZP (mV)	Z_{av} (nm)	PdI
TST-AgNPs	-23.15±0.65	151.7±7.10	0.298±0.07
TST-AgNPs-LP	-11.03±0.33	276.7±11.42	0.401±0.08

The ZP values of uncoated and pectin-coated *Cirsium*- derived AgNPs were -23.15 and -11.03 mV, respectively. Thus, TST-AgNPs showed a moderate physical stability assured by repulsive forces between silver particles carrying negatively-charged functional groups (like carboxylate arising from proteins, aminoacids) belonging to capping agents on the TST-AgNPs' surface, as proved by UV-Vis spectra (Figure 9.2).

After pectin addition to TST-AgNPs, ZP values shifted to smaller ones since ($ZP_{TST-AgNPs-LP}$ = -11,03 mV), large molecules of pectin polysaccharide tend to adsorb at TST-AgNPs' surface through the –COO^- and –OH groups, decreasing the ZP magnitude, without affecting the stabilization properties [163,218].

9.4. Morphological aspects of materials obtained from Cirsium arvense

Size and morphological aspects of thistle - derived materials were monitored by DLS and SEM analyses.

DLS results (Table 9.2) revealed mean diameter of 151.7 nm for TST-AgNPs, and of 276 for TST-AgNPs- LP. After addition of pectin to thistle-derived AgNPs, the dimensions increased, and also the polydispersity index increased from 0.298 to 0.401.

SEM images of "green" developed nanoparticles are displayed in Figure 9.3. The images correspond to AgNPs obtained from thistles which are uncoated (Figure 9.3a) and covered with pectin (Figure 9.3b).

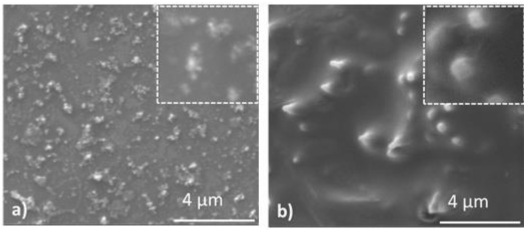

Figure 9.3. SEM images of AgNPs: a) obtained from thistle (TST-AgNPs) and b) obtained from thistle and AgNPs, coated with pectin (TST-AgNPs-LP). It is inserted on SEM images of 2x2 µm the image of the enlarged sample [133]

One can notice that TST-AgNPs showed small particles with diameters ~60 nm, which are organized in irregular shape clusters with sizes between 200 and 400 nm.

However, when pectin was added, the particles become larger, with diameters between 200 to 400 nm, and a more round in shape.

SEM images are in agreement with DLS results, and UV-Vis absorption spectra. The difference between particle dimensions obtained from DLS and SEM could be explained by the fact that DLS provides a mean hydrodynamic diameter that is slightly bigger than physical diameter [219].

9.5. Evaluation of the biological action of materials obtained from Cirsium arvense

Antioxidant profile of the developed *Cirsium* - based materials is displayed in Figura 9.4.

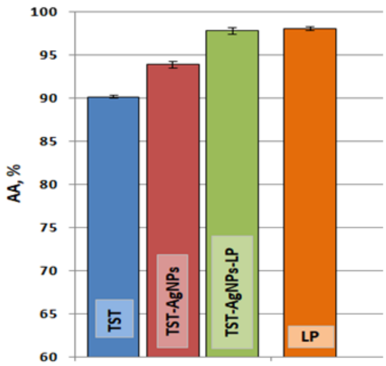

Figure 9.4. Antioxidant activity of Cirsium arvense-based materials and their biocomponents (Data are presented as mean ± SD) [133].

TST-AgNPs presented strong antioxidant properties (AA = 93,85%) as compared to vegetal extract, TST (AA = 90,10%), this behaviour of phyto-generated silver nanoparticles being demonstrated by previous studies [164, 169, 176]. Addition of pectin resulted in the formation of biohybrids (TST-AgNPs-LP) with superior antioxidant activity (AA = 97,75%).

Results obtained for the antimicrobial susceptibility test of *Cirsium* – nanosilver- materials on the organism showed MBCs of 100 µg/mL against *Escherichia coli*. The results presented in Table 9.3.

Tabel 9.3. Antimicrobial susceptibility of *E. coli* microorganism, to the pectin-based biohybrid [133]

Sample concentration (TST-AgNPs-LP µg/mL), MBC-uri	***Escherichia coli***											
	400	200	**100**	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195
TST-AgNPs-LP	S	S	**S**	R	R	R	R	R	R	R	R	R

Key: R - resistant; S - Susceptible / Sensitive

The antibacterial activity of the samples obtained from *C. arvense* was evaluated by a commonly used Petri dish method, presented in Chapter 4.

The antibacterial activity of *Cirsium* – derived samples was evaluated by a spread plate method. These results indicate that the silver content in the hybrids is good enough for antibacterial effect.

In the samples without AgNPs, a more pronounced growth of *Escherichia coli* was noticed (>600 CFUs/mL versus <100 CFUs/mL for treated sample).

At concentration of 100 μg/mL of AgNPs, the silver nanoparticle susceptibility constant, Z value (see Table 9.4), was determined by equation presented in Chapter 4.

Tabelul 9.4. *E. coli* colony forming units, bactericidal ratio and sensitivity constant (Z) for *Cirsium arvense* samples [133]

Test code	**CFU, *E. coli***	**Bactericidal ratio (%)**	**Z (mL/μg)**
TST	656±3.2	NBR	NBR
TST-AgNPs	97±2.1	85	0.01911
TST-AgNPs-LP	85±3.5	87	0.0204

* NBR - No bactericidal ratio

The strong antioxidant and antibacterial action of the biohybrids TST-AgNPs-LP could be attributed to their composition including thistle molecules, *Cirsium* – nanosilver and lemon pectin. Moreover, pectin is a polysaccharide with antioxidant and antibacterial properties [190, 191, 220], that facilitates adhesion to bacterial membrane, disturbing them and enhancing the effect of antibacterial agents due to delivery-promoting action [163, 221-222].

9.6. Partial conclusions

1. Vegetal extract of *Cirsium arvense* leaves was used for “green” development of nanomaterials (based on AgNPs coated or not with lemon pectin) with amplified properties.

2. As shown by the SEM investigations, it was proved that small silver nanoparticles with diameters below 100 nm were obtained, whereas after addition of pectin, particles with diameters above 100 nm can be produced.

3. The results showed that bio-active nanomaterials phyto-generated from weed herb *Cirsium arvense* contain active ingredients against *E. coli* bacterium. Results obtained for the antibacterial susceptibility test of *Cirsium*–nanosilver–materials on the tested bacterium showed MBCs of 100 μg/mL against *Escherichia coli*.

4. Also, we found that bio-active nanomaterials phyto- generated from weed herb *Cirsium arvense* have strong free radical scavenging potential, having high values of antioxidant activity (90-98%).

5. Our findings are promising, the obtained biomaterials could be used in biomedical applications, as antibacterial or antioxidant agents. On the other hand, the UV-Vis absorption spectra showed that our developed *Cirsium*-derived materials absorb the radiation from the visible range, so they could be good candidates for biophotonic applications.

II. CONCLUSIONS

C.1 GENERAL CONCLUSIONS

The objectives of the paper were materialized by obtaining original results as a result of the research conducted and summarized below.

- ✓ The study of 15 plants, from the local and international flora, from the point of view of the general characteristics, of the presence of some phytochemical constituents and of their effects, mentioned in the speciality literature. Among the studied plants, a number of 6 (*Salvia sclarea, Arctium lappa, Artemisia abrotanum, Asparagus officinalis, Cirsium arvense, Harpagophytum procumbens*) were selected for the phyto-generation of silver nanoparticles and the obtaining of bio-nanocomposites and bio-nanohybrids .
- ✓ The capacity of all the studied plants was analyzed to synthesize silver nanoparticles, finding an optimal volumetric ratio, plant extract: $AgNO_{3\,(aq,\,1\,mM)}$ = 1:100 (mL/mL).
- ✓ The following nano-systems were obtained: 12 phyto-generated suspensions through the 6 plants used; 3 types of bio-nanohybrids (BH1, BH2, BH3) composed of biomimetic lipid membranes (MLV), labeled with chlorophyll a (Chla) and phyto-synthesized silver nanoparticles through the plant extract of *Artemisia abrotanum*; 4 types of bio-nanohybridis (nSL1, nSL2, nSLC1, nSLC2) consisting of liposomes, vitamin C and phytogenic silver nanoparticles from aqueous extract of *Arctium lappa* and bio-nanohybrids based on lemon pectin and phytogenerated silver nanoparticles from aqueous extract of *Cirsium arvense* (TST-AgNPs-LP).
- ✓ Realization of a total number of 37 samples, which depending on their type, were characterized by: spectral techniques using UV-Vis absorption spectroscopy, fluorescence, by measurements of dynamic light scattering (DLS), followed by monitoring the size and morphology of nanomaterials by SEM analysis and evaluating the physical stability of silver phyto-nanoparticles, bio-nanocomposites and bio-nanohybrid obtained, evaluation of antioxidant activity by chemiluminescence method and ABTS method, evaluation of antimicrobial / antibacterial activity and antibacterial quantitative) against the *Escherichia coli* microorganism of the products obtained.
- ✓ The phyto-nanosystems obtained showed surface plasmon resonance band (SPR) specific to the synthesis of silver nanoparticles, located at 438 nm for phytosynthesized silver nanoparticles using *Arctium lappa* extract (AgNPs-Br) and SPR band for phyto-generated AgNPs by means of *Artemisia abrotanum* extract (AgNPs-LD) located at 467 nm; the other prepared preparations showed SPR band at 448 nm for AgNPs-TST, 449 nm for AgNPs-He, 452 nm for AgNPs - ISI and 454 nm for AgNPs-Asp. The obtained bio-nanohybrid hybrids showed specific SPR band, depending on the biocomposites present in their structure.
- ✓ Bio-nanohybrid hybrids obtained by *Artemisia abrotanum* extract, composed of liposomal biomimetic membranes (MLV) marked with chlorophyll a (Chl*a*) and AgNPs (BH1, BH2, BH3) showed the characteristic spectral fingertprint of bioporphyrin Chl*a* at 668m n, respectively 663 nm.
- ✓ Aut of all the 6 plants studied for the phytogeneration of AgNPs, it should be noted that, through the Lord's Wood extract, AgNPs with the smallest average diameter (Zav = 51 nm) were phyto-synthesized, and through the plant Thistle extract of AgNPs with the largest average diameter were phyto-synthesized (Z_{av} = 151.7).
- ✓ For the phytosyntheses of silver nanoparticles, the application of the ultrasound process determines the decrease of their average diameter.

- ✓ AgNPs obtained buy different plant extracts *Salvia sclarea*, *Arctium lappa*, *Artemisia abrotanum*, *Asparagus officinalis* showed a good physical stability, with values between: ξ (AgNP-Asp) = -22,00 ± 0,50 mV și ξ (AgNP-ISI) = -24,00 ± 1,20 mV and moderate physical stability ξ = -17,20 ±1,42 mV, for phytosynthesized AgNPs via *A. lappa* extract.
- ✓ All phytosynthesized AgNPs through plant extracts, which were selected for this research showed a higher antioxidant activity than the plant extract alone.
- ✓ The studies carried out and presented in this Thesis highlighted distinct aspects, specific to each plant and the products obtained:
    - The results obtained in the characterization of phytogenerated AgNPs from *Artemisia abrotanum* extract and bio-nanocomposites (phytogenerated AgNPs from *Cirsium arvense* leaves and lemon pectin) lead us to consider that they could be successfully used in phytotherapeutic formulations to reduce the given disorders the presence of free radicals, daily and / or organic oxidative stress, xenobiotics and cosmetics with dermal tropism.
    - Due to the found properties, bio-nanohybrids obtained from phytogenerated AgNPs from *A. abrotanum* extract and liposomal biomimetic membranes (MLV), we appreciate that new carrier-type formulations with protective, antioxidant properties could be incorporated.
    - Antibacterial activity against *E. coli* of phytosynthesized AgNPs from *Asparagus officinalis* and bio-nanohybridis formed from AgNPs-TST and lemon pectin, reloaded in these studies, leads us to consider that they could be useful in product recipes for infection prevention specific microbial, in the relief of urogenital diseases, in skin care products.
    - AgNPs-Br showed good antioxidant activity, over 80% compared to short-lived free radicals and over 45% compared to long-lived free radicals, which suggests that the nanoparticles obtained would produce important antioxidant effects in disorders caused by oxidative stress such as neoplastic, inflammatory, bacterial, dermatological, geriatric diseases.
    - Nanoformulations AgNPs-Burdock – liposomes – vitamin C, due to the properties found, are supposed to be used in formulations aimed at reducing inflammatory processes, protection and proper functioning of the liver cell, reducing disorders due to oxidative stress; could be useful in making new nanostructural formulations with hydro and lyopophilic elements (vitamin A, E, D, etc.). intended for the reconstruction of the dermis, the epidermis.

The novelty aspects in the field of research that emerge from this paper are given by the new nanoformulations prepared from phytogenerated AgNPs, liposomes, vitamin C and pectin, as well as by the advantages given by the properties of the obtained bio-nanohybrids.

C.2 ORIGINAL CONTRIBUTIONS

The original contributions brought by the research studies, presented in the doctoral thesis entitled: **"Development of new biohybrids based on silver nanoparticles and plant extracts using green chemistry"** can be summarized as follows:

- ➢ Extending the range of plants that can be used in the phytosynthesis of silver nanoparticles and highlighting the qualities of each plant used for this purpose, noting that in the literature there are no the use of the 6 plants selected for phyto-nanosynthesis of silver and their subsequent incorporation into bio-nanohybrid hybrids composed of biomimetic membranes, vitamin C, pectin.
- ➢ Highlighting the fact that each plant studied has different synthesis capabilities of AgNPs of different sizes and degrees of stability.

- Valorization of plant materials and plant extracts in the field of nanotechnology to obtain new bio-nanohybrid, consisting of phytogenerated AgNPs, biomimetic membranes, vitamin C and pectin, with proven antioxidant and antibacterial properties, is a novelty in the field of bio-nanotechnology.
- Obtaining phyto-nanocomposites and bio-nanohybrids with much improved antioxidant properties compared to the constituents of nanoformulations taken separately.
- The use of plant extracts in the synthesis of silver nanoparticles has led to the formulation of new products with increased antibacterial properties against the microorganism *Escherichia coli*.
- It is found the development of antimicrobial properties in nanoformulations obtained through phyto-extracts, properties that are not found to be present in the plant extract.

C.3 PERSPECTIVES FOR FURTHER DEVELOPMENT

The prospects for further development can be summarized as follows:

- Deepening the research carried out by continuing specific determinations, using UV-Vis spectroscopy, FT-IR, microscopy (TEM, SEM, AFM), determinations of biological activities (anti-inflammatory, anticancer, antitumor, etc.) in order to highlight and other properties specific to developed bio-nanohybrids or similar so there.
- Development of new bio-nanohybrid formulations based on bio-active systems that could be composed of AgNPs, phyto-nanosynthesized AuNPs, polysaccharide compounds such as pectin, vitamins (A, C, D, E), protein structures, nucleic acids, aiming at developing and highlighting new therapeutic, bio-medical properties (antioxidants, anti-inflammatory, antitumor, anticancer, healing, anti-aging, etc.) or improving those already known.
- Highlighting the lipid bio-nanohybrids obtained as carriers for various substances with therapeutic role or as membranes or thin films for applications in optoelectronic or biophotonic devices.
- Development of new products (cosmetics, phyto-medical, food supplements) in the composition of which to find some of the bio-nanostructures made by these researches, products that aim to contribute to improving functioning of the immune system, preventing the occurrence of various dysfunctions of the body, maintaining the integrity of the dermis and epidermis, reducing the disorders caused by coli bacilli and, in particular, *Eschericia coli*, and those caused by other pathogens, toning the body's functions and improving its general condition.

ANNEXES

A. LIST OF PAPERS PUBLISHED IN THE FIELD OF DOCTORAL THESIS

1. M. E. Barbinta-Patrascu, N. Badea, C. Ungureanu, **D. Besliu**, S. Antohe, Bioactive phyto-nanosilver particles “green” synthesized from Clary Sage, Burdock, Southernwood and Asparagus, ***Rom. Rep. Phys***. 72(3), 606, **2020**; WOS:000562620700017; **IF = 2.147/ 2019**

2. D. Besliu, M. E. Barbinta-Patrascu, N. Badea, I. Rau, A. Meghea, Lipo-nanosilver composites biogenerated using *Artemisia abrotanum L.* aqueous extract, ***Mol. Cryst. Liq. Cryst.*** 694(1),40-48, **2020**; DOI: 10.1080/15421406.2020.1723895; WOS:000531061100004; **IF = 0.512/ 2019**

3. M. E. Barbinta-Patrascu, **D. Besliu**, A. Meghea, Antioxidant silver-based biogenic systems generated from *Arctium lappa* leaves, ***Revista de Chimie***, Volume: 71, Year: 2020, Issue: 4, https://doi.org/10.37358/RC.20.4.8049

4. M. E. Barbinta-Patrascu, C. Ungureanu, **D. Beșliu**, A. Lazea-Stoyanova, L. Iosif, Bio-active nanomaterials phyto-generated from weed herb *Cirsium arvense*, ***Optoelectronics and Advanced Materials-Rapid Communications*** 14(7-10), 2020;**IF = 0.445/2019**

5. **D. V. Beșliu**, Aurelia Meghea, Optimization of the silver nanoparticle synthesis method using *Harpagophytum procumbens* root extract, ***U.P.B. Sci. Bull.***, 2021 Series B, Vol.83, Iss.4, 2021.

FACTOR DE IMPACT CUMULAT: 2,147 + 0,512 + 0,445 = **3,104**

B. LIST OF COMMUNICATIONS DURING THE DOCTORAL INTERNSHIP PERIOD

1. **D. Besliu**, M. E. Barbinta-Patrascu, N. Badea, I. Rau, A. Meghea, ,,Lipo-nanosilver composites biogenerated using Artemisia abrotanum L. aqueous extract ", ***15th International Conference on Frontiers of Polymers and Advanced Materials (ICFPAM 2019)****, Penang, Malaysia, 17th – 21st June* ***2019****.*

The thesis summary contains chapters 5-9 of the original contributions. The number of chapters, subchapters and tables corresponds to that of the thesis. Significant bibliographic references are presented.

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