# Bio-active nanomaterials phyto-generated from weed herb *Cirsium arvense*

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"Green" nanotechnology is in spotlight of scientific research, over last decades. This paper describes a "green" method for weed herbs valorization. Thus, *Cirsium arvense* was converted into two type of materials with high biological value: silver nanoparticles (AgNPs) coated and no-coated with lemon pectin. AgNPs were phyto-generated using *Cirsium arvense* plant extract as a reducing and stabilizing agent, with superior inactivation activity against *Escherichia coli (E. coli).* The obtained samples were characterized by the following methods: UV-Vis absorption spectroscopy, DLS, SEM, and zeta potential measurements. *Cirsium* – derived AgNPs showed mean diameters of 60 nm, while pectin-coated particles presented higher size values ranging from 200 to 400 nm, evaluated by SEM investigations. The antioxidant activity assayed by chemiluminescence technique, ranged between 93.85 and 97.75% for the obtained *Cirsium* –silver materials. The silver containing materials caused a sharp decrease in bacterial cell growth from >600 CFUs/mL to <100 CFUs/mL, *E. coli.* Our findings are promising, the developed materials could be applied in bio-photonics and biomedical fields.

(Received August 26, 2020; accepted October 21, 2020)

*Keywords*: "Green" silver nanoparticles, Bio-design, Lemon pectin, *Cirsium arvense*, Bio-activites (antioxidant and antibacterial activity)

## 1. Introduction

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 [1-5].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, where many commercially available synthetic antibiotics are becoming less efficient against several human diseases [6].

*Cirsium arvense* is a medicinal herbaceous plant, a perennial species from the family *Asteraceae*, often found as noxious weed in grasslands and riparian [7], widespread in Europe and parts of North Africa and Asia [8], but having various therapeutical uses. In folk medicine the medicinal applications include the treatment of peptic ulcer, metrorrhagia, syphilis, leukaemia, diabetes, eye infections, skin sores, gonorrhoea, some mouth diseases and tuberculosis [7]. The various therapeutic uses attributed to *Cirsium* species prompt 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-glucopyranoside) from leaves, apigenin, luteolin, 3-O-methyl kaempferol, cosmosiin, and acacetin 7-β-D-glucopyranosid uronic acid from flowers have been detected [9]. Studies on the therapeutic effects of *Cirsium arvense* highlight the inhibitory capacity on cholesterol biosynthesis [9], the vulnerary use [8], and also its hemostatic, anti-inflammatory and antioxidant effect [10-11], encouraging *in vitro* antimicrobial activity of their crude extracts against some Gram negative (*P. aeruginosa*, *Enterobacter*, *Micrococcus luteus*) and Gram positive (*E. coli, K. pneumoniae, S. aureus*) bacteria, and also promising antifungal, and antiproliferative potential [11].

Additionally, biosynthetic AgNPs, obtained in our research from natural aqueous extract of *Cirsium arvense*, could be used in various and significant applications, ranging from human and veterinary medicine, pharmacology, water purification, to biophotonics. For a long time silver has been known to have a disinfecting effect, even the Greeks used it for cooking and to keep water safe. The first recorded medicinal use of silver was reported during 8$^{th}$ century [12], however only much later, in the 17th century, it was recognized the lethal potential of silver ions and silver-based compounds, against microorganisms [13]. In last decade, preparation of silver

nanoparticles (AgNPs) has attracted considerable attention due to promising antimicrobial properties, which are slow but long-term and persistent, and avoiding the production of hazardous disinfection by-products [14]. Many reports highlighted the excellent inhibitory and bactericidal properties of AgNPs against both Gram-positive and Gram-negative bacteria including *Staphylococcus bacillus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [15], several phytopathogenic fungi (e.g., *Alternaria alternate*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinereal* and *Curvularia lunata*) [16], as well as human pathogenic fungi (e.g., *Candida* and *Trichoderma sp*) [17], respectively efficient inhibitory activities against several viruses including human immunodeficiency virus, hepatitis B virus, herpes simplex virus, human parainfluenza virus [18] and not only. Numerous methods have been adopted for the synthesis of AgNPs in order to meet these increasing requirements. Nowadays, biosynthesis of AgNPs had gained so much attention in developed countries due to development demand of "green" technology for material synthesis and its benefits against conventional physical and chemical synthesis method such as the availability of a vast array of biological resources, a decreased time requirement, high density, stability, and the ready solubility of prepared nanoparticles (NPs) in water [19].

Last but not least, a novel approach of this study was made by using pectin extracted from lemon peel. 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. Due to its content in bioactive substances, lemon peel has inhibitory properties on the growth of microorganisms, but the inhibition efficiency is related to the extraction process such as alcoholic and aqueous route [20-21]. Several compounds as flavanones, flavanone glycosides and polymethoxylated flavones are unique to *Citrus*, and occur relatively rare in other plants [22], and other ones are common like pectin, which is a structural polysaccharide consisting of a linear chain of linked galacturonic acid, which is found in *Citrus*, apples, grapes, plums, etc., although it differs from its composition and quantity from one species to another. It has been reported that pectin decreased blood lipid level and peroxidative status, and showed antioxidant activities in kidney toxicity induced by octylphenol [23].

The surprising *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 few studies started to report new pectin-based substances with high bactericidal activity [24].

Reduction of the huge amount of *Citrus* wastes is another important issue nowadays, and their efficient valorization is an interesting challenge to develop new materials with unusual properties [25-26].

The *Cirsium*-derived materials developed in our study, were investigated by various biophysical methods: Ultraviolet–Visible (UV-Vis) absorption spectroscopy, Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), and also by Zeta Potential (ZP) measurements. The biological action of these materials were evaluated in terms of antioxidant (chemiluminescence method) and antibacterial (*in vitro* tested against *Escherichia coli*) activities.

## 2. Materials and methods

### 2.1. Materials

Silver nitrate ($AgNO_3$), peptone, Tris (tris (hydroxymethylaminomethane base)), hydrochloric acid (HCl), hydrogen peroxide ($H_2O_2$), luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), were purchased from Merck (Germany). Sodium chloride (NaCl) was supplied from Sigma Aldrich (Germany). Agar was obtained from Fluka (Switzerland) and the yeast extract was purchased from Biolife. Fresh leaves of weed herb: *Cirsium arvense* were acquired from a local garden.

### 2.2. Sample preparation

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

*i) Preparation of thistle extract*. An amount of washed fresh thistle leaves was crumbled and immersed in boiling distilled water (in a mass ratio of vegetal material:water of 1:5), for 15 minutes; the obtained extract (TST) was then filtered through Whatman filter papers No. 1.

ii) *Phyto-mediated preparation of silver-based materials*. Equal volumes of 1 mM $AgNO_3$ aqueous solution and thistle extract were mixed, under continuous magnetic stirring, resulting in silver nanoparticles (TST-AgNPs); the bioreduction reaction was completed after 24 h. Fresh lemon peels were washed in distilled water and then chopped, and used to prepare pectin (LP) by using the method previously described in [27]. Equal volumes of LP and TST-AgNPs were mixed, under continuous stirring, and then, the resulted biohybrids (TST-AgNPs-LP) were further subjected to ultrasonic irradiation (ultrasound bath Elmasonic S60H Elma Schmidbauer GmbH, Singen, Germany) for 5 minutes.

### 2.3. Characterization methods

**UV-Vis absorption spectra** of the *Cirsium*-based samples were recorded in the 200-800 nm wavelength range, (at the resolution of 1 nm) on double beam UV-Vis-NIR Spectrophotomer V670, Jasco (Tokyo, Japan).

**Zeta potential** (ZP, mV) values were measured at 25°C, in triplicate, using a special dispositive of Zetasizer Nano ZS (Malvern Instruments Ltd., UK) by applying an electric field across the analyzed aqueous suspensions.

The average size, $Z_{ave}$, as hydrodynamic diameter of the particles (*the particle diameter + the double-layer thickness*), and also the polydispersity index, PdI (parameter indicating the particle population uniformity) were estimated by **Dynamic Light Scattering (DLS)**. The particles were analyzed on Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK), as described in [28].

Surface morphology investigations were done with a scanning electron microscope (**SEM**), a FEI Inspect model S50 apparatus. The SEM images make use of a 10 mm working distance, 50 up to 50 000x magnifications and 5 kV acceleration voltage. Samples preparation, prior to SEM images, includes a 5 nm Au thin film deposited onto our samples. The layer was obtained by a Cressington 108 auto sputter coater apparatus that has a Cressington mtm 20 thickness controller. The particles clusters' sizes were measured with the freeware ImageJ programme.

**Chemiluminescence (CL) technique** was used for ***in vitro* evaluation of antioxidant properties** of the samples. Reactive oxygen species (ROS) were generated by a system containing luminol ($10^{-3}$ M), $H_2O_2$ ($10^{-5}$ M), and TRIS-HCl buffer solution (pH 8.6). These experiments were carried out on a Chemiluminometer Turner Design TD 20/20 (USA), and the values of *in vitro* antioxidant activity (AA%) was expressed as:

$$AA\% = [(I_0\text{-}I)/ I_0] \cdot 100\% \quad (1)$$

where $I_0$ and $I$ represent the maximum CL intensity at t = 5 s, for the reaction mixture without and with the sample, respectively [29]. Three different experiments were carried out for each sample.

For **antibacterial assay** of the tested samples we used *Escherichia coli* ATCC 8738 bacterium. *Escherichia coli* ATCC 8738 was cultured in Luria Bertani Agar (LBA, peptone (Merck), 10 g/L; yeast extract (Biolife) 5 g/L, NaCl (Sigma-Aldrich) 5 g/L and agar (Fluka) 20 g/L) plates [30] at 37°C. Negative control used was water.

Minimum bactericidal concentration (MBC) of AgNPs - based samples against *Escherichia coli* was evaluated by broth dilution technique [31]. Serial dilutions of AgNPs ranging from 400 μg/mL to 0.195 μg/mL were inoculated with *Escherichia coli* bacterium and incubated at 37°C for 18 hours.

Antibacterial effect of *Cirsium* - derived samples against *Escherichia coli* was determinated by both qualitative and quantitative methods:

i. Agar well diffusion method (qualitative assessment) [32-33]. The presence of a clear zone after incubation (ZI) indicates the antimicrobial effect of the samples.

ii. Percentage reduction test (quantitative assessment) [34].

The antibacterial effect was evaluated by bactericidal ratio, R (%), which was determinated as follows:

$$R\ (\%) = \frac{CFU_{\text{control group}} - CFU_{\text{experimental group}}}{CFU_{\text{control group}}} \cdot 100 \quad (2)$$

The nanoparticle susceptibility constant is suggested as a quantitative parameter for the estimation of antimicrobial effect. The nanoparticle susceptibility constant Z (mL/μg) is computed by the equation [35]:

$$Z = \frac{-\ln(N / N_0)}{C} \quad (3)$$

where N is the bacterial colony forming units (CFUs) on the agar plate (containing nanoparticles), $N_0$ is the CFUs on the pure agar plate, and C is the concentration of nanoparticles (μg/mL). The survival fraction ($N/N_0$) can be predicted using the Z value and a given C value. A higher Z value shows that the microorganisms are more sensitive to the materials.

The values of ξ, $Z_{ave}$, PdI, antioxidant activity, and antibacterial results, were reported for each sample, from three individual measurements, as mean values ± standard deviations (SD) calculated as the square root of variance using STDEV function in Excel 2010.

## 3. Results and discussion

### 3.1. Optical characterization of the obtained *Cirsium* – based particles

A comparative presentation of UV-Vis absorption spectra of all samples (Fig. 1) 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 [26, 36]; between 315-327 nm – assigned to the flavonoids [37].

The *Cirsium* – mediated synthesis of AgNPs was firstly demonstrated by the apparition of a single SPR band located at 448 nm, as shown in Fig. 1, Inset. This band is characteristic for spherical shaped silver nanoparticles [38].

### 3.2. Evaluation of Zeta potential of the pectin-coated materials

The silver-based particles have negative surface charge, quantified by zeta potential measurements (Table 1). 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 (section 3.1).

After pectin addition to TST-AgNPs, ZP values shifted to smaller ones since 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 [39-40].

*Table 1. Zeta potential values, Size and Polydispersity index of materials containing Cirsium – derived AgNPs*

| Sample | ZP (mV) | Zave (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 results are reported on the basis of mean ± SD.)*

### 3.3. Size and morphological aspects of *Cirsium arvense* – derived materials

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

DLS results (Table 1) 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 Fig. 2. The images correspond to AgNPs obtained from thistles which are uncoated (Fig. 2 a) and covered with pectin (Fig. 2 b).

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 [41].

### 3.4. Evaluation of the biological action of the *Cirsium* – derived materials

Antioxidant profile of the developed *Cirsium* – based materials is displayed in Fig. 3.

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 our previous studies [39, 42-44].

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 were presented in Table 2.

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 3), was determined by equation (3).

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 [3, 26, 45], that facilitates adhesion to bacterial membrane, disturbing them and enhancing the effect of antibacterial agents due to delivery-promoting action [39, 46-47].

*Table 2. Antimicrobial susceptibility of the microorganisms to pectin-based biohybrid*

| Concentration of TST-AgNPs, (μg/mL) | *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 |

*Keys*: R – Resistant; S - Susceptible/Sensitivity

*Table 3. Colony-forming units of E. coli, bactericidal ratio, and susceptibility constant (Z) for Cirsium – derived samples*

| Specimen | CFUs/mL, *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

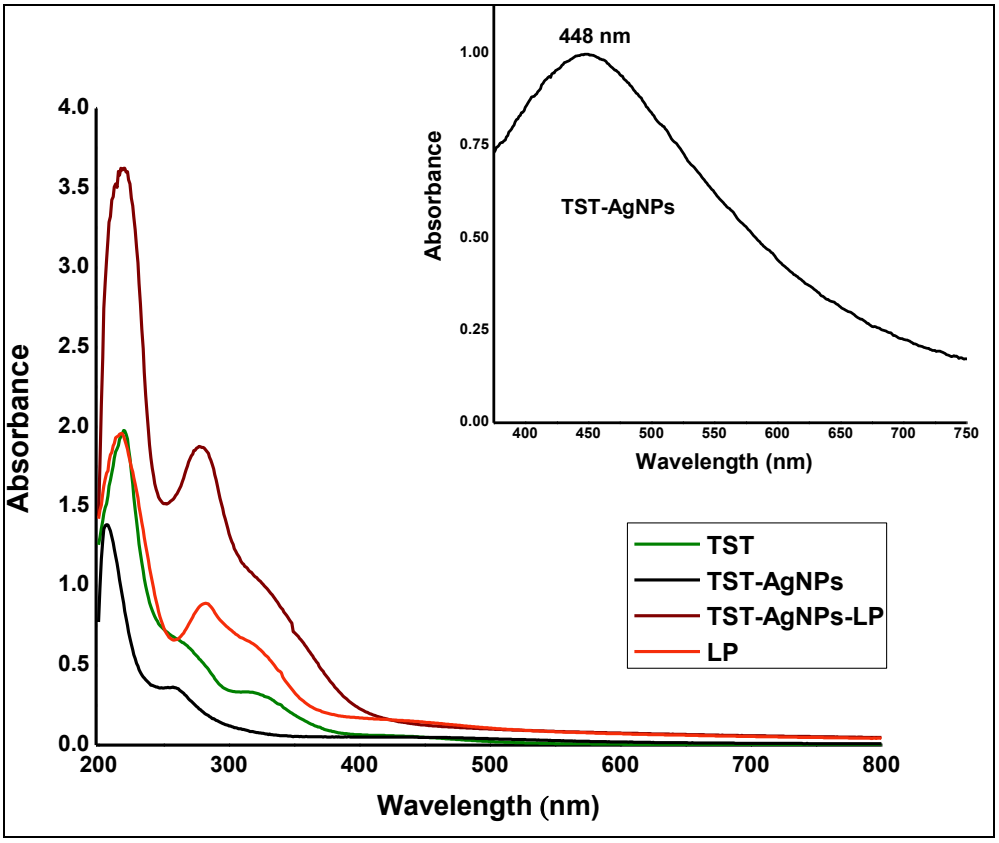


*Fig. 1. The absorption spectra of the obtained samples. Inset: SPR band of Cirsium - derived AgNPs (color online)*

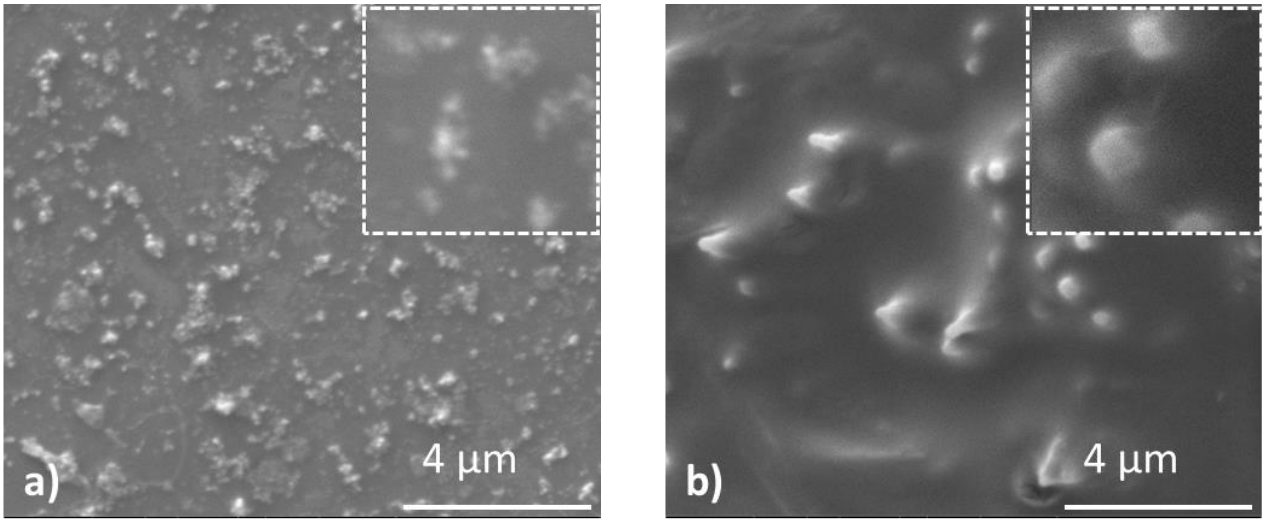


*Fig. 2. SEM images of the AgNPs: a) obtained from thistles (sample code TST-AgNPs) and b) obtained from thistles and covered with pectin (sample code TST-AgNPs-LP). SEM image insets of 2x2 µm$^2$ showing magnified samples' images*

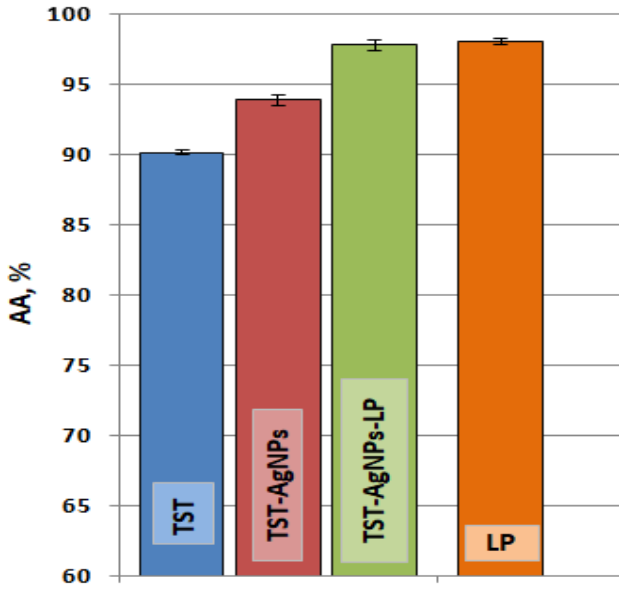


*Fig. 3. The antioxidant activity of the Cirsium -based materials and their components (The data are presented as mean values ± SD) (color online)*

## 4. Conclusions

Vegetal extract of *Cirsium arvense* leaves was used for "green" development of nanomaterials (based on AgNPs coated or no with lemon pectin) with amplified properties.

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.

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.*

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%).

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.

### Acknowledgements

The present study was supported by the Projects JINR – Romania: Project No.48/ 2020 (Theme No.04-4-1121-2015/2020) and Project No. 75/ 2020 (Theme No. 04-4-1141-2020/2022) (IUCN ORDER no.269/20.05.2020) of JINR – Romania (University of Bucharest) collaboration. A. L.-S acknowledges the financial support of the Nucleu Programme at I.N.F.L.P.R., LAPLAS IV, contract no. 16N /08.02.2019.

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