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BIOACTIVE PHYTO-NANOSILVER PARTICLES "GREEN" SYNTHESIZED FROM CLARY SAGE, BURDOCK, SOUTHERNWOOD AND ASPARAGUS

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Abstract. This study reports the "green" synthesis and biophysical characterization of silver nanoparticles (AgNPs) generated from natural extracts of *Salvia sclarea (*Clary sage), *Arctium lappa* (Burdock), *Artemisia abrotanum* (Southernwood) and *Asparagus officinalis* (Asparagus), for biomedical purposes. UV–Vis absorption spectroscopy revealed SPR bands for AgNPs, between 438 and 467 nm. DLS measurements showed the nano-dimensions of particles ranging from 51.05±0.99 to 123.6±2.05 nm. Zeta potential values of these biogenic AgNPs revealed their good physical stability. The developed AgNPs exhibited impressive antioxidant potential (evaluated by chemiluminescence and ABTS methods) and also antibacterial effect against *Escherichia coli* bacterium. These materials could be exploited in biomedical field to fight against bacteria and to combat oxidative stress by scavenging short-life & long-life free radicals.

Key words: Silver nanoparticles, phytosynthesis, medicinal plants, bio-activities.

1. INTRODUCTION

Nowadays, it is a real concern regarding the valorization of vegetal wastes with beneficial impact on human health and on the environment. Plants were used from ancient times in folk medicine, for the treatment of many diseases. It is well known the role played by the chemical composition of plants in conferring their biological activity. Herbal materials contain many bioactives with high biological value and good potential to reduce metallic ions resulting in metal nanoparticles with interesting bio-properties [1–5]. Phytogenic silver nanoparticles (AgNPs) have gained increasing/ special interests due to their interesting bio-properties like antioxidant and antibacterial efficacy. The converting plant materials in AgNPs is a

good idea to keep clean the environment, to recycle and to valorise vegetal wastes and turn them into novel materials with high therapeutic potential value.

Our research brings new insights in developing novel antioxidant & antibacterial nanosystems based on AgNPs phyto-generated from aqueous extracts of aerial parts of Clary sage (*Salvia sclarea*), Burdock (*Arctium lappa*), Southernwood (*Artemisia abrotanum*) and Asparagus (*Asparagus officinalis*), for biomedical purposes. These plants were chosen due to their high therapeutic significance and great potential in pharmacy and phytotherapy. Thus, *Salvia sclarea* (known as clary sage) is an herbaceous perennial species, widely appreciated as cooking additive and also for its therapeutic potential as antioxidant, anti-inflammatory, antimicrobial, antiemetic & neuroprotective agent, and in treatment of gastrointestinal disorders, arthritis, and rheumatism [6]. *Arctium lappa* (burdock) is a popular plant traditionally used as depurative, diuretic, carminative, antioxidant, antibacterial, anti-inflammatory, and anti-tubercular agent, or in treatment of hypertension, hepatitis, atherosclerosis, skin disorders, and geriatric diseases due to their polyphenolic constituents with antioxidant properties such as quercetin, quercitrin, rutin, and luteolin [7–9]. *A. abrotanum* L. (southernwood) is used as an aromatic plant or in folk medicine for the treatment of a variety of diseases, such as: dermatological disorders [9], allergic rhinitis [10], infections of the upper respiratory tract [11], or as antimicrobial agent [12]. The high biological value of this plant is due to its chemical composition rich in phenolic compounds, such as: ferulic acid, sinapic acid, rutin, patuletin and luteolin [13]. *Asparagus officinalis* (asparagus) is a perennial plant with large amounts of phenolic acids (ferulic acid) and flavonoids (such as rutin, quercetin) or saponins (protodioscin) which are healthy biofunctional components with anti-inflammatory, antioxidant, antitumor, and antibacterial properties [14–15].

The obtained AgNPs were spectral characterized 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.

2. MATERIALS AND METHODS

2.1. MATERIALS

Silver nitrate was supplied by Gatt Koller-GmbH Austria, and Tris (hydroxymethylaminomethane base), H_2O_2, HCl, luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), were acquired from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2 azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and potassium persulfate were

purchased from Sigma Aldrich (Munich, Germany). The vegetal materials were bought from a local Romanian market.

2.2. SAMPLE PREPARATION

Preparation of biogenic silver nanoparticles. An appropriate amount of fresh leaves of each plant was inserted into hot water (in a mass ratio *vegetal material*: *water* = 1:5, w/w) and boiling for 15 minutes, in order to release the intracellular bio-active compounds into extracellular medium. After cooling, this infusion was filtered on Whatman paper no. 1, resulting in clear extracts (samples ISI, Br, LD, and Asp). A volume of 400 μL of each vegetal extract was poured into 40 mL of 1 mM $AgNO_3$ solution, and kept in dark under continuous stirring for 24 h (VIBRAX stirrer – Milian USA, Ohio 43230 USA 200 rpm); the colour of each sample turned from pale yellowish to brown after 45 min., due to the formation of silver nanoparticles (samples AgNP-ISI, AgNP-Br, AgNP-LD, and AgNP-Asp).

A description and abbreviation of the prepared samples is summarized in Table 1.

Table 1

Description and abbreviations of the obtained specimens

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

2.3. PHYSICO-CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF AgNPs

The **UV-Vis absorption spectra** of the prepared specimens were recorded on a double beam UV–Vis 670 Jasco spectrophotometer, in the 350–800 nm wavelength range, operated at a resolution of 0.5 nm.

The mean particle size ($\boldsymbol{Z_{av}}$) and **the size distribution** (**PdI**) of biogenic AgNPs were determined by **Dynamic Light Scattering (DLS)** measurements using a Malvern Zetasizer ZS 90 (Malvern Instruments Inc., UK), equipped with a solid-state laser (670 nm), working at 25°C temperature and at a scattering angle of 90°. Samples were prepared by dispersing the AgNPs samples in appropriate amount of

deionized water before conducting the experiment. The particle size data were evaluated using intensity distribution. Each value of Z_{av} and PdI was given as average of three individual measurements.

Zeta potential (ξ) values were measured in triplicate, at 25°C, using a special dispositive of Zetasizer Nano ZS (Malvern Instruments Ltd., UK) by applying an electric field across the analyzed AgNPs samples; the mean values were reported.

The *in vitro* antioxidant activity was evaluated by two methods:

i) *Chemiluminescence assay.* The ability of the obtained AgNPs to scavenge short-life free radicals was evaluated by chemiluminescence technique, on a Chemiluminometer Turner Design TD 20/20 (USA). The system responsible for the chemiluminescence (CL) generation contains 10^{-3} M luminol, 10^{-5} M H_2O_2, and TRIS-HCl buffer solution pH = 8.6. These investigations were performed in triplicate, and the values of *in vitro* antioxidant activity AA[%] of the samples was expressed as:

$$AA[\%] = [(I_0 - I_s) / I_0] \cdot 100\% \quad (1)$$

where I_0 is the maximum CL intensity at t = 5 s, for the standard, and I_s is the maximum CL intensity for each sample at t = 5 s [16].

ii) *$ABTS^{\bullet+}$ assay.* Antioxidant activity of vegetal extracts and AgNPs has been determined by ABTS radical cation ($ABTS^{\bullet+}$) assay. The radical $ABTS^{\bullet+}$ was produced by reacting ABTS solution 7 mM with 2.45 mM potassium persulfate water solution and kept in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution was normalized to absorbance 0.700 (±0.1) at 734 nm. Samples were prepared by addition of 3.0 mL diluted $ABTS^{\bullet+}$ solution to 1 mL vegetal extract/AgNPs solution and brought to a 5 mL flask with distilled water [17]. By adding an antioxidant in the reaction media, $ABTS^{\bullet+}$ is reduced and the solution is discolored. Absorbance of samples was measured using UV–Vis–NIR Spectrophotometer Type V670, (Jasco, Japan) at exactly 4 min after initial mixing using ethanol as reference. The blank solution was prepared in the same manner but without samples solution. The scavenging of $ABTS^{\bullet+}$ was calculated as inhibition [%] using the equation:

$$\text{Inhibition}[\%] = \frac{A_0 - A_s}{A_0} \times 100. \quad (2)$$

A_0 is the absorbance of the blank (unscavenged radical cation solution), while A_s is the absorbance after the addition of the sample. Three different experiments were performed for each sample, and mean values with standard deviations were reported.

***In vitro* antibacterial activity**. For evaluation of *in vitro* antibacterial activity of the obtained metallic nanoparticles, we used the agar well diffusion assay as described in detail in previous studies [18–19]. *In vitro* antibacterial activity to the tested sample was established by the diameter of the inhibition zones according to Ponce *et al.* (2003) [20] as not sensitive (total diameter of well under 8 mm), sensitive (total diameter of well from 9 to 14 mm), very sensitive (total diameter of well from 15 to 19 mm), and extremely sensitive (total diameter of well above 20 mm). The biological strains used for this investigation was *Escherichia coli* ATCC 8738 grown in Luria Bertani Agar (LBA, Miller), at 37°C with the following medium composition: 10 g/L casein enzymatic hydrolysate, 10 g/L sodium chloride, 5 g/L yeast extract, and 15 g/L agar. Briefly, sterile LBA dishes were prepared by pouring the sterilized media in sterile Petri plates under aseptic conditions. *E. coli* bacterium (1 mL) was spread on agar plates and wells were made at the size of 6 mm diameter, in the agar plates using the sterile borer. The wells were inoculated with 50 μL of tested sample; then, LBA plates are incubated at 37°C for 24 h. The antimicrobial agent diffuses into the LBA and inhibits growth of the test bacteria and then the diameters of inhibition zones are measured (ZOI).

3. RESULTS AND DISCUSSIONS

3.1. SPECTRAL CHARACTERIZATION OF THE OBTAINED NANO-METALLIC PARTICLES

The phytosynthesis of AgNPs was proved by UV-Vis absorption spectroscopy and Dynamic Light Scattering.

After mixing natural extracts (ISI, Br, LD, and Asp) with 1 mM $AgNO_3$ solution, a strong SPR band appeared at 452, 438, 467 and 454 nm for AgNP-ISI, AgNP-Br, AgNP-LD, and AgNP-Asp, respectively (Fig.1 a, b, c & d, respectively), indicating the formation of silver nanoparticles [3, 21]. Fig. 1e 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 increase as follows: size(AgNP-LD) ~ size(AgNP-ISI) < size(AgNP-Asp) < size(AgNP-Br). These assumptions were further confirmed by DLS measurements (Fig. 2) showing the nano-scaled dimension of the prepared AgNPs those average diameters presented the following values: 51.05 ± 0.99 (AgNP-LD), 74.52 ± 6.71 (AgNP-ISI), 122.3 ± 8.13 (AgNP-Asp) and 123.6 ± 2.05 (AgNP-Br). All the obtained AgNPs presented a monomodal distribution having a polydispersity index PdI < 0.35.

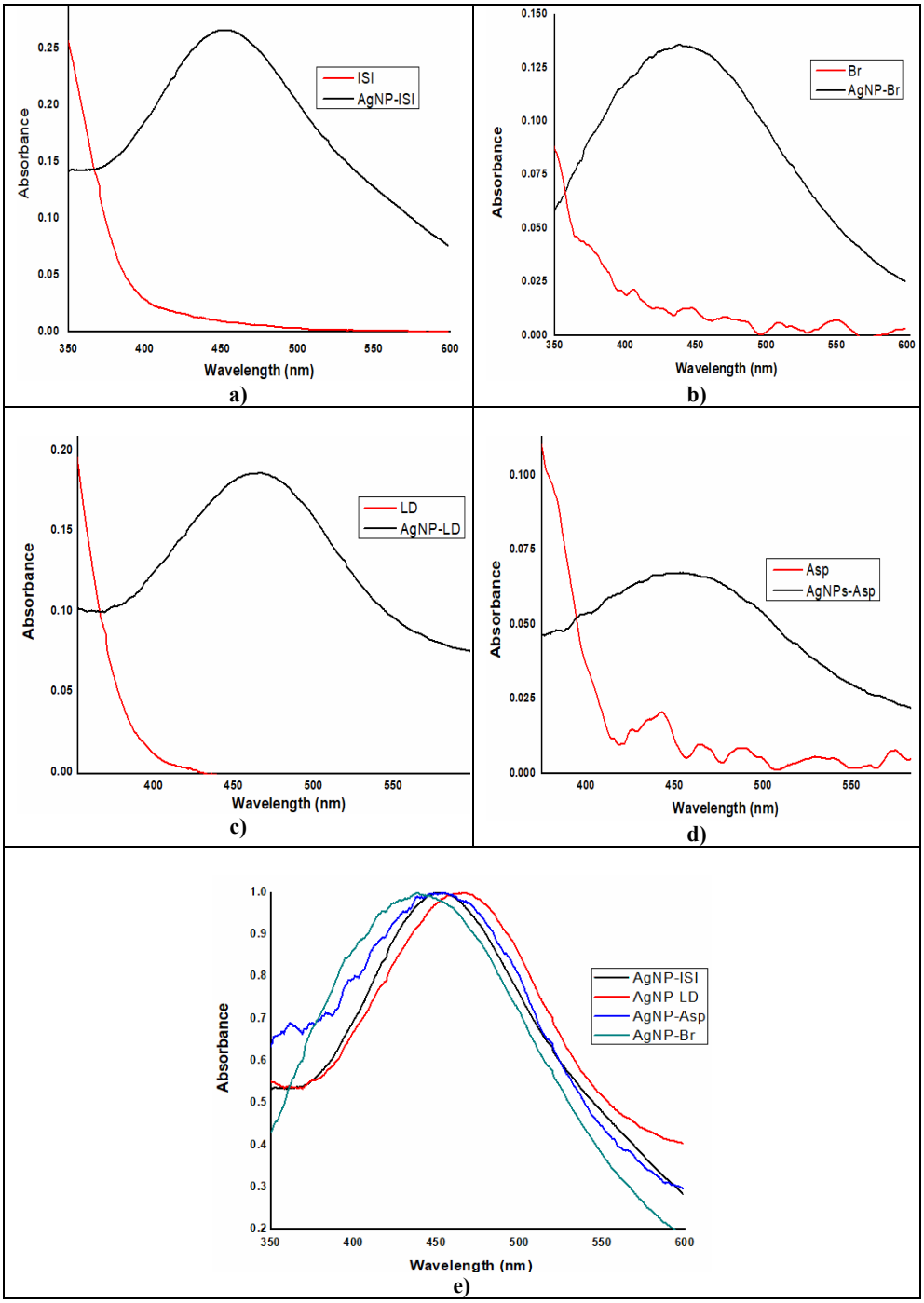

Fig. 1 – The SPR band of "green" synthesized AgNPs.

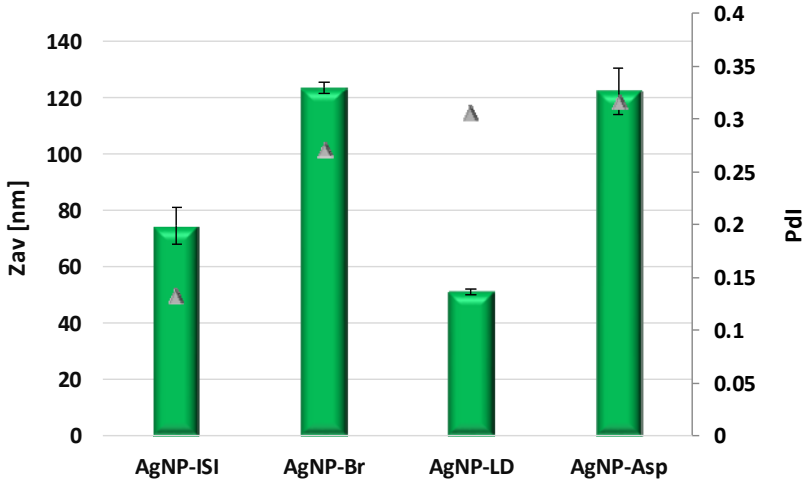

Fig. 2 – Size determination of the phytosynthesized AgNPs.

AgNP-ISI showed the smallest polydispersity index (PdI = 0.132), therefore the most uniform population of particles, as seen in size distribution profile displayed in Fig. 3.

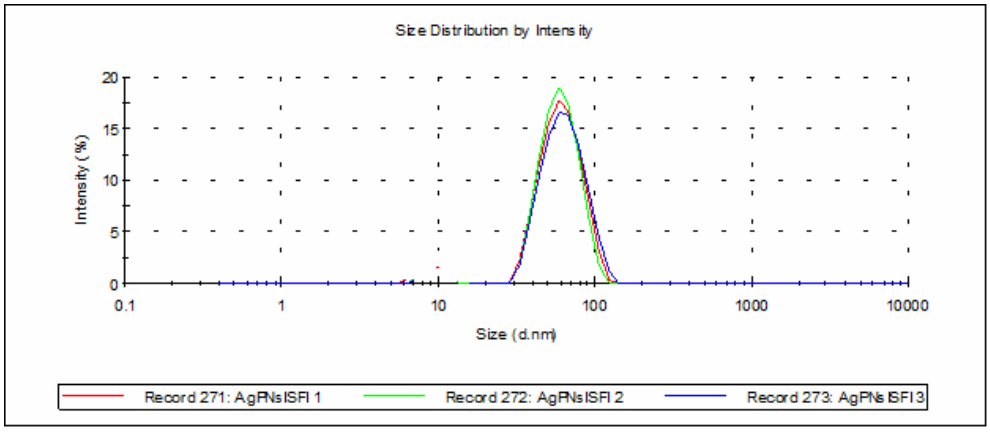

Fig. 3 – Size distribution profile of silver nanoparticles phytogenerated from *Salvia sclarea.*

3.2. EVALUATION OF PHYSICAL STABILITY OF BIOGENIC NANOSILVER PARTICLES

The physical stability of the phytogenerated silver nanoparticles was evaluated in terms of Zeta Potential (ξ) which is a parameter close related to the surface charge of particles. ξ was calculated based on electrophoretic mobility, by using the Helmholtz–Smoluchowski equation:

$$\xi = EM \cdot \frac{4\pi\eta}{\varepsilon}, \quad (3)$$

where ξ is zeta potential, EM is the electrophoretic mobility, η is the viscosity of the dispersion medium and ε is the dielectric constant [22].

As observed in Fig. 4, 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.

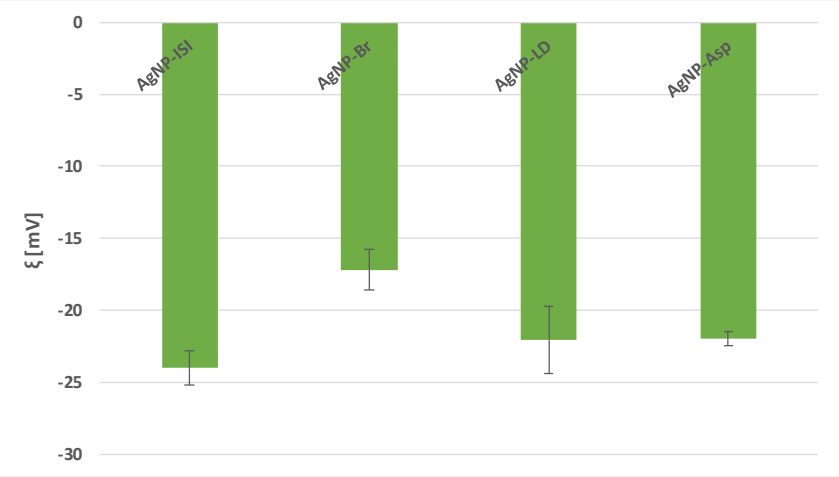

Fig. 4 – Zeta potential values of the samples (the results are reported on the basis of mean ± SD).

3.3. EVALUATION OF THE BIOLOGICAL ACTIVITIES OF THE DEVELOPED BIOGENIC 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 section 2.3).

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 [3, 4, 23].

Chemiluminescence results (Fig. 5) revealed high capacity for scavenging short-life free radicals, in the following sequence:

$$AA_{AgNP\text{-}LD} > AA_{AgNP\text{-}ISI} > AA_{AgNP\text{-}Asp} > AA_{AgNP\text{-}Br}.$$

$ABTS^{\bullet+}$ method (Fig. 6) 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•$^{+}$, with values of ABTS•$^{+}$ capturing ranging between 32.44 and 61.26%.

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.

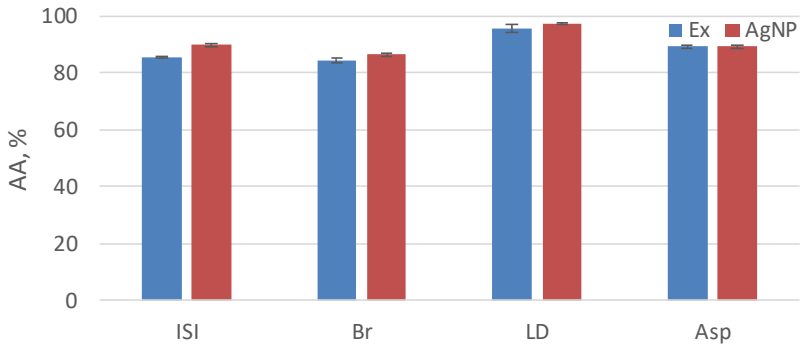

Fig. 5 – The antioxidant activity (evaluated by chemiluminescence technique) of the biosynthesized materials and their building blocks (the data are presented as mean ± SD).

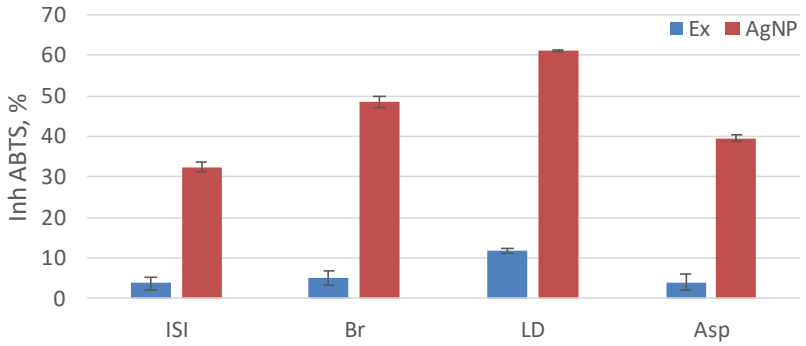

Fig. 6 – The antioxidant activity (evaluated by ABTS method) of the biosynthesized materials and their building blocks (the data are presented as mean ± SD).

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. Thus, **ISI** (clary sage) is rich in *phenolic acids* (3-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, *p*-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid, *p*-coumaric acid, chlorogenic acid, rosmarinic acid, ferulic acid, ellagic acid), and *flavonoids* (salvigenin, galangin, chrysin, luteolin, myricetin and pinocembrin) [24–26]. **Br** contains antioxidant phytocompounds, such as: phenolic acids (chlorogenic acid; 1,5-di-O-caffeoylquinic acid), flavonoids (baicalin; luteolin; rutin; quercitrin; quercetin and its derivatives; astragalin),

lignans (arctiin, arctigenin and its derivatives), sesquiterpene lactones (arctiopicrin and onopordopicrin) [7]. Research studies reported the presence of ferulic acid, hydroxycinnamic derivatives, sinapic acid, artemisin, luteolin, rutin and patuletin in **LD** (Southernwood *A. abrotanum*) [13]. **Asp** is also a good source of *phenolic acids* (ferulic acid) and *flavonoids* (rutin, protodioscin, quercetin and isorhamnetin) [15, 27].

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. The pictures of Petri dishes showing the halos of these zones are illustrated in Fig. 7.

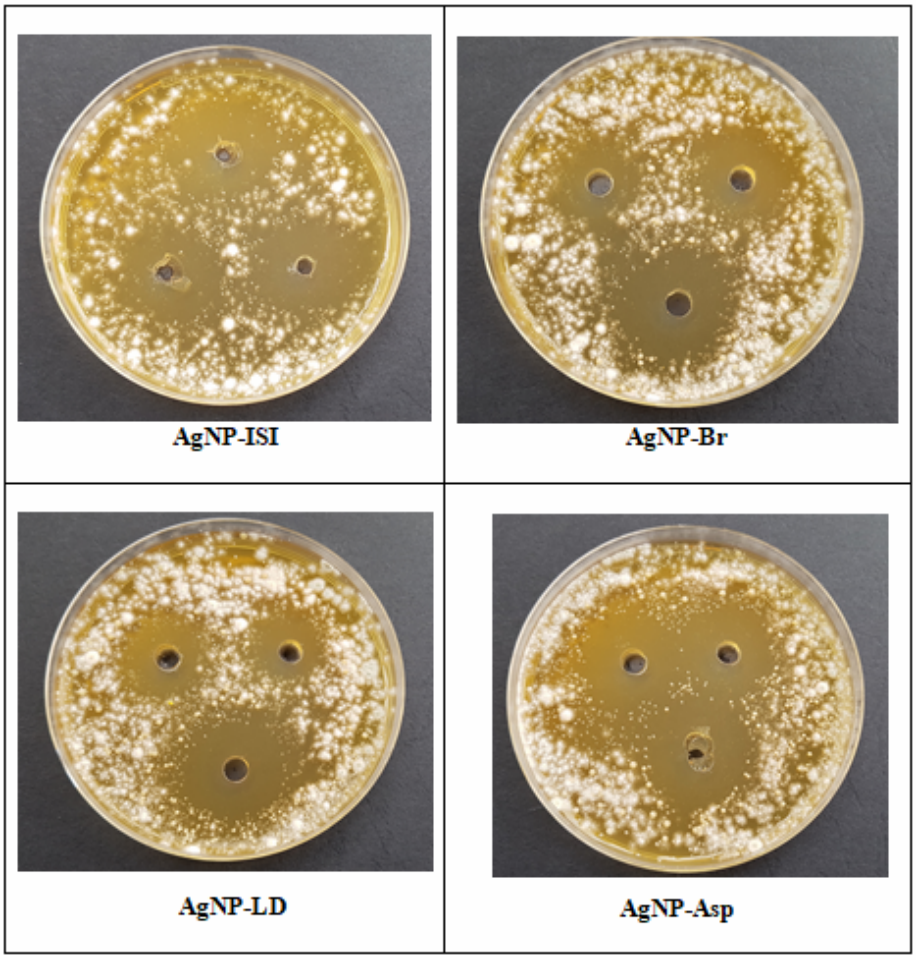

Fig. 7 – Growth inhibition zone of *Escherichia coli* ATCC 8738 measured around the wells loaded with phytosynthesized AgNPs.

Figure 8 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.

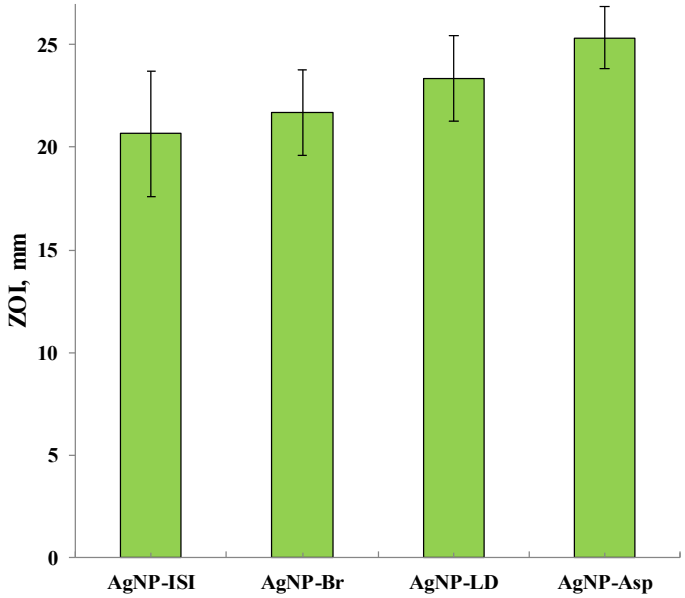

Fig. 8 – The ZOI diameters (in mm) exhibited by the obtained biogenic silver nanoparticles against *Escherichia coli* ATCC 8738 (the data are presented as mean ± SD).

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 [1].

4. 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 ± 0.99 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*, 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.

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