OPTIMIZATION OF GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING *HARPAGOPHYTUM PROCUMBENS* ROOT EXTRACT

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This paper reports on biosynthesis of silver nanoparticles (AgNPs) by a phytosynthesis method using an aqueous extract from Harpagophytum procumbens (Devil's claw) root for biomedical application. For AgNPs synthesis two ratios of vegetal extract: AgNPs 1mM solution have been used. Time evolution of AgNPs formation has been monitored by means of SPR band of UV-Vis spectroscopy, while nanometric size of these nanoparticles has been confirmed by DLS measurements. Antioxidant properties have been evaluated by $ABTS^+$ and chemiluminescence techniques, demonstrating an 18.3 % capacity for $ABTS^+$ radicals capture and 75.5 % quenching of short-lived radicals.

Keywords: phyto-nanosynthesis, AgNPs, *Harpagophytum procumbens (Devil,s claw)*, antioxidant activity

1. Introduction

Capitalization of vegetal extracts in nanotechnology domain has shown a particular development during the last period by obtaining a large variety of nanomaterials with tailored properties like: antioxidant [1], antimicrobian [2], anti-inflammatory [3], photo-protective [4], antitumor [5], anticarcinogenic [6] and optic [7]. Encapsulation of natural extracts within silica matrix [8], lipid matrix [9], polymer matrix [10] or their use for phyto-synthesis of metallic nanoparticles [11] has demonstrated that the bioactive properties of vegetal extracts can be amplified at nanometric scale.

Moreover, research studies reported during the last decades highlighted that in some conditions, many biomolecules (polyphenols, flavones, flavonoids, etc.) existent in plants or vegetal extracts, in the presence of silver ions, exhibit specific alterations at secondary structure level accompanied by silver ions reduction and formation of silver nanoparticles (AgNPs). At present, there is a

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high interest in finding new plants and vegetal extracts endowed with a bio-reducing character of silver ions, with the aim to optimize the existent methods for obtaining AgNPs and to improve previous bio-medical products and also to develop new products with antioxidant properties. In this respect, the following aspects are envisaged: improving the yield of AgNPs biosynthesis, enhancing bioactivity of synthetized nanoparticles and increasing their bioavailability, while maintaining a healthy environment and successfully use of products obtained in various domains of industry, medicine and pharmacy, in different natural/traditional therapies.

The aim of this study is to highlight the capacity of *Harpagophytum procumbens* (*H. procumbens*), known by its popular name of *Devil's claw*, containing 5% harpagozides, to synthetize silver nanoparticles by using a phyto-chemistry method, and to demonstrate the antioxidant capacity of synthetized silver nanoparticles.

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. In traditional phytotherapy both root and tubers are used.

Pedaliaceae family includes several plant species, like *H. zeyheri H. pinatifidum, H. peglearea, H. abbreviatum, H. buchellii, H, gramdidieri*, etc. Among these, *H. zeyheri* can be cultivated, but its phyto-therapeutic effects are diminished as compare to that of *H. procumbens*, probably due to a lower content in bioactive compounds. This aspect needs further investigation.

Other similar plants belonging to the same *Pedaliaceae* family, for instance *Sesamum indicum* (sesame), can be found in tropical regions of South-East Africa, like Madagascar, and also in Australia, India, South America. They are well known for their application as functional food with multiple biological, phyto-therapeutic and antioxidant activities, being useful in disease prevention and improving life quality, even for life time prolongation. [12]. Among beneficial effects can be mentioned their actions as antiallergenic [13], anti-hyperlipidemic, anti-obesity [14], analgesic, anti-carcinogenic, anti-rheumatoid [15], protection of hepatic and renal tissues [16]. However, there are no reported studies using these plants in metallic phyto-nanosynthesis.

Moreover, from the same *Pedaliaceae* family are also other plants belonging to the *Josephina* genus (*Josephinia eugeni, Josephinia Africana, Josephinia celebrica*) and Linariopsis genus (*Linariopsis cheriopsis, Linariopsis prostate*), that can be found in Africa territories. These plants are currently used by aborigens in various therapeutic practices, but they have not been identified to be used in metallic nanosynthesis. *Pedalium mirex* from *Pedaliaceae* family has been reported as being a useful remedy in renal disease, by anti-lithiasic activity

[16], and as a result of its use in phyto-synthesis of cerium oxide nanoparticles important antimicrobian activity has been evidenced [17].

In specialty studies on *Devil's claw*, the anti-inflammatory effects of the phyto-compounds contained by this plant are mentioned such as: glicozide iridoides – harpagoside (esther of cinnamic acid), harpagides, phenylethanoides (verbacosides and isoverbacosides), phenols and flavonoids, and also lipids, fibers, proteins, polyphenols, carbohydrates [18]. Among other properties of this plants are the following activities: antioxidant, antimutagenic [19], hepatoprotective, hemodynamic, antimicrobian, antiinflamatory, antitumor [20], antiallergenic, antiarthritic [21]; it can be used in the therapy of degenerative diseases of bony and muscular systems; prevents osteoporosis [22], confers protection against bon losses induced by ovarictemy [23]; stimulates uterotonic spastic activity, being used in inducing/accelerating travail and placenta expulsion after birth.

The above mentioned characteristics specific for *H. procumbens*, corroborated with its presence in composition of many phytotherapeutic products and food additives in both Europe and Romania on one hand, and the absence of any research reported on the phytosynthesis of silver nanoparticles on the other hand, determined us to select this plant for metal phytosynthesis, with perspectives to use the resulted phyto-nanocompounds to obtain new products with enhanced phytotherapeutic activities, that might be further included into cosmetic products or food additives as complementary contribution to indigenous products. It is worthwhile to mention that on Romania territory there are many plants containing in their composition similar phytochemical compounds like glicozide iridoides, verbacosides, which confer antiinflamatory, antiallergenic effects. However, we were also interested to investigate the behavior during metal nanosynthesis of some plants nonspecific to European or Romanian areas, this being another factor to motivate our selection for *Harpagophytum procumbens* in realization of this study. 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.

2. Materials and methods

2.1. Materials

$AgNO_3$ was obtained from Gatt Koller-GmbH (Austria), 5-Amino-2,3-dihydro-1,4-phthalazinedione (Luminol), Tris[hydroxymethyl] aminomethane, 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox)

were obtained from Sigma Aldrich (Germany). *Harpagophytum Procumbens* root powder was obtained from the company Bionatura Plant S. R.L, Romania.

2.2. Sample preparation

2.2.1. Obtaining *Harpagophytum procumbens* extract

For obtaining the aqueous extract of *Harpagophytum procumbens* root an amount of 10 g vegetal powder has been placed into 50 mL volume of boiled water. The mixture obtained has been continuously stirred by using a VIBRAX - Milian USA, OHIO 43230 SUA system (200 rpm) and then boiled for 5 minutes. After this, the mixture has been left to cool in dark at room temperature. After cooling the extract obtained has been filtered through a Whatman no.1 paper (Fig 1a). The filtered extract, denoted HE, has been kept in the fridge.

2.2.2. Phytosynthesis of silver nanoparticles by using the *Harpagophytum procumbens* aqueous extract

For the bio-nanosynthesis of AgNPs an optimal volume of extract has been mixed (under continuous stirring, at dark and room temperature for 24 hours) with a $AgNO_3$ 1 mM solution in two volumetric ratios extract : $AgNO_{3(aq)}$ of 1:10 and 1:100 (mL/mL), resulting into two suspensions of metal nanoparticles, denoted S1 and S2, respectively. After a while, the color change of the mixture has been observed from pale-yellow to redish-brown (Fig. 1), demonstrating formation of silver nanoparticles under the action of vegetal extract of *Harpagophytum procumbens* root, which acted as a bio-reductant agent for silver ions and as stabilizer for metal nanoparticles formed.

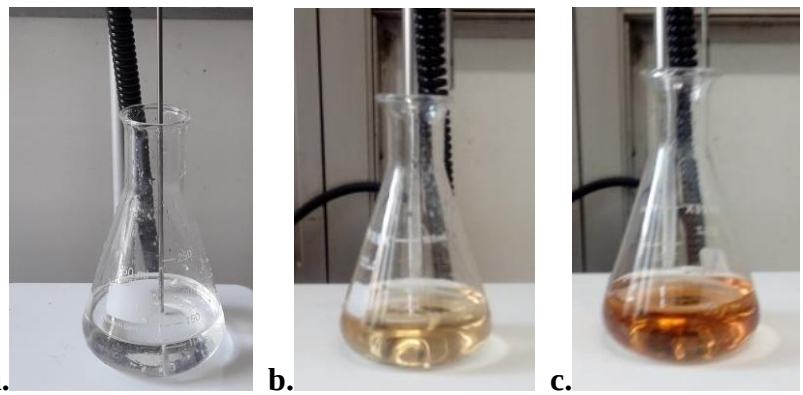


Fig. 1. Photos of the samples obtained: a) Aqueous extract of *Harpagophytum procumbens* root; b) mixture formed from 0.3 mL vegetal extract + 30 mL $AgNO_3$ 1 mM solution (at initial moment after mixing); c) suspension of AgNPs bio-synthetized (after 24 hours)

Monitorization of AgNPs synthesis has been followed by using UV-Vis absorption spectroscopy.

2.3. Characterization techniques

Characterization by UV-Vis absorption spectroscopy has been performed by using a Jasco UV 670 spetrophotometer with double beam, operating on 400-800 nm wavelength interval, at a 0.5 nm resolution.

The average size ($Z_{average}$) of biogenerated silver nanoparticles has been evaluated by means of dynamic light scattering (DLS) measurements using a Malvern Zetasizer ZS 90 (Malvern Instruments Inc., U.K.) instrument, at 25°C working temperature. Each sample has been analyzed in triplicate.

2.4. Antioxidant activity

2.4.1. Antioxidant activity by chemiluminescence method

The *in vitro* evaluation of the antioxidant activity (AA%) of AgNPs by the chemiluminescence method used a Turner Design TD 20/20 chemiluminometer (Sunnyvale, California, USA) and a chemiluminescent system composed by luminol (10^{-5}M) - H_2O_2 (10^{-5} M), buffer solution Tris-HCl at pH = 8.6. The antioxidant activity calculated by equation 1 of silver nanoparticles was compared to that of plant extract:

$$AA\,\% = \frac{I_0 - I_s}{I_0}.100 \quad (1)$$

where I_0 is the maximum chemiluminescence for the standards at t = 5 s and I_s is the maximum chemiluminescence for the samples at t = 5 s.

2.4.2. The antioxidant activity measured by ABTS method

The antioxidant capacity of the samples was also evaluated by ABTS method, using the UV–Vis-Nir Spectrophotometer type V670 Jasco, (Japan). The cation radicals $ABTS^{\bullet+}$ resulted by reaction of ABTS (2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) solution with potassium persulfate solution (dark conditions for 16 h, at 25°C). A volume of 3 mL $ABTS^{\bullet+}$ solution was added into 1 mL vegetal extract/silver nanoparticles solution and then distilled water was added until the volume was 5 mL. All determinations were carried out against a blank sample prepared with 3 mL ABTS solution and 2mL of distilled water. The percentage of ABTS inhibition was calculated by following equation:

$$\%\, Inhibition\ ABTS^{+\cdot} = \frac{A_o - A_s}{A_o} \text{x} 100 \quad (2)$$

A_o = absorbance of the blank (unscavenged radical cation solution);

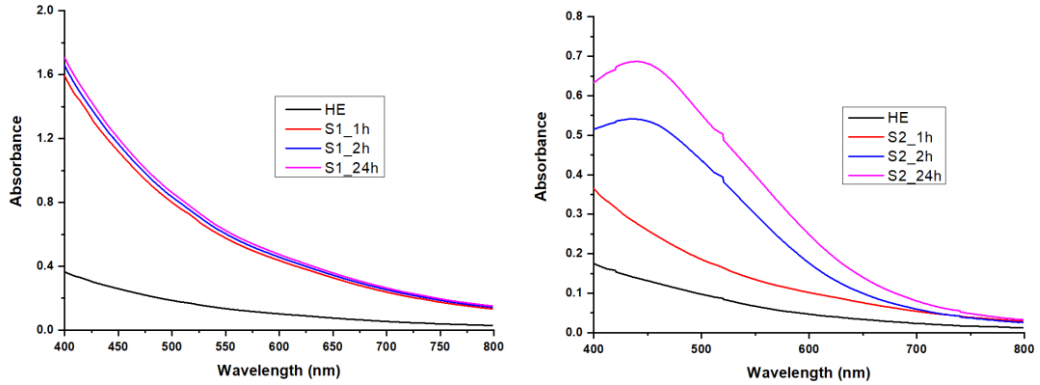
A_s = absorbance after the addition of the vegetal extract/AgNPs samples.

The experimental results were recorded at 4 minutes for each solution, by measuring their absorbance.

3. Results and discussion

3.1. Spectral monitoring of silver nanoparticle formation

Fig. 2 illustrates the evolution in time of metal nanoparticles suspensions prepared using two volumetric ratios extract: $AgNO_{3(aq)}$ of 1:10 and 1: 100 (mL/mL).

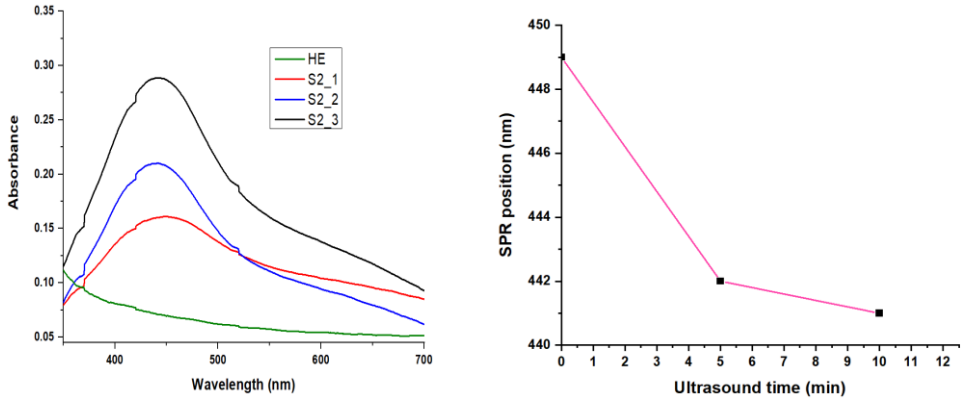


a) b)

Fig. 2. Time evolution of metallic nanoparticles suspensions by using two volumetric ratios of extract: $AgNO_{3(aq)}$: a) 1:10 (mL/mL) and b) 1:100 (mL/mL)

One can be noticed 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 silver nanoparticles obtaining. Thus, the AgNPs suspension has been centrifuged for 30 minutes, at 4^{o}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). Fig. 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 (Fig. 4). 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.



a) b)

Fig. 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

One may conclude that the optimal sonication time is of 5 min, a longer time producing insignificant effects.

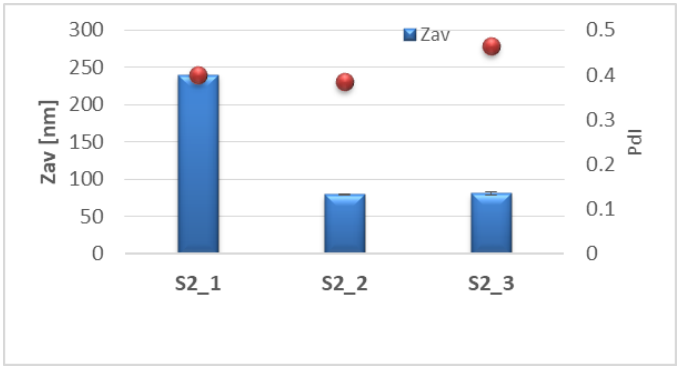


Fig. 4. Nanoparticles size of samples S2 by DLS measurements

3.2. Estimation of antioxidant properties of silver 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 (Fig. 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.

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. 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).

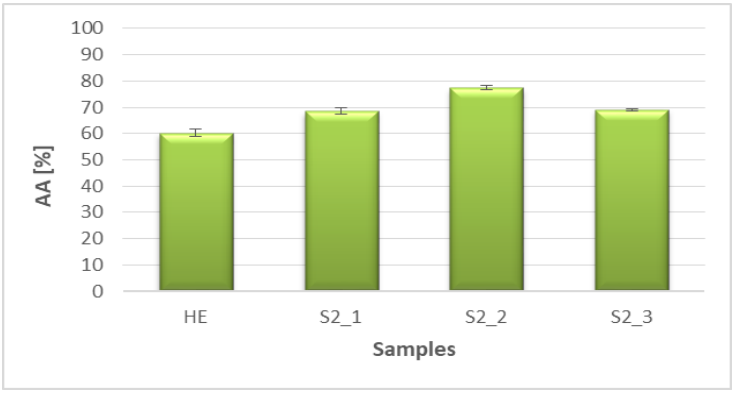


Fig. 5. Evaluation of antioxidant activity by chemiluminescence

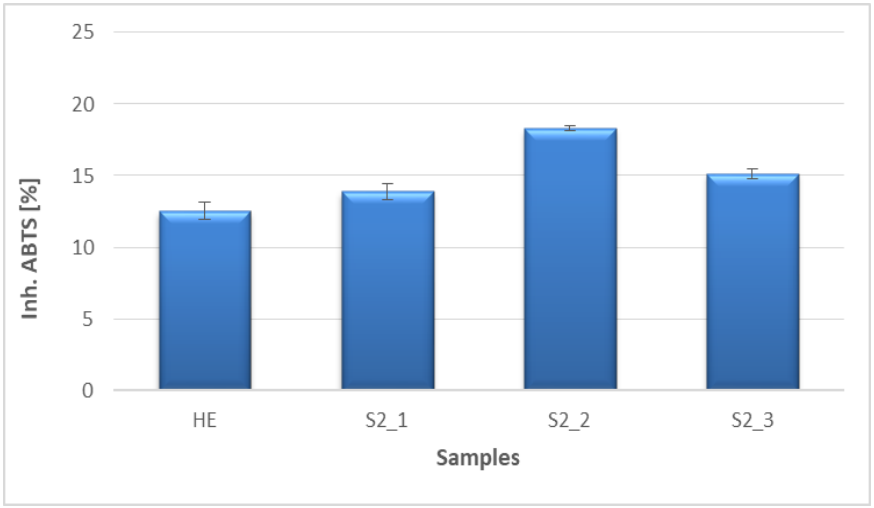


Fig. 6. Evaluation of antioxidant activity by ABTS method

6. 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.

R E F E R E N C E S

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