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CHARACTERIZATION OF SILVER NANOPARTICLES OBTAINED BY *LAVANDULA ANGUSTIFOLIA* EXTRACT

Ioana Raluca BUNGHEZ,[a] Radu Claudiu FIERASCU,[a] Ovidiu DUMITRESCU,[b]
Irina FIERASCU[a,*] and Rodica Mariana ION[a]

[a] National R&D Institute for Chemistry and Petrochemistry – ICECHIM, 202 Spl. Independentei, Bucharest, 060021, Roumania
[b] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Polizu Str., Bucharest, 011061, Roumania

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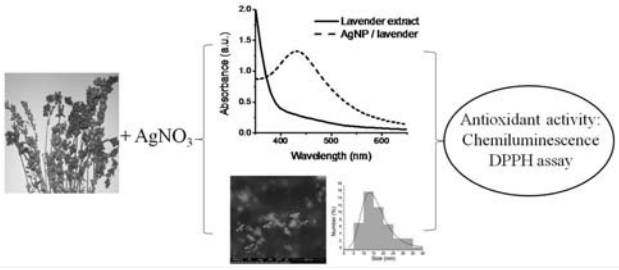
The nanotechnology is an area of outmost importance for researchers worldwide. In the present paper is presented a **green** and **eco-friendly** method for obtaining nanoparticles in presence of plants extracts. This study presents the use of lavender flowers (well-known for their use in homeopathic applications, due to antioxidant and antifungal properties) for the biosynthesis of silver nanoparticles with enhanced antioxidant properties. The formation and characterization of silver nanoparticles (AgNPs) in lavender extract was confirmed by UV-Vis spectroscopy, X-ray fluorescence (XRF) and scanning electron microscopy (SEM). The antioxidant activity was carried out using two methods: chemiluminescence and DPPH assay.



INTRODUCTION

Lavender is a heavily branched short shrub, growing to approx. 60 cm, original to the Mediterranean mountain zone, encountered today all over the world. Plant or essential oils extracted from *Lavandula angustifolia* Mill. have been used, for long time ago in a lot of areas, for medicinal purposes, fragrances or pharmaceutical industries therapeutically properties, such as anticonvulsive, antiseptic, anti-inflammatory, antidepressant, antiviral and antibacterial activity.[1,2] In our days, aromatherapy and alternative medicine use lavender essential oil due to its clinical benefits on the central nervous system.[2]

One of the most important properties of lavender extract is its antioxidant activity, which has attracted the interest of many scientific papers,[3-7] and also will be discussed in our research.

The synthesis of metal nanoparticles is widely discussed in the scientific literature due to their properties (small sizes, large surface area, unique physical and chemical properties), which have many potential applications.[8-10]

In this paper, a green synthesis route was followed, in order to obtain silver nanoparticles with possible utilization for medical devices, like antimicrobial plasters.

MATERIAL AND METHODS

1. Material

Silver nitrate (10^{-3} M $AgNO_3$), TRIS (tris(hydroxymethyl)-aminomethan, ≥99.5) and HCl (37%) (0.2 M, pH 8.6), luminol (5-amino-2,3

* Corresponding author: dumitriu.irina@yahoo.com

dihydrophthalazine-1,4-dione) and hydrogen peroxide (H_2O_2) were purchased from Merck (Germany). DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical) was supplied by Sigma-Aldrich (Germany) and ethanol (C_2H_6O) by Scharlau (Spain). Bidistilled water was obtained in our laboratory, using GFL 2102 water still. *Lavandula angustifolia* was purchased from Bucharest, from a commercial source.

2. Methods

2.1. Preparation of lavender extract

Lavandula angustifolia flowers were dried at room temperature to constant mass, in order to remove excess water. After that, 1 g of dried flowers was transferred into a 25 mL Berzelius flask containing bidistilled water and boiled for 10 minutes in order to release the intracellular material into solution. The lavender aqueous extract obtained, was cooled and filtered through a filter paper to obtain a clear pink extract.

Fig. 1 – Lavender flowers.

2.2. Synthesis of silver nanoparticles

An aqueous solution of silver nitrate (10^{-3} M) was prepared and used for the synthesis of silver nanoparticles.[11] For the nanoparticle synthesis, 20 mL of lavender aqueous extract was mixed with 20 mL of $AgNO_3$ aqueous solution and ultrasounds irradiated (40 kHz) in ultrasound bath at 30°C, for 10 minutes.

2.3. Characterization analytical methods

The absorption spectra of the plant extracts and of the silver nanoparticles were obtained using a M400 Carl Zeiss Jena UV-VIS spectrophotometer, in the wavelength range of 350-650 nm. The energy-dispersive X-ray fluorescence (EDXRF) determinations have been carried out in Helium atmosphere, for a period of 300 seconds, without any filter, at 20 kV and proper current intensity. The apparatus used was a PW4025 – MiniPal – PANalytical type spectrometer with a Si(PIN) detector. For Scanning Electron Microscopy (SEM) determinations was used a Quanta FEI 200 microscope. Analytical results were processed using professional data analysis software (Origin 8.0 Pro).

2.4. Antioxidant activity

Chemiluminiscence method

The chemiluminescence (CL) was measured on a Turner Biosystems Modulus. For the assay, the sample was mixed in TRIS-HCl buffer (0.2 M, pH 8.6), with hydrogen peroxide (5 mM) and luminol (8 mM). The buffer, TRIS-HCl was obtained in the laboratory. The antioxidant activity of each sample was calculated using the mathematical expression:[12,13]

$$AA\ (\%) = [(I_0 - I)/I_0] \times 100$$

where: I_0 is the maximum CL intensity for blank and I is the maximum CL intensity for sample at t = 5 s after the initiation of the reaction.

DPPH method

For the DPPH reduction assay, 0.5mL of lavender extract was mixed with 1 mL of 0.02 mg/mL DPPH solution. After that, the mixtures were tested by reading the absorbance at 517 nm. As a blank, it was prepared 0.5 mL of bidistilled water with 1 mL of 0.02mg/mL DPPH solution, which was read at the same wavelength.[14-16]

The antioxidant activity (AA %) percentage was calculated using the formula:

$$AA\% = [(A_C - A_S)/ A_C] \times 100$$

where: A_C is the absorbance of a DPPH solution without sample and A_S is the absorbance of the sample mixed with 0.02 mg/mL DPPH solution.

RESULTS AND DISCUSSION

Reduction of silver ions was first observed visually. It was observed the change of colour extract after the $AgNO_3$ it was added (Fig. 2).

The reduction of silver metal ions to silver nanoparticles was analyzed by UV-Vis spectrometry technique, between 350-650 nm wavelengths.[17] The

reduction of silver ions in the aqueous solution of AgNP-lavender extract, presented a strong absorption at 435 nm as shown in Fig. 3.

X-ray fluorescence analysis, was used to identify the silver presence in the AgNP-lavender extract sample (Fig. 4).

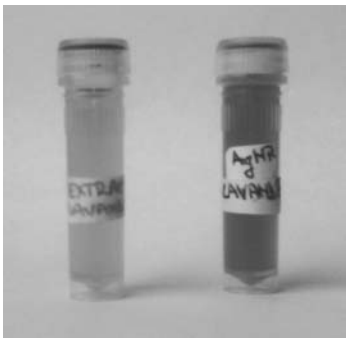


Fig. 2 – Lavender extract and AgNP-lavender colours.

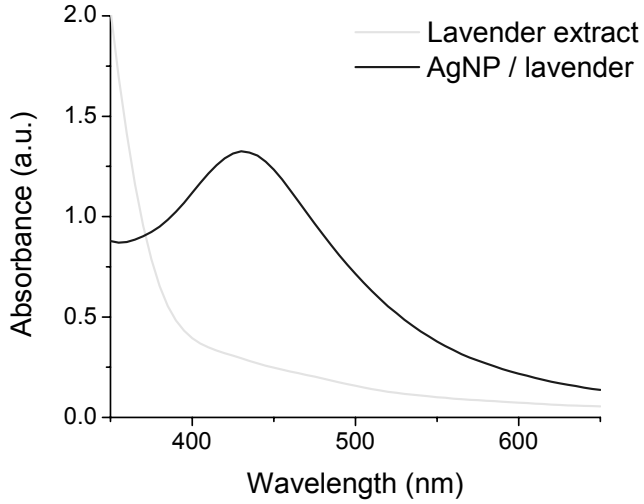


Fig. 3 – UV-VIS spectrum of lavender extract and AgNP-lavender.

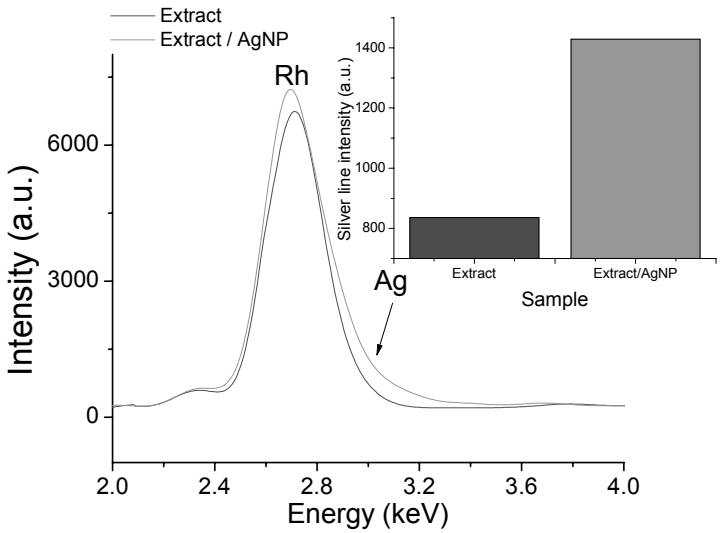
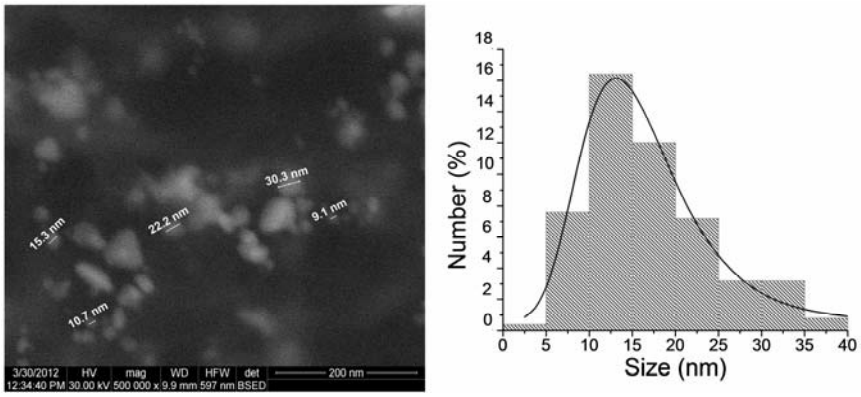


Fig. 4 – EDXRF spectrum of the two samples in the region of interest (2-4 keV).
Inset –intensity of the specific bands of silver for the extract and for AgNP/extract.

Table 1

Antioxidant activity results obtained by two methods

Method/Sample	CL	DPPH
Lavender extract	88.66	86.693
Lavender-AgNP	98.86	88.466



A **B**

Fig. 5 – SEM micrograph of AgNP-lavender (A) and size distribution of the obtained nanoparticles (B).

The samples (lavender extract and AgNP-extract) antioxidant activity was tested using the two methods (CL-chemiluminescence and DPPH) described in the Materials and Methods chapter; the samples exhibited strong antioxidant properties ranging between 86.69 and 88.66% for plant extract and 88.46 and 98.86%, for silver nanoparticles.

The differences between the results are most probably due to different reaction mechanisms, and different time periods at which the antioxidant activity is estimated.

The solution mixture was ultrasonicated for 20 min and then was characterized by SEM in order to establish the morphological nature of the nanoparticles.

The morphology details, size and shape of AgNP-lavender extract were certified by using Scanning Electron Microscopy. The SEM image (Fig. 5A) showed a high density of silver nanoparticles synthesized by using *Lavandula angustifolia* flower extract. It was observed the development of silver nanostructures uniform in size and shape with a narrow size distribution (Fig. 5B).

CONCLUSIONS

An aqueous lavender plant extract solution was used to synthesize and obtain silver nanoparticles. *Lavandula angustifolia* Mill. plant extract demonstrated to have reducing ability to obtain an *"eco-friendly"* new method of silver nanoparticles. The color of AgNP-lavender extract solution became brown and presented an intense absorption band at 430 nm. SEM analysis of AgNP-lavender extract sample presented the formation of spherical silver nanoparticles between 10-30 nm diameters. Also, X-ray fluorescence analysis demonstrated the presence of silver in the AgNP-lavender extract sample.

In order to check the antioxidant properties, the samples *Lavandula angustifolia* extract and lavender-silver nanoparticles were subjected to an oxidative stress simulated *in vitro* using the chemiluminescence (CL) and DPPH assay, which confirmed the fact that AgNP-extract has a higher antioxidant activity than the lavender extract.

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