CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF PHYTOSYNTHESISED SILVER NANOPARTICLES USING *CALENDULA OFFICINALIS* EXTRACT

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Abstract

The present paper describes an eco-friendly bottom-up approach to design metallic nanoparticles with biomedical potential. The aim of the study was to characterize in terms of antioxidant activity the silver nanoparticles obtained using *Calendula officinalis* petals extract. First of all, a visual inspection confirmed the formation of silver nanostructures by colour changing of the sample. The bioreduction of silver ions was analyzed by modern analytical techniques (UV–VIS, FTIR (Fourier transformed IR spectroscopy), XRF (energy-dispersive X-ray fluorescence) spectroscopy and SEM (Scanning Electron Microscopy)). Antioxidant activity of the silver nanostructures was evaluated using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method. The antioxidant activity of the phytosynthesised nanoparticles proved to be superior to the results of the extract.

Rezumat

Lucrarea descrie o abordare *eco-friendly*, de tip *bottom-up*, pentru obținerea nanoparticulelor metalice. Scopul acestui studiu a fost de a caracteriza din punct de vedere al activității antioxidante nanoparticulele de argint obținute, folosind extract de petale de *Calendula officinalis*. În primul rând, vizual s-a confirmat formarea de nanostructuri de argint prin schimbarea culorii probei. Bioreducerea ionilor de argint a fost analizată prin tehnici analitice moderne (UV-VIS, FTIR (spectroscopie IR u transofrmata Fourier), XRF (*energy-dispersive X-ray fluorescence*) și SEM (microscopie electronică de baleiaj)). Activitatea antioxidantă a nanostructurilor de argint a fost evaluată prin metoda DPPH (2,2-difenil-1-picril-hidrazil-hidrat). Activitatea antioxidantă a nanoparticulelor fitosintetizate s-a dovedit a fi superioară celei obținute în cazul extractului.

Keywords: *Calendula officinalis*, Silver nanoparticles, green synthesis, Antioxidant activity.

Introduction

From ancient times, plants have been used intuitive for medicinal purposes. Many of them show high antioxidant activity and are used to treat various diseases [1, 2]. A large number of plants have been investigated and various species have been reported to exhibit antioxidant activity, including *Asteraceae, Pteridophitae* and so on [3-5].

Marigold flower (*Calendula officinalis*), belonging to the *Asteraceae* family, is a medicinal plant which contains oleanolic acid and other compounds, which present considerable potential health benefits, protective effects against the development of cancer, chemotherapy and radiation therapy adverse effects, inhibition of existing tumour cells, anti-inflammatory activity, antioxidant activity, cardiovascular protective and antiviral effects [6, 7].

Calendula officinalis is a good source of natural antioxidants, which contains many different radical scavenger components providing protection against harmful-free radicals and so is associated with lower incidence and mortality rates of cancer and heart diseases in addition to a number of other health benefits [6]. It is used for a long time for the treatment of inflammation and skin wounds [6, 7].

The phytosynthesis of silver nanoparticles (AgNP) using *Calendula officinalis* extract was firstly observed by visual inspection (appearance of a yellow colour), confirmed by modern spectroscopic techniques and the nanoparticles/extract complex was characterized in terms of antioxidant activity.

Materials and Methods

Materials

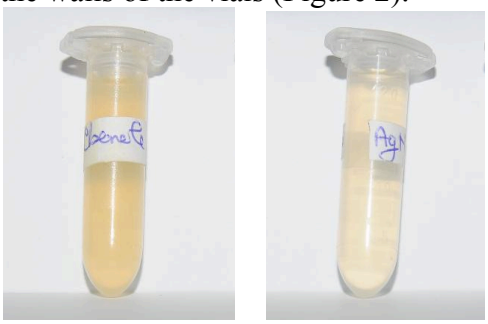
Silver nitrate ($AgNO_3$) was purchased from Merck (Germany), 2,2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical (DPPH) was supplied by Sigma-Aldrich and ethanol by Scharlau. The hydroquinone was purchased from Merck and bidistilled water obtained in our laboratory used was throughout the experiment. Marigold flowers were harvested from the natural environment, as they are part of spontaneous native flora.

Methods

The marigold (Figure 1) petals were exhaustively extracted in ethanol for 24 hours at room temperature, and the macerate was kept in a brown volumetric flask at 6ºC, in order to avoid degradation.

Figure 1
Marigold flower

The AgNP/marigold were prepared by mixing 1 mL of marigold extract with 5 mL of aqueous solution of 1 mM $AgNO_3$ and kept overnight at room temperature. Visually, the formation of silver nanoparticles was evidenced by changes occurred in the mixture colour after addition of $AgNO_3$, from yellow opaque to yellow transparent due to excitation of surface plasmon vibrations in the metal nanoparticles. It was also observed a mirror like film on the walls of the vials (Figure 2).



a) *b)*

Figure 2
Samples of: a) marigold extract, b) AgNP- marigold

Characterization Methods

The absorption spectra of the samples were recorded using a double beam M400 Carl Zeiss Jena UV-VIS spectrophotometer from 400 to 800 nm, at the resolution of 1 nm, with 1 nm slit width and 0.3 nm/s scan rate.

Fourier transformed IR spectroscopy (FTIR) spectra were collected using a Perkin Elmer Spectrum GX instrument. Scans in the range of 4000–600 cm^{-1} were recorded for each spectrum at a spectral resolution of 4 cm^{-1}.

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 rhodium anode.

For Scanning Electron Microscopy (SEM) determinations, a Quanta FEI 200 microscope was used.

In order to establish the antioxidant activity of the studied samples, their inhibitory effect against free radicals was evaluated using the DPPH method [8].

For experimental procedure, 0.5mL of each sample 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 on a UV-VIS Specord M 42 spectrophotometer. As a blank, it was used a solution prepared by mixing 0.5 mL of bidistilated water with 1 mL of 0.02mg/mL DPPH solution and reading at the same wavelength. The antioxidant activity (AA%) percentage was calculated using the formula [8]:

$$AA\% = [(A_{Control} - A_{Sample} / A_{Control}] \times 100$$

where: $A_{Control}$ is the absorbance of a DPPH solution without sample, A_{Sample} is the absorbance of the sample mixed with 0.02 mg/mL DPPH solution.

Results and Discussion

The phytosynthesis of silver nanoparticles was confirmed by modern analytical techniques (UV-VIS, FTIR, EDXRF, SEM). The marigold extract was used as a reducing agent for Ag as well as a capping agent for silver nanoparticles [9].

In the obtained AgNP marigold sample X-ray fluorescence technique was performed in order to identify the silver existence.

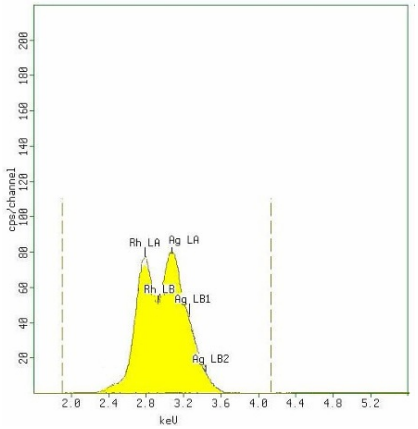


Figure 3
XRF spectrum of AgNP-marigold extract sample

The reduction of the Ag^+ ions was detected by UV-VIS spectrometry for marigold extract and AgNP-marigold samples (Figure 4). One of the

main characteristics of silver nanoparticles is the extraordinary efficiency to absorb and scatter light; unlike many other dyes and pigments, the silver nanoparticle solution have a colour that strongly depends upon the size and the shape of the particles. Thus, UV-VIS spectroscopy is a very useful tool for evaluating the physical state of the nanoparticles [10].

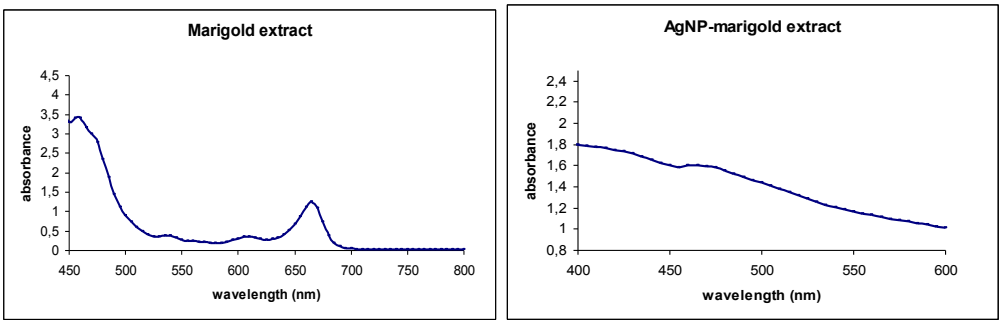


Figure 4

The UV–VIS absorption spectra of the a) *Calendula officinalis* extract (left) and b) AgNP-*Calendula officinalis* (right)

All types of marigold contain a lot of carotenoids. In our marigold extract sample, it was observed, using a spectrophotometric method, peaks specific to carotenoid pigments, at 446-450 nm and chlorophylls, at 648-655 nm (Figure 4, left). [6, 11]. The AgNP-marigold sample was measured between 400-800 nm and it was observed the specific peak of silver nanoparticles, at 465 nm (Figure 4, right) [12 - 14]. The presence of the absorbance peak at 465 nm is a clear indicator for the formation of nanoparticles with dimensions around 65 nm [10].

This conclusion was confirmed by the SEM analysis, presented in figure 5.

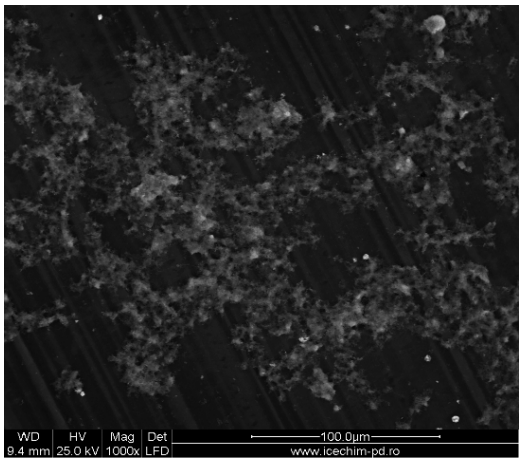


Figure 5

SEM analysis of the AgNP-marigold samples

FTIR spectroscopy was used to identify the possible molecules responsible for obtaining the silver nanoparticles synthesized by the marigold extract (Figure 6). In order to obtain FTIR spectrum of marigold extract and AgNP marigold extract samples, the spectra were recorded in the region 4000 - 600 cm^{-1}.

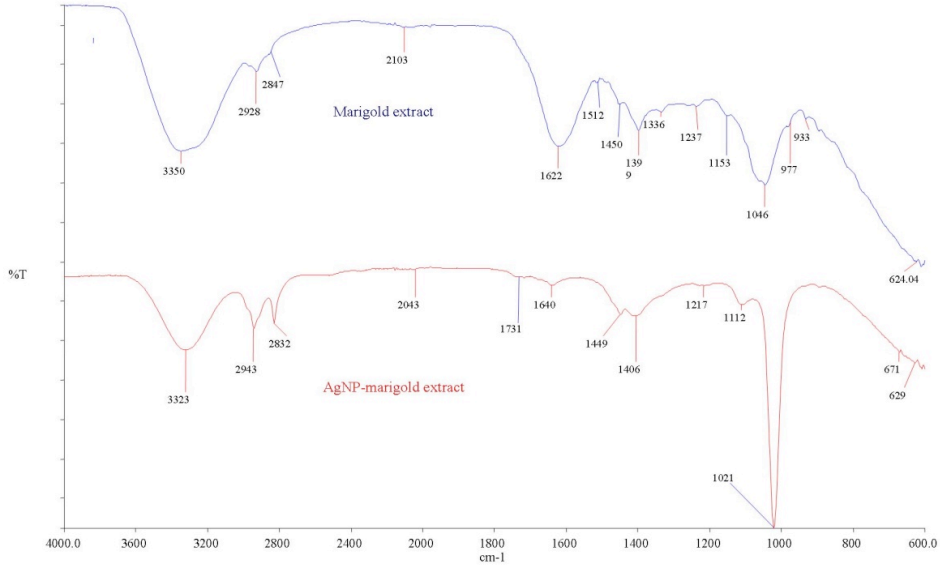


Figure 6
FTIR spectra of marigold extract and AgNP-marigold samples

Some common peaks were observed for both samples, which shows that metal nanoparticles have occurred in the structure of the plant. The bands at 3350 cm^{-1} (for the range of marigold extract) and at 3323 cm^{-1} (for the range of AgNP-marigold) are assigned to OH groups. At 2928 cm^{-1} in the spectrum of marigold extract and 2943 cm^{-1} in the spectrum of AgNP-marigold, exist alkyl and CH groups. At 1622 cm^{-1} (marigold extract) and 1640 cm^{-1} (AgNP-marigold) there are amides of type I. The band 1406 cm^{-1} (AgNP-marigold) is specific to silver. Bands of the 1237 cm^{-1} (marigold extract) and 1217 cm^{-1}(AgNP-marigold) are attributed to amide type III.

Several intense bands (2928 cm^{-1}, 2847 cm^{-1}, 1622 cm^{-1}, 1450 cm^{-1}, 1399cm^{-1}, 1046 cm^{-1}) in marigold have been observed and discussed using literature data [6, 15, 16]. At 1153 cm^{-1} are situated ether bonds. Registered spectra shown a higher intensity band near 1450-1399 cm^{-1} that corresponds to higher amounts of β-carotene pigment (1450 $cm^{-1}$with a near band at 1399 cm^{-1}), at 1622 cm^{-1} band corresponding for some chlorophylls, which are reflected in the colour of this flower. These pigments were confirmed by

UV-VIS analysis, which means that these pigments prevail in *Calendula officinalis*.

The bands observed at 1021 and 840 cm^{-1} present in the AgNP-marigold spectrum are specific for silver and as expected it weren't found in marigold plant extract.

Antioxidant activity was assessed using the DPPH method. The antioxidant activity of these extracts was compared with standard solutions of hydroquinone. The experiments were performed in triplicate. The results are given as mean value. DPPH produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured ethanol solutions. The use of DPPH provides an easy way to evaluate the antioxidant potential.

The samples presented a higher antioxidant activity than other medicinal plants widely used in alternative medicine presented by the literature data [17-19].

Sample	AA%
Marigold extract	80.05
AgNP-Marigold extract	84.27

Conclusions

Marigold extract was used to phytosynthesize silver nanoparticles. Marigold extract proved to have strong reducing power for "green synthesis" of silver nanoparticles. The phytosynthesis of marigold-AgNPs was firstly observed by visual inspection by appearance of a different colour from that of marigold extract, a yellow transparent colour which was confirmed by analytic studies (EDXRF, UV-VIS, FTIR, SEM) that revealed the presence of the carotenoids and chlorophylls in the plant extract, bioactive compounds responsible for the reduction of silver ions and for the stabilization of AgNPs.

It is obvious that the silver nanoparticle-marigold extract could be used as a good natural source of antioxidants due to the high antioxidant activity and can be used in the development of valuable products for biomedical or cosmetic purposes.

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