Silver Nanoparticles Obtained via *Morus Nigra* Extract
Synthesis and antioxidant activity

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The fruits of mulberry (Morus nigra) are used in herbal therapies due to their tonic and depurative properties. They are used to treat lung and throat diseases, thrush, diabetes, gastric and duodenal ulcers. Some research studies have revealed high content of beneficial acids (phenolic, ascorbic - vitamin C), antioxidant and anti-inflammatory properties of mulberry fruits. In the present paper, we have developed a simple green and economical method for synthesis of silver nanoparticles (AgNPs) using this type of fruits. The analytical techniques (UV-Vis, SEM, DLS and FTIR) studies suggested that the mulberry fruits have played an important role in the reduction and stabilization of silver nanoparticles. Using UV-Vis method, it was observed an intense peak at 465 nm specific for silver nanoparticles. SEM revealed the formation of spherical nanoparticles with size less than 40 nm, after ultrasonication. DLS method was used to determine the size of particles and FTIR spectra indicate the appearance of peaks in the amide I and II regions (characteristic to proteins), which have been found to be possible responsible for capping and efficient stabilization of the metal nanoparticles synthesized by the mulberry fruit. The eco-friendly silver nanoparticles presented strong antioxidant properties, so AgNP-mulberry sample (AA%=93.23) has a higher activity than mulberry extract sample (AA%=88.25).

Keywords: green chemistry, silver nanoparticles, Morus Nigra, antioxidant activity

A lot of methods used for nanoparticle synthesis involve the use of hazardous chemicals, so, it is necessary to develop a new environmentally friendly, low cost nanoparticles synthesis and production methods. Actually, it is very important to create "*green chemistry*" methods, without using toxic chemicals [1].

Also, the synthesis of noble metal nanoparticles (Au, Ag, Pt) [2 - 4] is a beneficial research area due to the potential applications for the development of new technologies, having antioxidant and antimicrobial properties [2, 3]. It is known the fact that silver nanoparticles have important properties which help in molecular diagnostics, in therapies that are used in several medical procedures, taking into account that the silver metal has proved antibacterial activity, good conductivity or chemical stability [5].

As AgNP form, silver presented potential applications in different types of areas (medicine, cosmetics, pharmaceuticals, environmental or biomedical devices) [6]. Biomolecules existent in extracts are used to reduce metal ions to nanoparticles in a single-step eco friendly synthesis process [7].

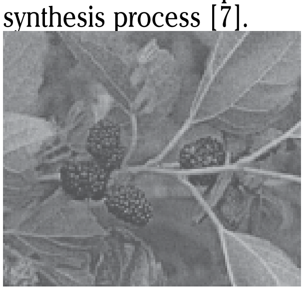

Fig. 1 Mulberry fruits (Akl M. Awwad, Nida M. Salem, 2012)

Mulberry (*Morus Nigra*) fruits are an important source of natural antioxidants which are fighting against cancer and heart diseases and have a great contribution for a number of other health benefits. A lot of studies have been published about antioxidant activity, physical and chemical properties of these fruits, reporting important remedies for asthma, cold, cough, diarrhea, dyspepsia, edema, fever, headache, hypertension or wounds [8-10].

Antioxidant activity of mulberry is attributed to phenolic compounds (anthocyanins: cyanidine-3-glucoside, cyanidine-3-rutinoside, cyanidine-3-sophoroside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside) present in fruits, which have a very important inhibitory effect on migration and invasion of lung cancer cells [11, 12].

The synthesis of mulberry silver nanoparticles was observed firstly by visual inspection, which was confirmed by change of sample color (from yellow to orange-brown) and then confirmed by UV-Vis spectroscopy. DLS (Dynamic light scattering) analysis was used to provide information about the morphology of the synthesized silver nanoparticles. FTIR (Fourier Transform Infrared) spectroscopy was used to measure the particle formation and to identity the elements from the samples and to study the nature of surface adsorbents in nanoparticles. The morphology like size and shape of the fruit silver nanoparticles was rated by SEM (Scanning Electron Microscopy) that presented the formation of spherical silver nanoparticles with size less than 40 nm, after ultrasonication.

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Experimental part

The silver nitrate ($AgNO_3$) and hydroquinone were purchased from Merck (Germany), 2,2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical (DPPH) was supplied by Sigma-Aldrich and ethanol by Scharlau. It was used distilled water, obtained in our laboratory. Mulberry fruits were harvested from nature, as they are part of spontaneous native flora.

Synthesis of Morus Nigra Silver Nanoparticles

Morus Nigra fruits were washed and dried at room temperature. Then 50 g of these fruits were placed into an Erlenmeyer flask with 250 mL of distilled water and boiled for 5 min in order to release the intracellular material into solution. The aqueous extract thus obtained were filtered through a filter paper to obtain a clear yellow extract.

The AgNP-mulberry sample were obtained by mixing 5 mL of fruits extract with 5 mL of aqueous solution of 1 mM $AgNO_3$ and kept over night 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 to orange-brown, due to excitation of surface plasmon vibrations in the metal nanoparticles.

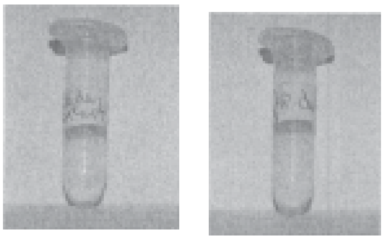


Fig. 2. Extract colours of: a) mulberry extract, b) AgNP-mulberry

Characterization Methods

The absorption spectra of the samples were carried out using a M42 Carl Zeiss Jena UV-VIS spectrophotometer from 200 to 600 nm wavelength.

Fourier transformed IR spectroscopy (FTIR) spectra were performed using a Perkin Elmer Spectrum GX instrument with Attenuated Total Reflectance (ATR). The scans were in the range of 400–4000 cm^{-1}.

Scanning Electron Microscopy (SEM) morphology (size and shape) of the fruit silver nanoparticles was investigated using a FEI Quanta 200 SEM in a low vacuum working mode, with a resolution of 1.2 nm. One drop of each sample was placed on an aluminum support and left to dry overnight. SEM images were recorded using a secondary electron detector.

The physical stability of samples for zeta potential (ξ-potential) was collected using a Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.) by measuring the electrophoretic mobility of the samples in an electric field.

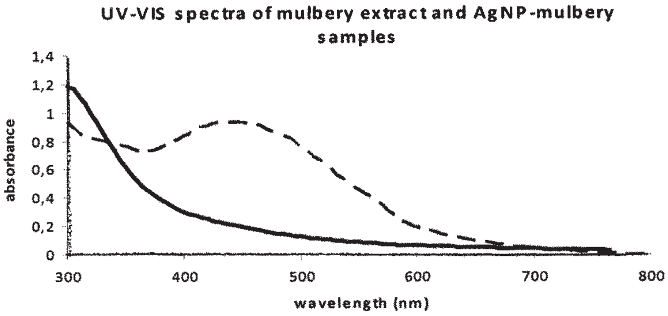


Fig. 3. The UV–VIS absorption spectra of the —— mulberry extract and - - - AgNPs-mulberry sample

The antioxidant activity (AA%) of the extracts was evaluated spectrophotometrically using the DPPH method represented by other articles research [13].

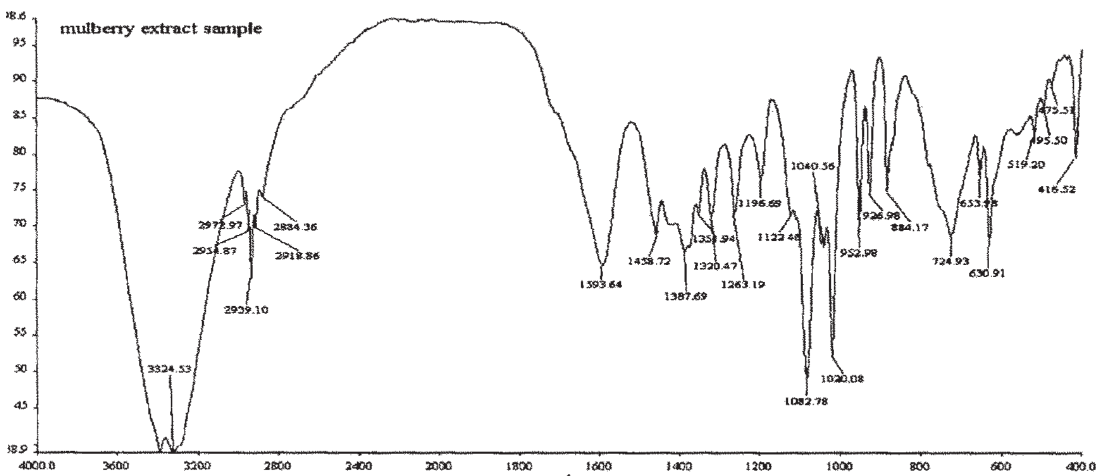
Results and discussions

The silver nanoparticles synthesis was confirmed using UV-Vis and ATR-FTIR analyses. The aqueous mulberry fruit extract was used as a reducing agent for Ag^+ as well as a capping agent for silver nanoparticles.

The absorption bands between wavelengths of 300–350 nm at mulberry extract sample presented specific peaks of phenolic acids and flavonoids [12], while the UV-Vis absorption spectrum of *Morus Nigra*-AgNP extract sample (fig. 2) was observed at 465 nm [14].

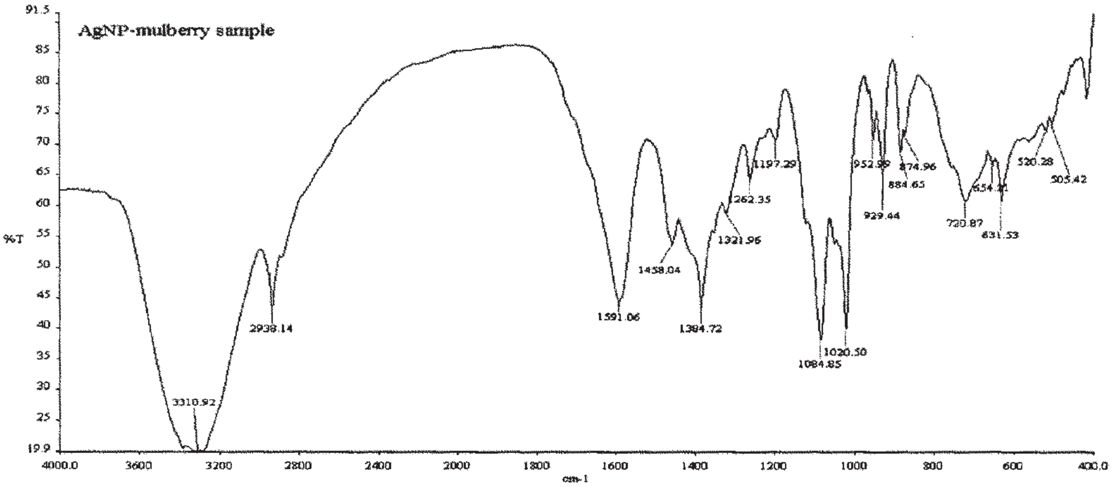
FTIR spectra (fig. 4,a) are specific for mulberry extract, due to strong bands at 3325 cm^{-1} assigned to hydroxyl groups. The band from 2940 cm^{-1} is characteristic to methine groups CH. The bands C = C and C = O could be identified in the spectra in the 1594-1458 cm^{-1} region. The aromatic group of the amide of type I and II are identified in the region between 1387 and 1320 cm^{-1}. The CO groups, esters, hidroxiflavones, catechins and amides of type III are found in the 1263-1122 cm^{-1}, and the aliphatic amine functional groups are visible in the 1083-1020 cm^{-1} region [15-17].

Bands (fig. 4,b) between 1500-1300 cm^{-1} region, could be attributted to amides, proteins, enzymes which are responsible for the reduction of metal ions who occurs due nanoparticles obtained in fruits extract. FTIR spectrum of AgNP-mulberry sample revealed strong interactions with the fruit extract during their synthesis. Some IR bands, common to mulberry extract, appeared in AgNP-mulberry, but the transmittance level of the fruit bands was weakened after interaction with silver nanoparticles and shifted to: 3311 cm^{-1} (O–H stretching), 2938 cm^{-1} (alkyl and CH groups), 1591 cm^{-1} (assigned to amide I, arising due to carbonyl stretch in proteins), 1262 cm^{-1} (amide III,



a) mulbery extract

Fig. 4a. ATR-FTIR spectra of mulberry extract



b) AgNP- mulberry samples

Fig. 4b. ATR-FTIR spectra of AgNP-mulberry samples

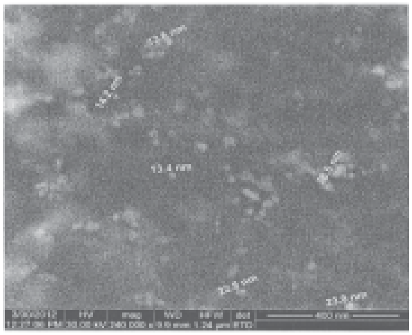


Fig. 5. SEM micrograph for AgNPs obtained using mulberry extract

Table 1
ANTIOXIDANT ACTIVITY OF MULBERRY EXTRACT AND AgNP-MULBERRY

Sample	AA%
Mulberry extract	88.25
AgNP- mulberry	93.23

C-O bending: esters, hidroxiflavones, catechines), at 1197 cm^{-1} are situated ether linkages. So, ATR-FTIR results certified the fact that composition of mulberry extract (proteins and polyphenols) is responsible for silver reduction [2, 18].

The morphological aspects of these silver nanoparticles obtained in presence of mulberry fruits were realized by SEM analysis. Using this technique, it was confirmed the synthesis of nanoscaled silver particles and detailed important morphological details about mulberry-AgNP sample. SEM micrograph was made after the sample was ultrasonicated. After that, the nanoparticles presented values between 13-40 nm.

The average diameter of these silver nanoparticles was more than 100 nm, with a polydispersity index PDI = 0.587, which indicates a population of nanoparticles uniform. Also, zeta potential (ξ-potential) is 29.2 nm which showed good physical stability of the sample.

The antioxidant activity of mulberry extract and AgNP-mulberry samples were measured using the DPPH method and compared with standard solutions of hydroquinone. DPPH solution has become violet in ethanol, which is reduced in the presence of an antioxidant molecule, giving rise to uncoloured ethanol solutions. The DPPH method provides an easy modality to evaluate the antioxidant activity. For doing the experimental part, it was mixed 0.5mL of each sample with 1 mL of 0.02 mg/mL DPPH solution. Then, the mixtures were tested by reading the absorbance at 517 nm using a UV-Vis Specord M 42 spectrophotometer. For the blank sample, it was used a solution prepared by mixing 0.5 mL of distilled water with 1 mL of 0.02mg/mL DPPH solution and reading at the same wavelength. The antioxidant activity (AA %) percentage it was calculated using this formula:

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

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.

The AgNP-mulberry sample has a higher antioxidant activity than mulberry extract sample. This fact confirms the good utilization of silver in alternative medicine or future pharmaceutical analysis [14].

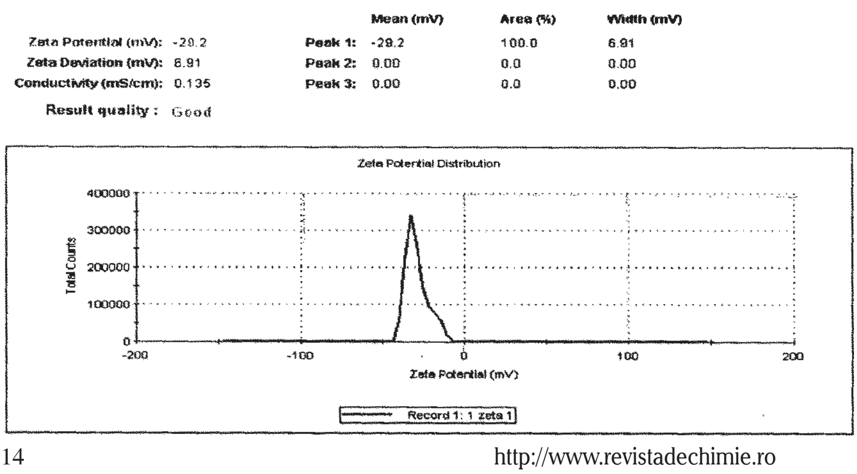


Fig. 6. Zeta potential analysis of AgNP-mulberry sample

Conclusions

An aqueous mulberry fruit extract was used to synthesize silver nanoparticles and it was proved to have strong reducing power for "green" synthesis. It was confirmed the fact that chemical and physical properties of silver nanoparticles increase the beneficial efficacy of silver. The synthesis of mulberry-AgNP sample was initial observed by visual inspection because it was changed the color (from yellow to orange-brown). The second base of this study was confirmed by spectral studies (UV-VIS absorption and ATR-FTIR spectroscopy) that revealing the presence of the polyphenols and proteins from the mulberry extract, which are bioactive compounds responsible for bioreduction of silver ions and for stabilization of AgNPs. The particle sizes of these silver nanoparticles were obtained using DLS analysis. Also, the morphological aspect of AgNP sample was obtained by SEM technique. These silver nanomaterials presented high values of antioxidant activity, so that bioconstructs could be used in the development of valuable products for medical, biological environmental applications.

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