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Silver nanoparticles from leafy green extract of Belgian endive (*Cichorium intybus* L. var. *sativus*): Biosynthesis, characterization, and antibacterial activity

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ABSTRACT

We report for the first time a green, simple, and low-cost synthesis of silver nanoparticles (AgNPs) by mixing $AgNO_3$ solution with the aqueous leaf extract of Belgian endive, a variety of *Cichorium intybus* L., without any harmful reducing and capping agents. The biosynthesis of AgNPs was observed by the color charge from colorless (metal salt solution) to a yellowish brown (nanoparticle colloidal dispersion), which was confirmed by UV–vis spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD). UV–vis spectra showed the surface plasmon resonance signature of AgNPs around 420 nm, TEM revealed that nanoparticles were quasi-spherical with an average diameter ranging from 19 to 64 nm depending on the metal salt concentration, and XRD pattern indicated that the biosynthetic process produced face-centered cubic AgNPs. Surface-enhanced Raman spectroscopy analysis showed that the AgNPs were capped with bioactive molecules from the leaf extract, which are also believed to be responsible for the bio-reduction of silver ions. The antibacterial activity of the biosynthesized AgNPs was studied using both the disk diffusion and minimum inhibitory concentration methods against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa,* and they were found to be effective at picomolar concentration levels.

1. Introduction

Synthesis of silver nanoparticles (AgNPs) is an expanding research area due to the unique chemical, physical and biological properties of them [1,2], which leads a wide range of applications in spectroscopy, sensors, electronics, catalysis, and pharmaceutical sciences [3–5]. Several approaches (chemical, physical, and biological) have been used in order to prepare silver and other metal nanoparticles [2,3,6–9]. Actually, there is a growing interest in the biosynthesis of AgNPs due to the advances in eco-friendly technologies in materials science. Biosynthesis pathways are cost effective and do not use toxic chemicals, high pressure, and temperatures [10]. For this purpose, the use of plants and plant extracts is potentially advantageous over other biological methods, mainly microorganisms, due to the ease of improvement and it needs no elaborate process of maintaining cell cultures [6,11]. A variety of plant extracts such as *Cinnamomum camphora*, *Aloe vera*, *Azadirachta indica*, *Coriandrum sativum*, *Chrysanthemum indicum*, *Melia azedarach*, *Vitex negundo*, *Skimmia laureola*, *Grewia flavescens* [12–20], and other species [6] have been used for the synthesis of AgNPs. However, there are only a few published reports about the use of leafy green vegetables as reducing and capping agents for nanoparticles synthesis [21,22]. Within this context, the present study describes for the first time the biosynthesis of AgNPs using the aqueous leaf extract of Belgian endive (*Cichorium intybus* L. var. *sativus* Bishoff), their physicochemical characterization, and their *in vitro* antimicrobial effect against both Gram-negative and Gram-positive bacteria. Belgian endive is a typical Mediterranean vegetable but it is now largely cultivated in many countries [23]. Due to this leafy green vegetable contains a large amount of natural antioxidants, mainly phenolic acids [23], it is available in markets and is inexpensive; Belgian endive is a potential candidate for green synthesis of AgNPs.

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2. Materials and methods

2.1. Preparation of leaf extract and biosynthesis of AgNPs

Leaf pieces (5 g) of Belgian endive obtained from a local market were placed in a 250 mL Erlenmeyer flask with 100 mL of ultrapure water, boiled for 5 min, and filtered to obtain the aqueous extract. For a typical biosynthesis of AgNPs, 5 mL of Belgian endive leaf extract was added to 50 mL of $AgNO_3$ solution with constant stirring at 75–80 °C in a 250 mL Erlenmeyer flask. Three different final concentrations of $AgNO_3$ were used (0.10 mM, 1.00 mM and 10.00 mM) for each biosynthesis. $AgNO_3$ (99%) was obtained from J.T. Baker and used as received.

2.2 Characterization of biosynthesized AgNPs

The formation of nanoparticles was monitored by UV–vis spectroscopy using a Shimazdu UV-1700 PharmaSpec spectrophotometer, and the transmission electron microscopy (TEM) images were obtained using a JEM-JEOL 1120 EXII. X-ray diffraction (XRD) measurements were carried out in a PANalytical X-Pert Pro X-ray diffractometer with Cu K-alpha radiation, and surface enhanced Raman spectroscopy (SERS) experiments were performed using a Horiba LabRaman confocal microscope with a 5X (NA = 0.12) objective in the backscattering geometry.

2.3. Antibacterial activity of biosynthesized AgNPs

ATCC reference strains and clinical isolates (CI) of Gram-positive and Gram-negative were used in this work. Antibacterial efficacy of nanoparticles was assayed using both the standard disk diffusion and minimum inhibitory concentration (MIC) methods. Experimental details are described in the Supporting Information.

3. Results and discussion

Bio-reduction of Ag^+ ions to colloidal nanoparticles was visually observed by color change from colorless ($AgNO_3$ solution) to yellowish brown (AgNPs) and confirmed by UV–vis spectral analysis. Fig. 1 (first line) displays the spectral evolution during the synthesis performed with the Belgian endivia leaf extract (9% v/v) for the three different $AgNO_3$ concentrations (0.10 mM, 1.00 mM, and 10.00 mM) used in the experiments. The absorption band centered at about 420 nm in Fig. 1 arise due to the excitation of surface plasmon in the biosynthesized AgNPs [24]. It can be observed in Fig. 1 (first line) that the absorbance intensity increases with increasing reaction time up to 40 min. Moreover, increasing concentrations of $AgNO_3$ led to a red-shift of the absorption maxima and a wider peak indicating a larger size of biosynthesized AgNPs. This was confirmed by TEM images showed in Fig. 1 (second line), where the nanoparticles appeared to be predominantly spherical in shape and dispersed with an average diameter (19, 56 or 64 nm) that depends on the metal salt concentration-to-reducing agent volume (extract) ratio.

Fig. 2a shows the XRD pattern of the biosynthesized AgNPs offering details regarding their crystalline nature. The diffraction peaks at 2θ values 38.1°, 44.2°, and 64.5° were assigned as (111), (200), and (220) lattice planes of a face-centered cubic structure, respectively, using the powder diffraction database of JCPDS No. 04-0783. This result confirmed that the AgNPs obtained are crystalline in nature, which was found to be in agreement with those biosynthesis of AgNPs reported previously in the literature [19,20]. SERS spectrum of AgNPs obtained with the Belgian endive leaf extract is shown in Fig. 2b, which was analyzed to identify the active biomolecules of the extract involved in the biosynthetic process. Previous studies reported that hydrophilic extracts obtained from fresh aerial parts of Belgian endivia show chicoric acid (around 75%) and chlorogenic acid as characteristic compounds in this variety of *Cichorium intybus* L. [23]. Prominent bands are observed at 1605, 1342, 1250, 1135, 1041, and 960 cm^{-1} (Fig. 2b). The bands around 1605 cm^{-1} and 1250 cm^{-1} were attributed to the phenyl ring and phenyl C-O stretching vibrations, respectively; while the bands around 1135 cm^{-1} and 1041 cm^{-1} may correspond to the in-plane C—H bending motions and phenyl ring breathing modes, respectively [25]. A band around 1342 cm^{-1} and a peak at 960 cm^{-1} can be assigned to the carboxylate bending and stretching motions, respectively [26]. These bands indicate an interaction of the phenolic acids present in the Belgian endive extract with the surface of biosynthesized AgNPs, and that the bio-

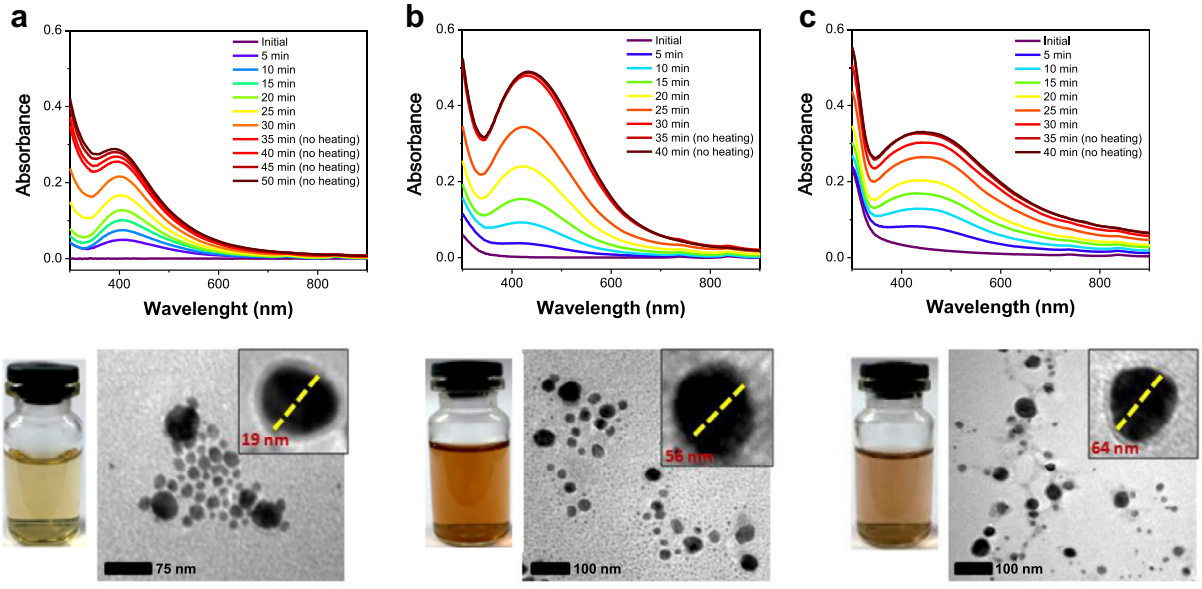


Fig. 1. Temporal evolution of the UV–vis absorption spectrum (first line) of the biosynthesized AgNPs, and a representative TEM image (second line) for the different $AgNO_3$ concentrations used in the synthesis: a) 0.10 mM, b) 1.00 mM, and c) 10.00 mM.

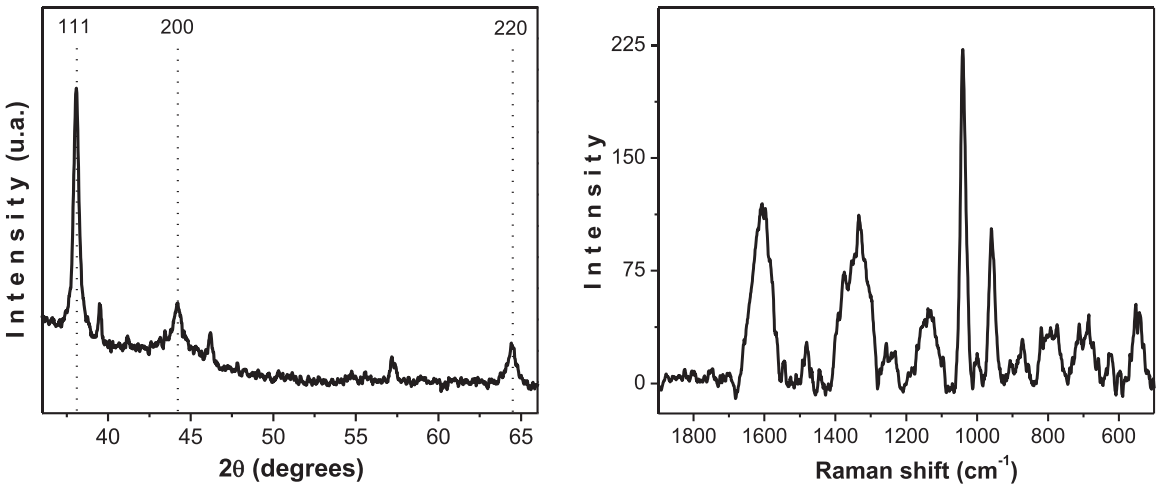
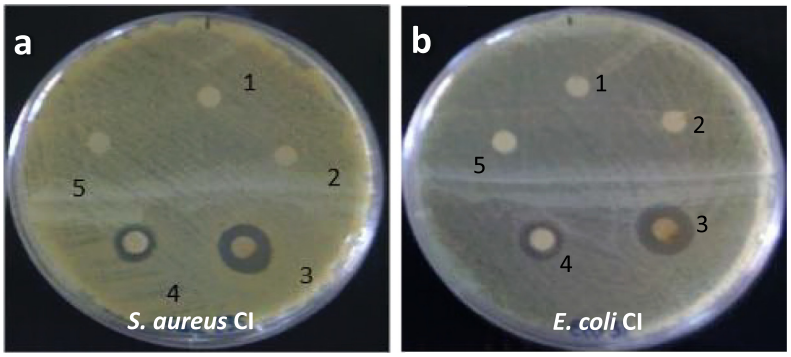


Fig. 2. (a) XRD pattern and (b) Raman spectrum of Ag-NPs biosynthesized.

molecules could perform dual functions of Ag^+ reduction and stabilization of formed nanoparticles.

Antibacterial effect of AgNPs was studied against ATCC reference strains and clinical isolates (CI) of *S. aureus*, *E. coli*, and *P. aeruginosa* using both the disk diffusion and minimum inhibitory concentration methods. The results obtained are displayed in Fig. 3 and summarized in Table 1. These results demonstrate that AgNPs at very low levels of concentration (picomolar levels) have a notable inhibitory activity against both Gram-positive and Gram-negative bacteria, and they are more bioactive than ciprofloxacin (a conventional antibiotic) at micromolar levels. The absence of growth of bacteria around the disks with Ag^+ or aqueous leaf extract clearly indicates that the antibacterial effect is due to the AgNPs biosynthesized. Currently, the mechanism of antibacterial action of AgNPs remains to be understood. Several studies have highlighted that AgNPs adhere to the bacteria cell wall affecting its membrane properties, permeability, and respiration functions [27]. Likewise, AgNPs could exert their toxic effect on bacterial structures *via* an oxidative stress mechanism that lead to oxidative damage of macromolecules (lipids, proteins, and DNA) and consequently, the bacterial death [28].



1: Belgian endive extract. **2:** 1 μM Ag^+. **3: 10 pM AgNPs**. **4: 5 pM AgNPs**. **5.** 1 μM Ciprofloxacin.

Fig. 3. Antibacterial activity of biosynthesized AgNPs against both (a) *S. aureus* and (b) *E. coli* clinical isolates (CI) tested by disk diffusion method.

Table 1
Antibacterial activity of the biosynthesized AgNPs.

Target microorganism	Zone of inhibition (mm)					Minimum inhibitory concentrationof AgNPs (pM)
	Belgian endive extract (9% v/v)	AgNPs (5 pM)	AgNPs (10 pM)	Ag^+ (1 μM)	Ciprofloxacin (1 μM)	
S. aureus ATCC 29213	0.0	9.0	13.0	0.0	0.0	4.8
S. aureus CI	0.0	9.0	13.0	0.0	0.0	5.2
E. coli ATCC 25922	0.0	11.0	14.0	0.0	18.5	4.7
E. coli CI	0.0	10.0	13.0	0.0	0.0	4.4
P. aeruginosa ATCC 27853	0.0	6.5	10.0	0.0	0.0	8.4
P. aeruginosa CI	0.0	11.5	15.0	0.0	0.0	5.1

4. Conclusion

In this work, the reducing and capping properties of a Belgian endive leaf extract were successfully used for the biosynthesis of stable AgNPs. These nanoparticles were characterized combining UV–vis spectroscopy, TEM, XRD, and Raman spectroscopy. Moreover, this green, simple, and rapid procedure for preparing nanoparticles can be adopted for large-scale production and accepted as a cost effective alternative for antimicrobial, biomedical, and nanotechnological applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.matlet.2017.03.141.

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