Antimicrobial Activities of Silver Nanoparticles Synthesized by Using Water Extract of *Arnicae anthodium*

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Abstract Green synthesis of nanoparticles has gained significant importance in recent years and has become the one of the most preferred methods. Also, green synthesis of nanoparticles is valuable branch of nanotechnology. Plant extracts are eco-friendly and can be an economic option for synthesis of nanoparticles. This study presents method the synthesis of silver nanoparticles using water extract of *Arnicae anthodium*. Formation of silver nanoparticles was confirmed by UV–vis spectroscopy, Fourier transform infrared spectroscopy and total reflection X-ray fluorescence analysis. The morphology of the synthesized silver nanoparticles was verified by SEM–EDS. The obtained silver nanoparticles were used to study their antimicrobial activity.

Keywords Nanotechnology · Nanoparticles · Silver nanoparticles · *Arnicae anthodium* · Antimicrobial activity

Introduction

A green approach to the biosynthesis of nanoparticles, myconanotechnology, has been presented as a novel field of study in nanotechnology. Metal nanoparticles have achieved meaningful attention due to their technological interest [1]. The use of metallic nanoparticles is promising because they

show excellent antibacterial properties due to their large surface area to volume ratio. This is very interesting for researchers due to the growing microbial resistance to metal ions, antibiotics and the development of resistant strains [2]. The chemical and physical synthesis of nanoparticles is expensive and often involves the use of toxic hazardous chemicals which may pose environmental risks [3]. Biological methods are cost-effective and do not involve the use of toxic chemicals, high pressure, energy and temperatures [4, 5]. Furthermore, nanoparticles are synthesized using various biosources such as bacteria, fungi, yeast and plant extract. Moreover, silver nanoparticles were produced by marine actinomycetes strain [6]. Nanoparticles have high potential of absorption, adsorption, penetration and availability, which makes them essential antibiofilm agents [7]. Silver nanoparticles demonstrate the application in optical receptors, bio-labeling, intercalation materials for electrical batteries, filters, antimicrobial agents and sensors [8]. Moreover, these nanoparticles are also reported to possess anti-fungal, anti-inflammatory, anti-viral, anti-angiogenesis and anti-platelet properties [9].

Recently, the development of silver nanoparticles has expanded. The green synthesis of silver nanoparticles and their antimicrobial activities have been reviewed by Sharma et al. [10]. Mohanpuria et al. [11] have reviewed the synthesis of silver nanoparticles using plants. Gupta et al. [12] synthesized silver nanoparticles (AgNPs) using the methanolic extract of *Syzygium cumini* leaf. The prepared nanoparticles inhibited biofilm formation in a wide range of AgNP concentrations. Significantly, cytotoxicity assays showed good compatibility of AgNPs with human embryonic kidney cells (HEK 293). Velmurugan et al. [13] obtained AgNPs synthesized using the natural cashew nut shell liquid against bacterial fish pathogens. Mahanty et al. [14] used phyto extracts-synthesized silver nanoparticles as

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cost-effective and ecofriendly therapeutic agents against *Aeromonas hydrophila*-stimulated diseases in aquatic animals.

In this study, the extract of *Arnicae anthodium* was used to synthesize silver nanoparticles. *Arnicae* is popular in mountainous regions and foothills in almost entire Europe. In Poland it is protected. *Arnica* is a medicinal plant broadly used in the cosmetic and liqueur industry [15, 16]. The main active compounds of *Arnicae* are flavonoids. Moreover, *Arnicae* contains triterpenes, sesquiterpene lactones and essential oils. Sesquiterpene lactones exhibit antioxidant and cytotoxic effects [17]. The present study reports a new methodology to synthesize silver nanoparticles using the water extract of *A. anthodium*.

Materials and Methods

The synthesis of silver nanoparticles solution may be easily observed through the use of ultraviolet–visible absorption (UV–VIS) spectroscopy. The optical property of silver nanoparticles was analyzed by means of ultraviolet and visible absorption spectroscopy (spectrophotometer Cary E 500) in the range of 350–800 nm. The morphology of the plant-synthesized silver nanoparticles was examined by means of scanning electron microscopy (SU3500, Hitachi with spectral imaging system Thermo Scientific NSS (EDS), the tape of detector (BSE-3D), acceleration voltage (15.0 kV), working distance (11.6 mm), pressure (40 Pa). Fourier transform infrared (FTIR) spectra of the samples were measured using Perkin-Elmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. Powder samples for the FTIR spectroscopy were prepared similarly as for powder diffraction measurements. The presence of silver nanoparticles in the water extract of *A. anthodium* was confirmed through the use of X-ray fluorescence spectrometer Bruker S2 total reflection X-ray fluorescence analysis (TXRF) Picofox (50 kV and 600 μA).

The in vitro antimicrobial activity of the silver nanoparticles was determined using (1) bacterial strains *Staphylococcus aureus* ATCC 4163, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 6749, and (2) yeast *Candida albicans* ATCC 10231. The microorganisms were obtained from American Type Culture Collection (ATCC). Bacterial and yeast strains were stored in Microbank cryogenic vials (ProLabDiagnostics, Canada) at $-70 \pm 10\text{ }^{\circ}\text{C}$. Bacterial cultures were grown in Brain Heart Infusion broth (BHI, BioMerieux, France) at $34\text{ }^{\circ}\text{C}$ for 18 h, and yeast cultures were grown in Sabouraud dextrose broth (SDB, Merck) at $34\text{ }^{\circ}\text{C}$ for 18 h. After incubation each culture was diluted in suitable liquid medium (bacteria—Mueller–Hinton broth (MHB; Oxoid, UK); *C. albicans*—Sabouraud dextrose broth

(SDB, Merck, Germany) to obtain a final suspension containing about 10^6 CFU/mL. The antimicrobial activity of the examined silver nanoparticles was studied by employing the macrodilution method in accordance with EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines with modifications, using: Mueller–Hinton broth (MHB)—bacteria, and SDB—fungi. The solution of silver nanoparticles was two-fold serially diluted in a culture broth to concentrations ranging from 40 to 0.3125 %. One mL of each dilution were distributed in test tubes, as well as sterility control and growth control tubes (containing culture broth). Each test and growth control tube was inoculated with 1 mL of microbial suspension. The test tubes were incubated at $34 \pm 1\text{ }^{\circ}\text{C}$ for 18 h. The MIC was defined as the lowest concentration at which visible growth was inhibited. After performing MIC test and recording the MIC end point, every tube that demonstrated no growth (concentration equal to and greater than MIC) was subcultured onto an agar medium: Trypticase soy agar (TSA; BioMerieux)—bacteria; Sabouraud dextrose agar (SDA; Merck, Germany)—yeast. The plates were incubated at $34 \pm 1\text{ }^{\circ}\text{C}$ for 18 h. The MBC/MFC was defined as the lowest concentration at which no growth was observed. Amikacin for bacteria and nystatin for fungi were used as a reference positive control. All tests were performed in duplicate.

Preparation of Silver Nanoparticles

4.5 g of powder of *A. anthodium* were boiled for 60 min at $80\text{ }^{\circ}\text{C}$ with 100 mL of doubly distilled water. For synthesis of silver nanoparticles, 90 mL of AgNO_3 (1 mM) was reacted with 10 mL of extract and stirred at $25\text{ }^{\circ}\text{C}$ for 24 h. A change in the color of the solution was observed during the stirring process. The solution was filtered by $0.45\text{ }\mu\text{m}$ Millipore membrane filter and followed by $0.2\text{ }\mu\text{m}$ Millipore membrane filter. Prepared silver nanoparticles solution was stored for 72, 96, 120 and 168 h in dark conditions. Figure 1 present solutions of AgNO_3 (1 mM) before and after 24 h of stirring.

Results and Discussion

UV–VIS Spectra Analysis

The UV–VIS spectroscopy is an indirect method to examine the bioreduction of silver nanoparticles from aqueous AgNO_3 solution. The complete reduction of silver ions was observed after 24 h of continuous stirring at $25\text{ }^{\circ}\text{C}$. The appearance of color change from yellow to reddish brown is a clear indication of the formation of silver nanoparticles in the reaction mixture. The absorption spectra were taken at different time intervals. Absorbance

Fig. 1 Solutions of 1 mM of AgNO_3 **a** before and **b** after 24 h stirring

was measured 1, 24, 72, 96, 120 and 168 h after preparation of the solution. Figure 2 shows the UV–VIS absorption spectrum of the synthesized silver nanoparticles. The

Fig. 2 UV-Vis absorption spectrum of obtained silver nanoparticles from water extract of *Arnicae anthodium*

Fig. 3 Representative SEM showing the size of synthesized obtained silver nanoparticles from water extract of *Arnicae anthodium*

peak was observed at 458 nm, which is a characteristic band for silver. Apart from that, no other peak was observed in the spectrum, which confirms that the synthesized products are silver only. To sum up, an increase in absorbance at a wavelength of 458 nm with increasing storage time was observed. The increase in absorbance could stem from the increasing number of nanoparticles formed as a result of reduction of silver ions presented in the water solution. In recent studies, Patel et al. [18] immobilized laccase on SiO_2 nanoparticles.

Scanning Electron Microscopy (SEM)

The morphology of the synthesized silver nanoparticles was verified by SEM–EDS. Figures 3a, b, 4a, b show the SEM images of silver nanoparticles from water extract of *A. anthodium* after stirring for 24 h at 25 °C. Figures 3a, b demonstrate that the average size was from 90 to 118 nm. The larger sizes stem from the agglomeration of the

Fig. 4 Scanning electron micrographs (SEM) of synthesized silver nanoparticles

Fig. 5 SEM of synthesized obtained silver nanoparticles from water extract of *Arnicae anthodium* and EDS profile (the scale bar is 20 μm)

particles. Moreover, Figs. 4a, b show individual silver particles as well as a number of aggregates. The silver nanoparticles were evaluated using EDS on the SEM [19]. EDS profile demonstrates the evidence of elemental silver. The peak at 3 keV is typical for the absorption of metallic silver nanoparticles. This proves the presence of nano crystalline elemental silver [20]. Figure 5b presents four peaks between 1 and 4 keV. Figure 6b shows three peaks between 1 and 4 keV. The results indicate that the reaction product was composed of high-purity silver nanoparticles.

Fourier Transform Infra-Red Spectroscopy (FT IR)

Fourier transform infra-red spectroscopy is the measurement of the absorption of IR radiations by a sample plotted against the wavelength. The interpretation of the IR spectrum

involves the correlation of the absorption bands (vibrational bands) with the chemical compounds in the sample. This way, the biomolecules present in plant extracts that are responsible for the reduction and stabilization processes of the green synthesis of nanoparticles can be identified [21]. The strong IR bands (Fig. 7) were observed at 3,284, 2,923, 2,853, 1,735, 1,622, 1,370, 1,240, 1,027, 430 and 395 cm^{-1} .

The peaks at 3,284, 2,923 and 2,853 cm^{-1} correspond to $-\text{OH}$ stretching and aliphatic $-\text{C}-\text{H}$ stretching [22]. The strong bands at 1,735 and 1,622 cm^{-1} are attributed to the $\text{C}=\text{C}$ stretch in aromatic ring and the $\text{C}=\text{O}$ stretch in polyphenols [21]. The absorption bands located at 1,370, 1,240 cm^{-1} and 1,027 cm^{-1} may be attributed to $-\text{C}-\text{O}$ and $-\text{C}-\text{O}-\text{C}$ stretching modes [8]. The intense broad bands at 430, 395 cm^{-1} related to silver nanoparticles banding with oxygen from hydroxyl groups.

Fig. 6 SEM of synthesized obtained silver nanoparticles from water extract of *Arnicae anthodium* and EDS profile (the scale bar is 25 μ m)

Fig. 7 FTIR spectrum of obtained silver nanoparticles from water extract of *Arnicae anthodium*

Total Reflection X-Ray Fluorescence Analysis (TXRF)

The sample was determined by X-ray fluorescence spectrometer TXRF Bruker S2 Picofox, operated at 50 kV and 600 μ A. The signal at 2.82 keV suggests that silver nanoparticles were the major element from the water extract of *A. anthodium*. Figure 8 presents TXRF spectrum of the synthesized silver nanoparticles obtained from the water extract of *A. anthodium*.

Antimicrobial Activity

The in vitro antimicrobial activity of silver nanoparticles was assessed quantitatively by determining the MIC and MBC/MFC as given in Table 1. The result showed positive inhibitory activity of silver nanoparticles against all tested bacterial strains as well as against *C. albicans*. MBC values for bacteria were equal or one concentration greater than MIC, whereas for *C. albicans* MFC the value was greater

Fig. 8 TXRF spectrum of obtained synthesized silver nanoparticles from water extract of *Arnicae anthodium*

Table 1 Antimicrobial activity of silver nanoparticles

Microorganisms	MIC; MBC/MFC					
	Silver nanoparticles		Amikacin		Nystatin	
	MIC ($\mu\text{g mL}^{-1}$)	MBC/MFC ($\mu\text{g mL}^{-1}$)	MIC ($\mu\text{g mL}^{-1}$)	MBC ($\mu\text{g mL}^{-1}$)	MIC ($\mu\text{g mL}^{-1}$)	MFC ($\mu\text{g mL}^{-1}$)
<i>Staphylococcus aureus</i> ATCC 4163	0.1	0.2	1	1	–	–
<i>Pseudomonas aeruginosa</i> ATCC 6749	0.1	0.1	2	2	–	–
<i>Escherichia coli</i> ATCC 25922	0.1	0.2	–	–	–	–
<i>Candida albicans</i> ATCC 10231	0.2	>0.2	–	–	8	16

MIC Minimal inhibitory concentration, MBC minimal bactericidal concentration, MFC minimal fungicidal concentration

than the highest tested concentration. Moreover, silver nanoparticles show excellent antimicrobial activity against the tested bacterial strains. The synthesis of effective antibacterial substances is very important as many antibiotics were found to have become ineffective within a few years of their launch, as bacteria were found to have developed resistance to them [23].

Conclusion

The current work presents the eco-friendly method of synthesizing silver nanoparticles from the water extract of *A. anthodium*. The silver nanoparticles obtained in this work

have the average size of 90–118 nm. Therefore, *A. anthodium* seems to be a very good material for the synthesis of silver nanoparticles. The larger sizes of silver nanoparticles stem from the agglomeration of the particles. This work used UV–VIS spectroscopy, FTIR spectroscopy and TXRF. The morphology of silver nanoparticles was verified by means of SEM–EDS. The obtained silver nanoparticles demonstrated excellent antimicrobial activity against all tested bacterial strains (*S. aureus* ATCC 4163, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 6749) as well as against *C. albicans*.

To sum up, the present work proves that water extract of *A. anthodium* is a new useful method using cheap precursors for the preparation of silver nanoparticles which exhibits excellent antimicrobial activity.

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Conflict of interest The authors declare that they have no conflict of interest.

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