BIOSYNTHESIS OF SILVER NANOPARTICLES USING EXTRACT FROM VINE CANES AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY AGAINST *PSEUDOMONAS AERUGINOSA*

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Abstract

One of the many interesting properties of silver nanoparticles is their antimicrobial activity. Since these properties are related to the morphology of the particles, it is interesting to pay attention to the antimicrobial effects of various metal nanostructures, which differ in the method of synthesis, size, or shape. One of the very interesting methods of synthesis of metal nanoparticles, which seems to be very advantageous because of its low cost, efficiency, and ecology, is the synthesis using plant extracts. For this work, woody parts of the *Vitis vinifera* were selected as material for the preparation of the extract. The canes of this plant are an agricultural waste containing many bioactive substances, which are not used for other significant purposes, making it a promising material for the preparation of metal nanoparticles with antimicrobial potential. The biosynthesized nanoparticles were detected and characterized using UV-Vis spectrophotometry, transmission electron microscopy (TEM) and dynamic light scattering (DLS). TEM showed that the obtained nanoparticles have a heterogeneous shape with a size 4–51 nm. The DLS analysis supported that the Z-average was 113.8 nm and 0.165 PDI value. The use of biosynthesized nanoparticles showed an excellent growth inhibition property against two strains of the opportunistic pathogenic bacteria *Pseudomonas aeruginosa* (ATCC 10145 and ATCC 15442). Furthermore, a bactericidal concentration (BC) 40% v/v of AgNPs was found for *P. aeruginosa* ATCC 15442, while no BC was found in the range of tested concentrations for *P. aeruginosa* ATCC 10145.

Keywords: Biosynthesis, plant extract, nanoparticles, antimicrobial activity

1. INTRODUCTION

Metal nanoparticles are very popular nanomaterials in both scientific and commercial spheres. Nanoparticles have unique chemical, physical, and biological properties, which are closely related to specific characteristics of the nanoparticle, such as shape, size, and distribution [1]. Among the most widely used NPs are silver nanoparticles, which have many interesting applications, for example, in biomedicine. For many applications of silver nanoparticles, their key property is antimicrobial activity. Even historically, silver has been known for its disinfecting effects. In recent times, many studies have reported the antimicrobial activity of AgNPs against a variety of clinically important microorganisms (e.g., Gram-positive methicillin-resistant *Staphylococcus aureus* [2], Gram-negative *Escherichia coli*, funghi *Fusarium oxysporum* [3] or yeast *Candida albicans* [4]).

As interest in metal nanoparticles has grown significantly in recent decades, it is important rational choice of methods for their production. Therefore, in recent years, biological methods are becoming increasingly popular, using the principle of so-called green chemistry, which uses natural raw materials for the synthesis of metal nanoparticles (plants, plant extracts, microorganisms, biomolecules…). The use of plant extracts is an extremely practical solution, which is often the case preferred for the simplicity of the process, rapid reaction time, and high efficiency. The ability of plant extracts to reduce metal ions and stabilize the resulting nanoparticles is caused by the presence of a large number of different active metabolites (e.g., phenols, ketones, carboxylic acids, aldehydes, amides, proteins and others) [5].

In this study, we biosynthesized silver nanoparticles using an extract from agricultural waste. *Vitis vinifera* is well-known plant which is nontoxic and has many applications. Specifically, "wooden parts" – canes from *Vitis vinifera* plant were used. This material was chosen because it is easily available (waste parts of vine) and additionally it is full of many active metabolites, which could mediate the formation and stabilization of the nanoparticles. Prepared nanoparticles were detected using UV-Vis, TEM, and DLS. Subsequently, the antimicrobial potential against the opportunistic pathogenic bacteria *Pseudomonas aeruginosa* was also studied.

2. EXPERIMENTAL

2.1. Chemicals and materials

Vitis vinifera canes (wine-growing region of Čechy), silver nitrate ($AgNO_3$, Sigma-Aldrich, USA), ethanol (96%, Penta, CZ), distilled water, Phosphate-buffered saline (pH 7.4, PBS), Lysogeny broth medium (LB) – 10 g/L Trypton (Oxoid, UK), 5 g/L Yeast extract (Carl Roth, DE), 10 g/L sodium chloride (NaCl, Penta, CZ), LB agar (addition of 20 g/L of agar to liquid LB medium).

2.2. *Vitis vinifera* extract production

The dried *Vitis vinifera* canes were cut into small pieces and then crushed to powder. After that, 75 g of the prepared material was mixed with 150 ml of 40 % ethanol and extracted in the dark for 24 h. The mixture was then filtered through a laboratory filter and additionally through a filter membrane (0.22 µm size) to give the final extract for the synthesis of nanoparticles. The extract was stored in the dark at 4 °C until further use.

2.3. Biosynthesis and detection of silver nanoparticles

For the nanoparticle synthesis, the ethanolic extract of *V. vinifera* prepared in the previous step was mixed with silver nitrate to give a 1 mM solution. The extract made up 10 % of the final volume (dilution with distilled water). Reaction occurred in a 50 ml Erlenmeyer flask. Subsequently, the mixture was heated in a water bath at 60 °C for 30 min. After heating, the flask was incubated for the next 48 h at room temperature in the dark. The final solution was stored at 4 °C for future use.

The formation of silver nanoparticles was detected preliminarily by the colour change of the solution. Furthermore, the formation of AgNPs was confirmed by UV-Vis spectrophotometry at 260–700 nm with 10 nm resolution. The morphology of the prepared nanoparticles was determined by transmission electron microscopy. The size of the particles was measured using ImageJ software. The hydrodynamic diameter and the index of polydisperzity (PDI) were observed using dynamic light scattering (DLS).

2.4. Evaluation of antimicrobial activity of the nanoparticles

The activity of biosynthesized nanoparticles was evaluated against two bacterial strains of *Pseudomonas aeruginosa* (ATCC 10145 and ATCC 15442). For this analysis, the cryopreserved bacterium was inoculated in sterile LB medium and incubated for 24 h at 150 rpm and 37 °C. The culture was then centrifuged (9,000 rpm, 10 min, 10 °C) and resuspended in fresh LB medium to prepare the final inoculum with the optical density (OD600 nm) 0.100 ± 0.020.

The antibacterial assay investigating the ability of nanoparticles to inhibit cell growth was performed using sterile 100-well microtiter plates for a BioscreenC microculture device. The wells were filled with antimicrobials at a concentration range of 1.25 to 40.0 % v/v (two-fold dilution). Subsequently, 160 µl of sterile LB medium and 30 µl of inoculum from the previous step were added to each well. A control was performed for each experiment without the addition of antimicrobials. The microtiter plates were incubated using the BioscreenC device (37 °C) for 24 h and the optical density of each well was measured in 30-min intervals. Each experiment was done in 10 parallels.

Moreover, the ability of nanoparticles to kill bacteria (bactericidal) was investigated. To determine the bactericidal concentration of the nanoparticles, 5 µl of the grown cell suspension from each well was transferred to sterile Petri dishes with LB agar, which were incubated for another 24 hours at 37 °C.

3. RESULTS AND DISCUSSION

3.1. Nanoparticles biosynthesis and detection

As shown in **Figure 1**, the formation of silver nanoparticles could first be detected by the colour change of the solution from light brown to dark orange.

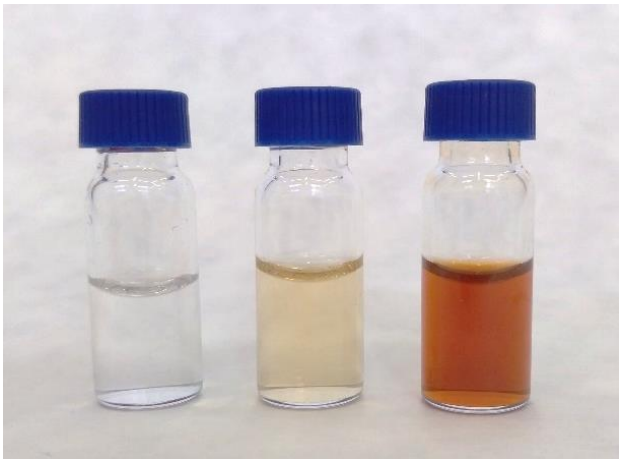

Figure 1 Formation of silver nanoparticles accompanied by colour change: *from the left* – 1 mM silver nitrate, 10 % v/v *V. vinifera* extract, AgNPs (60 °C, 30 min)

The presence of silver nanoparticles was also detected by measuring the UV-Vis spectra of the colloid (**Figure 2**). The obtained absorbance band with maximum at 450 nm together with the mentioned colour change are characteristic for the formation of silver nanoparticles [6,7]. Moreover, λ_{max} at 450 nm corresponds usually to nanoparticles of sizes 70–80 nm (when spherical) [8].

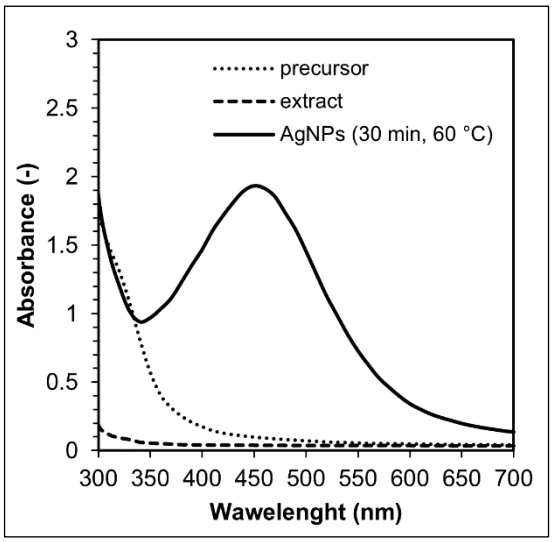

Figure 2 UV-Vis spectra of biosynthesized nanoparticles and controls

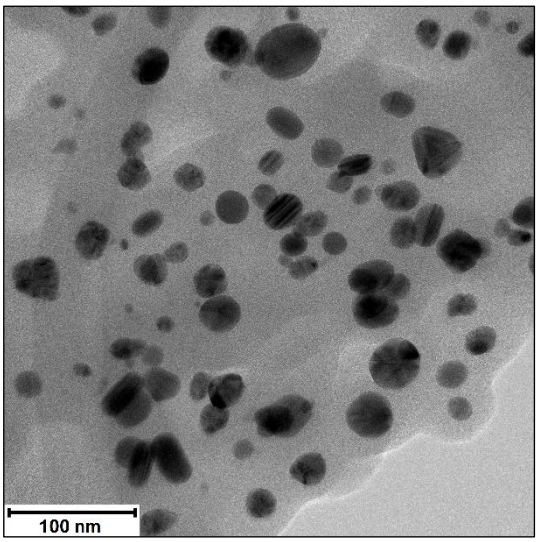

Figure 3 TEM image of silver nanoparticles biosynthesized using *V. vinifera* cane extract

Additionally, TEM was done for further characterization of the prepared nanoparticles (**Figure 3**). TEM analysis showed that the biosynthesized nanoparticles have a heterogeneous shape and are 4–51 nm in size. We supported our results by DLS measurement to observe the hydrodynamic diameter and PDI of the synthesized AgNPs. We found the Z-average 113.8 nm and 0.165 PDI value. The difference in size determined by TEM and DLS could be explained by the fact that the DLS method includes also an organic coating of the nanoparticle surface when determining the size. An electrostatic attachment of biomolecules from plant extract to the nanoparticle surface is desirable, because it improves the stability of nanoparticles. High stability of plant extract mediated metal nanoparticles is one of the advantages of this approach. However, for the possibility to use nanoparticles prepared in this way (e.g., in biomedicine), it is necessary to analyse the surface of nanoparticles and determine which biomolecules surround it. Techniques such as XPS or FTIR could be used for it [9], and it is also planned in our research.

3.2. Antimicrobial activity against planktonic bacteria

The results confirmed that the biosynthesized nanoparticles have excellent bacterial growth inhibition property against both strains of *P. aeruginosa*. The growth of planktonic cells of *P. aeruginosa* (ATCC 10145, ATCC 15442) was completely inhibited using 2.5 % v/v of AgNPs (**Figure 4** and **Figure 5**). However, even the lowest tested concertation of NPs (1.25 v/v) affected the growth dynamics in both strains of *P. aeruginosa* by prolongation the lag phase. Similar extend of the lag phase was observed in bacteria when using low concentrations of antimicrobials (e.g., antibiotics, metal nanoparticles) [10]. Similar results were investigated by Otari et al. who tested the antimicrobial activity of biologically synthesized silver nanoparticles (5-50 nm) against *P. aeruginosa*. The lowest concentration of NPs (10 mg/L) used by authors extended the lag phase from 5 to 10 hours, while by using higher concentrations of AgNPs (50 mg/L) the growth of bacteria was completely inhibited [11].

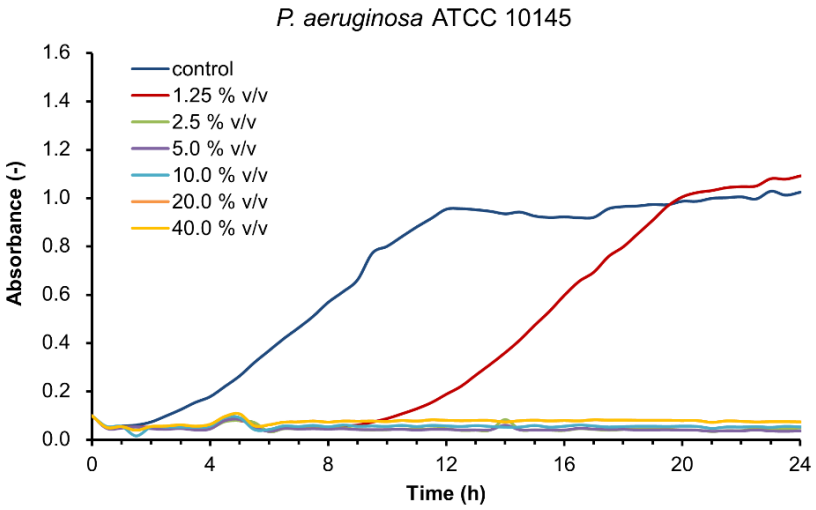

Figure 4 Growth curves of *Pseudomonas aeruginosa* ATCC 10145 in the presence of silver nanoparticles (% v/v) prepared using *V. vinifera* cane extract

We also studied the ability of biosynthesized AgNPs to kill bacteria by transferring an aliquot of grown bacterial culture from the microtiter plate to sterile LB agar, which was incubated for another 24 h. The results showed that the bactericidal concentration value of AgNPs against selected bacteria varied, which may be due to their different phenotypic characteristics. While no bactericidal concentration was found in *P. aeruginosa* ATCC 15442, the bactericidal concertation of the second microorganism tested (*P. aeruginosa* ATCC 10145) was 40 % v/v of the nanoparticles. It shows that AgNPs synthesized using viticultural waste are promising antibacterial agents.

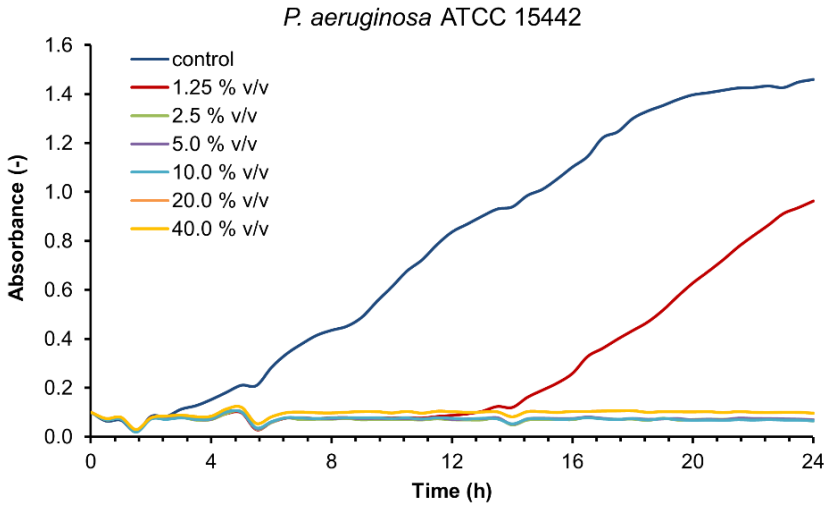

Figure 5 Growth curves of *Pseudomonas aeruginosa* ATCC 15442 in the presence of silver nanoparticles (% v/v) prepared using *V. vinifera* cane extract

Antibacterial activity of silver nanoparticles produced using plant extracts has been already described in literature. Using an extract from *Origanum vulgare* L. plant were prepared polydisperse silver nanoparticles with antibacterial activity against *P. aeruginosa, S. aureus*, *Staphylococcus epidermidis and E. coli* [12]. In another study, silver nanoparticles with heterogeneous shape and size from 5–20 nm were synthesized using *Murraya koenigii* extract and their antibacterial activity was proved against *S. aureus* and *E. coli* [13].

4. CONCLUSION

The present work describes the biosynthesis of silver nanoparticles using an extract from the waste parts of the agriculturally important plant *Vitis vinifera*. The extract from this material proved to be able to reduce silver ions and stabilize the resulting nanoparticles. TEM revealed that the prepared nanoparticles have a heterogeneous shape and size in the range of 4–51 nm. This data were supported by DLS analysis, which showed that the Z-average of nanoparticles was 113.8 nm and PDI was 0.165. Furthermore, it was found that these nanoparticles have the ability to inhibit the growth of the clinically important opportunistic pathogenic bacteria *P. aeruginosa*. This study shows that AgNPs synthesized using viticultural waste could find interesting applications as antimicrobial agents.

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