ANTIOXIDANT ACTIVITY OF SILVER NANOPARTICLES PREPARED BY GREEN SYNTHESIS

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Abstract

At present, great attention is given to silver nanoparticles (AgNPs), which thanks to its unique properties, such as good electric conductivity, photoelectrochemical activity and antimicrobial activity are widely used. Green synthesis of nanoparticles uses biological molecules from living organisms. Biological extracts may contain molecules which exhibit significant antibacterial, antiviral and cytotoxic effects. The aim of this work was to study the diverse AgNPs synthesized from 10 different types of plant extracts. Extracts from dried plants (0.5 g/25 mL 18 MΩ water) were prepared at 70 °C by heating for 20 minutes. After filtration, the leachates were mixed in the 1:1 ratio with 0.1 M $AgNO_3$ and allowed to stir at room temperature for 18 hours. They were then mixed in the 1:1 ratio with methanol, shaken for 5 minutes on the rotary mixer, and centrifuged at 12,000 g for 30 minutes. After removing the supernatant, the pellet was dried at 60 °C for 24 hours. After weighing, the purified AgNPs were dissolved in 18 MΩ water. AgNPs were yellow, orange and brown. The extraction efficiency was monitored by organic solvents (methanol, ethanol, acetone, propanol). The yields of AgNPs ranged from 15 to 5 %, and the most suitable solvent was methanol with an average AgNPs yield of about 10 %.AgNPs were characterized spectrally (spectral maxima were in the range of 300-500 nm) by determining the zeta potential and the size of nanoparticles (30-80 nm). Furthermore, antioxidant activity was monitored using ABTS (670 nm), DPPH (517 nm), and FRAP (595 nm) methods. Two methods (ABTS and DPPH) based on the elimination of synthetic radicals and the FRAP method based on the reduction of iron complexes were used to monitor the antioxidant activity. Antioxidation assays were evaluated using calibration curve equations in which the standard was gallic acid. The results for the ABTS and DPPH methods were also expressed as percentage of inhibition of the radicals and in the FRAP method as percentage of reduction activity. The results were calculated using the ABTS method in the range (25.9 - - 84.9 %), in the DPPH method (19.2-86.6 %) and in the FRAP method (8.5 - 93.2 %). Most AgNPs prepared by green synthesis showed significant antioxidant activity.

Keywords: Nanomedicine, silver nanoparticles, green synthesis, antioxidant activity

1. INTRODUCTION

The synthesis of silver nanoparticles (AgNPs) attracts an increasing interest due to their new and different characteristics that allow applications in various fields of medicine and biotechnology such as antimicrobials, and anticancer agents [1]. The properties of AgNPs as a high surface area, very small size, and high dispersion

make them one of the most commonly used nanomaterials. AgNPs are also known to have antioxidant properties [2]. Several techniques have demonstrated that AgNPs can be synthesized using chemical and physical methods, however due to the fact of usage of a huge amount of toxic chemicals and high temperature conditions, a search for alternative methods began. Synthesis of nanoparticles by biological methods, using microorganisms, enzyme and plant or plant extract, has been suggested as possible eco-friendly alternatives to chemical and physical methods [3]. The use of plant extracts to produce nanoparticles is one of environmental friendly green processes. Nanoparticles produced from plant extract, because of their medicinal properties, could be used in drugs, targeted drug delivery and cosmetic applications [4]. The various biomolecules present in the plant extract such as enzymes, proteins, flavonoids, terpenoids, and cofactors act as both reducing and capping agents [5]. The plant-mediated synthesis of nanoparticles is relatively fast as there is no need of maintaining specific media and culture conditions, unlike microbial synthesis [5].

Most of the oxidative diseases are due to oxidative stress resulting from free radicals [6]. Free radicals such as superoxide anion, hydroxyl radicals and non-radical species such as hydrogen peroxide and singlet oxygen are different varieties of activated oxygen constituting reactive oxygen species [7]. An active antioxidative defense system is needed to balance the output of free radicals. Antioxidant therapy by the curing of these diseases has an enormous importance. Nanotechnology is an interdisciplinary approach in biochemical applications and focusing on synthesis of nanoparticles have improved antimicrobial and antioxidant properties against the degenerative diseases and cancer [8]. Antioxidant activity in plant extract is due to the redox potential of phytochemicals [9], which can play an important role in quenching singlet and triplet oxygen, decomposing the peroxides or neutralizing the free radicals. Therefore, it is assumed that higher antioxidant activity of nanoparticles might be due to the preferential adsorption of the antioxidant material from the plant extract onto the surface of the nanoparticles. In our study we used ten types of greenly synthesized AgNPs from medicinal plants or food of plant origin and we tested them for their antioxidant activity using DPPH, ABTS and FRAP methods.

2. MATERIAL AND METHODS

2.1. Nanoparticle synthesis

For preparation of AgNPs extracts from 10 different plants were used. They were the following: AgNPs 1 - black tea (*Camelia sinensis*), AgNPs 2 - green tea (*Camelia sinensis)*, AgNPs 3 - coffee (*Coffea arabica)*, AgNPs 4 - common thyme (*Thymus vulgaris*), AgNPs 5 - red clover (*Trifolium pratense*), AgNPs 6 - red raspberry (*Rubus idaeus*), AgNPs 7 - absinthe wormwood (*Artemisia absinthium*), AgNPs 8 - common agrimony (*Agrimonia eupatoria*), AgNPs 9 - garden strawberry (*Fragaria ananassa*), AgNPs 10 - purple crownvetch (*Securigera varia)*. Plants except tea and coffee were harvested in the Boskovická brázda area in May 2018. After collection, they were washed in distilled water and dried in a 60 ° C oven for 48 hours followed by grinding to about 1 mm particle size. Extracts from dried plants (0.5 g/25 mL 18 MΩ water) were prepared at 70 ° C by heating for 20 minutes. After filtration, the leachates were mixed in the 1:1 ratio with 0.1 M $AgNO_3$ and allowed to stir at room temperature for 18 hours. The prepared AgNPs solution was stored at 4 ° C in closed containers and spectrally characterized. AgNPs solutions were also used for determination of antioxidant activity.

2.2. Characterization of nanoparticles

The absorbance spectra of nanoparticles were recorded within the range from 350 to 700 nm using an UV-3100PC UV-VIS spectrophotometer (VWR, Germany). The average nanoparticles' size and size distribution were determined by quasielastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments, Worcestershire, UK). Nanoparticle distilled water solution of 1.5 mL (1 $mg.mL^{-1}$) was put into a polystyrene latex cell to measure the following properties such as: detector angle 173°, wavelength 633 nm, refractive index 0.30, real refractive index 1.59, and temperature 25 °C.

2.3. Antioxidant properties of nanoparticles

Photometric measurements were carried out using an chemical analyser BS-300 (Mindray, China). For detection itself, the following range of wavelengths can be used - 340, 380, 412, 450, 505, 546, 570, 605, 660, 700, 740 and 800 nm. The DPPH test is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical to react with hydrogen donors. A 150 µL volume of reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl - DPPH•) was incubated with 15 µL of sample. Absorbance was measured at 505 nm for 12 minutes and output ratio was achieved by difference of absorbance at the last (12th) minute and second minute of the assay procedure. The ABTS radical method is one of the most used assays for the determination of the concentration of free radicals. It is based on the neutralization of a radical-cation arising from the one-electron oxidation of the synthetic chromophore 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS): ABTS• - e- ABTS•+. This reaction is monitored spectrophotometrically by the change of the absorption value. A 150 µL volume of reagent 7mM ABTS• (2,2´-azinobis 3-ethylbenzothiazoline-6-sulfonic acid and 4.95 mM potassium peroxodisulphate) is poured with 3 µL of sample. Absorbance is measured at 660 nm. For calculating of the antioxidant activity, difference between absorbance at the last (12th) minute and second minute of the assay procedure was used. The FRAP method (Ferric Reducing Antioxidant Power) is based on the reduction of complexes of 2,4,6-tripyridyl-s-triazine (TPTZ) with ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), which are almost colorless, or eventually slightly brownish. This chemical forms blue ferrous complexes after its reduction. Reagent preparation: Solution 1: 10 $mmol.L^{-1}$ solution of TPTZ in 40 $mmol.L^{-1}$ of hydrochloric acid. Solution 2: 20 $mmol.L^{-1}$ solution of ferric chloride hexahydrate, in ACS water. Solution 3: 20 $mmol.L^{-1}$ acetate,buffer pH 3.6. These three solutions (TPTZ, $FeCl_3$, acetate buffer) are mixed in a 1:1:10 ratio. A 150 µL volume of reagent is injected into a plastic cuvette with subsequent addition of a 3 µL sample. Absorbance is measured at 605 nm for 12 minutes. Difference between absorbance at the last (12th) minute and second minute of the assay procedure was used for calculating of the antioxidant activity.

3. RESULTS

3.1. Characterization of nanoparticles

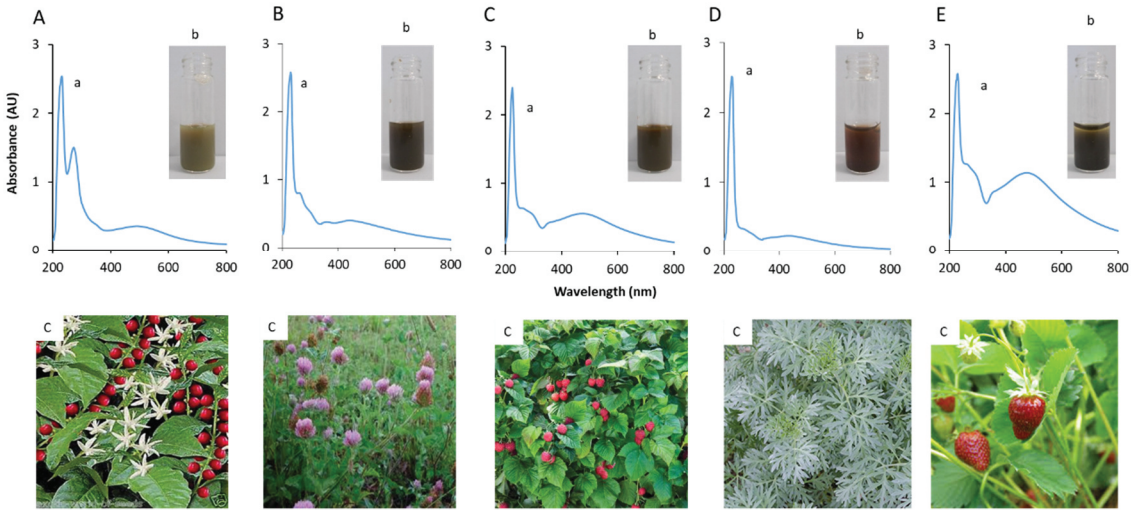

Figure 1 Characterization of AgNPs synthesized by green synthesis (A - AgNPs 3, B - AgNPs 5, C - AgNPs 6, D - AgNPs 7, E - AgNPs 9. AgNPs were prepared using following plant extracts: AgNPs 3 - coffee (*Coffea arabica,* AgNPs 5 - red clover (*Trifolium pratense*), AgNPs 6 - red raspberry (*Rubus idaeus*), AgNPs 7 - absinthe wormwood (*Artemisia absinthium*), AgNPs 9 - garden strawberry (*Fragaria ananassa*). AgNPs were characterized by absorption spectra (a). The relevant characteristic images of the nanoparticles are shown in the pictures marked with b. Pictures of plants used for the preparation of plant extracts are marked with c.

The AgNPs were characterized by absorbance spectra (**Figures 1, A, B, C, D, E, F, G, H, I, J-a**) with the two characteristic absorption maxima that ranged between 470-480 nm (AgNPs B) (**Figures 1, A-Ja**). **Figures 1, A-Jb** illustrates the relevant images of the AgNPs prepared by green synthesis. Size of nanoparticles ranged between 30 and 80 nm.

3.2. Antioxidant activity of nanoparticles

The antioxidant activity was analyzed by DPPH, ABTS and FRAP methods for AgNPs prepared by green synthesis and the results were expressed in both DPPH and ABTS methods as percentage of radical scavenging and in FRAP method as percentage of Fe^{3+} reduction as well as in all methods as gallic acid equivalent (GAE) in $mg.L^{-1}$. Percentage of DPPH and ABTS radical scavenging by AgNPs prepared by green synthesis is shown in **Figures 2A,B** and percentage of Fe^{3+} reduction measured using FRAP method is shown in **Figure 2C**. Results of antioxidant activity of AgNPs determined by DPPH, ABTS and FRAP method and expressed in GAE are shown in **Figures 2D,E,F**, respectively.

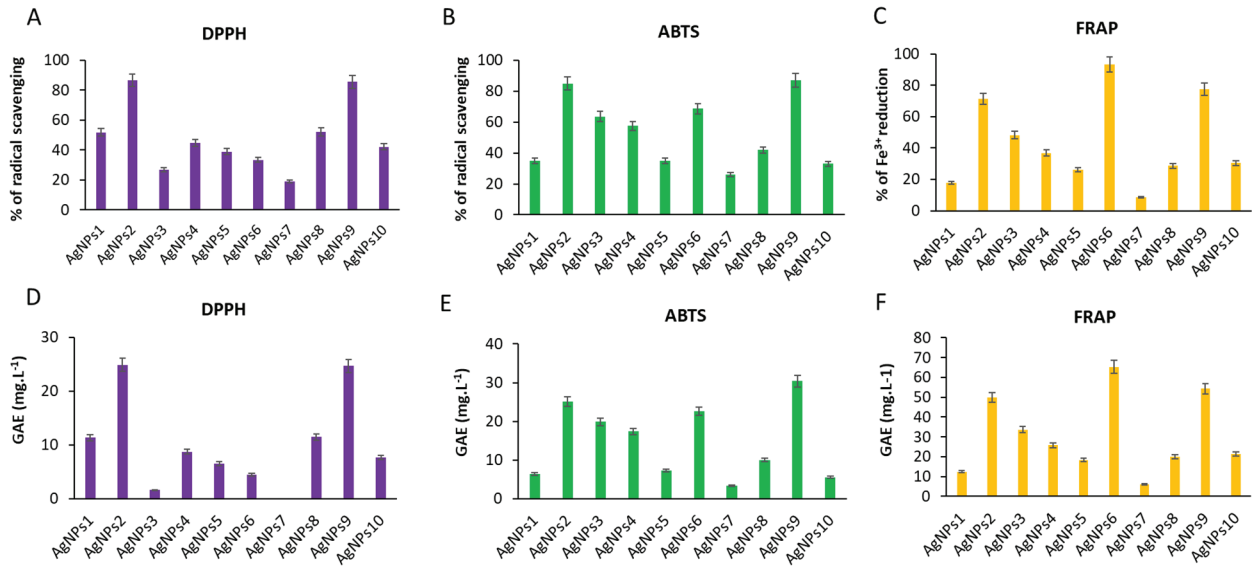

Figure 2 Antioxidant activity of AgNPs synthesized by green synthesis determined by DPPH method (A, D), ABTS method (B, E) and FRAP method (C, F). In Figures 2A and 2B, the antioxidant activity is expressed as percentage of radical extinction, in Figure 2C as a percentage of Fe^{3+} reduction, and in figures 2D, E and F, in GAE. AgNPs were prepared using following plant extracts: AgNPs 1 - black tea (*Camelia sinensis*), AgNPs 2 - green tea (*Camelia sinensis)*, AgNPs 3 - coffee (*Coffea Arabica)*, AgNPs 4 - common thyme (*Thymus vulgaris*), AgNPs 5 - red clover (*Trifolium pratense*), AgNPs 6 - red raspberry (*Rubus idaeus*), AgNPs 7 - absinthe wormwood (*Artemisia absinthium*), AgNPs 8 - common agrimony (*Agrimonia eupatoria*), AgNPs 9 - garden strawberry (*Fragaria ananassa*), AgNPs 10 - purple crownvetch *Securigera varia*.

The highest antioxidant activity was determined in AgNPs 2 (prepared using green tea extract) and in AgNPs 9 (prepared using garden strawberry extract) using DPPH and ABTS methods. Results of antioxidant activity of these samples ranged between 85-87 % of radical scavenging and 24-30 GAE ($mg.L^{-1}$). When FRAP method was used, the highest antioxidant activity was determined in AgNPs 6 (prepared using red raspberry extract). Result of antioxidant activity of the sample was 93 % of radical scavenging and 65 GAE ($mg.L^{-1}$).

4. CONCLUSION

Ten different AgNPs were prepared by green synthesis and spectrophotometrically characterized. The spectral maxima ranged between 470-480 nm. The determination of antioxidant activity using DPPH, ABTS and FRAP

methods showed, that the highest antioxidant activity was determined in AgNPs 2 (prepared using green tea extract), AgNPs 9 (prepared using garden strawberry extract), and AgNPs 6 (prepared using red raspberry extract). The anticancer properties of these AgNPs will be investigated.

ACKNOWLEDGEMENTS

This work has been funded by the grant IGA GREEN-NANO 316/2018 /FaF.

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