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DEPENDENCE OF ANTIBACTERIAL PROPERTIES OF SILVER NANOPARTICLES ON THEIR SURFACE MODIFICATION

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Abstract

Nanosilver, in the form of colloidal silver, has been used for many years. In recent years, the development of efficient green chemistry methods for the synthesis of metal nanoparticles by organisms has become a major focus of researchers. The different forms of nanoparticles prepared by green synthesis using plants are dependent on the structure as well as the potential reactions of molecules present in plant extracts. These forms of nanoparticles can exhibit antibacterial activity to any bacterial strain. The surface of silver nanoparticles (AgNPs) prepared by green synthesis using plants is modified with polyphenols, terpenoids and flavonoids that increase their antibacterial activity. Five types of AgNPs using inorganic synthesis as well as five types of AgNPs using green synthesis were successfully prepared. The AgNPs generated by inorganic synthesis differed in various concentrations of reducing agent ($NaBH_4$, gallic acid). In addition, the AgNPs prepared by green synthesis are easily identified according to the plant extract entering into the synthetic reactions. Extracts of *C. sinensis* (green tea 1 and 2), *T. erecta* (Marigold), *H. perforatum* (St.John's wort) and *A. cepa* (onion) were utilised for the green synthesis. Green synthesized AgNPs had a higher ability for quenching of radicals. Antibacterial activity of AgNPs was determined on bacterial cultures *S. aureus* and '*E. coli*. AgNPs synthesized using green tea showed the highest antibacterial activity which was for *S. aureus* 96 % and for *E. coli* 95 %. The changes in bacterial biochemical parameters were also determined. AgNPs synthesized using St. John's wort caused the highest numbers of biochemical changes (9 cases) in comparison with control. Changes in bacterial biochemical parameters due to effect of AgNPs is a significant discovery which will be worth of further investigation.

Keywords: Nanosilver, antibacterial activity, *Staphylococcus aureus*, *Escherichia coli*

1. INTRODUCTION

Utilization of nanoparticles is widespread in various industrial fields [1]. Nanotechnology and its utilization as a potential antimicrobial component plays a significant role against harmful pathogenic bacteria which received the resistance to current antibiotic agents by their own evolution. Nanosilver, in the form of colloidal silver, has been used for many years. The significant increase of hospital infections' resistance combined with the failure of an effective therapeutic outcome on antibiotics brings a health problem as well as an increment in mortality [2]. Various types of materials have been prepared and tested for their antimicrobial effect [3-5]. In recent

years, the development of efficient green chemistry methods for the synthesis of metal nanoparticles by organisms has become a major focus of researchers. Among these organisms plants seem to be the best candidates as they are suitable for large-scale biosynthesis of nanoparticles [6]. Nanoparticles produced by plants are more biologically active. Nanosilver can be found in a huge variety of different forms and sizes. The forms are dependent on the structure and potential reactions of molecules present in plant extracts in the case of green synthesis [7]. The green synthesis of silver nanoparticles (AgNPs) begins after incubation of the silver ions with plant extract. The process of reduction of silver ions takes place in the presence of phytochemicals (terpenoids, phenols, flavonoids, alkaloids, amino acids, vitamins and polysaccharides) [8]. Surfaces of AgNPs synthesized by green synthesis are modified with a coating derived from phytochemicals, so they are biologically active and exhibit increased antimicrobial activity [9]. In our study we used inorganic and organic "green" synthesis [7] where five types of inorganic and five types of green synthesized AgNPs were prepared and their antibacterial properties were compared.

2. MATERIAL AND METHODS

2.1. Nanoparticle synthesis

Synthesis of AgNPs A, C, E and I: 0.1 M $AgNO_3$ was mixed with distilled water. Then, 1650 µL of 1% tri-sodium citrate was added dropwise. Subsequently, four different types of nanoparticles were synthesised using 20 mM $NaBH_4$ for (AgNPs A) or 10 mL of 15 mM $NaBH_4$ for (AgNPs I), 10 mL of 10 mM $NaBH_4$ for (AgNPs C) and 10 mL of 5 mM $NaBH_4$ for (AgNPs E). Those amounts were added and the solution was stirred for 1 hour to colour change to yellow which indicated the particle formation. Synthesis of AgNPs B, D, F, H and J: 2 g of green tea 1 (AgNPs B) or green tea 2 (AgNPs D) or St John's wort (AgNPs F) or Marigold (AgNPs H) or onion (AgNPs J) was dipped in distilled water. The solution was heated, cooled and filtered. The filtrate was obtained as a reducing agent. Synthesis of AgNPs G: 0.001 M $AgNO_3$ was mixed with 10 mL of gallic acid (1 mg/mL) and the solution was stirred for 1 hour until the color changed to yellow.

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) 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

The DPPH test is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors [4]. The ABTS radical method is one of the most employed assays for the determination of free radicals concentration. 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): $ABTS^{\bullet-}$ e^- $ABTS^{\bullet+}$ [10].

2.4. Determination of antibacterial properties

To investigate the antimicrobial effect of AgNPs the absorbance was measured using Tecan Infinite 200 PRO (TECAN, Switzerland). In the microplate *S. aureus* and *E. coli* was mixed with AgNPs. The concentrations of AgNPs samples A - G were: 0, 1.25, 10, 40 and 160 µM and from samples H and I were 0, 0.065, 0.52, 2.1 and 8.3 mM [18]. The STAPHYtest16 was utilised to determine the utilized substrates.

3. RESULTS

3.1. Characterization of nanoparticles

The AgNPs were characterized by absorbance spectra (**Figures 1, A, B, C, D, E, F, G, H, I, J-a**) with an absorption maxima ranged from 270 nm (AgNPs B) (**Figure 1B-a**) to 405 nm (AgNPs I) (**Figure 1I-a**). The nanoparticles size and the zeta potential were also characterized. **Figure 1A - J-b** shows, that the nanoparticles size of AgNPs A, B, C, E (**Figures 1A, B, C, E-b**) was found to be 55 ± 3 nm. The zeta potentials of AgNPs A, B, C, D, E, F, G, H, I, J-b was -19 mV. **Figure 1A - J-c** [11] illustrates the relevant images of the nanoparticles in different solutions.

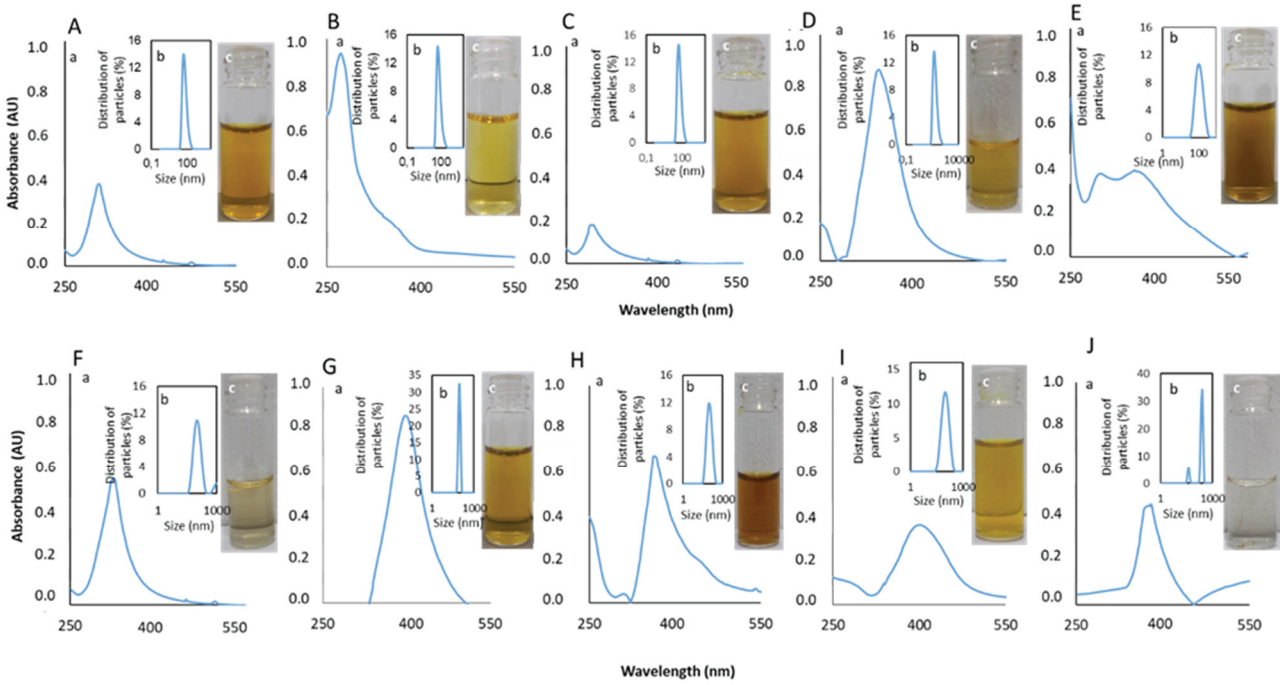

Figure 1 Characterization of synthesized silver nanoparticles (A - AgNPs A, B - AgNPs B, C - AgNPs C, D - AgNPs D, E - AgNPs E, F - AgNPs F, G - AgNPs G, H - AgNPs H, I - AgNPs I, J - AgNPs J) by absorption spectra (A - J-a). The nanoparticles size distribution measurements were performed by Zetasizer. Measuring conditions were: detector angle 173°, wavelength 633 nm, refractive index 0.30, a real refractive index 1.59, and a temperature 25 °C (A - J-b). The relevant characteristic images of the nanoparticles in solutions are shown in the pictures marked with c.

3.2. Antioxidant and antibacterial activity of nanoparticles

Percentage expression of the quenching of ABTS or DPPH radicals by AgNPs prepared by inorganic synthesis is shown in **Figure 2A** and by the AgNPs prepared by green synthesis in **Figure 2B**. Nanoparticles prepared by green synthesis showed greater efficiency in quenching of radicals. This effect was most evident by the DPPH method. **Figure 2 C** depicts the application of AgNPs prepared by inorganic synthesis on bacterial strains *S. aureus* and *E. Coli*. It shows that the AgNPs E was the most effective inhibitor of *E. coli* (95% inhibition effect) whereas AgNPs G exhibits 74% inhibition of *S. aureus* growth. AgNPs B illustrates the highest and the most incomprehensible inhibitory effect of all prepared nanoparticles showed with an inhibition of 96 and 95 % for *S. aureus* and *E. coli*, respectively (**Figure 2D**). The AgNPs F, H, J inhibited gram-negative *E. coli* with higher effect than gram-positive *S. aureus*. The most potent inhibitory effect was given by AgNPs B which were prepared using extract from green tea 1.

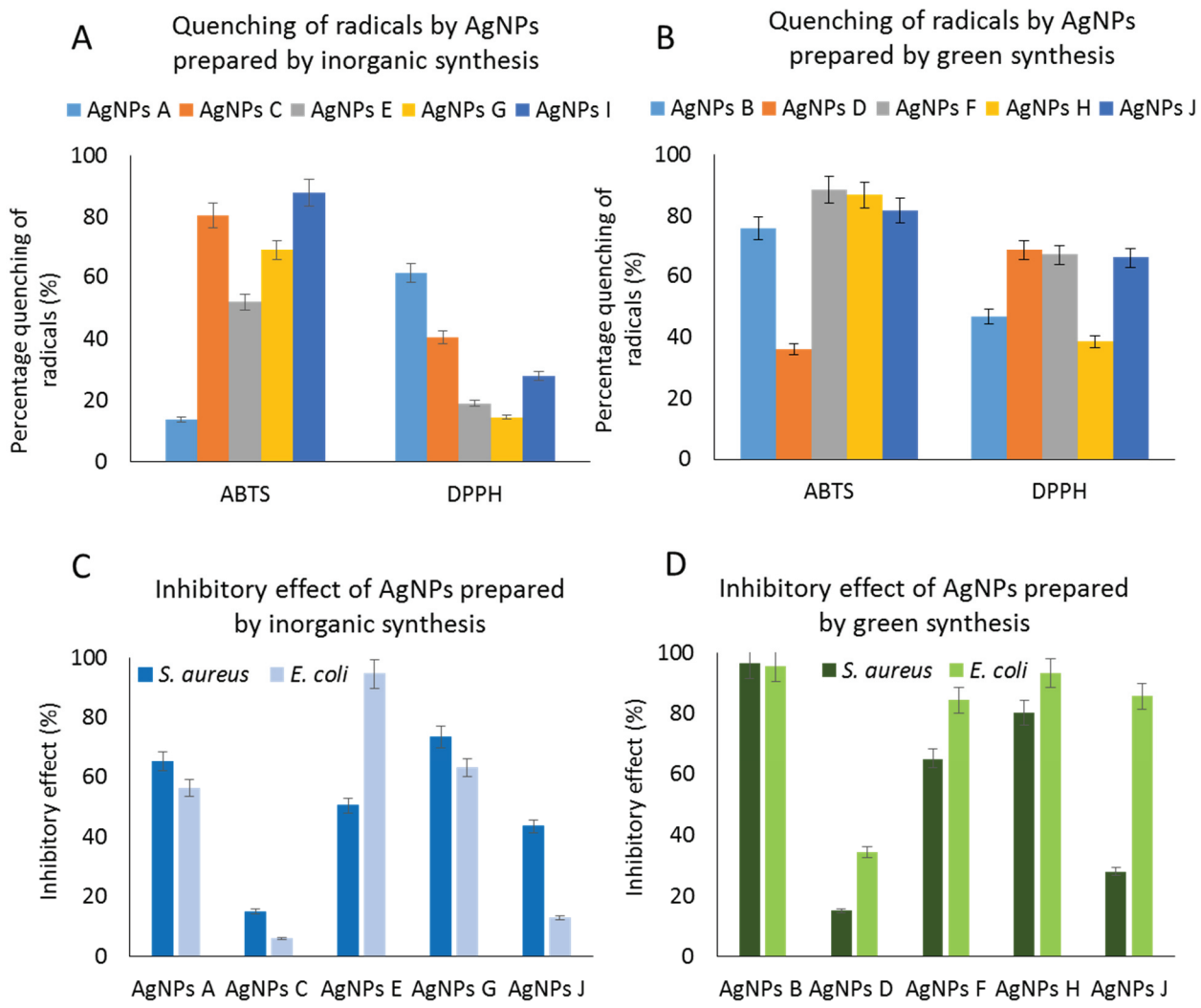

Figure 2 Percentage expression of the ability to quench ABTS or DPPH radicals by AgNPs prepared by inorganic (A), and green synthesis (B). Percentage of inhibitory effect of AgNPs on bacterial strains *S. aureus* and *E. coli* using AgNPs prepared by inorganic synthesis (C) and by green synthesis (D). Inhibitory effect 0% represents control value and 100% represents total inhibition of bacterial growth.

3.3. Biochemical changes of S. aureus after incubation with selected AgNPs

AgNPs with an average inhibition effect for testing changes in biochemical parameters of *S. aureus* were selected. The AgNPs B and H inhibited *S. aureus* growth totally, therefore, we focused on nanoparticles with partial inhibition of bacterial growth which were AgNPs J, G, F, C, A. **Figure 3A** illustrates biochemical test with *S. aureus* and with selected AgNPs. *S. aureus* without nanoparticles was used as a control test. *S. aureus* culture used for biochemical test was diluted to absorbance value 0.1. **Figure 3B** depicts the biochemical tests in individual well position. The most numerous biochemical changes occurred after an application of AgNPs F (synthesized using St John's wort extract) to *S. aureus*. The changes occurred in all cases except for phosphatase, esculin, β-glucuronidase, β-galactosidase, ornithine, urease and xylose metabolism**. Figure 3C** shows different values of absorbance of individual tests over the time 0, 4, 8, 12, 16 and 20 hours. The increasing or decreasing trend for AgNPs F was the most obvious. Silver nanoparticles synthesized using St John's wort caused the highest numbers of biochemical changes (9 cases) in comparison to control.

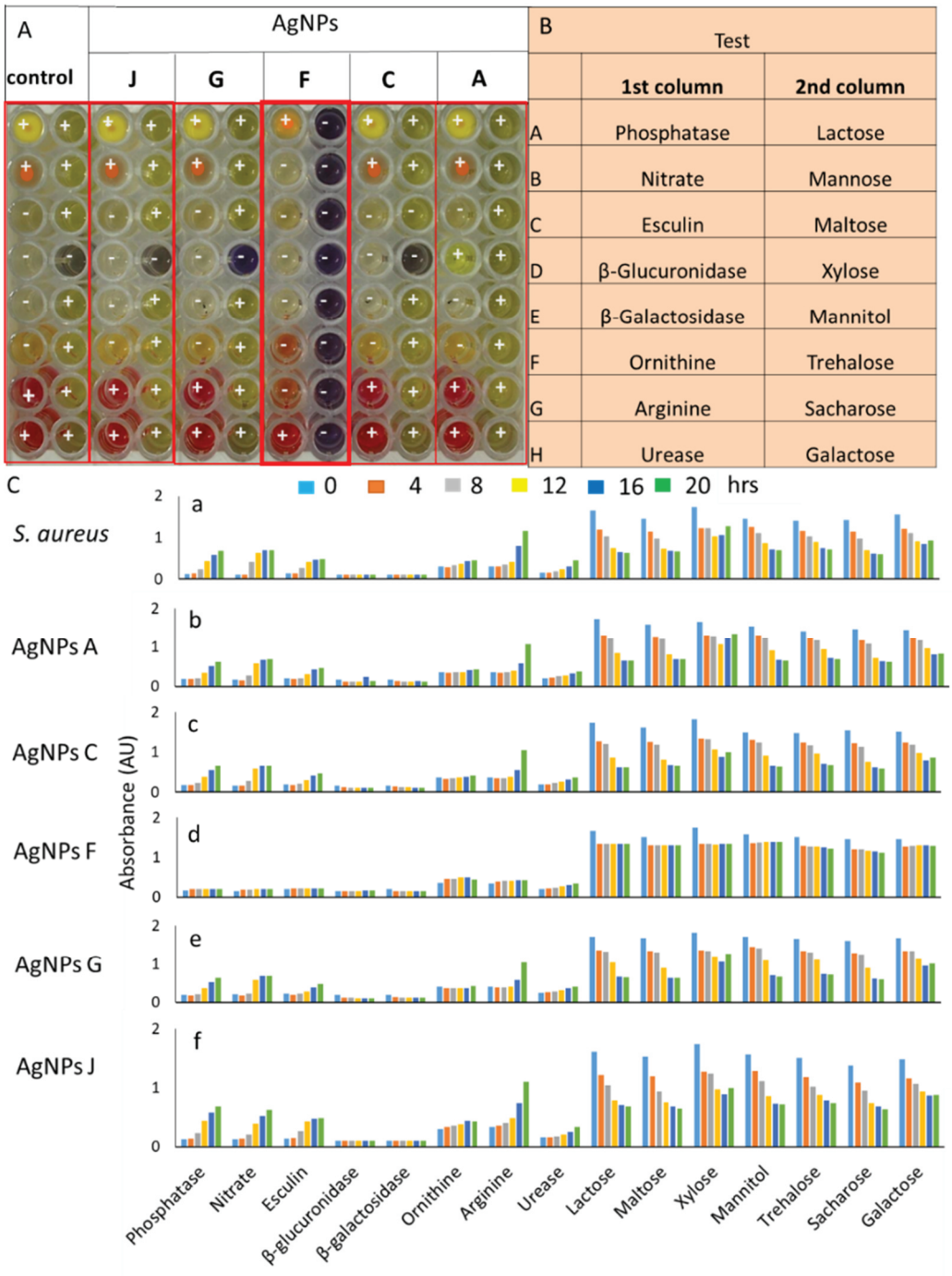

Figure 3 The changes in biochemical parameters of *S. aureus* after AgNPs (8 mM) addition were observed by STAPHYtest 16. A) 16 well microplates with *S. aureus* control and *S. aureus* incubated with 8 mM AgNPs J (onion extract), G (gallic acid), F (St John's wort extract), C (10 mM $NaBH_4$) and A (20 mM $NaBH_4$); B) List of biochemical tests with different substrates for bacteria in individual well positions; C) Biochemical reactions observed by intensity of colour change (absorbance value).

4. CONCLUSION

This study compared the antimicrobial activity of silver nanoparticles prepared by green synthesis and inorganic synthesis. Silver nanoparticles prepared by green synthesis showed higher antimicrobial activity as well as higher ability for quenching of free radicals. Silver nanoparticles synthesized using St John's wort

caused the highest number of changes in bacterial biochemical parameters. Silver nanoparticles, which were prepared by green synthesis, appear to be potent biogenic nanoparticles with antimicrobial activity due to the modification of their surface by phytochemicals.

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