Antimicrobial treatment for textiles based on flavonoid-mediated silver nanoparticles dispersions

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ABSTRACT – REZUMAT

Antimicrobial treatment for textiles based on flavonoid-mediated silver nanoparticles dispersions

*The use of "green" reagents for silver nanoparticles (AgNPs) synthesis represents an attractive alternative to conventional methods, due to the abundance of available biomass with reducing properties. Herein, a flavonoid compound, hesperidin, was used to produce AgNPs, and the resulting dispersion was applied to textile fabrics and tested for its antimicrobial properties. Different ratios of flavonoid to the silver precursor (v:v) were prepared. The formation of the nanoparticles was indicated by the presence of the SPR specific band in the UV–Vis spectra. For the synthesis optimization, the DLS technique was used to determine the size and polydispersity of the AgNPs, while zeta potential measurements were performed to assess their physical stability. For the dispersion with the optimal ratio, particles with the average size (Z_{av}) of 93.55±3.51 nm were obtained, with a polydispersity index (PdI) of 0.259. For this ratio, the value of the zeta potential (ξ.) was –28.80±1.54 mV, indicating that the dispersion is physically stable. The nanoparticles were further characterized using SEM-EDS techniques to confirm the particles' nature and evaluate their morphology. The AgNPs were deposited on textile samples, by direct soaking into the dispersion. The distribution of the AgNPs on the textile samples fibres was evaluated by SEM and the chromatic parameters L*a*b* were determined compared to the untreated samples. Antimicrobial tests were conducted against Escherichia coli, Staphylococcus aureus, and Bacillus subtilis strains, according to the ISO 20743:2013 standard, and percentages of bacterial reduction up to 99.99% were obtained.*

Keywords: *silver nanoparticles, flavonoids, hesperidin, dispersions, antimicrobial textiles*

Tratament antimicrobian pentru textile pe bază de dispersii de nanoparticule de argint mediate de flavonoizi

*Utilizarea de reactivi „verzi" pentru sinteza nanoparticulelor de argint (AgNPs) reprezintă o alternativă atractivă la metodele convenționale, datorită abundenței biomasei disponibile cu proprietăți reducătoare. În lucrarea de față, un compus flavonoid, hesperidina, a fost folosit pentru a produce AgNPs, iar dispersia rezultată a fost aplicată pe țesături textile și testată pentru proprietățile sale antimicrobiene. Au fost preparate diferite rapoarte de flavonoid la precursor de argint (v:v). Formarea nanoparticulelor a fost indicată de prezența benzii specifice SPR în spectrele UV-Vis. Pentru optimizarea sintezei, tehnica DLS a fost utilizată pentru a determina dimensiunea și polidispersitatea AgNPs, în timp ce măsurătorile potențialului zeta au fost efectuate pentru a evalua stabilitatea lor fizică. Pentru dispersia cu raportul optim, s-a obţinut AgNPS cu dimensiunea medie de particule (Z_{av}) de 93,55±3,51 nm, cu un indice de polidispersitate (PdI) de 0,259. Pentru acest raport, valoarea potențialului zeta (ξ.) a fost de –28,80±1,54 mV, ceea ce indică faptul că dispersia este stabilă din punct de vedere fizic. Nanoparticulele au fost caracterizate în continuare, folosind tehnicile SEM-EDS, pentru a confirma natura particulelor și pentru a evalua morfologia lor. AgNPs au fost depuse pe probele textile, prin îmbibarea directă în dispersia de AgNPs. Distribuția AgNPs pe fibrele din probele textile a fost evaluată prin SEM și au fost determinați parametrii cromatici L*a*b* comparativ cu probele netratate. Au fost efectuate teste antimicrobiene pe tulpini de Escherichia coli, Staphylococcus aureus și Bacillus subtilis, conform standardului ISO 20743:2013 și s-au obținut procente de reducere bacteriană de până la 99,99%.*

Cuvinte-cheie: *nanoparticule de argint, flavonoizi, hesperidină, dispersii, textile antimicrobiene*

INTRODUCTION

Textile fabrics represent an excellent media for the growth of microorganisms, such as fungi, moulds, and bacteria. The metabolism products of these pathogens lead to the degradation of the fibres, the generation of unwanted odour, and an increased risk of contamination [1, 2]. Even though if the research of antimicrobial textiles is not a new topic, the context of the SARS-CoV-2 pandemic brought attention to the continuous need for developing innovative treatments for textiles disinfection [3]. The current antimicrobial reagents include quaternary ammonium salts, polyhexamethylene biguanide, regenerable N-halamines and peroxyacids, chitosan and chitosan derivatives etc. [1, 4]. Apart from chemicals, there are reports about achieving the manufacturing of antimicrobial textiles by using natural compounds, such as curcumin, chitosan, or essential oils [3]. The field of nanomaterials gained the attention of the research community for its remarkable properties and applications [4, 5]. Ag, ZnO, MgO, CaO, CuO, and TiO_2 nanoparticles are among the nanomaterials assessed for exhibiting antimicrobial properties [6–10]. Silver nanoparticles (AgNPs) present the capacity to inactivate bacteria due to the interaction with the sulfur contained in the constituent amino acids of the proteins from the bacterial cell membranes. Moreover, the interaction of the silver ion with the DNA phosphoric groups leads to the inhibition of the enzyme's activities [11]. The advantage of using AgNPs consists of the ease to produce them, at low costs, and by environment-friendly methods when using green synthesis [12]. Otávio Augusto L. dos Santos investigated the antimicrobial potential of AgNPs applied on cotton gauze. The AgNPs treatment led to microbial growth inhibition from 50% to 90% against *E. coli*, *S. epidermidis*, *S. aureus* and the fungal strain of *C. albicans* [13]. In another study, published by Fatma A., the antimicrobial effect of AgNPs on linen textile samples was investigated. The antibacterial tests against *E. Coli* and *S. aureus* demonstrated that the treated samples maintained their antibacterial properties even after 20 washing cycles [14].

In this study silver nanoparticles (AgNPs) have been synthesized using hesperidin as a reducing agent. Hesperidin is a flavonoid compound present in many plant parts. Its potential as a reducing agent arises from the phenolic groups in its structure [15]. Different ratios of hesperidin to silver ions were tested and the formation of the AgNPs was revealed by recording the UV-Vis absorption spectrum, due to the presence of the specific absorption band called the surface-plasmon resonance (SPR) band. Synthesis optimization consisted of determining the average size, polydispersity, and physical stability of the nanoparticles, by performing dynamic light scattering (DLS) measurements and by determining the zeta potential, respectively. The size and nature of the particles were confirmed by scanning electron microscopy (SEM) coupled with an X-ray detector (energy dispersive X-ray spectroscopy – EDS). The optimal dispersion of AgNPs was tested on textile samples (cotton and wool) for its antimicrobial effect against *Escherichia coli, Staphylococcus aureus, and Bacillus subtilis strains.* The distribution of AgNPs on the textile fibres was assessed by SEM and their nature was confirmed by EDS. Moreover, the chromatic parameters of the treated and untreated samples were determined and compared, and a total colour change was quantified.

EXPERIMENTAL

Material and methods

The source of silver ions consisted of silver nitrate, which was purchased from Anal-R NORMAPUR. The rest of the substances were purchased from Merck. The cotton and wool textile samples were provided by the National R&D Institute for Textiles and Leather (INCDTP, Bucharest, Romania). The antibacterial tests were conducted using *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, and *Bacillus subtilis* ATCC 6633 strains. The culture media consisted of: TSA (Casein Soya Bean), TSB (Tryptic Soy Broth), NB (Nutrient broth), EA (Enumeration Agar), Digest Agar, and SCDLP (Casein Soya Bean Digest). The pretreatment of the samples for performing the DLS tests consisted of adding 300 µl of AgNPs dispersion to 20 ml of distilled water. For the zeta potential measurements, an increase in the conductivity of the samples was necessary. Hence, 50 µl of NaCl 0.9% solution was added to the 20 mL of water and 300 µl of AgNPs dispersion.

Before the AgNPs morphology evaluation, 300 µl of dispersion was deposited, by pipetting, on a SiO_2 slide. After the evaporation of the liquid, the SiO2 slide was subjected to analysis

Synthesis and optimization

Aqueous solutions of hesperidin 1 mM were prepared, while adjusting the pH to 11.80 with NaOH, for completing the dissolution of flavonoid compounds. For the pH monitorization, the Crison GLP 21+ pH meter (Barcelona, Spain) was used.

For optimization, different ratios flavon:$AgNO_3$, were used - 1:1, 1:3, 1:5 and 1:9 (v:v). The first indicator of the AgNPs formation consisted of the dark colour of the reaction mixtures, after adding the silver precursor to the flavonoid solutions. After two hours, the reaction mixtures were centrifuged at 5000 rpm for 30 minutes. The supernatant was collected for characterization and used in further experiments.

A Lambda 950 UV-Vis spectrophotometer, from Perkin Elmer, was used to record the absorption spectra of the AgNPs dispersion. The presence of the AgNPs was confirmed by the characteristic SPR (surface plasmon resonance) absorption band [16, 17].

The size, polydispersity, and physical stability of the dispersions were evaluated using a Zetasizer Nano ZS instrument from Malvern Instruments Ltd. (Worcestershire, UK). Dynamic Light Scattering (DLS) technique was conducted for determining the average size (Z_{av}) and polydispersity index (PdI), which indicates the distribution of the particles size populations. The physical stability of the AgNPs dispersions was determined by performing zeta potential (ξ) measurements, which is an indicator of physical stability. A dispersion is considered stable when the absolute value of its zeta potential exceeds 25 mV [18].

Characterization of produced AgNPs

Scanning Electron Microscopy (SEM) was performed to evaluate the morphology of the produced AgNPs. For this purpose, a FEI Quanta 200 microscope was used, which was provided with an Everhart-Thornley (ET) detector. Moreover, the nature of the nanoparticles was confirmed by performing X-ray dispersive spectroscopy (EDS), using an EDAX-AMETEK X-ray detector coupled to the electron microscope instrument. The dispersion with the optimal ratio was deposited on a SiO_2 slide and, after evaporation of the liquid, the sample was subjected to the SEM-EDS characterization.

Effect of AgNPs dispersions on textile samples

The treatment of the textile samples (10 × 10 cm) consisted of directly soaking the cotton and wool samples into the silver nanoparticles dispersions. The samples were left to dry at room temperature, then they were characterized by SEM, to evaluate how the AgNPs were distributed on the textile fibres. Their nature was confirmed by performing EDS measurements.

The chromatic parameters, expressed in the CIE L*a*b* system of colours, of the treated and untreated textile samples (the reference fabrics) were measured, using the Datacolor spectrophotometer, from DKSH Holding Ltd. (Zurich, Switzerland), equipped with a D65/10 lamp).

The antibacterial tests were conducted according to the ISO 20743:2013 standard, which involves quantitative test methods to determine the antimicrobial activity of finished antimicrobial samples. The absorption method was used, which involved the direct inoculation of the test bacteriological inoculum directly onto the treated samples.

RESULTS AND DISCUSSION

Characterization of the AgNPs dispersions

Spectral characterization

The reduction of the silver is firstly indicated by the brownish colour of the reaction mixture. The UV-Vis spectra recorded for the hesperidin solution and the AgNPs dispersions prepared in different ratios of hesperidin solution to silver ions are presented in figure 1. The bands present at 280 nm and 350 nm correspond to hesperidin, while the bands at 430 nm are attributed to the reduced silver and appear due to the specific surface plasmon resonance (SPR) of AgNPs [19–21]. The width of the bands is an indicator of the size and polydispersity of the AgNPs.

Size, polydispersity, and physical stability of the AgNPs dispersions

The average particle size (Z_{av}) determined by DLS measurements (figure 2, *a*) of the AgNPs varied from 65 to 155 nm (from the ratio 1:1 to the ratio 1:9).

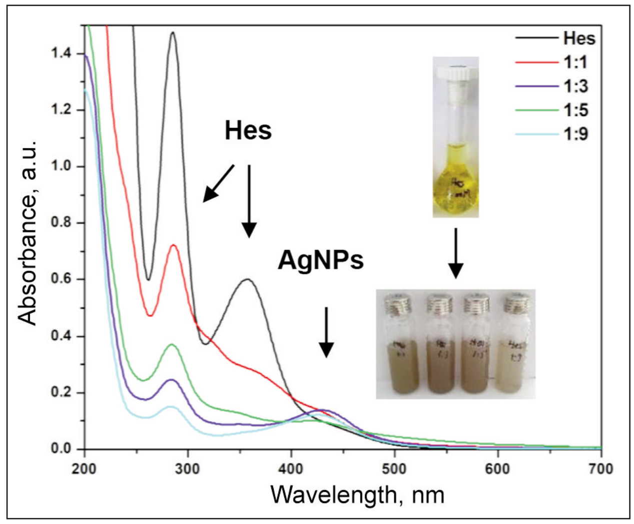


Fig. 1. UV-Vis spectra of hesperidin solution and AgNPs dispersions

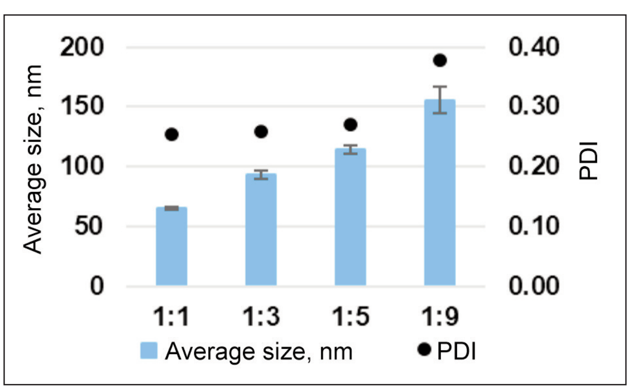
While the average particle size underwent a linear growth, the polydispersity index was constant for the first three ratios, and, at values 0.255–0.271, and had the value 0.378 when the ratio was 1:9. Moreover, while for the ratios 1:1 and 1:3 the particle size is situated in the nanoscale, the size of the particles produced using 1:5 and 1:9 ratios exceed this scale (the particle size exceed 100 nm).

The absolute values of the zeta potential (figure 2, *b*) exceeded 25 mV, when the ratio was 1:3, 1:5, and 1:9, indicating that this dispersion is physically stable. When correlating the variation of the size, polydisperisy and physical stability of the dispersions, it is observed that large and polydisperse silverpartiles lead to the stabilization of the dispersions.

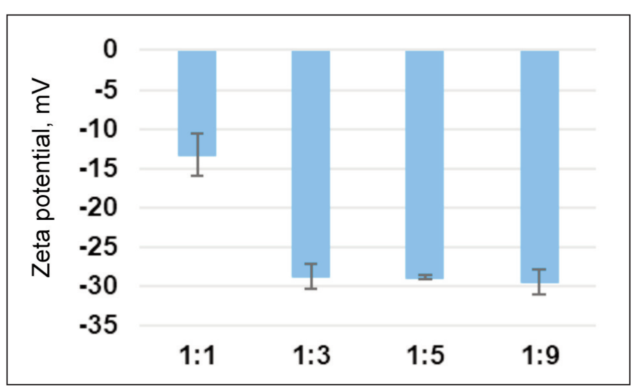
The optimal ratio selected for further characterization is 1:3.

Morphological characterization of AgNPs

The SEM-EDS results are illustrated in figure 3. The DLS values are confirmed by the SEM measurements. The silver nanoparticles agglomerate into clusters of different sizes and shapes. The nature of the particles was confirmed by the EDS spectrum.



a



b

Fig. 2. Graphical representations of: *a* – the average size (Z_{ave}) and polydispersity index (PdI) of AgNPs, evaluated using the Dynamic Light Scattering (DLS) technique; *b* – zeta potential measurements of the AgNPs dispersions

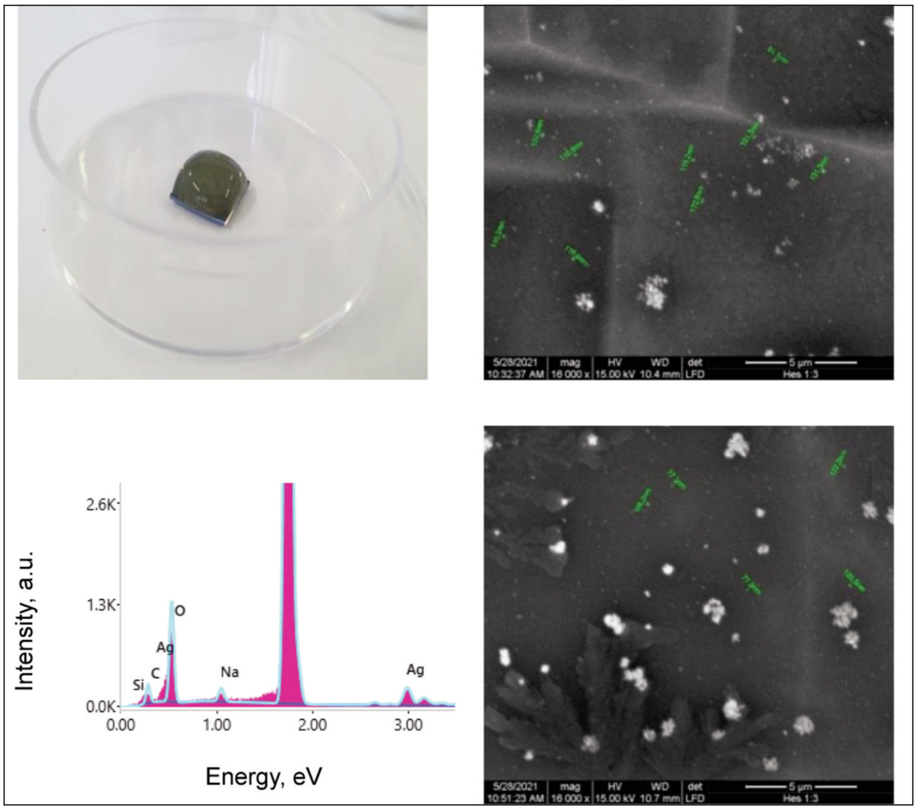


Fig. 3. SEM-EDS results of the AgNPs fabricated using the ratio Hes:$AgNO_3$ of 1:3

Characterization of textile samples treated with AgNPs dispersions

Morphological evaluation and nature confirmation of the AgNPs deposited on textile

The morphology of the treated textile fibres is presented in figure 4 and the EDS spectra in figure 5. The results suggest a uniform distribution of the silver nanoparticles on the surface of the fibres.

Chromatic evaluation

The values of the chromatic parameters L* a* b* are listed in table 1. The total colour change is quantified through the value of ΔE*.

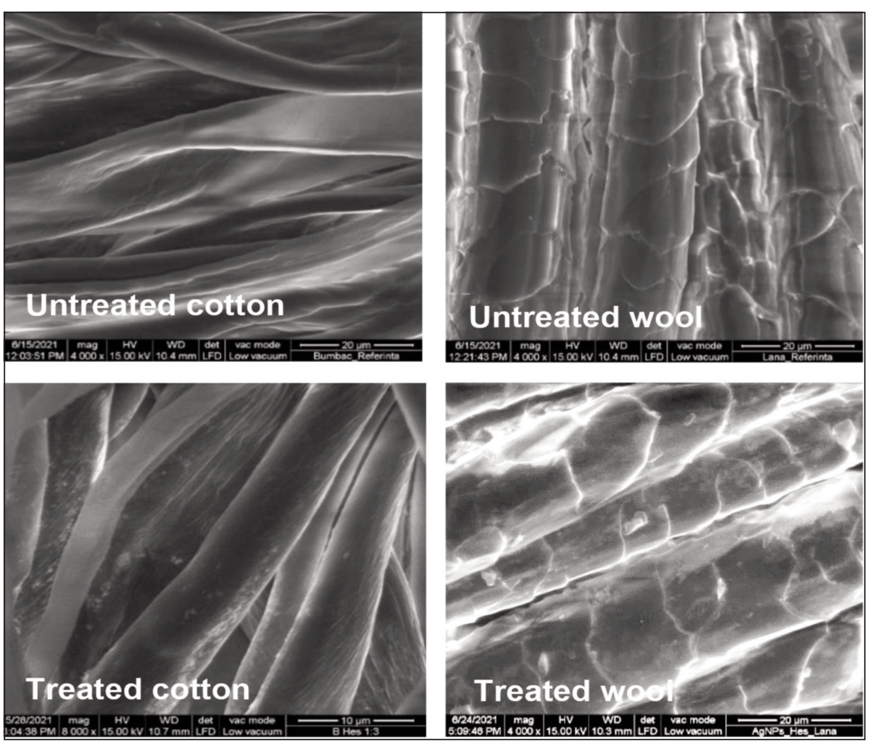


Fig. 4. SEM micrographs of textile fibres before and after applying the AgNPs dispersions

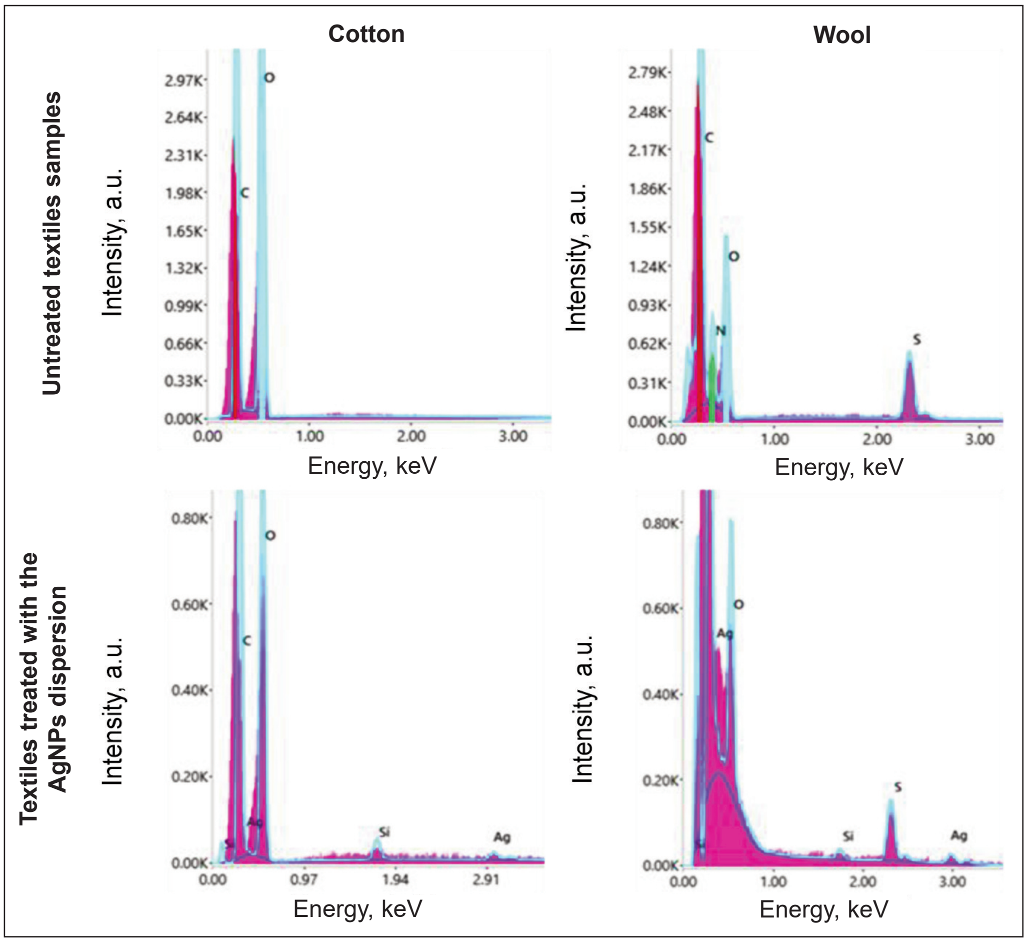


Fig. 5. EDS spectra of textile fibres before and after applying the AgNPs dispersions

While the colour of the cotton sample is visibly affected by the AgNPs dispersion, having a colour difference ΔE* of 6.18, the wool sample is not chromatically affected by the treatment (ΔE* = 0.83). Compared to our previous study where the cotton sample colour was affected in terms of luminosity, the current treatment produces an increase of the b* parameter, from 3.19 to 9.07, meaning that the sample becomes more yellow [22].

Antimicrobial activity

The percentage of bacterial reduction exceeded 99% in all cases (table 2). The treatment has been effective against the tested bacteria strains for both cotton and wool samples.

Table 1

CHROMATIC PARAMETERS OF THE TEXTILE SAMPLES BEFORE AND AFTER APPLYING THE AGNPS DISPERSIONS

Textile sample	**Cotton**							**Wool**						
Chromatic parameter	**L***	**a***	**b***	**ΔL***	**Δa***	**Δb***	**ΔE***	**L***	**a***	**b***	**ΔL***	**Δa***	**Δb***	**ΔE***
Untreated textile samples	93.58	–0.01	3.19	-	-	-	-	85.32	–0.04	12.07	-	-	-	-
Textile samples treated with AgNPs	91.86	–0.82	9.07	–1.73	–0.81	5.88	**6.18**	84.51	–0.24	12.18	–0.80	–0.20	0.11	**0.83**

Table 2

PERCENTAGES OF BACTERIA REDUCTION OF THE TEXTILE SAMPLES (COTTON AND WOOL) TREATED WITH AGNPS SYNTHESIZED WITH HESPERIDIN

Bacteria strains	***Escherichia coli***	***Staphylococcus aureus***	***Bacillus subtilis***
Cotton	99.99%	99.99%	100.00%
Wool	99.99%	99.99%	100.00%

CONCLUSIONS

The present study demonstrated the effectiveness of green synthesized AgNPs for antibacterial textile application. Hesperidin, a flavonoid compound, was used in the synthesis as a reducing agent of the silver ions. Stable nanoparticles (ξ = –28.80±1.54 mV), with an average diameter of 93.55±3.51 nm and a polydisperse index of 0.259 were obtained for the optimal ratio used for the synthesis (1:3). The average size was confirmed by SEM characterization and the nature was confirmed by EDS technique. The textile samples (cotton and wool) treated with the dispersion with the optimal ratio (1:3), Hes: $AgNO_3$ (v:v), were characterized by SEM-EDS and by determining the colour change and the antibacterial effectiveness. ΔE* was 0.83 for the wool sample and 6.18 for the cotton sample, with b* as the main parameter affected, leading to the acquirement of a yellow colour. The treatment has been proven to be effective against the *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis* bacteria strains for both cotton and wool samples, with the percentage of bacterial reduction exceeding 99%.

The present study demonstrated both the effectiveness of the AgNPs synthesis using hesperidin as a reducing agent and the superior antibacterial performances of the resulting dispersion for obtaining antimicrobial textiles.

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