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ORIGINAL PAPER

DETERMINATION OF ANTIBACTERIAL PROPERTIES OF SILVER NANOPARTICLES WITH AQUEOUS EXTRACTS OF *BRASSICA OLERACEA* L. VAR. *ACEPHALA* D.C. IN COTTON TEXTILES

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Abstract

Silver nanoparticles (AgNPs) were synthesized by using leaf extracts of Brassica oleracea L. var. acephala D.C. (Kale), and the antibacterial character of cotton fabric loaded with these nanoparticle solutions was tested against four different bacteria. The conditions for preparing nanoparticle solutions were optimized by adjusting time, volume and concentration of silver nitrate solutions. UV-Vis spectroscopy, SEM-EDS, FT-IR and scanning transmission electron microscopy (STEM) were used for the characterization of AgNPs. The synthesized nanoparticles from Kale have spherical shapes with an average size of 25±5 nm. The antibacterial effect of nanoparticles on 4 different bacteria (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Enterococcus faecalis) was determined using the Kirby-Bauer Disc diffusion and broth microdilution methods. AgNPs obtained from Kale leaves were found to have the highest activity towards E. coli (15 mm) and E. faecalis (15 mm), and nanoparticles synthesized from Kale petioles showed the most effective antibacterial activity against P. aeruginosa (14 mm). Cotton fabric loaded with these nanoparticles displayed high antibacterial activity against E. coli (18 mm) and S. aureus (18 mm). Although cotton fabrics that were treated with AgNPs were washed with ultra pure water for 20 min, they still exhibited good durability in terms of antibacterial character. In conclusion, cotton fabric containing AgNPs showed an antibacterial activity against E. coli, E. faecalis, P. aeruginosa and S. aureus. AgNPs synthesized from aqueous extracts of Kale can be employed for economical production of antimicrobial cotton fabrics.

Keywords: antibacterial activity, *Brassica oleracea* L. var. *acephala* D.C., cotton textiles, silver nanoparticles.

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INTRODUCTION

Cotton textiles are used in everyday life owing to their softness, affinity to skin, capability to absorbe moisture and the ability to protect body warmth. However, cotton textiles provide an appropriate medium for the growth of microrganisms when they are in contact with oxygen, humidity, body temperature and exudates. The widespread use of antibiotics has led to the emergence of antibiotic-resistant bacteria, which has become a worldwide problem. Silver ions and silver nanoparticles at low concentrations are nowadays prominent for their antibacterial activities that reduce the risk of infection. Metallic nanoparticles such as silver and gold are more effective in treatment against antibiotic resistant bacteria (MOHAMMED et al. 2018). The manufacture of antibacterial textiles has gained importance since antibiotic resistant bacteria are the main cause of hospital infections which threaten human health. Antibacterial textiles can be also used in wounddressing applications for healing wounds and burns (EL-SHISHTAWY et al. 2011, MORITZ, GESZKE-MORITZ 2013).

Nanotechnology is a new branch of interdisciplinary science which combines biology, chemistry, physics, material science and engineering, and it is a field in which nano-sized particles are synthesized and used (DEP 2014, MORITZ, GESZKE-MORITZ 2013). Nanoparticles have a great number of aplications in biomedical, optical and electrical fields, etc. (PHANJOM et al. 2012). Owing to their antibacterial activity, nanoparticles are used to develop antibacterial agents; particularly, in food, textile, medical and other industries (MORITZ, GESZKE-MORITZ 2013). The size, shape and morphology are important factors affecting the antimicrobial activity of nanoparticles. Some of the nanoscale antimicrobials such as chitosan, titanium dioxide and noble metal nanoparticles have been mostly applied for fiber coatings. These nano-sized materials provide some advantages such as a greater surface area to volume ratio, adherence to the substrate and longer antimicrobial protection activity (KASHID et al. 2017). Since nanoparticles have higher surface areas, they penetrate into bacteria much more efficiently. Therefore, they maximize their antimicrobial reactivity (SATYAVANI et al. 2011, TAMILESWARI et al. 2015). Among the noble metal nanoparticles, gold and silver nanoparticles have attracted significant attention among researchers. Silver interacts with proteins in thiol (-SH) groups, where it binds to the bacterial cell wall and cell membrane. Damage observed in membranes that come into contact with silver nanoparticles causes leakage of cellular contents and thus cell death. Nanoparticles with a smaller diameter have a larger surface area and it has been observed that these particles have more antibacterial activity. These nanoparticles can reach the cytoplasm more readily than large nanoparticles, and the silver ion release from these nanoparticles is more intensive. After the nanoparticles and silver ions enter the cell, they also interact with cellular structures such as proteins, enzymes, DNA and ribosomes. These interactions will lead to cell death due to the disruption of morphological and functional properties of cellular structures. In addition, nanoparticles have been reported to increase the generation of reactive oxygen species in normal cells. ROS increase can cause respiratory inhibition, decrease in ATP production, lipid peroxidation and DNA damage (JALILIAN et al. 2020). Silver has been recognized for having good antimicrobial properties and inhibitory effects on microbes. It has been found that silver nanoparticles are effective against bacteria such as *E. coli, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* (GHOLAMREZA et al. 2014). Silver nanoparticles are known and used in textile and medical industries for their antimicrobial and wound healing properties (GHOLAMREZA et al. 2014, Roy et al. 2015).

Several methods are used to synthesize nanoparticles, such as chemical, physical and biological methods. The most popular methods to produce nanoparticles are chemical approaches (ZIA et al. 2016). When compared with chemical methods in which toxic and harmful chemicals are used, it was observed that biological methods of nanoparticle synthesis using plant extracts have many advantages due to their cost effectiveness and ecofriendly nature (PHANJOM et al. 2012, ANJUM et al. 2013, DEP 2014). Organic and inorganic components of leaf extracts can be used as reducing and capping agents when they react with metal ions, although the formation of nanoparticles using plant extracts is not completely known (GAVARKAR et al. 2014, FIRDHOUSE, LALITHA 2015). Active biological compounds in plant extracts such as proteins, polysaccharides, vitamins, polyols, water soluble heterocyclic complexes, flavones, terpenoids and polyphenols were found to be responsible for the formation of metal particles via the reduction of metal ions (BARANI et al. 2012, GAVARKAR et al. 2014). These phytochemicals also act as agents that encapsulate nanoparticles, stabilizing them and preventing their clumping together (JALILIAN et al. 2020). Brassica oleracea L. var. acephala D.C. (Kale) is a green plant which is a member of the Brassicaceae family, which also includes cabbage and broccoli. In Turkey, especially in the Eastern Black Sea Region, Kale is consumed as traditional food. It is a rich source of minerals (iron, calcium, potassium, etc.), vitamines (vitamin A, vitamin C, vitamin B_e , vitamin E, vitamin K) and it also contains lutein, zeaxanthin and carotene, which are flavonoid antioxidants (ANJUM et al. 2013, DEP 2014). Since the components of Kale have strong antioxidant capacity, Brassica vegetables have beneficial effects on human health owing to their chemopreventive characteristics against stomach, colon and lung cancer (GIORGETTI et al. 2018). The purpose of this work is to synthesize silver nanoparticles (AgNPs) using aqueous extracts of Kale and to prepare antibacterial cotton textile with the application of AgNPs onto cotton fabric. To the best of our knowledge, there is no study about the synthesis of silver nanoparticles by using extracts of *Brassica oleracea* L. var. acephala D.C. and their applications on cotton fabric.

MATERIALS AND METHODS

Chemicals

Analytical reagent grade $AgNO_3$ was purchased from Sigma-Aldrich. Cotton textiles and Kale plant were provided from local suppliers in Samsun, Turkey. Ultra pure water was chosen as the reaction medium and it was produced using an Elga PURELAB Option-Q instrument.

Preparation of fresh Kale extracts

Fresh Kale was purchased from local markets and Kale leaves and petioles were used to prepare aqueous extracts. Kale leaves in the amount of 5 g were washed thoroughly in ultra pure water. Then, they were dried, cut into small pieces and boiled in 32 mL of ultra pure water for 5-6 mins. The extract was filtered through Whatman No.1 filter paper and centrifuged at 2500 rpm for 10 mins. The supernatant was kept in a refrigerator at 4°C and used in 2-days' time. The aqueous extract of petiole parts from fresh Kale plant was prepared using the same extraction procedure as mentioned above.

Synthesis of silver nanoparticles

For the synthesis of AgNPs, the volumes and concentrations of silver nitrate solutions were optimized. 1mM and 3 mM aqueous silver nitrate solutions were prepared and mixed with Kale extracts for the production of silver nanoparticles.

1 ml of Kale leaf extracts was added into twelve test tubes containing 1, 2, 4, 6, 8, 10 ml of aqueous solutions of 1 mM and 3mM silver nitrate. The test tubes containing leaf extracts and silver nitrate solutions were incubated for about 20 min at room temperature.

1ml of Kale petiole extracts was also added into other twelve test tubes containing 1, 2, 4, 6, 8, 10 ml of aqueous solutions of 1 mM and 3 mM silver nitrate. The solutions were incubated for about 15 min at room temperature.

Characterization of silver nanoparticles

The proof for nanoparticle formation was investigated by monitoring the change of colour in solutions from yellow to yellowish brown. In addition, the characteristic absorbance peaks of AgNP solutions were analyzed in wavelengths between 190-800 nm by using a Thermo Array Evolution UV-Vis Spectrophotometer (USA). The surface morphology of AgNPs and cotton fabric loaded with AgNPs was analyzed by using JEOL-JSM-7001F equipped with EDS. The SE detector, high vacuum mode and 5-15 kV accelerating voltages were used during the experiments with EDS Oxford Instruments. Samples for scanning transmission electron microscopy (STEM) experiments were prepared by drying a drop of silver nanoparticle aqueous solutions on carbon-coated copper grids at room temperature. The histogram showing the size distribution of nanoparticles and average particle diameter of nanoparticles was obtained by measuring about 2300 nanoparticles from STEM micrographs using the ImageJ Software.

The application of AgNPs onto cotton fabrics

Cotton textile samples (5x5 cm, 0.3 g) were put in conical flasks. 2 ml plant extract and 3 mM 8 mL $AgNO_3$ solution were added to every cotton sample and heated until boiling. After boiling, the heating process continued for 60 min at low heat to fix AgNPs on cotton. Afterwards, cotton samples were air-dried at room temperature.

Determination of antibacterial activity

The antibacterial activity of synthesized AgNPs against 4 different pathogenic bacteria (*Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC27853, and *Enterococcus faecalis* ATCC29212) was determined using Kirby-Bauer Disc Diffusion and broth microdilution methods. The standard bacterial strains used in the study were obtained from the University of Ondokuz Mayıs, Faculty of Veterinary Medicine, Department of Microbiology, Turkey. For the determination of antibacterial activity, the isolates of *E. coli*, *S. aureus*, *E. faecalis* and *P. aeruginosa* were inoculated onto 5% blood agar and incubated for 24 h aerobically at 37°C. A pure colony of each isolate was inoculated onto Tryptic Soy Broth and incubated for 24 h aerobically at 37°C. After incubation, broth cultures were adjusted to 0.5 McFarland for the antibacterial activity tests.

The Kirby-Bauer Disc Diffusion method was carried out against each bacterium using Kale leaves and petioles. To this aim, blank test discs (0.5 cm diameter) were soaked with synthesized silver nanoparticles of Kale leaves and petioles separately and left for 12 h. Then, the soaked discs were incubated and dried for 12 h. Dried discs were used for the Kirby-Bauer Disc Diffusion method according to NCCLS interpretive standards (COCKERILL et al. 2013, BALOUIRI et al. 2016).

Each bacterium that was adjusted to 0.5 McFarland was inoculated and spread onto Mueller Hinton agar in 0.1 ml volume and the loaded discs of Kale leaves and petioles were placed onto agar. After the incubation period (24 h) conducted aerobically at 37°C, the zones of bacterial growth inhibition were measured.

The broth microdilution method was carried out by using microplates (BALOUIRI et al. 2016). The two-fold dilutions (180 μ l) of Kale leaves and petioles (from 1/1 to 1/32) were prepared in microplate wells separately for each bacterium. Then, pure broth cultures (pre-adjusted to 0.5 McFarland standard) of each bacterium (20 μ l) were inoculated onto each well. The microplate was incubated for 24 h aerobically at 37°C. After the incuba-

tion period, the inhibition of bacterial growth was evaluated, and the lowest concentration of the agent that completely inhibited the growth of organism in wells was determined as the minimum inhibitory concentration (MIC) for Kale leaves and petioles against the tested bacteria.

AgNPs loaded test discs and cotton samples were used for the Kirby-Bauer Disc Diffusion method according to NCCLS interpretive standards (BALOUIRI et al. 2016). After the incubation period conducted aerobically at 37°C for 24 h, the zones of bacterial growth inhibition were measured.

RESULTS AND DISCUSSION

Characterization of silver nanoparticles

In the synthesis of AgNPs using Kale aqueous extracts and silver nitrate solutions, colour changes occured after 20-25 min from colourless to yellowish brown and honey brown, respectively, indicating the reduction of silver ions (Ag⁺¹) to AgNPs by the plant extract. The surface plasmon resonance (SPR) bands of silver nanoparticles for different parts of Kale plant were found to be close to ~460 nm for leaves and petioles (Figures 1*a* and 1*b*). In agreement with previous reports (KUMARI et al. 2016), the SPR peaks that were observed between 410 and 450 nm confirmed the synthesis of spherical AgNPs. Polyphenols, flavonoids, vitamins E and C and glucosinolates are the major components of *Brassica* vegetables. These compounds have functional groups which play important roles in the reduction of Ag⁺¹ ions and stabilization/capping of AgNPs (GIORGETTI et al. 2018). It was observed that AgNPs can be formed by using two different concentrations of silver nitrate solutions.



Fig. 1. The UV-Vis spectra of silver nanoparticle samples from leaves at concentrations of (a) 3 mM ${\rm AgNO}_3$ and (b) 1 mM ${\rm AgNO}_3$



Fig. 2. The FTIR spectra of fibers loaded with AgNPs and with Kale extract (without AgNPs)

Figure 2 presents the FTIR spectra of fibers loaded with and without AgNPs showing strong vibration bands between 3000 cm⁻¹ and 3600 cm⁻¹ (specifically at 3331 cm⁻¹ and 3360 cm⁻¹), which are attributed to stretching vibrations of -N-H and -O-H groups. A vibration band appearing at 2900 cm⁻¹ is responsible for -CH stretching vibration. The bands that appeared at 1427 cm⁻¹ and 1426 cm⁻¹ are attributed to $-CH_2$ bending vibrations while the band at 1314 cm⁻¹ is attributed to -CH₂ wagging vibration. The bands recorded at 1159 cm⁻¹ and 1160 cm⁻¹ indicate the presence of a C–O–C pyranose ring. The bands at 1104 cm⁻¹ and 1108 cm⁻¹ showed the presence of C-N (aliphatic amine) stretching vibration while the bands located at 662 cm⁻¹ and 663 cm¹ are attributed to the out-of-plane bending vibrations of C-H groups. The occurrence of these peaks shows the presence of secondary plant metabolites such as phenolic compounds and flavonoids. The presence of -NH, -OH and -CH groups in FTIR results indicated that the leaf extract contained flavonoids which were substituted with the hydroxyl and amine groups. The fabrics had similar peaks in their spectra; the intensity of the spectrum associated with the fabric loaded with the Kale extract was lower than that of the spectrum of the fabric loaded with nanoparticles, which showed the involvement of -OH groups in the production of AgNPs. Consequently, flavonoids acting as reducing and stabilizing agents help to reduce Ag⁺ ions to Ag⁰ in the generation of AgNPs (BALASHANMUGAM, KALAICHELVAN 2015, SADANANDA et al. 2017). In the study conducted by JAILIAN et al. (2020), a decrease in peak intensities in the FTIR spectrum was reported after nanoparticle formation. This decrease shows that these groups play an active role in the formation and stabilization of nanoparticles.

The SEM images in Figures 3a and 3b showed that AgNPs synthesized from fresh Kale extracts were almost spherical in shape. The STEM



Fig. 3. The SEM images of AgNPs synthesized from (a) leaves and (b) petioles of Kale plant



Fig. 4. Lower magnification STEM micrograph of AgNPs (*a*), higher magnification STEM micrograph of AgNPs (*b*), the histogram showing the particle size distribution of AgNPs (*c*)



Fig. 5. The SEM images of cotton fabric (a) unloaded and (b) loaded with AgNPs

micrographs and the corresponding histogram showing the particle size distribution of AgNPs are shown in Figures 4 (*a-c*). STEM images of AgNPs (Figures 4*a* and 4*b*) clearly show the formation of spherical silver nanoparticles with a uniform particle size of 25 ± 5 nm.

Surface morphology of the cotton fabric loaded with AgNPs was investigated by electron microscopy. The deposition of AgNPs onto cotton fabric was confirmed by these SEM images (Figures 5a and 5b). EDS results of cotton fiber loaded with AgNPs (Figure 6) and cotton fiber loaded with AgNPs which was washed with ultrapure water (Figure 7) confirmed the presence of AgNPs on the cotton fiber.

Antibacterial activity

The results of the Kirby-Bauer Disc Diffusion method of testing silver nanoparticles which were synthesized by using Kale leaves and petioles are shown in Table 1. According to these results, the zones of bacterial growth inhibition by AgNPs that were synthesized from leaves were observed to be the highest against *E. coli* (15 mm) and *E. faecalis* (15 mm). Nanoparticles which were synthesized from petioles were found to exhibit the highest antibacterial activity against *P. aeruginosa* (14 mm).

Table 1

The zones of bacterial growth inhibition by AgNPs synthesized from Kale leaves and petioles applied against pathogenic bacterial strains

Bacterial strains	Kale leaves (mm)	Kale petioles (mm)
E.coli	15	12
E. faecalis	15	10
P. aeruginosa	13	14
S. aureus	12	13



Fig. 6. EDS results of cotton fabric loaded with AgNPs



Fig. 7. EDS results of cotton fabric loaded with AgNPs and then washed with ultrapure water

The results of the broth microdilution method applied to AgNPs synthesized from Kale leaves and petioles are shown in Table 2. The MIC values of AgNPs from Kale leaves were determined as $\frac{1}{2}$, $\frac{1}{2}$, 1 and 1 against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus*, respectively. The MIC values of AgNPs from Kale petioles were determined as $\frac{1}{2}$, 1, 1 and 1 against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus*, respectively. According to these results, $\frac{1}{2}$ dilutions of the AgNPs synthesized from Kale leaves showed the antibacterial activity against *E. coli* and *E. faecalis*. Also, $\frac{1}{2}$ dilutions of the AgNPs synthesized from Kale petioles showed the antibacterial activity against *E. coli*.

As shown in Table 3, silver nanoparticles and cotton fabric samples treated with these AgNPs showed strong antibacterial efficacy. AgNP loaded

Table 2

Bacterial strains	Kale leaves	Kale petioles
E.coli	1/2	1/2
E. faecalis	1/2	1
P. aeruginosa	1	1
S. aureus	1	1

MIC values of AgNPs synthesized from Kale leaves and petioles against pathogenic bacterial strains

Table 3

The zones of bacterial growth inhibition in cotton fabric samples treated with AgNPs against pathogenic bacterial strains

Cotton fabric samples	Escherichia coli (mm)	Staphylococcus aureus (mm)
Plant extract loaded	0	0
AgNPs loaded	18	18
AgNPs loaded and washed	16	14

textile showed the antibacterial activity against *E. coli* (18 mm) and *S. aureus* (18 mm). The antibacterial character of fabrics exhibited good durability even though textile was washed with ultra pure water for 20 minutes.

DADA et al. (2020) synthesized AgNPs with the biosynthesis method using a 0.001 M AgNO₃ solution and the plant extract of *Acalypha wilkesiana* at a temp. of 100°C at pH 9. They reported that the dimensions of the nanoparticles they obtained were in the range of 10-26 nm. The antibacterial efficacy of nanoparticles and plant extract was evaluated in the study. When the measured zone diameters were evaluated, no antimicrobial activity was detected in the plant extract, but it was observed that nanoparticles prevented bacterial growth in the range of 16-20 mm. It was observed that the efficiency of AgNPs against *S. aureus*, a gram negative bacteria, was higher than that against *E. coli* bacteria (DADA et al. 2020). In our study, the zone diameters measured against E.coli and S. aureus bacteria were also determined as 15 mm and 12 mm, respectively. As a gram positive bacterium, S. aureus is more resistant than E. coli, and when the antimicrobial test results were compared, it was determined that the MIC concentration obtained from AgNPs (synthesized using Kale leaves) against S. aureus $(200 \ \mu g \ mL^{-1})$ was higher than that against E. coli $(100 \ \mu g \ mL^{-1})$. MALAIKOZHUNDAN et al. (2016) synthesized silver nanoparticles using Momordica charantia fruit extract. When the TEM results were evaluated, it was observed that the average diameter of the particles was 16 nm. According to the antibacterial activity test results, $100 \ \mu g \ mL^1$ concentration of the AgNPs synthesized using plant fruit extract showed the highest antibacterial activity against E. faecalis (MALAIKOZHUNDAN et al. 2016). In our study 100 μ g mL⁻¹ concentration of AgNPs (synthesized using kale leaves) was also determined as the lowest concentration that could be effective in preventing the growth of E. faecalis. The MIC values of AgNPs from Kale petioles were determined as $\frac{1}{2}$ (100 µg mL⁻¹), 1 (200 µg mL⁻¹), 1 (200 µg mL⁻¹) and 1 (200 μ g mL⁻¹) against E. coli, E. faecalis, P. aeruginosa and S. aureus, respectively.

The analysis of the antibacterial activity of the AgNPs prepared using green tea extract and silver nitrate solution (at 10 mM) were conducted by NAKHJAVANI et al. (2017). The test results showed that smaller nanoparticles (25 nm) showed greater antibacterial activity than larger (45 nm and 75 nm) nanoparticles. For E. coli and S. aureus, 16 nm and 14 nm values were obtained, respectively, as a result of the diameter measurements at which bacterial growth was prevented (NAKHJAVANI et al. 2017). MOHAMMED et al. (2018) synthesized AgNPs using ethanolic and aqueous extracts of the fruit samples (Acacia nilotica, Ferula asafoetida and Phoenix dactylifera L.) The size of the synthesized nanoparticles ranged from 67.8 nm to 155.7 nm. The synergistic antibacterial activities of AgNPs with antibiotics (amoxicillin, cefuroxime, and ciprofloxacin) were evaluated. It was concluded that the combination of antibiotics and silver nanoparticles with a MIC concentration of 50 mg L^{-1} would reduce the use of antibiotics and the growth of antibiotic resistant bacteria. In a study carried out by JALILIAN et al. (2020), AgNPs were prepared by a green synthesis method using water extract of Allium *ampeloprasum*. When the electron microscope images were examined, it was determined that the nanoparticles were spherical and their dimensions were in the range of 8-20 nm. The antibacterial potential of the AgNPs and plant extract was evaluated using disc diffusion method and broth dilution method. The MIC values determined using the broth dilution method for the AgNPs were 18.75 (against P. aeruginosa and S. aureus) and 37.5 (against *E.coli*). The zones of inhibition of the AgNPs (300 μ g mL⁻¹) were measured at 23 mm and 27 mm against E. coli and P. aeruginosa, respectively (JALILIAN et al. 2020). As the diameter of the nanoparticles becomes smaller and their surface area expands, an increase in their antibacterial activity can be observed.

For the antibacterial activities of metal nanoparticles, different mechanisms were proposed. According to one suggestion, silver ions generated by AgNPs penetrate into the cells, causing damage to bacterial cell membranes. Silver ions may behave like 'mild acid' and enter into interactions with proteins, enzymes and DNA, sulfur and phosphorus containing parts of cells. Inhibition of the DNA replication activity causes the death of cells. Respiratory chain enzymes also may be inactivated by silver ions. In addition, the bacterial cells may undergo self-destruction due to the oxidative stress induced by reactive oxygen species (ROS), which are also known as free radicals. Parameters such as the concentration and size of nanoparticles or the initial bacterial number affect the zones of bacterial growth inhibition. Nanoparticles having small sizes and large surface areas show higher antimicrobial activity due to their higher degree of interaction with bacterial cells compared to larger particles (KUMARI et al. 2016, NAYAK et al. 2016).

CONCLUSIONS

The results of this study demonstrated that AgNPs were successfully synthesized using Kale plant extracts at room temperature. Kale plant can be evaluated as a bioreduction source for aqueous Ag⁺ ions. This biosynthesis method of AgNPs is costly efficient, rapid, free from organic solvents and toxic chemicals and therefore environmental friendly. The silver nanoparticles with the uniform particle size of 25 ± 5 nm showed strong antibacterial efficacy against some human pathogens. Cotton fabric containing AgNPs also exhibited antibacterial activity against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus*. AgNPs synthesized from aqueous extracts of Kale can be employed for economical production of antimicrobial cotton fabrics.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

- ANJUM A.N., GILL S.S., DUARTE C.A., PEREIRA E., AHMAD I. 2013. Silver nanoparticles in soil--plant systems. J. Nanopart. Res., 15: 1896-1898.
- BALASHANMUGAM P., KALAICHELVAN T.P. 2015. Biosynthesis characterization of silver nanoparticles using Cassia roxburghii DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity. Int. J. Nanomed., 10: 87-97.
- BALOUIRI M., SADIKI M., KORAICHILBNSOUDA S. 2016. Methods for in vitro valuating antimicrobial activity: A review. J. Pharmeut. Anal., 6(2): 71-79.
- BARANI H., MONTAZER M., SAMADIB N., TOLIYAT T. 2012. In situ synthesis of nano silver/lecithin on wool: Enhancing nanoparticles diffusion. Colloid Surface B, 92: 9-15.
- Cockerill F.R., Wikler A.M., Alder J., Dudley M.N., Eliopoulos G.M., Ferraro M.J., Hardy D.J.,

HECHT W.D., HINDLER J.A., PATEL B.J. 2013. CLSI: M02-A11 and M100-S23 Package-Performance standards for antimicrobial disk susceptibility tests; approved standard-eleventh edition and Performance standards for antimicrobial susceptibility testing; Twenty-Third Informational Supplement, US.

- DADA A.O., ADEKOLA F.A., DADA F.E., ADELANI-AKANDE A.T., BELLO, M.O., OKONKWO, C.R., INYINBOR A.A., OLUYORI, A.P., OLAYANJU A., AJANAKU K.O., ADETUNJI C.O. 2019. Silver nanoparticle synthesis by Acalypha wilkesiana extract: phytochemical screening, characterization, influence of operational parameters, and preliminary antibacterial testing. Heliyon, 5: e02517.
- DEP S. 2014. Synthesis and characterisation of silver nanoparticles using brassica oleracea capitata (cabbage) and phaseolus vulgaris (french beans): A study on their antimicrobial activity and dye degrading ability. Int. J. ChemTech. Res., 6(7): 3309-3917.
- EL-SHISHTAWY M.R., ASIRI M.A., ABDELWAHED N.A.M., AL-OTAIBI M.M. 2011. In situ production of silver nanoparticle on cotton fabric and its antimicrobial evaluation. Cellulose, 18(1): 75-82.
- FIRDHOUSE J.M., LALITHA P. 2015. Biocidal potential of biosynthesized silver nanoparticles against fungal threats. J. Nanostruct. Chem., 5(1): 25-33.
- GAVARKAR P.S., ADNAIK S.R., MOHITE K.S., CHANDRAKANT C.S. 2014. Green synthesis and antimicrobial activity of silver nanoparticles (snp) of cucumis melo extract. Int. J. Univ. Pharm. BioSci., 3: 392-396.
- GHOLAMREZA A., VARSHOSAZ J., SHAHBAZI N. 2014. Synthesis of silver nanoparticle using Portulaca oleracea L. extracts. Nanomed. J., 1(2): 94-99.
- GIORGETTI L., GIORGI G., CHERUBINI E., GERVASI G.P., CROCE D.M.C., LONGO V., BELLANI L. 2018. Screening and identification of major phytochemical compounds in seeds, sprouts and leaves of Tuscan black kale Brassica oleracea (L.) ssp acephala (DC) var. sabellica L. Nat. Prod. Res., 32(14): 1617-1626.
- JALILIAN F., CHAHARDOLI A., SADRJAVADI K., FATTAHI A., SHOKOOHINIA Y. 2020. Green synthesized silver nanoparticle from Allium ampeloprasum aqueous extract: Characterization, antioxidant activities, antibacterial and cytotoxicity effects. Adv. Powder Technol., 31: 1323-1332.
- KASHID S.B., LAKKAKULA J.R., CHAUHAN S.D., SRIVASTAVA R., RAUT W.R. 2017. Biocompatible antimicrobial cotton fibres for healthcare industries: a biogenic approach for synthesis of bio-organic-coated silver nanoparticles. IET Nanobiotechnol., 11(8): 1046-1051.
- KUMARI J., MAMTA B., AJEET S. 2016. Characterization of silver nanoparticles synthesized using Urtica dioica Linn. leaves and their synergistic effects with antibiotics. J. Rad. Res. Appl. Sci., 9(3): 217-227.
- MALAIKOZHUNDAN B., VASEEHARAN B., VIJAYAKUMAR S., SUDHAKARAN R., GOBI N., SHANTHINI G. 2016. Antibacterial and antibiofilm assessment of Momordica charantia fruit extract coated silver nanoparticle. Biocatal. Agricult. Biotechnol., 8: 189-196.
- MOHAMMED A.E., AL-QAHTANI A., AL-MUTAIRI A., AL-SHAMRI B., AABED K. 2018. Antibacterial and cytotoxic potential of biosynthesized silver nanoparticles by some plant extracts. Nanomaterials, 8: 382.
- MORITZ M., GESZKE-MORITZ M. 2013. The newest achievements in synthesis, immobilization and practical applications of antibacterial nanoparticles. Chem. Engin. J., 228: 596-613.
- NAKHJAVANI M., NIKKHAH V., SARAFRAZ M.M., SHOJA S., SARAFRAZ M. 2017. Green synthesis of silver nanoparticles using green tea leaves: Experimental study on the morphological, rheological and antibacterial behavior. Heat Mass Transfer., 53: 3201-3209.
- NAYAK D., ASHE S., RAUTA R.P., KUMARI M., NAYAK B. 2016. Bark extract mediated green synthesis of silver nanoparticles: Evaluation of antimicrobial activity and antiproliferative response against osteosarcoma. Mater. Sci. Engin. C, 58: 44-52.
- PHANJOM P., SULTANA A., SARMA H., RAMCHIARY J., GOSWAMI K., BAISHYA P. 2012. Plant-mediated synthesis of silver nanoparticles using Elaeagnus latifolia leaf extract. Digest J. Nanomater. Biostruct., 7(3): 1117-1123.

462

- ROY K., SARKAR K.C., GHOSH K. 2015. Single-step novel biosynthesis of silver nanoparticles using cucumis sativus fruit extract and study of its photcatalytic and antibacterial activity. Digest J. Nanomater. Biostruct., 10(1): 107-115.
- SADANANDA V., TIANB H., RAJULUC A.V., SATYANARAYANA B. 2017. Antibacterial cotton fabric with in-situ generated silver nanoparticles by one-step hydrothermal method. Int. J. Polymer Anal. Character., 22(3): 275-279.
- SATYAVANI K., GURUDEEBAN S., RAMANATHAN T., BALASUBRAMANIAN T. 2011. Biomedical potential of silver nanoparticles synthesized from calli cells of Citrullus colocynthis (L.) Schrad. J. Nanobiotechnol., 9: 43.
- TAMILESWARI R., NISHA H.M., JESURANI S.S. 2015. Green synthesis of silver nanoparticles using Brassica oleracea (Cauliflower) and Brassica oleracea Capitata (cabbage) and the analysis of antimicrobial activity. Int. J. Engin. Res. Technol, 4(4): 1071-1074.
- ZIA F., GHAFOOR N., IGBAL M., MEHBOOB S. 2016. Green synthesis and characterization of silver nanoparticles using Cydonia oblong seed extract. Appl. Nanosci., 6(7): 1023-1029.