JOURNAL OF SCIENCE
SAKARYA UNIVERSITY

Sakarya University Journal of Science

ISSN 1301-4048 | e-ISSN 2147-835X | Period Bimonthly | Founded: 1997 | Publisher Sakarya University | http://www.saujs.sakarya.edu.tr/

Title: Antimicrobial and antibiofilm activity of green synthesized silver nanoparticles by using aqueous leaf extract of Thymus serpyllum

Authors: Fatih Erci, Emrah Torlak
Recieved: 2018-07-18 00:00:00

Accepted: 2018-11-27 00:00:00

Article Type: Research Article
Volume: 23
Issue: 3
Month: June
Year: 2019
Pages: 333-339

How to cite
Fatih Erci, Emrah Torlak; (2019), Antimicrobial and antibiofilm activity of green synthesized silver nanoparticles by using aqueous leaf extract of Thymus serpyllum. Sakarya University Journal of Science, 23(3), 333-339, DOI: 10.16984/saufenbilder.445146
Access link
http://www.saujs.sakarya.edu.tr/issue/41686/445146

Sakarya University Journal of Science 23(3), 333-339, 2019

Antimicrobial and antibiofilm activity of green synthesized silver nanoparticles by using aqueous leaf extract of *Thymus serpyllum*

Fatih Erci*[1] Emrah Torlak[2]

ABSTRACT

Recently, metal nanoparticles have attracted the attention of researchers due to their unique properties when compared with bulk materials and have become used in many fields of application. In this study, green synthesis of Ag nanoparticles (AgNPs) was investigated by using the aqueous extract of *T. serpyllum* leaves. In addition, antimicrobial and antibiofilm activities of the synthesized AgNPs were evaluated in this study. Further, ultraviolet-visible spectroscopy (UV-Vis), fouirer transform infrared spectroscopy (FT-IR), dynamic light scattering (DLS), scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) and transmission electron microscopy (TEM) were used for characterization of the green synthesized AgNPs. The UV-Vis spectrum of the synthesized AgNPs had a maximum peak at 467 nm. Also, TEM analysis indicated spherical particles with an average size of 25.2 nm. The synthesized AgNPs have higher stability (zeta potential: -29.5 mV). The antimicrobial activity of the green synthesized AgNPs was investigated on both Gram-positive and Gram-negative bacteria, such as *Bacillus cereus* (*B. cereus*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) using agar well diffusion assay. According to the results of the study, Gram-positive bacteria showed larger inhibition zones compared to Gram-negative bacteria. Finally, the AgNPs were explored for the inhibition of *S. aureus* biofilms. AgNPs at 100 µg/mL concentration showed a high inhibition value of about 73% for *S. aureus* biofilm formation. So, it is concluded that the synthesized AgNPs might be potentially used in many applications due to their antimicrobial and antibiofilm properties.

Keywords: Green synthesis, silver nanoparticle, antimicrobial, antibiofilm

1. INTRODUCTION

Biotechnology and nanotechnology are two of the 21st century's most promising technologies. Generally, nanotechnology deals with structures with at least one dimension ranging from 1 to 100 nanometers [1]. In the meantime, biotechnology deals with the metabolic and other physiological processes of biological materials. Nanobiotechnology is an association between these two technologies, and life sciences play an important role in the development and implementation of many useful tools and technologies in the related research area [2]. Nanotechnology is now widely used due to its different properties, depending on the constituent elements of nanostructures, regardless of the bulk form [3]. Advances in nanotechnology have gained momentum with the development of unconventional synthesis protocols and characterization methods. Traditionally, NPs are synthesized by physical, chemical and biological procedures. There are usually

* Corresponding Author
[1] Necmettin Erbakan University, Faculty of Science, Department of Biotechnology, 42090 Meram, Konya, ferci@konya.edu.tr ORCID: 0000-0002-3044-7343
[2] Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics, 42090 Meram, Konya, etorlak@konya.edu.tr ORCID: 0000-0003-4636-7791

two approaches to produce NPs which are called bottom-up and top-down methods [4]. In recent years, NP synthesis has become a focus of interest by using biological methods that are nontoxic, less costly and environmentally compatible than physicochemical NP synthesis methods [4-6]. Synthesis of NPs under completely 'green' principles can be achieved using biologically compatible solvent systems with environmentally friendly reducing and stabilizing agents. Several ways have been developed for the biological or biogenic synthesis of NPs from the salts of the relevant metals [7, 8]. In terms of simplicity, the use of the plant itself, extract or plant tissue to reduce metal ions to nanoparticles has received considerable attention over the past 30 years [9, 10]. The process for synthesizing NPs using plant extracts is easier and has the advantage of being less costly than methods based on microbial processes or whole plant use [11-13]. The reaction temperature, pH, length of the incubation period and plant extract concentration can cause significant changes in the shape, size, stability and nucleation process of synthesized nanoparticles.

The precise mechanisms of antimicrobial or toxic activity of AgNPs are still unknown and have recently become one of the most popular topics. Silver must be in ionized form to have any antimicrobial properties. In its ionized form, silver is inert but releases silver ions when it meets moisture [14]. Similarly, AgNPs stop bacterial growth by destroying cell membranes and DNA, inhibiting vital metabolic enzymes, and producing reactive oxygen species (ROS) that destroy cellular components [15, 16]. Biofilms can be described as an organized organism group of microorganisms living in a matrix of self-produced polymeric substances attached to various surfaces [17]. These microbial collectives appear to be present in almost every environment. Biofilms are found on both biotic and abiotic surfaces [18]. It has been reported that various mechanisms that inhibit biofilm formation or cause degradation of biofilms in controlling biofilms. Among them, the bactericidal activity of nanomaterials provides an opportunity to combat bacterial biofilms. Various nanomaterials have been shown to exhibit antimicrobial activities [19, 20]. Small size and high surface area/volume ratio are unique features that allow nanomaterials to interact closely with microorganisms [21].

Here in, we report an efficient and sustainable route for the synthesis of silver nanoparticles and their characterization by using different spectroscopic and microscopic techniques. The aqueous extract of *T. serpyllum* leaves acts as reducing agent for the silver ions present in the solution of silver nitrate. Further we evaluated the antibacterial and antibiofilm activity of these biologically synthesized nanoparticles.

2. MATERIAL-METHOD

2.1. Preparation of plant extract

10 g of the leaves of the plant sample dried under the sunlight was taken for extraction and dust was removed from the plant material by washing thoroughly three times with double distilled water, before the synthesis of the nanoparticles. The powdered plant leaves placed in 100 mL of Milli-Q water (Millipore, 18.2 MΩ cm, USA), were then incubated at 80 °C for 60 minutes and then stirred at room temperature (22 °C) for 60 minutes in a magnetic stirrer. Finally, the resulting aqueous extract was filtered using filter paper (Whatman filter paper No. 1) and the filtrate was kept at 4 °C for further use.

2.2. Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$, 99.9%) used in the synthesis were obtained from Sigma-Aldrich Chemicals (USA). And, all other chemicals used were analytical grade. 5 mL of the aqueous leaf extract of *T. serpylum* with 100 mL of 1 mM $AgNO_3$ solution were mixed to initiate the synthesis of AgNPs. At room temperature, the reaction mixture was stirred at 350 rpm. The color change was recorded as the conversion of silver ions to AgNPs. Then, the synthesized nanoparticles were centrifuged at 10,000 rpm for 15 minutes at 4 °C. The resulting pellet of nanoparticles was subjected to 2 times washing steps using Milli-Q water to eliminate all residues. Finally, purified AgNPs were freeze-dried with the aid of a lyophilizer and then stored at 4°C.

2.3. Characterization of synthesized silver nanoparticles

Cary 60 UV–vis spectrophotometer (Agilent) was used for analysis UV-Vis. The black-brown colored AgNPs were sonicated for a while after dissolving in MilliQ water. The absorbance of the nanoparticles and extracts in a cuvette were recorded by AgNPs exposure to UV-visible radiation. FT-IR spectra from nanoparticle were collected in the 400–4000 cm^{-1} range by using Nicolet 380 ATR FT-IR instrument (Thermo Scientific). FT-IR spectroscopic studies were conducted to investigate the possible function of the metal reduction in the formation of metal nanoparticles. SEM and EDX studies were carried out by using SU-1510 SEM (Hitachi) associated with EDX analyzer. The result obtained by SEM have revealed the nature of AgNPs and EDX analysis gives chemical composition of the AgNPs that may have a role in their formation. The

characterization for size, shape and morphology of the synthesized AgNPs were performed by microscopic analysis using TEM 1400 (JEOL).

2.4. Antibacterial activities of the synthesized nanoparticles

Agar well diffusion experiment was used to evaluate the antibacterial susceptibility of the synthesized AgNPs [22]. Lyophilized cultures of tested bacteria (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus subsp. aureus* Rosenbach ATCC 25923), *Escherichia coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028) were obtained from Microbiologics Inc. (Saint Cloud, USA). Stock culture of working microorganisms were stored in Nutrient Broth (Merck) at −18 °C. Working cultures of microorganims were grown on Nutrient Agar (Merck) slants and kept at 4 °C The inoculums were prepared by adjusting the cell suspension of microorganisms to 0.5 McFarland turbidity standard representing approximately 1.5 x 10^8 colony forming units (CFU / mL). 100 µL of the inoculums were spread on Mueller Hinton Agar (Lab M) plates. With the aid of gel puncture, a well of approximately 7 mm in diameter was formed in the Muller-Hinton Agar plate. Aliquots of 50 µL of AgNPs were added to wells. After incubation at 35 °C for 24 hours, the diameters (mm) of the inhibition zones around the well were measured. The antibacterial activity of the AgNPs was determined comparative to the standard antibiotic of gentamicin (10 µg discs, BD, USA) and the tests were repeated three times.

2.4. Evaluation of antibiofilm activity of nanoparticles

Staphylococcus aureus subsp. *aureus* Rosenbach ATCC 35556 strain was used as a strain for the biofilm assay and inoculated into a total of 10 mL Muller Hinton Broth (Lab M)) followed by incubation at 37 ° C for 24 hours. The bacterial suspension was then diluted 1: 100 with fresh Muller Hinton Broth and 0.2 ml of dilution was added to 96-well microplates. 10 µL of bacteria and 10 µL of AgNPs solution at various concentrations were added to 180 µL working medium of the final volume. Only sterile Muller Hinton Broth was used as the blank. After incubation, the wells were washed four times with 0.2 mL phosphate buffered saline (PBS) (pH 7.2). The remaining biofilms in the wells were mixed with 2% sodium acetate (Sigma-Aldrich S2889) and stained with 0.1% crystal violet (Sigma-Aldrich C3886). Then, the excess dye was washed with deionized water and the wells were left to dry. After drying, 200 µL of 95% (v / v) ethanol was added to the wells. And, the optical density was measured at 620 nm in an ELISA microplate reader and the average OD values were assessed in the biofilm inhibition activity calculation based on the following formula.

The assessment was made in the following way:

$$\%\ \text{biofilm inhibition} = 1 - \frac{\text{OD620 (cells treated with AgNPs)}}{\text{OD620 (negative control)}}\ x\ 100 \quad (1)$$

3. RESULTS

The results show that the synthesis of the AgNPs by using *T. serpyllum* leaf extract is easy and reliable within 12 hours at room temperature; which does not require toxic chemicals or energy inputs. The clear peaks at 467 nm were obtained by UV-Vis spectra of the leaf extract-$AgNO_3$ mixture after 12 hours (Figure 1A). Surface plasmon resonance (SPR) bands were consistent with the previous studies for AgNPs [23].

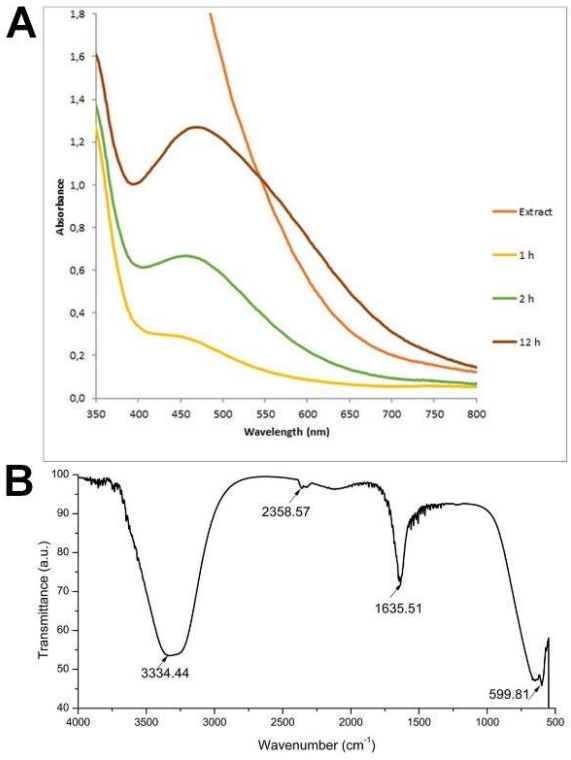

Figure 1. A. UV–visible absorption spectra of AgNPs synthesized by leaf aqueous extract of *T. serpyllum* at different reaction time, B. FT-IR spectra of synthesized AgNPs

The spectra of FT-IR were recorded for both AgNPs (Figure 1B). The intensive bands relating the functional groups were determined by comparing with the standards. Bands at 3334.44, 2358.57, 1635.51 and 599.81 cm^{-1} in the region of 400-4000 cm^{-1} were detected on AgNPs (Figure 1B). The band at 3334 cm^{-1} shows O-H stretching vibration. The peak at 2358 and

1635 cm^{-1} characterizes the N-H stretching vibrations of the amides and the C═C stretching vibrations of the alkenes or the C═O stretching vibrations of the amides, respectively [6]. The functional groups estimated according to the FTIR results may have significant roles in the green synthesis of AgNPs as both reducing and stabilizing agents. However, the possible mechanism which takes a role in the synthesis of the AgNPs is still unclear and needs further research.

SEM imaging analysis enabled to examine the morphology of AgNPs [24]. The results showed the synthesized AgNPs have a relatively spherical shape (Figure 2 and 3).

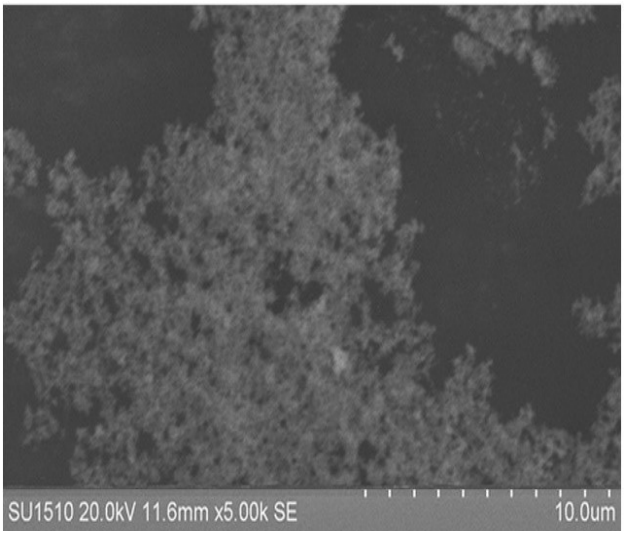

Figure 2. SEM micrographs of synthesized AgNPs using aqueous leaf extract of *T. serpyllum*

The EDX spectrometer spectrum obtained from SEM reveals a strong signal at about 3 keV in the silver region and confirms the formation of AgNPs. In general, metallic AgNPs exhibit a typical optical absorption peak at about 3 keV due to their surface plasmon resonance [25].

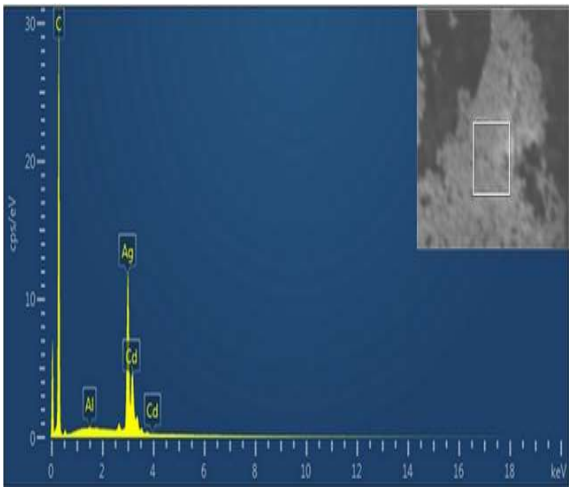

Figure 3. EDX analysis of AgNPs

Diameter measurements of the nanoparticles from TEM images showed the AgNPs diameter distribution ranged from 12 to 36.4 nm with an average particle diameter of 25.2 nm (Fig. 4). The morphology of AgNPs is almost spherical.

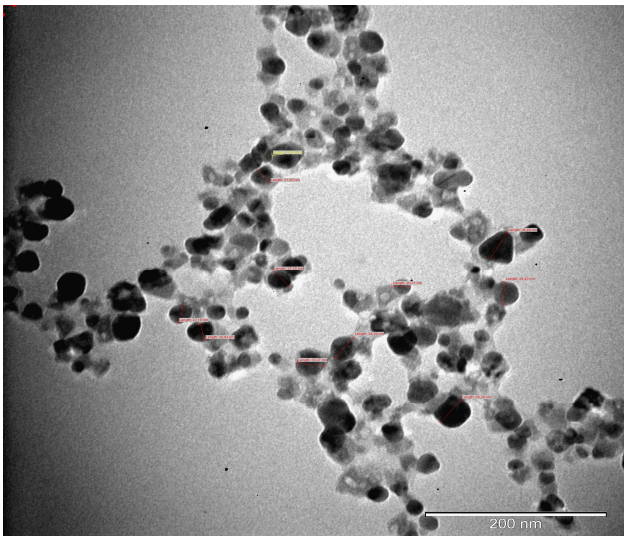

Figure 4. TEM analysis of AgNPs

DLS also known as photon correlation spectroscopy, used to analyze the average particle size, size distribution and polydispersity index (PDI) of AgNPs in the suspension. DLS studies have revealed that the average particle size distribution of the AgNPs was 30.5 nm, and the PDI was 0.261. The size difference observed between TEM and DLS may be due to the existence of bioactive molecules of plants on the surface of AgNP or by reason of the aggregation during the sample preparation [6, 26]. In addition, AgNPs dispersed in Milli-Q water showed zeta potential values of -29.5 mV for AgNPs (Figure 5B). The Zeta potential is also considered as an important parameter to determine the stability and dispersion of metal nanoparticles, while at the same time it indicates the total charge held by the nanoparticles in the solution. The results indicate that silver nanoparticles are coated with negatively charged biomolecules and that the electrostatic repulsion interaction between these nanoparticles prevents possible aggregation Also, this negative value may be responsible for the long-term stability of the AgNPs [27].

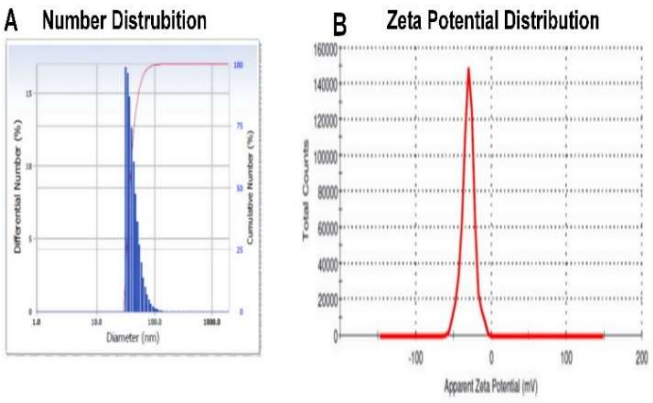

Figure 5. (A) DLS and (B) Zeta potential analysis of AgNPs

Antibacterial activity of selected Gram-positive bacteria (*B. cereus* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *S.* Typhimurium) to biosynthesized AgNPs was determined by agar well diffusion test. The diameter (mm) of the zone around the wells containing AgNPs is shown in Figure 6. According to the results obtained, the inhibition zone of AgNPs against *B. cereus* was 12.23 ± 0.54 while inhibition zone for *S. aureus* treated with AgNPs was observed to be 13.86 ± 0.58 mm. The inhibition zone diameters of AgNP for *E. coli* and *S.* Typhimurium were 9.98 ± 1.02 and 10.60 ± 0.53, respectively. In this study, The Gram-positive bacteria showed larger zones of inhibition, compared with the Gram-negative bacteria. These findings agree with previous studies that examined antibacterial activity of AgNPs synthesized by using aqueous extract of different plant leaves [6]. This may be due to the structural difference in the cell wall composition of Gram-positive and Gram-negative bacteria. The Gram-negative cell wall is covered with an outer lipid membrane that is more negatively charged than Gram-positive. As evidenced by the Zeta value in Figure 5B, the synthesized AgNPs have also negatively charge and electrostatic repulsion between the AgNPs and the Gram-negative bacteria, which inhibits particle attachment and entry into the cell [28]. It is also known that AgNPs can cause oxidative damage by producing reactive oxygen species (ROS). This results in irreversible damage to DNA replication by damaging the enzymes and proteins involved [29].

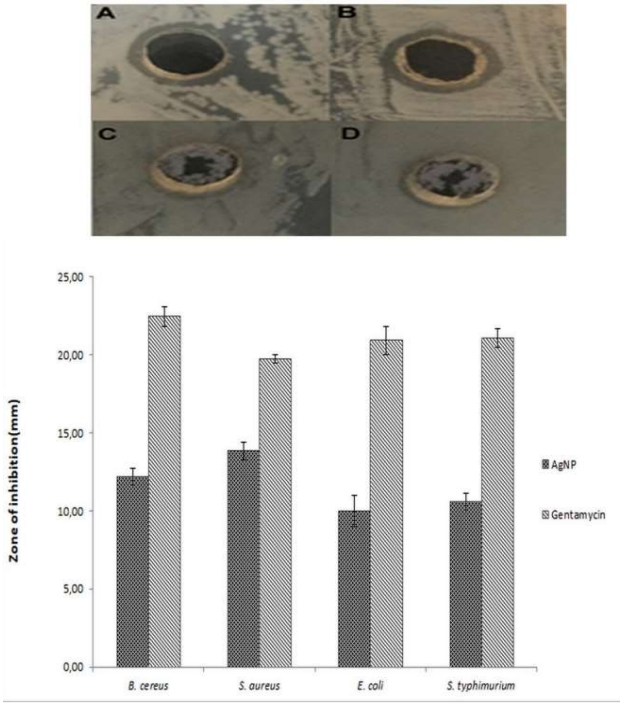

Figure 6. Zone of inhibition of silver nanoparticles against various microorganisms. A, B, C and D represent *B. cereus, S. aureus*, *E. coli* and *S.* Typhimurium, respectively

The results in Figure 6 show the inhibition of concentration-dependent biofilm formation of AgNPs. The data show percentages of biofilm inhibition after 24 hours of application of AgNPs at concentrations ranging from 6.25 and 100 µg/mL.

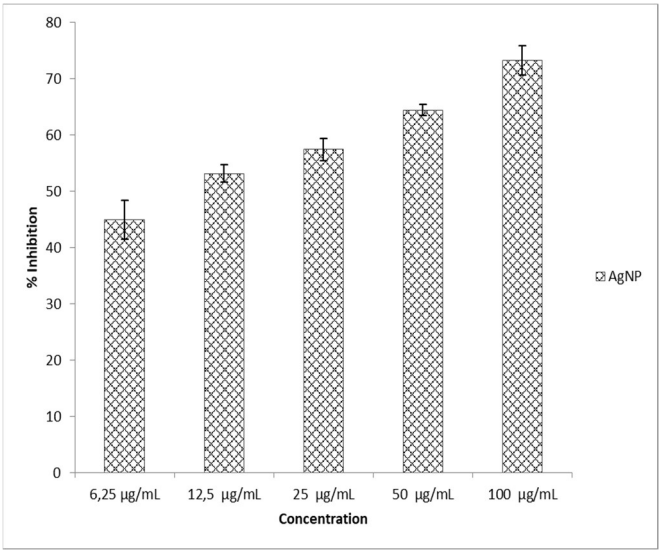

Figure 7. Antibiofilm activity of AgNPs

AgNPs showed the highest inhibition value around the 73% for the formation of biofilms formed by *S. aureus* at 100 µg / mL. At 6.25 µg / mL, inhibition value was found as 44.88 ± 3.47 %. The overall results show that the antibiofilm activity was significantly affected with increased concentrations of AgNPs.

4. CONCLUSIONS

The green synthesis of AgNPs was carried out using the leaves of *T. serpyllum* extract. This type of plant extract-mediated synthesis is more advantageous than traditional physical and chemical methods, as it is cost-effective, environmentally friendly and easy to use. It is estimated that the bioactive compounds existing in leaf extract of *T. serpyllum* play a role in the reduction and stabilization of AgNPs. These nanoparticles showed potent bacteriostatic effects against Gram-positive bacteria and showed significant inhibition effect on *S. aureus* biofilms. More studies on the mechanism of synthesis of AgNPs and their antimicrobial and antibiofilm activity mechanisms are needed to improve the biomedical properties of silver nanoparticles.

REFERENCES

[1] A. Nel, T. Xia, L. Mädler, and N. Li, "Toxic potential of materials at the nanolevel," *Science,* vol. 311, no. 5761, pp. 622-627, 2006.

[2] K. N. Thakkar, S. S. Mhatre, and R. Y. Parikh, "Biological synthesis of metallic nanoparticles," *Nanomedicine: Nanotechnology, Biology and Medicine,* vol. 6, no. 2, pp. 257-262, 2010.

[3] A. Albanese, P. S. Tang, and W. C. Chan, "The effect of nanoparticle size, shape, and surface chemistry on biological systems," *Annual review of biomedical engineering,* vol. 14, pp. 1-16, 2012.

[4] S. Sepeur, *Nanotechnology: technical basics and applications*. Vincentz Network GmbH & Co KG, 2008.

[5] J. L. Hernández-Pinero, M. Terrón-Rebolledo, R. Foroughbakhch, S. Moreno-Limón, M. F. Melendrez, F. Solís-Pomar, and E. Pérez-Tijerina, "Effect of heating rate and plant species on the size and uniformity of silver nanoparticles synthesized using aromatic plant extracts," *Applied Nanoscience,* vol. 6, no. 8, pp. 1183-1190, 2016.

[6] F. Erci, R. Cakir-Koc, and I. Isildak, "Green synthesis of silver nanoparticles using Thymbra spicata L. var. spicata (zahter) aqueous leaf extract and evaluation of their morphology-dependent antibacterial and cytotoxic activity," *Artificial cells, nanomedicine, and biotechnology,* pp. 1-9, 2017.

[7] H. Bar, D. K. Bhui, G. P. Sahoo, P. Sarkar, S. P. De, and A. Misra, "Green synthesis of silver nanoparticles using latex of Jatropha curcas," *Colloids and surfaces A: Physicochemical and engineering aspects,* vol. 339, no. 1-3, pp. 134-139, 2009.

[8] J. Parsons, J. Peralta-Videa, and J. Gardea-Torresdey, "Use of plants in biotechnology: synthesis of metal nanoparticles by inactivated plant tissues, plant extracts, and living plants," *Developments in environmental science,* vol. 5, pp. 463-485, 2007.

[9] M. Altikatoglu, A. Attar, F. Erci, C. M. Cristache, and I. Isildak, "Green Synthesis of Copper Oxide Nanoparticles Using Ocimum basilicum Extract and Their Antibacterial Activity," *Fresenius environmental bulletin,* vol. 26, no. 12 A, pp. 7832-7837, 2017.

[10] A. K. Mittal, Y. Chisti, and U. C. Banerjee, "Synthesis of metallic nanoparticles using plant extracts," *Biotechnology advances,* vol. 31, no. 2, pp. 346-356, 2013.

[11] V. Armendariz, I. Herrera, M. Jose-yacaman, H. Troiani, P. Santiago, and J. L. Gardea-Torresdey, "Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology," *Journal of Nanoparticle Research,* vol. 6, no. 4, pp. 377-382, 2004.

[12] S. Iravani, "Green synthesis of metal nanoparticles using plants," *Green Chemistry,* vol. 13, no. 10, pp. 2638-2650, 2011.

[13] G. S. Dhillon, S. K. Brar, S. Kaur, and M. Verma, "Green approach for nanoparticle biosynthesis by fungi: current trends and applications," *Critical reviews in biotechnology,* vol. 32, no. 1, pp. 49-73, 2012.

[14] U. Klueh, V. Wagner, S. Kelly, A. Johnson, and J. Bryers, "Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation," *Journal of Biomedical Materials Research,* vol. 53, no. 6, pp. 621-631, 2000.

[15] Q. L. Feng, J. Wu, G. Chen, F. Cui, T. Kim, and J. Kim, "A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus," *Journal of biomedical materials research,* vol. 52, no. 4, pp. 662-668, 2000.

[16] J. S. Kim, E. Kuk, K. N. Yu, J. H. Kim, S. J.Park, H. J. Lee, S. H. Kim, Young K.Park, Y. H.Park, C. Y. Hwang, Y. K. Kim, Y. S. Lee, D. H. Jeong, and M. H. Cho,"Antimicrobial effects of silver nanoparticles," *Nanomedicine: Nanotechnology,*

Biology and Medicine, vol. 3, no. 1, pp. 95-101, 2007.

[17] J. Hurlow, K. Couch, K. Laforet, L. Bolton, D. Metcalf, and P. Bowler, "Clinical biofilms: a challenging frontier in wound care," *Advances in wound care,* vol. 4, no. 5, pp. 295-301, 2015.

[18] M. E. Cortés, J. C. Bonilla, and R. D. Sinisterra, "Biofilm formation, control and novel strategies for eradication," *Sci Against Microbial Pathog Commun Curr Res Technol Adv,* vol. 2, pp. 896-905, 2011.

[19] A. J. Huh and Y. J. Kwon, "“Nanoantibiotics”: a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era," *Journal of controlled release,* vol. 156, no. 2, pp. 128-145, 2011.

[20] O. V. Singh, *Bio-nanoparticles: biosynthesis and sustainable biotechnological implications*. John Wiley & Sons, 2015.

[21] J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramírez, and M. J. Yacaman, "The bactericidal effect of silver nanoparticles," *Nanotechnology,* vol. 16, no. 10, p. 2346, 2005.

[22] A. Nanda and M. Saravanan, "Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE," *Nanomedicine: Nanotechnology, Biology and Medicine,* vol. 5, no. 4, pp. 452-456, 2009.

[23] V. babu Nagati, R. Koyyati, M. R. Donda, J. Alwala, K. R. Kundle, and P. R. M. Padigya, "Green synthesis and characterization of silver nanoparticles from Cajanus cajan leaf extract and its antibacterial activity," *International Journal of Nanomaterials and Biostructures,* vol. 2, no. 3, pp. 39-43, 2012.

[24] A. M. Awwad, N. M. Salem, and A. O. Abdeen, "Biosynthesis of silver nanoparticles using Olea europaea leaves extract and its antibacterial activity," *Nanoscience and Nanotechnology,* vol. 2, no. 6, pp. 164-170, 2012.

[25] K. Shameli, M. B. Ahmad, E. A. J. Al-Mulla, N A. Ibrahim, P. Shabanzadeh, A. Rustaiyan, Y. Abdollahi, S. Bagheri, S. Abdolmohammadi, M. S. Usman, and M. Zidan, "Green biosynthesis of silver nanoparticles using Callicarpa maingayi stem bark extraction," *Molecules,* vol. 17, no. 7, pp. 8506-8517, 2012.

[26] J. Liu and R. H. Hurt, "Ion release kinetics and particle persistence in aqueous nano-silver colloids," *Environmental science & technology,* vol. 44, no. 6, pp. 2169-2175, 2010.

[27] R. Gengan, K. Anand, A. Phulukdaree, and A. Chuturgoon, "A549 lung cell line activity of biosynthesized silver nanoparticles using Albizia adianthifolia leaf," *Colloids and Surfaces B: Biointerfaces,* vol. 105, pp. 87-91, 2013.

[28] A. Ahmad, Y. Wei, F. Syed, K. Tahir, A. U. Rehman, A. Khan, S. Ullah, and Q. Yuan, "The effects of bacteria-nanoparticles interface on the antibacterial activity of green synthesized silver nanoparticles," *Microbial pathogenesis,* vol. 102, pp. 133-142, 2017.

[29] K. Markowska, A.M. Grudniak, and K.I. Wolska, "Silver nanoparticles as an alternative strategy against bacterial biofilms," *Acta Biochimica Polonica,* vol. 60, no. 4, pp. 523-530, 2013.