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ASSESSING THE ANTIBACTERIAL AND ANTIOXIDANT PROFILE OF SILVER NANOPARTICLES DERIVED FROM JASMINUM ANGUSTIFOLIUM

Ramesh Muthusamy[a], Mounishwaran Kamalesan[a], Mohanraj Raja[a], Rameshkumar Neelamegam[b], Shashank S. Kamble[b], Krishnan Muthukalingan[c], Kayalvizhi Nagarajan[a*]

[a]Department of Zoology, School of Life Sciences, Periyar University, Salem 636 011, Tamil Nadu, India.

[b]Amity Institute of Biotechnology (AIB), Amity University, Maharashtra, Mumbai 410206, India.

[c]Vice Chancellor, Central University of Tamil Nadu, Thiruvarur, Tamil Nadu, 610 005 India.

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***Corresponding Author**
Kayalvizhi Nagarajan
Department of Zoology, School of Life Sciences, Periyar University, Salem 636 011, Tamil Nadu, India.

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ABSTRACT

Owing to their eco-friendliness and nontoxic nature, the green synthesis of metallic nanoparticles (NPs) has intrigued the scientific community. Additionally, plant extracts offer a valuable source of reductants and stabilizers for the production of these NPs. The current study used leaf extracts of *Jasminum angustifolium* for the green synthesis of silver nanoparticles (Ja-AgNPs). The characterization of these Ja-AgNPs was conducted utilizing ultraviolet-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Analysis of FT-IR spectra revealed the acyclic alcohol functional groups presence as capping and reducing agents for the Ja-AgNPs. Furthermore, SEM analysis of the Ja-AgNPs revealed a size distribution of approximately 30 nm (diameter). Antimicrobial assays using log phase cultures of *Staphylococcus aureus* revealed zones of inhibition (ZOIs) with diameters of 1.16 ± 0.15 mm at 50 µg/mL and 1.56 ± 0.05 mm at 100 µg/mL Ja-AgNP. These results indicate that the

biosynthesized Ja-AgNPs have promising antibacterial properties.

KEYWORDS: Ja-AgNPs; *Staphylococcus aureus*; Antimicrobial activity; Antioxidant Properties.

1. INTRODUCTION

In 1880, Sir Alexander Ogston in Scotland first identified *Staphylococcus aureus* in patients with open wounds. *S. aureus* is a gram-positive bacterium present in the normal microbiota of human skin and is the causative agent for a variety of infections.[1] The pathogen releases several enzymes and toxins as virulence factors that are frequently linked to many illnesses and poisoning in humans. *S. aureus* is responsible for a range of infections, including skin and soft tissue infections (SSTIs), osteoarticular infections, endocarditis, sepsis, and pulmonary infections.[2] A variety of antimicrobial nanoparticles (NPs) and nanoscale carriers designed for antibiotic delivery have demonstrated efficacy in combating infectious diseases, including those associated with antibiotic-resistant bacteria, in both in vitro and in vivo studies.[3]

Future research into nanotechnology is anticipated to lead to significant improvements and advancements in synthesizing better and more efficient nanotherapeutic agents.[4] Among metallic NPs, the synthesis of AgNPs is the least expensive. Moreover, there is sufficient experimental evidence supporting the enhanced antimicrobial activity of AgNPs over silver ions.[16] Although a variety of methods have been utilized for the synthesis of metallic nanoparticles, environmentally friendly techniques can yield nanoparticles with precisely controlled size and shape when conditions are optimized.[5] AgNPs are typically synthesized using hazardous and expensive chemical methods, thereby limiting their potential for biomedical applications.[6] The physical methods of synthesis, via evaporation- condensation, spark discharge, laser ablation and light irradiation, are also unsuitable due to their high energy requirements, which increase the process cost and decrease their applicability.[7] Alternate biological methods are comparatively safer due to the involvement of nontoxic components and are also quicker, indicating their suitability for many industrial and environmental applications.[8] Various biological sources, including plant extracts,[9] fungi,[10] bacteria[11] and algae, have been successfully employed for the biological synthesis of nanoparticles.[12]

The nanoparticles produced, with sizes ranging from 1 to 100 nm, demonstrate a broad spectrum of antibacterial,[13] anticancer and[14] antidiabetic activities.[15] In the past few years, several extracts, including *Peganum harmala,*[17] *Acacia nilotica,*[18] *Mangifera indica*[18] and *Carissa carandas*, have been utilized for the synthesis of AgNPs.[19] Various plant metabolites and phytochemicals, including proteins, peptides, terpenoids, flavonoids, tannins, and alkaloids, are recognized for their ability to reduce AgNO3 to AgNPs. In the present study, we used the Oleaceae family member *Jasminum angustifolium* for the green synthesis of AgNPs. There have been reports on the medicinal properties of *J. angustifolium*. The dry petals of *J. angustifolium* have been shown to relieve ocular redness, and methanol extracts of this plant exhibit hepatoprotective properties.[20] However, the synthesis of AgNPs by *J. angustifolium* and evaluation of their antibacterial and cytotoxic properties have not been previously reported. Therefore, there is a strong need for a detailed fundamental study to evaluate the biocompatibility of these Ja-AgNPs before proceeding toward clinical applications. This study thus investigated the antibacterial and antioxidant of Ja-AgNPs to assess their potential for future biomedical applications.

2. MATERIALS AND METHODS

2.1. Chemicals

Silver nitrate ($AgNO_3$) was purchased from Sisco Research Laboratories (SRL) Pvt. Ltd., Maharashtra, India. Luria Bertani broth and Agar–Agar type I were purchased from Himedia laboratories Pvt. Ltd., Chennai, Tamil Nadu, India. Double distilled water was used for all the assays.

2.2. Collection and preparation of plant leaves

Leaves of *J. angustifolium* were obtained from Periyar University, Salem, Tamil Nadu, India. The gathered leaves were washed with bi-distilled water to remove dust particles and other exterior impurities, allowing for a more precise assessment of their underlying chemical makeup while lowering the effect of surface pollution. Approximately 10 g of dry leaves was cut into small pieces and boiled in 100 mL of sterile distilled water for 15 min at 60°C. After boiling, the extract was filtered through Whatman no. 1 paper and stored at 4°C for further use.

2.3. Biosynthesis of AgNPs

Fifty millilitres of *J. angustifolium* aqueous leaf extract were mixed with 450 millilitres of 1 mM AgNO3 solution and swirled continuously on a magnetic stirrer at room temperature for

24 minutes. During stirring, a dark brown precipitate was obtained, which indicated the initial formation of AgNPs due to the reduction of silver ions by the aqueous leaf extract of *J. angustifolium*. The reaction mixture was centrifuged at 5000 rpm for 15 min. The pellets were collected and dried at 75°C using a hot air oven for 6-8 h. The dried pellets were stored and subsequently used for characterization and bioassays.

2.4. Characterization of AgNPs

The synthesis of AgNPs was observed at regular intervals using UV–Vis spectroscopy across a wavelength spectrum of 200–800 nm at a resolution of 1 nm, employing a Shimadzu spectrophotometer (Model UV 1700). Prior to each measurement, the samples were adequately diluted with water. In order to remove any unbound AgNP molecules, the supernatant obtained after centrifuging at 22,360 g for 15 minutes was discarded. The resulting pellet was then re-suspended in distilled water. This procedure was repeated three times. The purified pellets were then dried in a hot air oven set at 80 °C for 12 hours, after which the dried AgNPs were gathered for further characterization via FTIR spectroscopy. The FTIR spectrum was obtained using a Shimadzu8400S spectrophotometer, employing potassium bromide pellets at a resolution of 4 cm−1 in diffuse reflectance mode. X-ray diffraction analysis was conducted to investigate the crystallographic structure of the purified AgNPs. For the XRD sample preparation, a thin film of the sample was applied to a glass slide by depositing 100 µL of the sample and allowing it to dry for 30 minutes. The XRD pattern was captured using a Rigaku Miniflex 600 X-ray diffractometer operating at 40 kV with a 15-mA current. The samples were analyzed under copper K (alpha) (CuKα) radiation, equipped with a nickel monochromator in the 2θ range of 20–80°. An equivalent sample preparation technique was employed for scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) assessments. A scanning electron microscope (EVO MA18) combined with energy dispersive X-ray (EDX) (Oxford) was used for the evaluation.

2.5. Determination of antibacterial activity using a well diffusion assay

The antibacterial activity of the Ja-AgNPs was evaluated via a well diffusion assay.[21] with slight modifications. The cryopreserved culture of *S. aureus* was revived, inoculated in Luria Bertani broth and further incubated for 18 h in a rotary shaker incubator. After incubation, 100 µl of *S. aureus* culture was swabbed on Luria Bertani agar and spread evenly to obtain lawn growth. Using a stainless-steel cork borer, the wells were punched in LB. agar culture swabs, and different concentrations of Ja-AgNPs (25-100 µg/ml) were added. Cephalexin (25

µg/ml) was used as a positive control, whereas LB broth alone was used as a negative control. All agar plates were incubated for 24 h at room temperature, after which the zones of inhibition were measured.

2.6. Time Kill assay

The proliferation rate of S. aureus, whether in the presence or absence of Ja-AgNPs, was determined through a time-kill assay.[21] The Ja-AgNPs formulation was diluted with LB broth to obtain final concentrations of 20 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL in a total final volume of 200 µL in 96-well plates. LB broth inoculated with *S. aureus* culture served as a positive control, whereas LB broth alone was used as a negative control. All the samples were incubated for 24 h at room temperature. Bacterial cell viability was measured using a microplate reader at 595 nm with a constant gap of 3 h.

2.7. DPPH radical scavenging assay

The total free radical scavenging capacity of extracts from the various plant samples was evaluated using the stable DPPH radical, following the method of Brand-Williams et al. (1995).[22] with minor modifications. A 500 µM DPPH solution was prepared in methanol. For the DPPH scavenging assay, 100 µl of the DPPH solution was mixed with different concentrations (20–100 µg/ml) of Ja-AgNP samples, and the volume was adjusted to 1 ml with ethanol. The mixture was then incubated in the dark at room temperature for 30 minutes. The antioxidant activity was determined based on the reduction in the initial purple color of the DPPH solution, and absorbance was measured at 517 nm.

$$\text{Scavenging activity } (\%) = \left[\frac{\text{Absorbance (Control)} - \text{Absorbance(Sample)}}{\text{Absorbance (Control)}}\right] * 100$$

2.12. Statistical analysis

The mean standard deviations for all the experiments were calculated by using three independent replicate samples. The importance of differences among the means was evaluated through one-way ANOVA. P values ≤ 0.05 were regarded as indicative of statistical significance across all analyses.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Ja-AgNPs

The leaf extract of *J. angustifolium* was used to synthesize AgNPs from $AgNO_3$. The generation of a colloidal suspension of Ja-AgNPs was indicated by the colour shift.[23] The

color of the aqueous extract shifted from pale black to dark brown, signaling the formation of AgNPs from the *J. angustifolium* leaf extract in the presence of AgNO3. Previous studies have reported similar observations involving the use of *Azadirachta indica* leaf extracts to produce AgNPs.[24] Since the leaf extract acts as both a capping and reducing agent, the bioactive compounds in the plant extract are likely crucial in reducing silver ions to AgNPs. UV-Spectrum of the analyte was obtained over a range of 200-800 nm at various time intervals. Silver induces the production of free radicals during the bioreduction of 1 mM AgNO3, thus allowing a surface resonance of 436 nm (Fig. 1). These absorbance readings were taken after 24 min. Previously, Selvaraj et al.[23] demonstrated similar results, where AgNPs produced using a green synthesis approach exhibited plasmon absorbance peaks at 436 nm, which is characteristic of AgNPs.

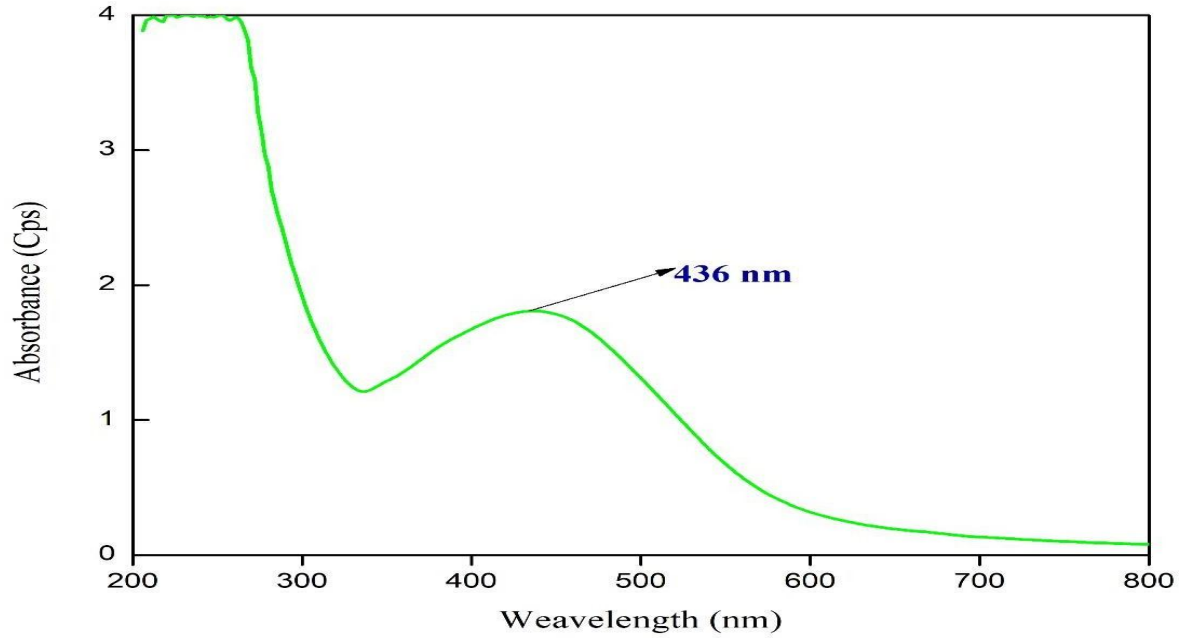

Fig.1 UV–visible spectrum of AgNPs synthesized from *J. angustifolium* leaf extract

Using *J. angustifolium* leaf extract, the biosynthesized silver nanoparticles were effectively stabilized, and their capping ability was evaluated by FTIR. Various absorbance peaks were visible in the FTIR spectrum at 1029, 1643, 21356 and 2623 cm-1 (Fig. 2). The peak at 1029 cm-1 was attributed to acyclic alcohols with C-O stretching vibrations; the band at 1643 cm cm-1 indicates the presence of a 2HC=CH2 group with C=C stretching vibrations; the peak at 2356 cm-1 indicates the presence of an NH=C group, and peak at 2923 cm-1 was attributed to a CH2 acyclic group. All these functional groups may facilitate the stabilization and capping of the Ja-AgNPs.

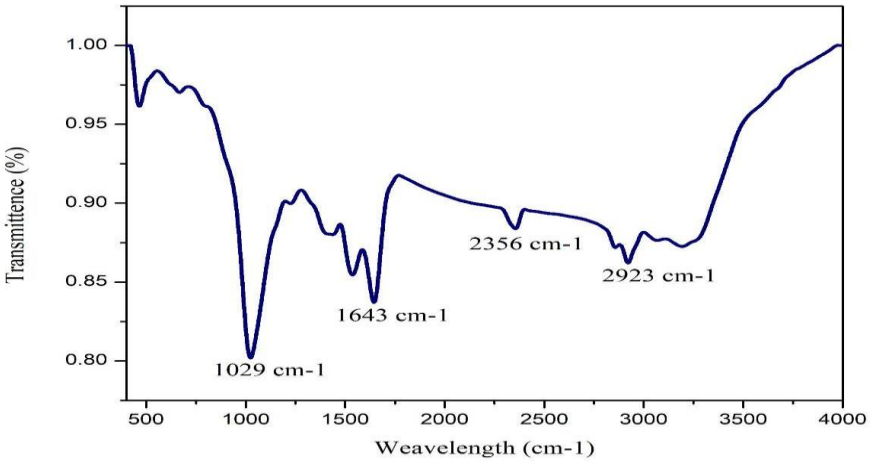

Fig.2 Fourier transform infrared spectroscopy of the Ja-AgNPs

X-ray diffraction analysis was utilized to explore the crystal structure of the Ja-AgNPs. The peaks obtained at 2θ values were 38.14, 44.08, 65.52 and 76.85, and their respective Bragg reflections were at (200), (111), (200) and (311). The precise peak positions for the FCC lattice surface of silver are provided in (Fig. 3). The crystalline nature of the Ja-AgNPs with a particle range of 26.65 to 58.32 nm was similar to that of AgNPs from *Carissa carandas*, with corresponding 2θ values of 38.06, 44.23 and 67.43.[19]

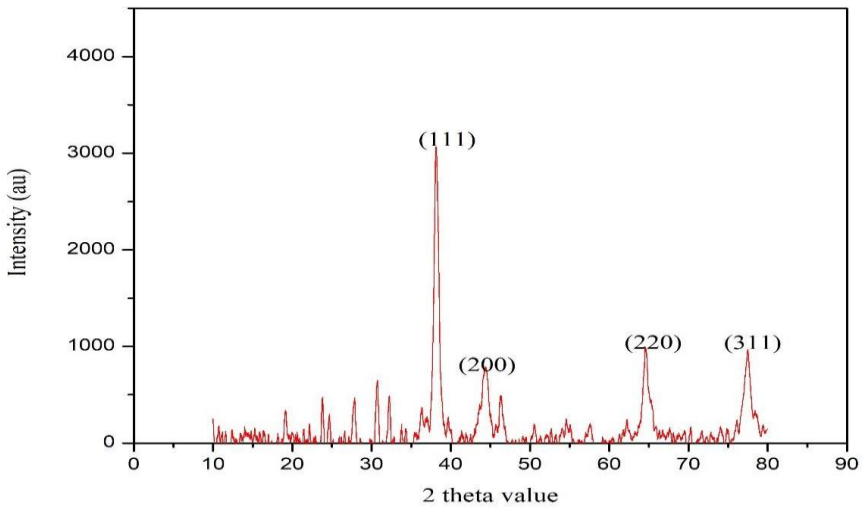

Fig.3 X-ray diffraction of the Ja-AgNPs

Scanning electron microscopy (SEM) images used to examine the structure and organization of the biosynthesized Ja-AgNPs showed spherical AgNPs grouped into clusters (Fig. 4). Recently, AgNPs synthesized from the leaf extracts of *Skimmia laureola* were shown to have irregular spherical shapes with an average particle size of 46 nm.[25] The particle size of the biosynthesized Ja-AgNPs was approximately 30 nm.

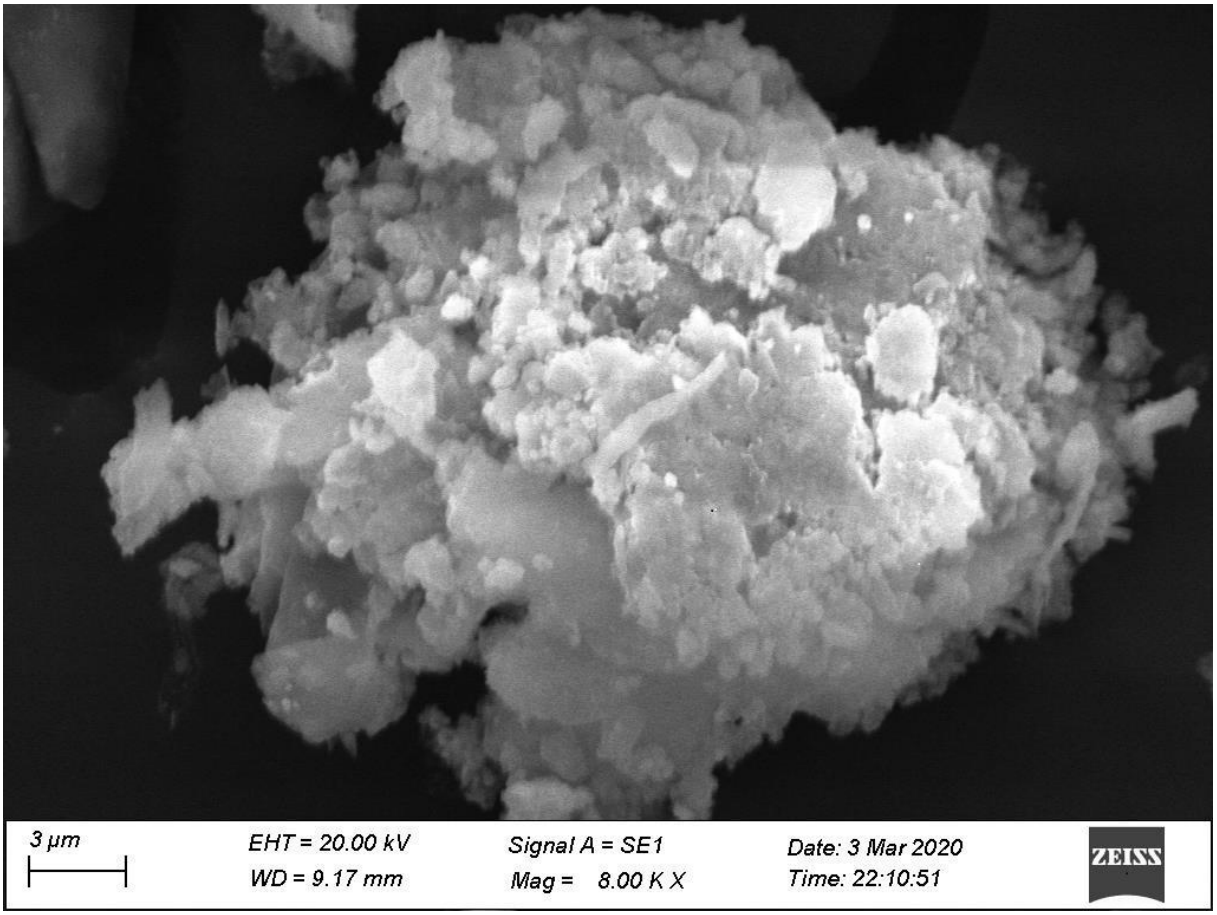

Fig.4 Scanning electron microscopic image of the Ja-AgNPs.

3.2. *In vitro* studies

3.2.1. Antibacterial activity

The biosynthesized compounds displayed strong dose-dependent antimicrobial activity against the Ja-AgNPs. Previous research has demonstrated that AgNPs disrupt the bacterial cell wall and release silver ions into the cell, ultimately causing cell death.[26] The size of AgNPs and the presence of active biomolecules that assist their binding to bacterial cell walls are important for the antimicrobial activity of AgNPs.[27] In our study, the antibacterial efficacy of Ja-AgNPs against *S. aureus* was evaluated using the well diffusion method. The log phase culture of *S. aureus* was exposed to different concentrations of AgNPs, such as 25, 50, 75 and 100 µg/ml, and the diameters of the zones of inhibition obtained were 0.96 ± 0.05 mm, 1.16 ± 0.15 mm, 1.36 ± 0.05 mm and 1.56 ± 0.05 mm, respectively. These zones of inhibition were compared to that of the positive control (Cephalexin (25 µg/mL), which was 1.16 ± 0.15 mm (Table 1). No inhibition zone was detected in the control well or in the wells containing the AgNO3 solution. Thus, coupling the inherent properties of *J. aestivum* extract with those of Ag NPs can minimize the dose needed for total microbial growth inhibition. An

earlier study[28] showed that biomolecule-encapsulated silver nanoparticles exhibited a greater zone of inhibition against *S. aureus* than did commercially available silver nanoparticles. Similar antibacterial activity was demonstrated using AgNPs biosynthesized from the extracts of *Zingiber zerumbet* against *S. aureus.*[29] Our results show the strong inhibitory activity of Ja-AgNPs against *S. aureus*.

Table 1: Antimicrobial activities of Ja-AgNPs against *S. aureus.*

Microorganism	Ja-AgNPs				Cephalexin (25 µg/mL)
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
Staphylococcus aureus	0.96 ± 0.05	1.16 ± 0.15	1.36 ± 0.05	1.56 ± 0.05	1.16 ± 0.15

3.2.2. Time-kill assay

A time-kill assay was conducted to assess bacterial viability following treatment with Ja-AgNPs and to determine the minimum duration required for an inhibitory or bactericidal effect. The time–kill curve of Ja-AgNPs against *S. aureus* is presented in Fig. 5, which shows significant differences in the bactericidal activity in a dose-dependent manner. The bactericidal activity gradually increased with exposure to increasing concentrations of Ag NPs. The results expressed the inhibition of *S. aureus* cells at various time intervals (3, 6, 9, 12, 15, 18, 21 and 24 h) at different concentrations of 20, 40, 50, 60, 80 and 100 µg/ml Ja-AgNPs. In the present study, 50 µg/ml Ja-AgNPs reduced bacterial cell viability.

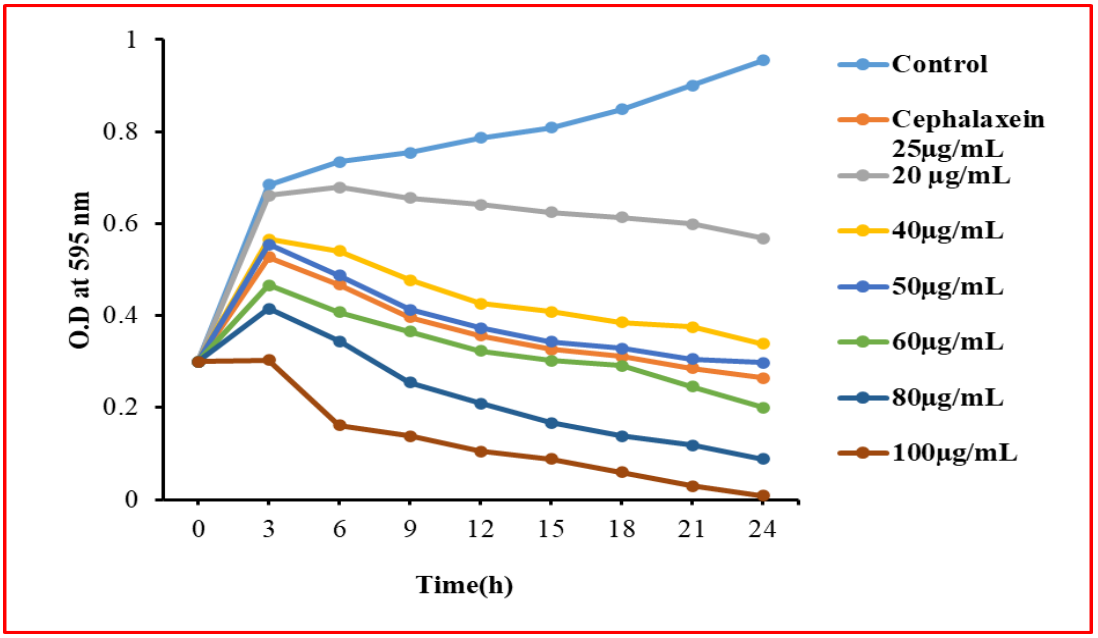

Fig.5 Time kill assay of Ja-AgNPs on *S. aureus* with 20 µg/mL, 40 µg/mL, 50µg/mL, 60 µg/mL, 80 µg/mL,100 µg/mL and Cephalaxein 25 µg/mL for 24 h at 3 hr interval.

3.3 DPPH radical scavenging assay

The DPPH radical scavenging activity of the Ja-AgNPs exhibited showed a clear concentration-dependent increase, indicating strong electron-donation and free-radical neutralization ability consistent with other green-synthesized AgNP systems (Fig.6). Similar patterns were reported for silver nanoparticles synthesized using *Dalbergia sissoo* leaf extract, which demonstrated notable antioxidant activity with an EC_{50} of 51.32 µg/Ml.[29] AgNPs synthesized using *Brachychiton populneus* also displayed concentration-dependent radical scavenging trends similar to the present results.[30] Furthermore, *Solanum xanthocarpum* fruit extract–derived AgNPs were shown to possess enhanced antioxidant activity due to phytochemical capping.[31] Overall, the DPPH scavenging efficiency of Ja-AgNPs falls within the activity range documented in the recent green-synthesis nanoparticle literature, supporting their potential biomedical relevance.

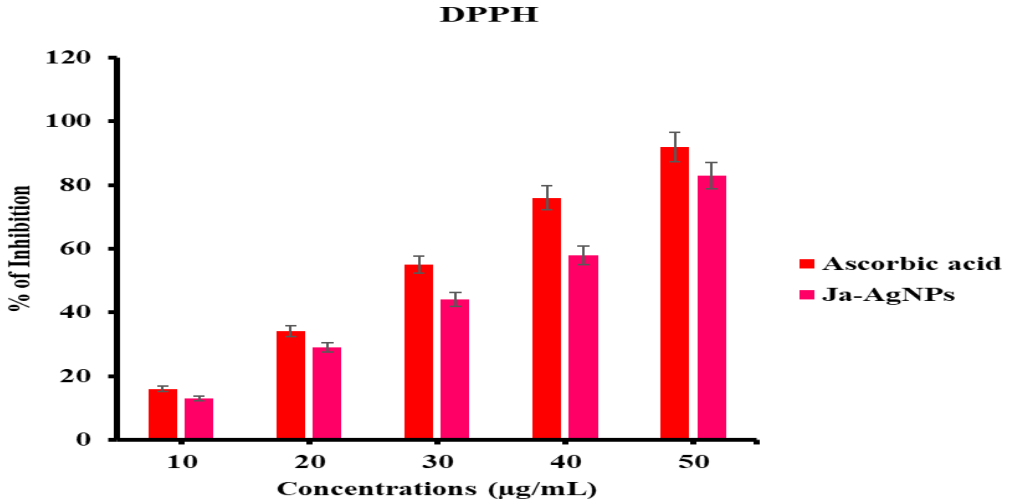

Fig.6 Antioxidant activity of the biosynthesized Ja-AgNPs.

4. CONCLUSION

In this study, silver nanoparticles were synthesized using a green method with the leaf extract of *J. angustifolium*. These Ja-AgNPs were characterized by various biophysical techniques. The antibacterial activity of Ja-AgNPs (100 µg/mL) was greater against *S. aureus*. Overall, the results suggest that the Ja-AgNPs have potential for the design of novel antibiotics against *S. aureus* and can be used for antioxidant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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