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Evaluation of the Antibacterial efficacy of green nanoparticles synthesized from *Lonicera japonica* plant against *Staphylococcus aureus*

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Green-synthesized metal nanoparticles primarily derived from plant sources, have attracted great interest due to their inherent biodegradability, speed, and cost-effectiveness. This investigation produced silver nitrate of nanoparticles (AgNPs) using an aqueous extract of *Lonicera japonica*. The biosynthesized AgNPs were subsequently characterized by ultraviolet-visible spectroscopy (UV-Vis), and Fourier transform infrared (FTIR) spectroscopy. The nanoparticles generated were then evaluated for antibacterial activity. The evaluation was carried out against seven Gram-positive strains. AgNPs. The effectiveness of AgNPs was also looked into, and the minimum inhibitory concentration (MIC) of AgNPs was found to be between 3.12 % and 6.25%. In the meantime, more tests were done on the well, diffusion assay to determine their role as antibacterial agents. Also, the average inhibition zone was (9-17 mm) against seven pathogenic bacteria (*S.aureus*). So, the results of biogenic AgNPs are new in that they can kill Gram-positive bacteria. Thus, this study presents biosynthesized AgNPs that can be employed as a safe alternative to synthetic chemicals and a possible candidate for antibacterial agents against various bacterial species.

Keywords: ***Biosynthesis; Lonicera japonica; antibacterial agents; Staphylococcus aureus; nanoparticle***

INTRODUCTION

The skin is the body's largest organ. It performs a variety of innate and adaptive immunological tasks, including defending the body against chemical, physical, and biological threats. Even though the cutaneous barrier has a powerful immune system, the skin promotes the colonization of bacteria. As a result, germs on the skin have a significant impact on how well human immunity works. As a result, the skin microbiota has sparked a lot of interest in trying to figure out what causes these diseases and how to find new and better treatments options (Wang and Fang , 2014; Sanford and Gallo, 2015). Acute bacterial skin and soft tissue infections (SSTIs) are a broad range of conditions that can affect the skin and underlying tissues. They can be superficial, such as impetigo, or deeper, such as cellulitis, cutaneous abscesses, erysipelas, and infected wounds, burns, or ulcers(May 2009,p.403-420; Pollack et al. 2015). The severity of SSTI can vary widely, from mild to life-threatening, depending on a number of factors. Despite variances in geographic distribution and severity, all of these illnesses are caused predominantly by Gram-positive bacteria, particularly *Staphylococcus aureus* (Russo et al. 2016; Principi et al. 2020; Hindy et al. 2022) .

S. aureus is a Gram-positive bacterium that is commonly found in the human body. It is most commonly found in the anterior nares, or the space between the nostrils. Around 20-25% of people carry *S. aureus* in their noses on a long-term basis, while the other 75-80% carries it only occasionally or not at all. Studies have shown that people who carry *S. aureus* in their noses are more likely to develop nosocomial infections, or infections that occur in hospitals (Archer et al. 2011 ; Davarpanah et al. 2022). The increasing antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) and community-acquired MRSA (CA-MRSA) strains obtained in hospitals has made it more difficult to treat these infections (Davarpanah et al. 2022) .

Lonicera japonica (Lj), also known as Japanese honeysuckle, is a traditional Chinese herbal medicine

that has been used for centuries to prevent and treat a variety of ailments. It is native to eastern Asia, including Korea, Japan, Taiwan, and northern and eastern China. *L. japonica* is utilized as a food, cosmetic, beautiful groundcover, and healthful beverage in addition to being a medical plant. *Lonicera japonica* has anti-inflammatory, antibacterial, antiallergy, and antioxidation properties. Bacillary dysentery, excessive blood pressure, lung infections, and acute urinary tract infections are all conditions that can be treated with this medication (Yang et al. 2021; Zhou et al. 2021). Fever syndrome, inflammation, febrile illnesses, diarrhea, carbuncles, and virulent swellings have all been treated with this herb (Zhao et al. 2015).

The unique physical and chemical properties of silver nanoparticles (AgNPs), which include optical, electrical, and thermal features as well as robust electrical conductivity and biological attributes, have led to their growing utilization in various industries such as medicine, health care, consumer goods, and manufacturing(Zhang et al. 2016). Nanoparticles have unique capabilities that are influenced by their size, shape, and morphology, allowing them to interact with plants, animals, and microbes. Silver nanoparticles (AgNPs) have been shown to possess antimicrobial properties that are effective against a broad range of bacteria. AgNPs are made in a variety of ways, with the goal of studying their morphology or physical properties (Siddiqi et al. 2018).

Plant extracts have been exploited in biomedicine to generate alternative green synthesis of AgNPs that is simple, cost-effective, safe, and ecologically friendly. Green synthesis is favoured over chemical synthesis in the production of nanoparticles because it is safer. Silver nanoparticles made using environmentally friendly technologies are frequently employed in antidiabetic, antibacterial, antioxidant, and anticancer applications (Balan et al. 2016).

In this study, a newly green synthesized AgNPs from *Lonicera japonica* was analysed using XRD, UV-DRS, FTIR, and SEM-EDX methods and evaluated for ability to inhibit the growth *S.aureus* isolated from skin infections.

The objective of this study was to assess the antibacterial effect of aqueous extracts of Lonicera japonica (Lj) and Lj-AgNPs against various isolates of S. aureus.

MATERIALS AND METHODS

Chemical studies

Sigma-Aldrich provided the silver nitrate AgNo3 used as a metal precursor to make AgNPs.

Collection of Plant leaves

L. japonica leaves were collected from Taif (21.275337, 40.397517), Saudi Arabia.

Preparation of aqueous *L. japonica* leaf extract

The L. japonica leaves were cleaned, dried, and then subjected to a thorough 10-minute wash in tap water, followed by a brief rinse in deionized water. Subsequently, a plant leaf broth solution was prepared using 10 g of the washed leaves. Leaves were mixed with 100 ml deionized water and then incubated at 60 ^{0}C for 5 min. (Balan et al. 2016).

Green synthesis of silver nanoparticles

Green synthesis of AgNPs was achieved using solution precipitation method to prepare *L. japonica* leaves aqueous extract using. After incubation, the solution was decanted. Different amount of the broth (50 µm, 100 µm,and 200 µm) was added respectively to 50 µm ,100 µm,200 µm of 0.1M aqueous $AgNO_3$ solution. This was followed by the addition of the different concentrations to different tubes with 5 ml of deionized water and incubated at room temperature until the colour become brownish. The extract was passed through a nylon mesh and then filtered using a Millipore hydrophilic filter (0.22 mm) before being utilized for subsequent experiments. In addition, a control setup was established without the L. japonica extract, and the color intensity of the extracts was measured at 435 nm over different time intervals (6, 12, 24, 48, and 72 h) using a spectrophotometer.

Microbiological Studies

Sample collection:

S. aureus were isolated from pus samples of patients suffering from SSTIs, including lesions that required incision and drainage, as well as those with spontaneously draining purulent fluid, carbuncles, boils, furuncles, chronic ulcers, cellulitis with purulent drainage, and deep wounds. The samples were collected from King Abdullah Medical Complex (K.A.M.C) in Jeddah, Saudi Arabia, by hospital staff such as phlebotomists and nurses. These samples were then delivered to the microbiology laboratory as pure cultures in Blood Agar medium for further analysis.

Growth of isolates on Mannitol Salt Agar (MSA):

All staphylococcal isolates were confirmed in the laboratory by inoculating onto mannitol salt agar (Hi-Media, India), followed by incubation at 37°C for 24-48 hours. The process of mannitol fermentation was then monitored and recorded. The color of the dish turns from red to yellow due to fermentation. Purification of the isolates was carried out using repeated streak culture on two selective media types, namely Blood and Mannitol salt agar. The purified samples were incubated for 24 hours at 37°C and then examined for bacterial growth.

Biochemical identification

Identification of bacterial strain using VITEK® 2 COMPACT System

VITEK® 2 COMPACT (BioMérieux, France) was used for the automated identification process. The needed materials were assembled such as; a) fresh culture plates, b) test tube rack, c) 75-millimeter clear polystyrene disposable test tubes and caps, d) 45% saline sodium chloride- pH: 5-7 , e) sterile sticks, f) the Densi CHEK plus instrument for measuring optical density of the microorganism suspension, g) pipette tips and fixed volume pipettor with fixed headers 145 and 280 milliliter, h) vortex, i) VITEK® 2 test ID cards GN and GP, j) , VITEK® 2 compact cassette. Second, the patient's ID was written on the top of the test tube with permanent marker then the dispense set was used. A 3.0 ml saline was dispensed into the tube, using the sterile sticks the colonies were removed from the culture to the saline tubes then mixed well to create homogenous suspension and the tube was capped and vortexed lightly. Furthermore, a densi CHEK plus instrument were used to check the turbidity and compare the type of bacteria to McFarland units that was required by the ID cards (GN: 0.5-0.63 – GP: 0.5-0.63) and provided by the laboratory (Szabados, 2017).The tube was put into the instrument, then the reading was generated, if the achieved number was too low more organisms were added, if it was too high the suspension was diluted. Afterwards, the tube was placed in the well of the rack in front of the test ID cards as well as the 49 transfer tube (blue colored transfer tube) which was placed into the suspension tube. Finally, the cassette of the identification was positioned into the instrument to be processed. It took 2- 8 hours to obtain the results. (Szabados, 2017)

The Antimicrobial susceptibility testing (AST) for isolates:

VITEK® 2 COMPACT (BioMérieux, France) was utilized for the fully automated Antimicrobial susceptibility testing (AST) of the isolates. As previously described in the identification process manual by VITEK® 2 kit (BioMérieux, France), AST was performed simultaneously with the identification process and in similar protocol with few changes. Hence, next to the ID test cards (GN, GP), suitable AST card (AST 91 and 92 for GN and AST 80 and 86 for GP) (BioMérieux, France) was added. However, the samples were prepared differently; one 3.0 ml of saline was added to the tubes, then if the AST card was for GN, a 145 ml pipettor was used to transfer from the previously prepared GN organisms suspension to the new saline tube, later the transfer tube was placed into the suspension. Also, if the AST card was for GP, a 280 ml pipettor was used to transfer from prepared GP suspension to the 3.0 ml saline tube, later the transfer tube (gray colored transfer tube) was placed into the suspension. Finally, the cassette was positioned into the instrument to be processed. It can take up to 12 hours to obtain the results (Szabados, 2017).

Molecular Identification of S. aureus Isolates

The total genomic DNA of the *S. aureus* isolates was extracted from bacterial pellets of an overnight culture using a modified version of the method described by Azcárate-Peril and Raya (2001). The bacterial pellets were mixed with lysozyme and TES buffer, incubated at 37°C, and then treated with proteinase K and sodium acetate. The mixture was then centrifuged, and the upper layer was transferred to a new Eppendorf tube and treated with isopropanol. The DNA was left to dry and then resuspended in distilled water. The isolated DNA was analyzed using agarose gel electrophoresis and stained with ethidium bromide.

The study used polymerase chain reaction (PCR) to amplify 16S rDNA from the bacterial isolates. Agarose gel electrophoresis was performed to separate DNA fragments, and sequencing of the 16S rRNA gene was carried out to identify the bacterial species. The PCR amplification was confirmed by ethidium bromide fluorescence, and the PCR amplicons were purified. The PCR product was sent to Korea (Macrogen) for sequencing. Upon sequencing the 16S rRNA gene, the resulting data were submitted to the genetic sequence database at the National Center for Biotechnology Information (NCBI), and the corresponding GenBank IDs (http://www.ncbi.nlm.nih.gov) of the strains. Subsequently, each unambiguous sequence was subjected to BLAST analysis by comparing with the sequences were in the GenBank database of NCBI, and phylogenetic trees were constructed using the MEGA software.

Detection of *pvl* gene in *S. aureus*:

Primers:

Pvl gene was detected in *S. aureus* after amplification using universal primers in a DNA thermo cycler. PCR amplification was performed with an initial denaturation step, followed by 30 amplification cycles and a final extension. Agarose gel electrophoresis was used to visualize the amplification product, and the amplified DNA was observed using UV transillumination (Bhatta et al. 2016).

Bioactivity of green synthesized AgNPs on identified *S. aureus* isolated

For this experiment, Mueller Hinton agar (MHA) media was poured into plates and allowed to dry. A bacterial inoculum containing approximately 10^{-3} CFU/ml was then evenly spread on the media using a cotton swab. Wells of 8 mm diameter were perforated using a

sterilized stainless steel cork borer. Following this, AgNPs at varying concentrations were added to each well. () and one well was filled with plant extract. and the plates were incubated at 37°C for 48h for bacterial plates.

Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The study determined the minimum inhibitory concentration (MIC) values of AgNP (200/200) µm sample solutions with antibacterial activity using microtiter plates. A serial dilution was performed, ranging from a concentration of 50 µg/ml to 0.1 µg/ml. Different wells were used as controls, including negative, sterility, and growth controls. Bacterial culture was added to each well, and the micro plates were incubated at 37°C for 12 hours. After incubation, a growth indicator, p-iodonitrotetrazolium (INT), was added to each well, and the plates were further incubated for 30 minutes. Colorless suspensions indicated growth inhibition, while violet-colored suspensions indicated growth (Patel et al. 2020).

MBC was determined …

RESULTS

Preparation of L. japonica AgNPs

Plant extract-capped silver nanoparticles (AgNps) are produced as a result of the reduction of silver nitrate in the presence of extract from leaves of the *L. japonica* plant. this is a qualitative evaluation of the reducing capability of extract *L. japonica* plant., The synthesized AgNps were validated by thechanging of color to brown. The process of preparing AgNPs involves observing a color change in the leaf extracts before and after they are reduced by the plant extract. This color change serves as confirmation of the formation of AgNPs, as the solution changes from pale yellow to brown (Figure 1). The stimulation of surface plasmon oscillations in the metal nanoparticles gave the synthetic silver nanoparticles their hue.

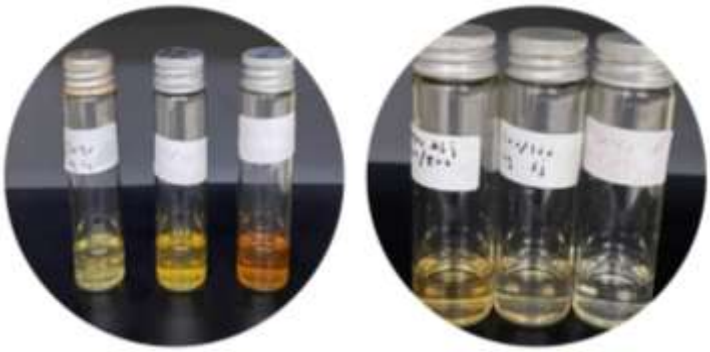

Figure 1: **Preparation AgNPs; Colour change of leaf extracts before and after reduction by plant extract.**

Characterization of *L. japonica* AgNPs

After synthesis, various techniques were employed to study the surface morphology, thermal stability, and physicochemical structural properties of the green synthesized AgNPs. The initial characteristics of the AgNPs were monitored using UV-vis absorbance spectra analysis. The study revealed that the peak absorbance occurred at 400 nm for different time intervals of AgNPs produced using *L. japonica* leaf extract. This was confirmed by the UV-VIS spectrograph of the colloidal solution of silver nanoparticles, which was recorded over time. The result of the UV-VIS spectra recorded from the reaction medium after 24 hours indicated that the absorption spectra of silver nanoparticles formed in the reaction media had an absorbance peak at 400 nm (Figure 2), confirming the formation of AgNPs. The broadening of the peak suggested that the particles were poly-dispersed.

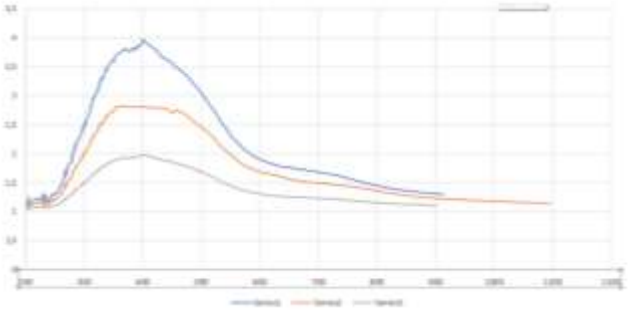

Figure 2: The UV-vis absorbance spectra were measured at various time points during the biosynthesis of AgNPs.

Confirmation of S. aureus isolated from SSSI

Seven bacterial strains of *S. aureus* isolated from SSSI were subsequently confirmed and purified

The selected isolates displayed identical morphological characteristics when grown on Blood and Mannitol salt agar (Figure 3a and b). Our findings indicated that all of the pathogenic bacteria (S. aureus) were gram-positive, as demonstrated in (Figure .4)

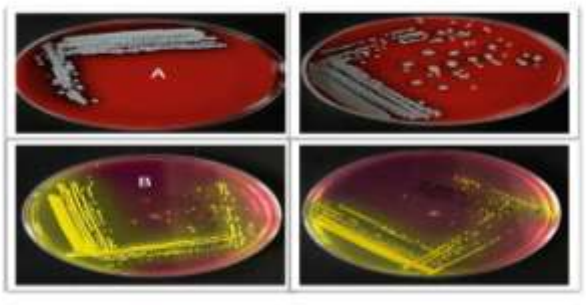

Figure 3: **Morphology of *S.aureus* on Blood agar (A) and Mannitol salt agar (B).**

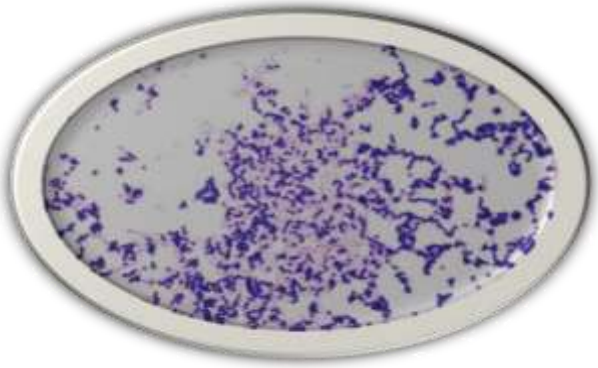

Figure 4: **Gram staining result showing appearance of *S.aureus*.**

Molecular characterization and identification of bacterial isolates:

The selected isolates were also identified using molecular characterization as *S.aureus*. the amplified 16s rRNA gene band was presented in figure 5. The similarity between the sequences obtained in this study and the sequences in the NCBI database is presented in table 1., as detailed in Table 1.

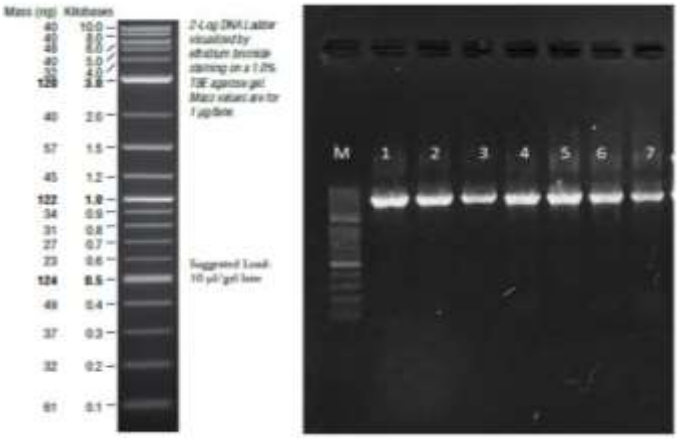

Figure 5: PCR product of 16s rRNA gene of *S.aureus* isolates, Lane M, DNA marker; lanes 1-7, 16s rRNA samples.

Table 1: Molecular characterization analysis (based on 16S rDNA sequence).

No	Closest species from GenBank	Sequence Similarity (%)
1	*S. aureus*	97% *S. aureus*
2	*S. aureus*	96% *S. aureus*
3	*S. aureus*	99% *S. aureus*
4	*S. aureus*	99% *S. aureus*
5	*S. aureus*	99% *S. aureus*
6	*S. aureus*	99% *S. aureus*
7	*S. aureus*	99% *S. aureus*

Detection of antibiotic resistance *pvl* gene in *Staphylococcus aureus*

The pvl gene detection was achieved successfully in all the deven isolates. The band which confirms the presence of the gene in the isolates was presented in Figure 6. Based on this finding, these isolates were identified as PVL-positive methicillin-resistant *S. aureus* (MRSA), whereas other isolates lacking the pvl gene were classified as PVL-positive methicillin-sensitive *S. aureus*.

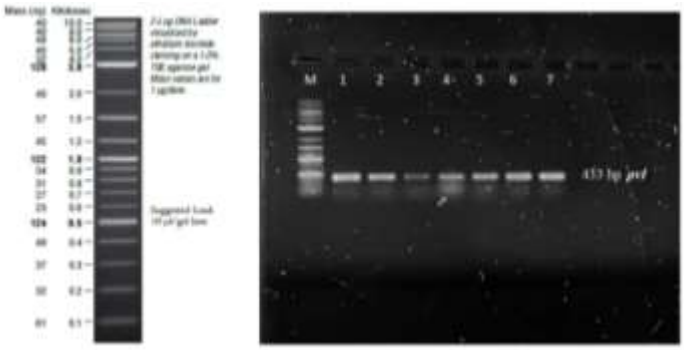

Figure 6: PCR product of *pvl* gene of *S.aureus* isolates, Lane M, DNA marker; lanes 1-7, 16s rRNA samples.

Antibacterial testing

Antibiotic sensitivity and resistance were done by VITEK® 2 to confirm these results. This study's finding indicated that most strains of *S.aureus* isolated were sensitive . Most of the isolates were sensitive to at least 9 antibiotics. The isolate 5 was sensitive to all 15 antibiotics used (15/15) 100%. The isolate 3 was sensitivity (14/15) 93.3%, followed by the isolates 1and 2 (13/15) 86.6%, then the isolates 4 and 7 were sensitive (12/15) 80%, whereas that the isolate 6 was sensitive to (9/15) 60%, while it was resistant to (6/15) 40% (Figure 7).

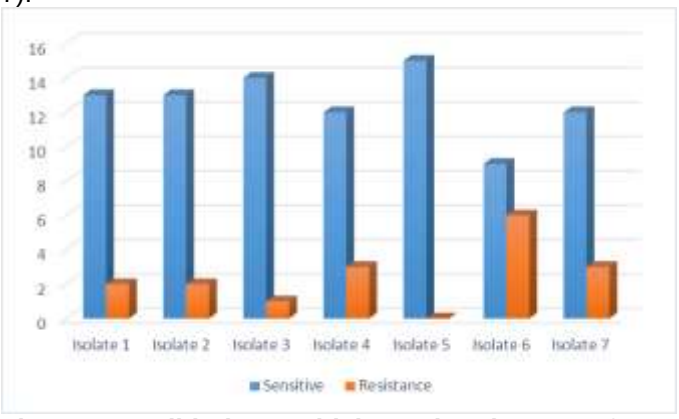

Figure 7: Antibiotic sensitivity and resistance of test (by VITEK® 2)

Bioactivity of the synthesized AgNPs

Minimum inhibitory concentration (MIC)

effect of aqueous extracts *L. japonica Lj*-AgNPs. The antibacterial activity of aqueous extracts *L. japonica* AgNPs was determined against seven Gram-positive pathogens *(S. Aureus*). The relative antibacterial efficacy of *Lj*-AgNPs compounds towards pathogens was studied qualitatively by well and disk diffusion and quantitatively in terms of minimum inhibitory concentration MIC. The broth's turbidity was visually inspected to study bacterial growth. The MIC of photoinduced bio-fabricated metal nanoparticles(*Lj*-AgNPs) was recorded as 3.12% of AgNo3 against *S. aureus,* while AgNPs (200/200) µm was 12.5%, whereas the MIC of *L. japonica* leaf extract was studied but it hasn't any activity against bacterial pathogens (Figure 8).

Figure 8: Determination of MIC for bacterial pathogens treated with Lj-AgNPs.

Antibacterial test using agar diffusion assay

For the Well diffusion test, a clear zone around the Lj-AgNPs suggested that the compounds possessed antibacterial activity, which can inhibit the growth of pathogenic bacteria. The findings of the Well-diffusion assay indicated that the inhibition zones range from a minimum of 9 ±0.5 mm to a maximum of 17±0.5 mm against pathogenic bacteria (Figure 9). The the results revealed that the highest activity was recorded against isolates 1, 2, and 3 (17.3 mm). In contrast, the lowest inhibition zone was isolate 7(9 mm) (Figure 9).

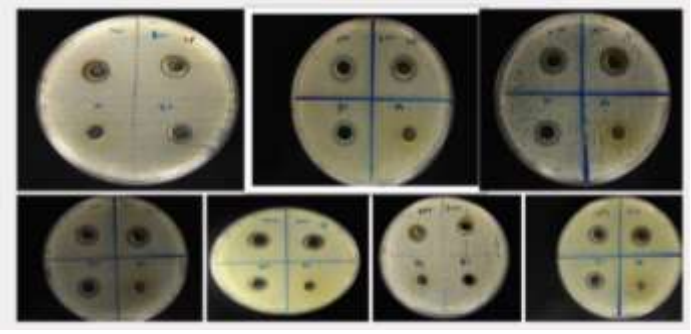

Figure 9a: Inhibition zone (mm) of Lj-AgNPs by agar Well diffusion method against *S.aureus* tested.

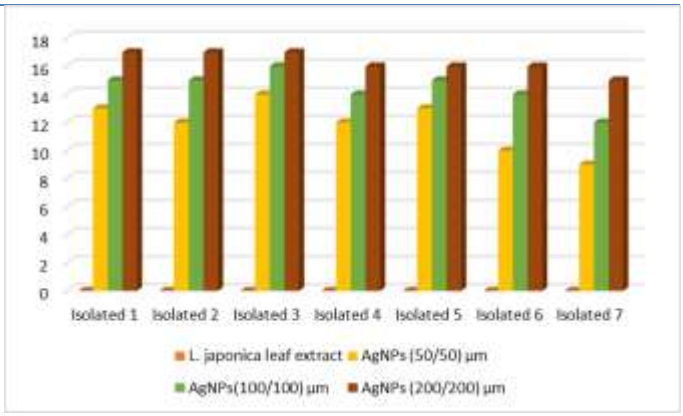

Figure 9b: Inhibition zone (mm) of Lj-AgNPs by agar Well diffusion method against *S.aureus* tested.

DISCUSSION

The current study was performed to explore the response of *S.aureus* which cause SSTIs towards AgNPs synthesized from *Lonicera japonica* plant and to investigate an effective, safe, and natural product alternative to the commercial antibiotics most of them *S.aureus* has resistance against them. effect of aqueous extracts *L. japonica*

The increasing use of nanoscale metals in fields including food, medical, and the environment has made the synthesis of these materials a popular area of study currently. However, most metal nanoparticles are created chemically, with unpredictable consequences like energy and environmental waste and significant health risks. In order to create silver nanoparticles in an environmentally conscious manner, it is important to utilize methods that are mild, non-toxic, and eco-friendly. Green synthesis is a process that was developed to meet these criteria by using plant extracts instead of manufactured chemicals to reduce metal ions. This approach offers numerous benefits, including affordability and safety for both human health and the environment. Recently, biomaterials such as fungi, bacteria, algae, and agricultural waste have become popular for producing silver nanoparticles. However, plant-based synthesis stands out due to its simplicity, diverse morphologies, and abundant natural secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and steroids, which act as reducing and stabilizing agents during the creation of metallic nanoparticles. These compounds can be found in various parts of plants, including roots, leaves, flowers, shoots, bark, fruits, stems, and seeds (Balan et al. 2016; Sorbiun et al. 2018; Bhardwaj et al. 2020; Alghamdi et al. 2021; Al-Maaqar et al. 2022; Guan et al. 2022; Qaeed et al. 2023a; Qaeed et al. 2023b; Malik et al. 2023).

Silver nanoparticles (AgNPs) are increasingly being utilized in a variety of biomedical treatments. Various methods, including biological, chemical, and physical methods, have been proposed for generating these

nanoparticles. In particular, the use of enzymes, proteins, microorganisms, and plant materials in biological approaches for synthesizing AgNPs offers several advantages, including biocompatibility, eco-friendliness, cost-effectiveness, and ease of large-scale synthesis (Sathishkumar et al. 2016a; Parlinska-Wojtan et al. 2016; Jadhav et al. 2016). The green synthesis of nanoparticles using plants is a highly effective approach that has numerous applications in modern medicine. Plant extracts can serve as both reducing and stabilizing agents during the synthesis of nanoparticles. Historically, plant materials have been used to alleviate a variety of ailments, including spasms, gastric complaints, bronchitis, gout, and giddiness (Sreelatha and Padma 2009; Inbathamizh et al. 2013; Sathishkumar et al. 2016b). In the present study, AgNPs were synthesized using an extract of *Lonicera japonica*.

The medical plants' antimicrobial activity against microbial infection has demonstrated that some medicinal plants may in fact be viable sources of new antimicrobial medicines, even for some types of bacteria that are resistant to some antibiotics. (Ilanko et al. 2019). In this research, the evaluation of the effect of *Lonicera japonica Thumb* with silver nanoparticles on *S. aureus* was done. The conventional method for isolating *S. aureus* from wound samples typically involves the use of media, such as blood agar. This method offers the advantage of being able to isolate other pathogens, such as streptococci, concurrently.(Perry et al. 2003)

The formation of AgNPs was confirmed by the observation of a color change in the solution from pale yellow to brown, as shown in Figure 1. The reaction mixture, which contained *L. japonica* and silver nitrate, also exhibited a similar color change, indicating the reduction of silver ions and the subsequent formation of AgNPs. The color change is attributed to the surface plasmon vibrations in AgNPs, which are surface-active molecules that contribute to the reduction and stabilization of the nanoparticles. This finding is consistent with previous research (Shankar et al. (2004), Donda et al (2013), Dubey et al. (2009), and Balan et al. (2016).

Ultraviolet-visible spectroscopy was employed to confirm the nanoscale size of the synthesized particles. The presence of an absorption peak around 300 nm in the UV–vis spectrum indicated the occurrence of silver surface plasmon resonance. Previous research has reported the appearance of an absorbance peak between 300-500 nm when using different plant leaf extracts in UV-VIS analysis, as in the study by Ameen et al. (2019). The maximum absorbance of AgNPs-LLJ was observed at 423 nm, which can be attributed to the presence of flavonoids in the aqueous leaf extract of plants that played a crucial role in reducing AgNO3 to form AgNPs. Sathishkumar et al. (2016a and b) reported that the hydroxyl and carbonyl groups of flavonoids act as reducing agents for the reduction of silver ions to AgNPs, while also acting as capping agents to prevent agglomeration.

The study's findings revealed that the pvl gene was present in all seven S. aureus isolates at a size of 433 bp, which is consistent with the findings of Bhatta et al. (2016). Their study found that PVL-producing organisms are the primary cause of skin and soft tissue infections (at a rate of 75.5%) due to the leucocidal activity of PVL, which provides a survival advantage to the bacteria.

The antibacterial activity of Lj-AgNPs was evaluated against seven Gram-positive pathogens (S. aureus) that were isolated in this study. The relative antibacterial efficacy of Lj-AgNPs towards the pathogens was determined qualitatively using well and disk diffusion techniques and quantitatively using the minimum inhibitory concentration (MIC) assay. The bacterial growth was assessed using visual inspection of the broth's turbidity. The MIC of photoinduced bio-fabricated metal nanoparticles (Lj-AgNPs) was recorded as 3.12% of AgNO3 against *S. aureus*, while AgNPs (200/200) µm was 12.5%. On the other hand, L. japonica leaf extract did not exhibit any activity against the bacterial pathogens (Figure 8). The well diffusion test was also conducted to evaluate the antibacterial activity of Lj-AgNPs. The presence of a clear zone around the NPs indicated their ability to inhibit the growth of pathogenic bacteria. The inhibition zones ranged from 9 ± 0.5 mm to 17 ± 0.5 mm against pathogenic bacteria, with the highest antibacterial activity observed against isolates 1, 2, and 3 (17.3 mm), and the lowest activity observed against isolate 7 (9 mm) (Figure 9). The Agar-well diffusion method was also used to investigate the microbial activity of the biosynthesized AgNPs and aqueous extract of Lonicera japonica leaf against S. aureus by zone inhibition. The biosynthesized nanoparticles using plant extract exhibited inhibition zones against all microbial species, and their antimicrobial activities were found to be higher than those of the aqueous plant extracts (used as controls). These findings are consistent with previous studies that examined the antimicrobial activity of AgNPs, such as those conducted by Yan et al. (2020) and Bachynskyj-Bilas et al. (2022).

CONCLUSIONS

. This study focuses on the antibacterial activity of green synthesized AgNPs against skin pathogenic bacteria, which can be used as emerging drug targets, especially for treating multidrug-resistant pathogens. We hypothesize that the newly synthesized photoinduced bio-fabricated metal nanoparticles (Lj-AgNPs) will possess antibacterial and can inhibit growth of pathogenic bacteria (S.aureus), leading to novel source of antibiotics with multiple drug targets.

Author contributions

Hind Saleh Alshaikh carried out all the experiments, contributed to drafting the manuscript, and analyzed the data. Hanah Ahmed Amer analyzed data from the experiments and contributed in writing the manuscript Roqayah Hassan Kadi conceived the idea, planned, designed, and coordinated the experiments.

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Institutional Review Board Statement

Not applicable

Informed Consent Statement

Not applicable.

Data Availability Statement

All data generated or analyzed during this study are included in this published article

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Conflict of interest

The present study was conducted without any conflicts of interest, according to the authors.

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