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Research Article
Anti-Biofilm Activities of Silver Nanoparticle Conjugated *Rhazya stricta* Phytocompounds in Periprosthetic Joint Infections by *Staphylococcus aureus*

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Abstract

Background and Objective: Periprosthetic Joint Infections (PJIs) caused by multi-drug resistant biofilm-forming *Staphylococcus aureus* make the treatment challenging. The present study makes an effort to find out the effect of AgNP-*Rhazya stricta* extract conjugate on biofilms associated with PJIs in total hip arthroplasties (THAs). **Materials and Methods:** The strains of *S. aureus* were identified and analyzed for their antibiogram against the antibiotics selected. The biofilm-forming abilities of the isolated strains were determined using the crystal violet method and the effect of green synthesized AgNP-*Rhazya stricta* extract conjugate coated on prosthetic femur head was analyzed using confocal microscopy. **Results:** Two isolates were identified as *S. aureus* and they showed resistance to all of the antibiotics tested and also have shown the ability to form biofilms. The AgNP-*Rhazya stricta* extract conjugate showed better antibacterial activity against O5 strain of *Staphylococcus aureus* compared with O3. The AgNP-*Rhazya stricta* extract-coated prosthetic femur heads were found to be reducing the biofilms on them. **Conclusion:** As the thickness of biofilms formed by MDR *S. aureus* was reduced on AgNP-*Rhazya stricta* extract conjugate coated metallic femoral head. Further studies are required on the AgNP-*Rhazya stricta* extract to make it available for its clinical use.

Key words: Total hip arthroplasty (THA), Periprosthetic Joint Infections (PJIs), femur head, biofilm, *Staphylococcus aureus*, *Rhazya stricta*, silver nanoparticles

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Total hip arthroplasty (THA) technique has been a milestone in medicine since it solved the sufferings of patients with hip joint complications resulting from many conditions including very late hip arthritis, osteoarthritis (OA), developmental dysplasia, Avascular Necrosis (AVN), tumor, pediatric hip diseases, septic and inflammatory arthritis, trauma etc., which will disturb the joint integrity causing severe hip pain and deformity of lower limb resulting in gradual loss of normal functioning[1,2]. Ever since its introduction, due to many reasons including the growing size of the aged population, the demand for THA has exponentially been increasing. But, unfortunately, in many cases, these arthroplasties have been followed by serious Periprosthetic Joint Infections (PJIs) resulting in extreme suffering of patients, challenges in the treatments, higher health care costs, prolonged hospitalizations, patient immobilization, functional and emotional morbidity etc., demanding surgical intervention and aggressive antimicrobial treatment[3,4]. The PJIs were found to have developed at a very early stage as short as less than three months postoperatively and their prevalence increased up to a recorded 5.6% ending up in revision surgeries[5,6]. The scenario has been predicted to worsen by 4% increase in PJIs both in revision and in primary knee and hip arthroplasties between the years 2005 and 2030[7,8]. The situation in Saudi Arabia is also the same as a study conducted in a tertiary hospital in Jeddah reported that there was an increase of 66.4% in THA surgeries performed between 2001 and 2015[1].

Most of the PJIs were found to be caused by biofilms of virulent strains of one or more pathogenic bacterial species. However, the formation of biofilms by multidrug-resistant bacteria, which are microbial (bacterial) communities adhered strongly to solid surfaces like prosthetic implants and these bacteria are inside a well-protected polysaccharide covering, makes the post-surgical life difficult[9]. Having a protective matrix of polysaccharides, these biofilms are found to have up to 8000-fold resistant capability against antibiotics than their planktonic forms and in about 50% of the cases, the causative organism was reported to be Methicillin-Resistant *Staphylococcus aureus* (MRSAs) strains[10,11]. As these pathogens are getting resistant to most of the antibiotics in use, the scientific world is in search of new agents including metal nanoparticles like Silver Nanoparticles (AgNPs) which have been found to be one of the potential alternatives with enhanced antimicrobial activity in suppressing and eliminating microorganisms including bacteria that form biofilms[12]. When these AgNPs are produced using the green synthesis technique, the final product was found to have an enhanced effect against bacteria including *Staphylococcus aureus.* On the other hand, various plant particles have been making headlines in the fight against MDR bacteria and *Rhazya stricta* (known as Asharmal in the Kingdom) has been a plant found in various parts of Saudi Arabia with proven biological activities from the common cold to parasitic infections, hyperglycemia and rheumatism to syphilis[13,14] and also has proven activity against different *S. aureus* including MRSA (Methicillin-Resistant *Staphylococcus aureus*) strains[13,15-20]. Based on the earlier reports, the AgNPs can be explored to invent coatings against biofilms on different biomedical devices. Therefore, the present investigation, evaluated the anti-biofilm activity of AgNP-*Rhazya stricta* extracts conjugate on multidrug-resistant *Staphylococcus aureus* biofilms formed on prosthetic femur heads.

MATERIALS AND METHODS

Study area and duration: The present study was performed in the Basic Medical Sciences Laboratories of the College of Medicine at the Prince Sattam bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia from November, 2022 to June, 2023.

Isolation and identification of *S. aureus* from clinical samples: The pathogenic bacterial strains were isolated using standard procedure from three patients at the PSAU hospital, Al-Kharj, Saudi Arabia. Without further delay, the transportation of the collected samples to the laboratory were done and processed and the pathogen *S. aureus* was identified based on various biochemical analysis including its growth on mannitol salt agar[21].

Resistance pattern of identified *S. aureus*: The isolated *S. aureus* (O1-O8) was analyzed for antibiotic-resistant patterns using the disc diffusion method. Briefly, the overnight cultures of isolated *S. aureus* were swabbed on the sterile Mueller-Hinton agar (MHA) plates and the disc of the following antibiotics ciprofloxacin, cefmetazole, ampicillin, tetracycline and streptomycin were placed on the surface and incubated for overnight. After the incubation for about 36 hrs at 37°C. The culture plates were analyzed for the zones of inhibition. Further, the *S. aureus* strains were selected based on their resistant pattern[22].

Determination of biofilm formation: To observe the biofilm-forming potentials of isolated MDR *Staphylococcus aureus* strains, the crystal violet method was used as described by Meiyazhagan *et al.*[23]. Briefly, the overnight *S. aureus* (O1-O8) culture was added into test tubes

containing MHB and allowed for 96 hrs. Then, the formed biofilms were washed, methanol-fixed and stained with 0.1% solution of crystal violet. Then, biofilms were destained with an ethanol acetone mixture and the strains were selected further based on the color intensity which indicates the biofilm-forming potentials of the MDR *Staphylococcus aureus*. The test tube with no biofilm served as the negative control.

Collection and extraction of *Rhazya stricta*: Three shrubs of the Saudi medicinal plant *Rhazya stricta* were collected from the suburbs of Al-Kharj City in the Central Region of the Kingdom. Before extraction, the plant was cleaned and air-dried. The cleaned plant was cut into small pieces and the plant extract was prepared by adding 20 g of plant material to a thimble and placed inside the Soxhlet apparatus (Macherey-Nagel, Germany) filled with methanol and the 60-65 °C temperature was set to run the cycles for a few hours till the colorless solvent obtained and the end product was used for synthesis of AgNPs[24].

Green synthesis of Silver Nanoparticles (AgNPs): The silver nanoparticles were synthesized using *Rhazya stricta* extract as a reducing agent by green synthesis as described earlier. In brief, for the synthesis, to 45 mL of 0.01 mM silver nitrate solution, 5 mL of plant extract was added and allowed to boil for 5 min. When the silver nitrate and plant extract mixer turn brown color indicates the formation of silver nanoparticles[25].

Characterization of green synthesized NPs: The silver nanoparticles which were synthesized by green procedure confirmed using UV-visible spectrophotometer (UV-2400 Shimadzu, absorbance between 200-800 nm), morphological characteristics were analyzed using FESEM (Field Emission Scanning Electron Microscopy) and analyzed for the zeta potential[26].

Conjugation of AgNPs with the herb extract: The silver nanoparticles were conjugated with the *Rhazya stricta* extract using a standard protocol in a ratio of 1:1 and these conjugated AgNPs were used further for all the studies[27].

Evaluation of antimicrobial potentials of AgNP-*Rhazya stricta* extract conjugate against *Staphylococcus aureus* isolate: The antimicrobial activity of synthesized AgNP-*Rhazya stricta* extract conjugate against isolated resistant *S. aureus* (O8) strains were analyzed using agar diffusion method as described by Gowri *et al.*[22]. Brief, the overnight cultures of isolated resistant *S. aureus* strains were swabbed on the sterile MH Agar plates and the wells were loaded with different concentrations (0.125, 0.25, 0.5 µg and 1 mg) of AgNP-*Rhazya stricta* extract conjugate and incubated. After incubation, the antimicrobial activity of AgNP-*Rhazya stricta* extract conjugate was found by inhibition zone measurement which was formed against the resistant *S. aureus* strains.

Determination of minimum inhibitory concentration: The broth dilution technique was used to find out the MICs of synthesized AgNP-*Rhazya stricta* extract conjugate against isolated resistant *S. aureus* (O3 and O5) strains as mentioned by Meiyazhagan *et al.*[23]. In brief, the silver concentration was serially diluted in Mueller Hinton broth and the overnight culture was added and incubated. After incubation, the resazurin dye was applied and incubated for 3 hrs. Then, the change in color from blue to pink indicated the growth of *S. aureus*.

Confocal microscopy: To evaluate the anti-biofilm potential of the synthesized AgNP-*Rhazya stricta* extract conjugate coated on the prosthetic femur head was analyzed using confocal microscopy. Briefly, before allowing the biofilm formation on the prosthetic femur head, the head was immersed in synthesized AgNP-*Rhazya stricta* extract conjugate suspension for 10 min and air dried. Then, the *S. aureus* biofilm formation on AgNP-*Rhazya stricta* extract conjugate coated prosthetic femur head was attained when the head was allowed for biofilm formation by immersing the tube in BHI broth containing overnight *S. aureus* culture suspension and allowed for 96 hrs. Then, the head was washed with phosphate-buffered saline stained with acridine orange for 10 min and air dried. The stained prosthetic femur head was allowed for confocal scanning microscopy[28]. An uncoated prosthetic femur head with biofilm formation serves as the control. The green fluorescence was considered as an indication of live cells and the fluorescence with red as an indication of the dead cells.

RESULTS

Identification and resistant pattern of isolated *S. aureus* strains: The isolated clinical samples (O1-O8) were identified as *S. aureus* based on their morphological observation on MSA plates and the growth pattern was shown in Fig. 1. As it is seen, out of eight isolates, two isolates showed growth on MSA plates and identified as *S. aureus*. Similarly, the resistant pattern of eight isolated isolates (O1-O8) was studied and the obtained result was presented in Fig. 2 and Table 1. As shown in the Table 1, all the isolates (O1, O2, O3, O5, O6, O7 and O8) showed resistance to ampicillin, three isolates (O3, O7 and O8) were resistant to all the tested antibiotics such as ciprofloxacin, cefmetazole, ampicillin, tetracycline and streptomycin.

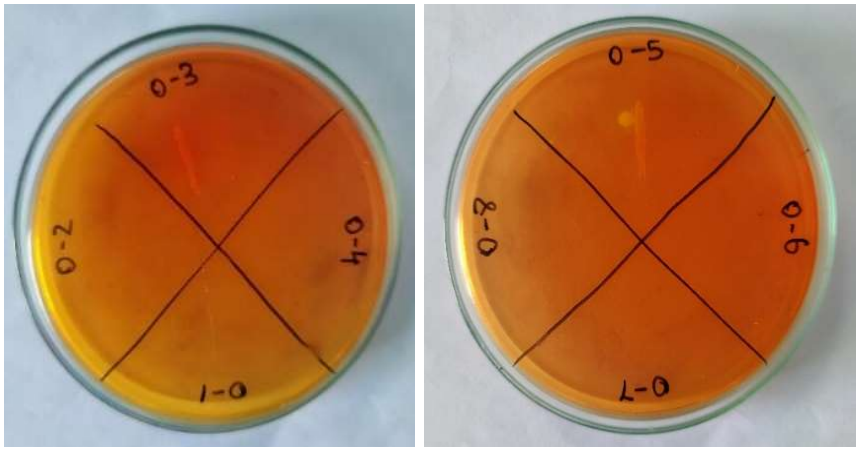

Fig. 1: Growth pattern of isolated strains (O1-O8) on mannitol salt agar (MSA)

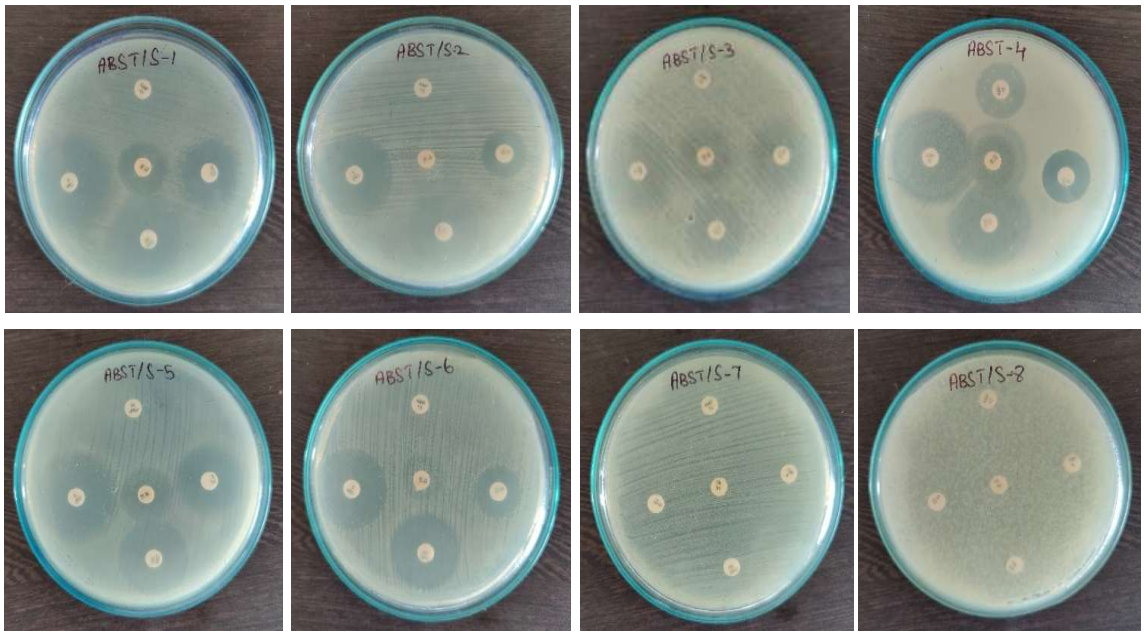

Fig. 2: Resistant pattern of tested antibiotics against isolated strains (O1-O8)

Table 1: Zone of inhibition of tested antibiotics against isolated strains (O1-O8)

	Zone of inhibition (mm)				
Bacterial strain	Ciprofloxacin	Cefmetazole	Ampicillin	Tetracycline	Streptomycin
O1	22	20	-	13	15
O2	21	18	-	-	12
O3	-	-	-	-	-
O4	-	-	-	-	16
O5	16	-	-	-	15
O6	20	18	-	-	12
O7	-	-	-	-	-
O8	-	-	-	-	-

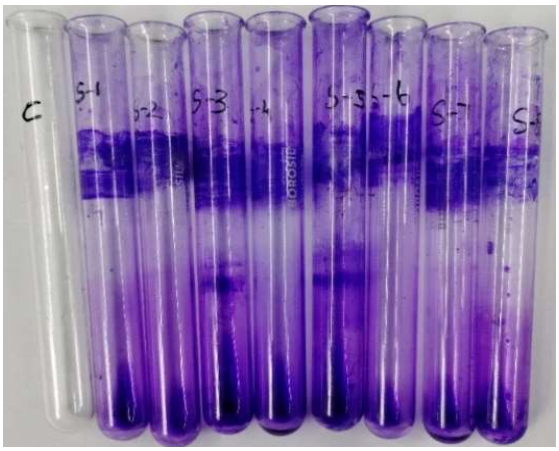

Fig. 3: Biofilm forming ability of isolated *S. aureus* strains (O1-O8)

Fig. 4: Antibacterial activity of AgNP-*Rhazya stricta* extract conjugate against resistant *S. aureus* strains (O3 and O5)

Determination of biofilm formation: Biofilm-forming potentials of the eight isolated *S. aureus* strains (O1-O8) were studied using the crystal violet method and the result was displayed in Fig. 3. As observed, all the eight isolates showed their biofilm-forming ability when compared to control. Based on the color intensity, two isolates such as O3 and O5 showed more potency to form biofilm on non-living things. Based on the growth on the MSA plate, resistant pattern and biofilm-forming ability of selected isolates, the isolates O3 and O5 were used for further studies.

Antibacterial activity of green synthesized AgNP-*Rhazya stricta* extract conjugate: The antimicrobial potentials of synthesized AgNP-*Rhazya stricta* extract conjugate against MDR *S. aureus* strains (O3 and O5) studied are presented in Fig. 4. The zones of bacterial growth inhibition for O3 strain against various plant extract concentration like 0.125, 0.25 and 0.5 µg; 1 mg are 12, 11, 14 and 18 mm, respectively. Similarly, the O5 strain also showed inhibition in growth by 13, 13, 16 and 19 mm, respectively against the plant extract concentrations as 0.125, 0.25, 0.5 µg and 1 mg. Thus, the AgNP-*Rhazya stricta* extract conjugate exhibited antibacterial activity in all the tested concentrations against resistant *S. aureus* strains (O3 and O5) and the results indicated that the antibacterial activity of AgNP-*Rhazya stricta* extract conjugate was increased when increasing the concentration.

Minimum inhibitory concentration (MIC) determination: The MICs of synthesized AgNP-*Rhazya stricta* extract conjugate against resistant *S. aureus* strains (O3 and O5) determined and were found to be 0.781 µg mL^{-1} against O3 and 0.195 µg mL^{-1} against O5 strains.

Confocal microscopy: The visualization of anti-biofilm effect on prosthetic femur head coated with synthesized AgNP-*Rhazya stricta* extract conjugate was studied using confocal microscopy and the obtained results were presented in Fig. 5a-d and 6a-d. As shown in Fig. 5a-b, two-dimensional

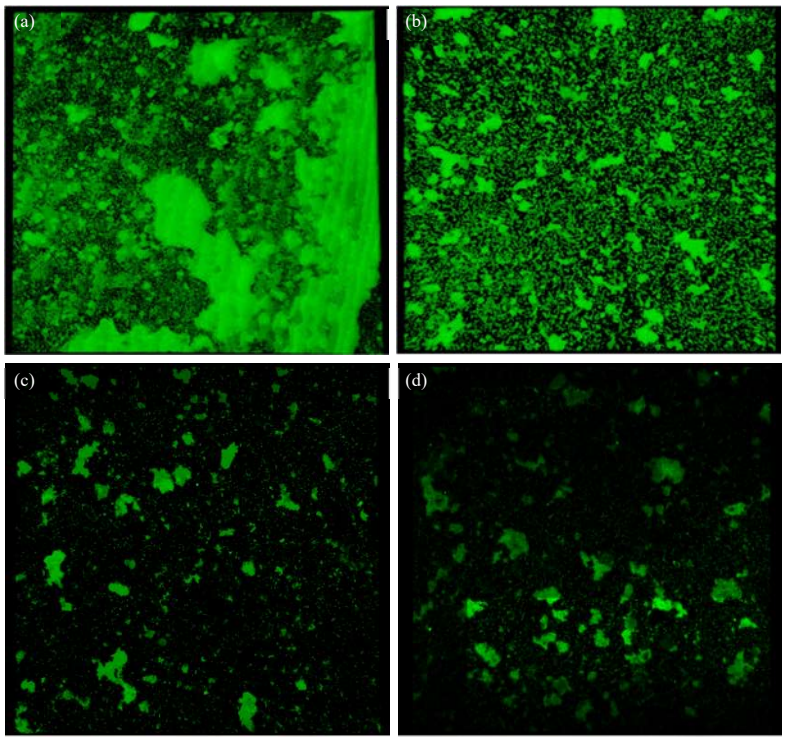

Fig. 5(a-d): 2D image of biofilm formation of *S. aureus* on the uncoated prosthetic femur head (a) O3, (b) O5 (Control), (c) 2D image of biofilm formation of *S. aureus* O3 on the coated prosthetic femur head and (d) O5

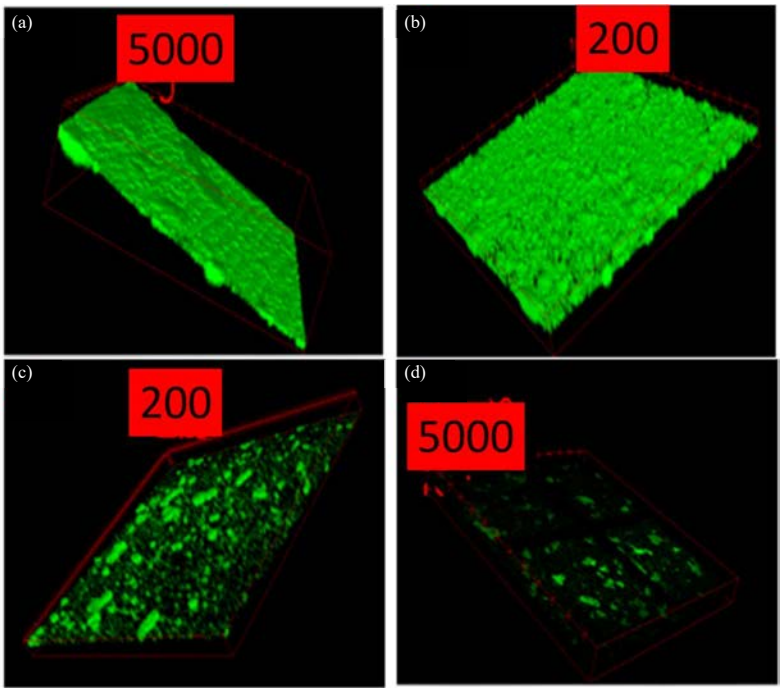

Fig. 6(a-d): 3D image of biofilm formation of *S. aureus* on the uncoated prosthetic femur head (a) O3, (b) O5 (Control), (c) 3D image of biofilm formation of *S. aureus* on the coated prosthetic femur head O3 and (d) O5

biofilm structures of resistant *S. aureus* strains (O3 and O5) were observed on an uncoated prosthetic femur head. Here, more green fluorescence indicates the live cells on biofilm structures. Similarly, two-dimensional biofilm structures of resistant *S. aureus* strains (O3 and O5) were observed on coated prosthetic femur heads with less green fluorescence (Fig. 5c-d). As observed in Fig. 6a-b, three-dimensional biofilm structures of resistant *S. aureus* strains (O3 and O5) were observed on uncoated prosthetic femur heads with more green fluorescence. Consequently, three-dimensional biofilm structures of resistant *S. aureus* strains (O3 and O5) were observed on coated prosthetic femur head with minimal green fluorescence as well as reduced biofilm thickness indicating the antibiofilm effect of synthesized AgNP-*Rhazya stricta* extract conjugate (Fig. 6c-d).

DISCUSSION

Staphylococcus aureus is an important and predominant causative agent for periprosthetic joint infections[29]. The biofilm-forming ability of *S. aureus* makes tolerance to many antibiotics resulting PJIs treatment very challenging[30]. Therefore, to overcome the existing problem, coating medical devices with antibacterial agents is an emerging approach. Hence, in the study, green synthesized AgNP conjugated with *Rhazya stricta* extract was investigated for its anti-biofilm potentials against isolated MDR *S. aureus*. Here, silver nanoparticles were synthesized using the green-reducing agent *Rhazya stricta* extract and they showed excellent antibacterial activity against MDR *S. aureus* strains. Moreover, the isolated strains were analyzed for their biofilm-forming ability because the *S. aureus* in PJI is mainly associated with biofilm. Similarly, a recent study investigated the antibacterial activity of TNP-2092, a hybrid drug consisting of quinolizinone pharmacophores and rifamycin against *S. aureus* involved in PJIs and found the promising *in vitro* activity against the tested organism[31].

Same way, studies have declared the prevalent causative agent for PJIs as *S. aureus* strains by determining the phenotypic and genotypic analysis as well as biofilm-forming ability in the isolated strains showed resistance to many commercially available antibiotics[32]. These studies proved the importance of causative agents and their genotype identification for treating PJIs. Moreover, *S. aureus* has the ability to form biofilm which makes treatment failure in many cases. Furthermore, biofilm formation was initiated when the bacterial attachment on the surfaces of the devices makes a favorable environment for biofilm maturation thereby treatment is crucial. Hence, the investigation was a trial to study the anti-biofilm potentials of AgNP-*Rhazya stricta* extract conjugate coated on a prosthetic femur head was evaluated. The present study revealed the potential anti-biofilm activity of AgNP-*Rhazya stricta* extract conjugate against isolated MDR *S. aureus* biofilm formation on prosthetic femur head and found the reduced biofilm thickness was observed. Similarly, the silver-containing hydroxyapatite coating was evaluated for its antibiofilm activity against methicillin-resistant and vancomycin-resistant *S. aureus* and showed a potent antibacterial effect which was evidenced in confocal microscopy[33]. The savarin *in vitro* anti-biofilm and antimicrobial activities against *S. aureus* were also found to be excellent in eliminating *S. aureus* infection[34].

CONCLUSION

The green synthesized AgNP-*Rhazya stricta* extract conjugate was evaluated for its anti-bacterial and antibiofilm activity against multi-drug resistant *S. aureus*. The isolated *S. aureus* strains were observed for their growth and resistant pattern and biofilm-forming ability. Mainly, the AgNP-*Rhazya stricta* extract conjugate-coated prosthetic femur head was analyzed for antibiofilm activity and visualized by confocal microscopy. Interestingly, the biofilm thickness was reduced in AgNP-*Rhazya stricta* extract conjugate coated prosthetic femur head indicating that AgNP-*Rhazya stricta* extract conjugate does not allow the biofilm formation when compared to control. Overall, the AgNP-*Rhazya stricta* extract conjugate can be a better coating agent for preventing biofilm formation in the prosthetic femur head.

SIGNIFICANCE STATEMENT

Even though the need for total hip arthroplasties (THA) is continuously increasing, the Periprosthetic Joint Infections (PJIs) caused by multidrug-resistant biofilm-forming bacterial pathogens like *Staphylococcus aureus* make the treatment and management more challenging. So, the present study analyzed a solution from nature-using a coating of silver nanoparticle conjugated Saudi medicinal plant (*Rhazya stricta*) extract on the metallic femoral head to find out, to what extent the conjugate can suppress the bacterial pathogens and their biofilms. The study found that, when the prosthetic femur part with induced biofilm is coated with the AgNPs conjugated plant extract, it could reduce the biofilms and we suggest detailed *in vitro* and *in vivo* analyses for its clinical applications.

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REFERENCES

1. Hamdi, A.S., D.M.M. Bakhsh and M. Al-Sayyad, 2017. Indications of total hip arthroplasty at a tertiary hospital in Jeddah. Saudi Surg. J., 5: 106-109.
2. Halawani, A.K., A.A. Ghaddaf, M.S. Alomari, K.H. Alaryani, A.O. Alibrahim and M.S. Alshehri, 2022. Post-operative clinical outcomes and complications of posterior versus lateral approach for primary total hip arthroplasty: A retrospective cohort study. J. Musculoskeletal Surg. Res., 6: 62-69.
3. Malchau, K.S., J. Tillander, M. Zaborowska, M. Hoffman and I. Lasa *et al*., 2021. Biofilm properties in relation to treatment outcome in patients with first-time periprosthetic hip or knee joint infection. J. Orthop. Transl., 30: 31-40.
4. Jacqueline, C. and J. Caillon, 2014. Impact of bacterial biofilm on the treatment of prosthetic joint infections. J. Antimicrob. Chemother., 69: i37-i40.
5. Springer, B.D., S. Cahue, C.D. Etkin, D.G. Lewallen and B.J. McGrory, 2017. Infection burden in total hip and knee arthroplasties: An international registry-based perspective. Arthroplasty Today, 3: 137-140.
6. Chalmers, B.P., T.M. Mabry, M.P. Abdel, D.J. Berry, A.D. Hanssen and K.I. Perry, 2018. Two-stage revision total hip arthroplasty with a specific articulating antibiotic spacer design: Reliable periprosthetic joint infection eradication and functional improvement. J. Arthroplasty, 33: 3746-3753.
7. Bozic, K.J., S.M. Kurtz, E. Lau, K. Ong, T.P. Vail and D.J. Berry, 2009. The epidemiology of revision total hip arthroplasty in the United States. J. Bone Joint Surg. Am., 91: 128-133.
8. Gbejuade, H.O., A.M. Lovering and J.C. Webb, 2015. The role of microbial biofilms in prosthetic joint infections. Acta Orthop., 86: 147-158.
9. Vos, T., C. Allen, M. Arora, R.M. Barber and Z.A. Bhutta *et al*., 2016. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet, 388: 1545-1602.
10. Jaśkiewicz, M., A. Janczura, J. Nowicka and W. Kamysz, 2019. Methods used for the eradication of staphylococcal biofilms. Antibiotics, Vol. 8. 10.3390/antibiotics8040174.
11. Albeshri, A., N.A. Baeshen, T.A. Bouback and A.A. Aljaddawi, 2021. A review of *Rhazya stricta* Decne phytochemistry, bioactivities, pharmacological activities, toxicity, and folkloric medicinal uses. Plants, Vol. 10. 10.3390/plants10112508.
12. Mohanta, Y.K., K. Biswas, S.K. Jena, A. Hashem, E.F. Abd_Allah and T.K. Mohanta, 2020. Anti-biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. Front. Microbiol., Vol. 11. 10.3389/fmicb.2020.01143.
13. Kabli, S.A.A., A.E.D.A. Hadhoud and M.N. Baeshen, 2012. An epidemiological survey of extended-spectrum β-lactamases producing bacteria genotypes and the evaluation of the antimicrobial effect of *Rhazya stricta* leaf extract. Microbiol. Res., Vol. 3. 10.4081/mr.2012.e16.
14. Park, S., T.A. Ruhlman, J.S.M. Sabir, M.H.Z. Mutwakil and M.N. Baeshen *et al*., 2014. Complete sequences of organelle genomes from the medicinal plant *Rhazya stricta* (Apocynaceae) and contrasting patterns of mitochondrial genome evolution across asterids. BMC Genomics, Vol. 15. 10.1186/1471-2164-15-405.
15. Bashir, A.K., A.A. Abdalla E.S. Hassan, I.A. Wasfi, M.A. Amiri and T.A. Crabb, 1994. Alkaloids with antimicrobial activity from the root of *Rhyzya stricta* Decn. growing in United Arab Emirates. Arab Gulf J. Sci. Res., 12: 119-131.
16. Sultana, N. and A. Khalid, 2010. Phytochemical and enzyme inhibitory studies on indigenous medicinal plant *Rhazya stricta*. Nat. Prod. Res., 24: 305-314.
17. Bukhari, N.A., R.A. Al-Otaibi and M.M. Ibhrahim, 2017. Phytochemical and taxonomic evaluation of *Rhazya stricta* in Saudi Arabia. Saudi J. Biol. Sci., 24: 1513-1521.
18. Khan, R., M.N. Baeshen, K.S. Saini, R.S. Bora, A.M. Al-Hejin and N.A. Baeshen, 2016. Antibacterial activities of *Rhazya stricta* leaf extracts against multidrug-resistant human pathogens. Biotechnol. Biotechnol. Equip., 30: 1016-1025.
19. Baeshen, M.N., R. Attar, T.A. Bouback, A.O. Albeshri and N.N. Baeshen *et al*., 2022. Assaying for antiviral activity of the folkloric medicinal desert plant *Rhazya stricta* on coronavirus SARS-CoV-2. Biotechnol. Biotechnol. Equip., 36: 68-74.
20. Alnabati, N.A., A.M. Al-Hejin, S.O. Noor, M.M.M. Ahmed, M. Abu-Zeid and N.T. Mleeh, 2021. The antibacterial activity of four Saudi medicinal plants against clinical isolates of *Propionibacterium acnes*. Biotechnol. Biotechnol. Equip., 35: 415-424.
21. Sharp, S.E. and C. Searcy, 2006. Comparison of mannitol salt agar and blood agar plates for identification and susceptibility testing of *Staphylococcus aureus* in specimens from cystic fibrosis patients. J. Clin. Microbiol., 44: 4545-4546.
22. Gowri, M., W.S. Beaula, J. Biswal, P. Dhamodharan and R. Saiharish *et al*., 2016. β-lactam substituted polycyclic fused pyrrolidine/pyrrolizidine derivatives eradicate C. *albicans* in an *ex vivo* human dentinal tubule model by inhibiting sterol 14-α demethylase and cAMP pathway. Biochim. Biophys. Gen. Subj., 1860: 636-647.

23. Meiyazhagan, G., R. Raju, S.B. Winfred, B. Mannivanan and H. Bhoopalan *et al*., 2015. Bioactivity studies of β-lactam derived polycyclic fused pyrroli-dine/pyrrolizidine derivatives in dentistry: *In vitro*, *in vivo* and *in silico* studies. PLoS ONE, Vol. 10. 10.1371/journal.pone.0131433.
24. Harley, B.K., A.M. Quagraine, D. Neglo, M.O. Aggrey and E. Orman *et al*., 2022. Metabolite profiling, antifungal, biofilm formation prevention and disruption of mature biofilm activities of *Erythrina senegalensis* stem bark extract against *Candida albicans* and *Candida glabrata*. PLoS ONE, Vol. 17. 10.1371/journal.pone.0278096.
25. Melkamu, W.W. and L.T. Bitew, 2021. Green synthesis of silver nanoparticles using *Hagenia abyssinica (Bruce) J.F. Gmel* plant leaf extract and their antibacterial and anti-oxidant activities. Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e08459..
26. Adinaveen, T., T. Karnan and S.A.S. Selvakumar, 2019. Photocatalytic and optical properties of NiO added *Nephelium lappaceum* L. peel extract: An attempt to convert waste to a valuable product. Heliyon, Vol. 5. 10.1016/j.heliyon.2019.e01751.
27. Khan, A., H. Jabeen, T. Ahmad, Najeeb Ur Rehman and S.S. Khan *et al*., 2022. Comparative efficacy of cephradine-loaded silver and gold nanoparticles against resistant human pathogens. Artif. Cells Nanomed. Biotechnol., 50: 312-321.
28. Muthu, D., M. Gowri, G.S. Kumar, V.S. Kattimani and E.K. Girija, 2019. Repurposing of antidepression drug sertraline for antimicrobial activity against *Staphylococcus aureus*: A potential approach for the treatment of osteomyelitis. New J. Chem., 43: 5315-5324.
29. Yu, J., F. Jiang, F. Zhang, Y. Pan and J. Wang *et al*., 2020. Virtual screening for novel SarA inhibitors to prevent biofilm formation of *Staphylococcus aureus* in prosthetic joint infections. Front. Microbiol., Vol. 11. 10.3389/fmicb.2020.587175.
30. Mandell, J.B., S. Orr, J. Koch, B. Nourie and D. Ma *et al*., 2019. Large variations in clinical antibiotic activity against *Staphylococcus aureus* biofilms of periprosthetic joint infection isolates. J. Orthop. Res., 37: 1604-1609.
31. Fisher, C.R., S.M. Schmidt-Malan, Z. Ma, Y. Yuan, S. He and R. Patel, 2020. *In vitro* activity of TNP-2092 against periprosthetic joint infection-associated staphylococci. Diagn. Microbiol. Infect. Dis., Vol. 97. 10.1016/j.diagmicrobio.2020.115040.
32. Chen, J.L., T.Y. Huang, W.B. Hsu, C.W. Lee, Y.C. Chiang, P.J. Chang and K.T. Peng, 2022. Characterization of methicillin-resistant *Staphylococcus aureus* isolates from periprosthetic joint infections. Pathogens, Vol. 11. 10.3390/pathogens11070719.
33. Hashimoto, A., H. Miyamoto, T. Kobatake, T. Nakashima and T. Shobuike *et al*., 2020. The combination of silver-containing hydroxyapatite coating and vancomycin has a synergistic antibacterial effect on methicillin-resistant *Staphylococcus aureus* biofilm formation. Bone Joint Res., 9: 211-218.
34. Pant, N., S. Miranda-Hernandez, C. Rush, J. Warner and D.P. Eisen, 2022. Effect of savirin in the prevention of biofilm-related *Staphylococcus aureus* prosthetic joint infection. Front. Pharmacol., Vol. 13. 10.3389/fphar.2022.989417.