Background: In the past few years, the rates of morbidity and mortality are increasing due to methicillin-resistant Staphylococcus aureus (MRSA) infection so, studies in controlling this infection is greatly required. Silver nanoparticles antimicrobial activity were well known but rapid and green synthesis of silver nanoparticles having stupendous antimicrobial activity against MSRA is needed.

Aim: Synthesis and evaluation of antibacterial activity of silver nanoparticles against MRSA.

Methods: The pigment produced by Streptomyces coelicolor was used to reduce AgNO₃ in solution to yield silver nanoparticles using photo-irradiation. The synthesized silver nanoparticles were characterized by UV–visible spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). The synthesized silver nanoparticles were tested for antibacterial activity against MRSA, and the synergetic effects, of the combination of silver nanoparticles and antibiotics were evaluated.

Results: We developed rapid synthesis for silver nanoparticles using S. coelicolor pigment by photo-irradiation within 20 min. The UV–visible spectroscopy result show maximum absorption between 400 and 450 nm a preliminary confirmation for nanoparticles synthesis. The XRD result confirms the crystalline nature of silver nanoparticles. The TEM image shows the particles are irregular having the size in a range of 28–50 nm. The FTIR result indicates the pigment as the probable reducing agent. The silver nanoparticles alone and in combination with antibiotics, exhibited antibacterial activity against MRSA.

Conclusions: The rapid synthesis method developed in this study for the synthesis of silver nanoparticles has distinct advantages over traditional synthesis as the process is less time consuming. The kinetics of silver nanoparticles synthesis using S. coelicolor pigment with photo-irradiation indicates an appropriate way to develop green technology for the bulk synthesis. Furthermore, these biosynthesized silver nanoparticles, alone and in combination with antibiotics, exhibited excellent antimicrobial activity against a MRSA which could be an alternate drug of choice.

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1. Introduction

Staphylococcus aureus (S. aureus) resistant to methicillin is a major problem that the world is now facing. The antibiotic era, barely 60 years old, is also threatened because of the increase in resistance of this organism against different antibiotics. So, studies are desperately required in finding out new antimicrobial agents against methicillin resistant Staphylococcus aureus (MRSA). Silver antimicrobial properties were identified based on morphological, biochemical tests and susceptibility test as per the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI-2012).

2. Materials and methods

2.1. Isolation and identification of MRSA

S. aureus isolates have been isolated from different sources like pus, blood, and other exudates from different hospitals and health care centers of Gulbarga region. The preliminary identification of S. aureus was done using mannitol salt agar (differential media) which was detected by change in color of the medium from red to yellow due to mannitol fermentation. The S. aureus identified based on morphological, microscopic, and biochemical tests Table 1a among the identified 5 S. aureus the MRSA was detected using antibiotic susceptibility test as per the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI-2012).

2.1.1. Isolation and identification of S. coelicolor

Soil samples from different areas of Gulbarga, Karnataka; India were collected and screened for pigment producing actinomycetes by serial dilution method using starch casein agar (SCA) medium and incubated at 27 °C for seven days. On the basis of the pigment production, the isolate klmp33 was selected and maintained on fresh SCA medium checked for its purity and stored at 4 °C for further work. The isolate klmp33 was identified as S. coelicolor based on 16S rRNA sequencing and the sequences were submitted to Gene Bank under the accession number JQ27722.

2.1.2. Pigment analysis and synthesis of silver nanoparticles

The synthesis of silver nanoparticles, 15 ml of AgNO₃ (10⁻³ M) solution was treated with the 1 ml actinorhodin and exposed to direct sun light. A color change from colorless to brown took place within few minutes indicating the formation of silver nanoparticles. A yield about 1.4 g of silver nanoparticles per liter was obtained from the above method. Further, the same silver nanoparticles were used for antimicrobial studies.

2.2. Characterization of silver nanoparticles

2.2.1. UV-visible spectroscopy

The synthesis of silver nanoparticles was preliminary confirmed by UV-visible spectroscopy, which is an important technique to verify the formation of metal nanoparticles provided that surface plasmon resonance exists for the metal. The UV-visible spectroscopy was analyzed for period of 20 min, conducted on Systronics double-beam UV-visible spectrophotometer 2200, operated at 0.1 nm resolution with scanning rate 270 nm/min.

2.2.2. X-ray diffraction (XRD)

The synthesis of silver nanoparticles was further confirmed using XRD. The X-ray diffraction patterns for the synthesized silver nanoparticles were recorded using a Rigaku Ultima 4 XRD instrument. The radiation used was Cu-Kα (0.154 nm) at 40 kV and 35 nm with scanning rate of 2°/min.

2.2.3. Transmission electron microscopy (TEM)

The TEM technique was employed to determine the size and shape of the silver nanoparticles. The TEM image was obtained using a Philips CM200 instrument. Sample for this analysis was prepared by coating carbon-coated copper grid with aqueous silver nanoparticles. After 5 min, the extra nanoparticles were washed with distilled water and dried before the analysis.
solution was removed using blotting paper, and then the film on the grid was dried under IR light.

2.2.4. Fourier transform infrared spectroscopy (FTIR)
A powder of silver nanoparticles was prepared by centrifuging the solution of synthesized silver nanoparticles at 10,000 rpm for 20 min. The solid residue was then washed with distilled water to remove any unattached biological moieties from the surface of the nanoparticles. The resultant residue was then dried completely, and the powder was used for FTIR measurement, which was performed on a NICOLET iS5 with Diamond ATR. The FTIR peaks were identified and expressed in wave numbers (cm$^{-1}$). To know the probable reducing agent the actinorhodin was also analyzed.

2.3. Antibacterial activity of silver nanoparticles against MRSA
To evaluate antimicrobial property of silver nanoparticles against MRSA we determined the minimum inhibitory concentration (MIC). To determine MIC different volumes of synthesized silver nanoparticles (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µL) and MRSA culture (maintained at $10^6$ CFU/ml) were added in to lactose broth medium and was incubated at 37°C for 18 h. The MIC was determined by measuring the optical density at 625 nm.

2.4. Synergistic effect of synthesized silver nanoparticles with antibiotics
The synergistic effect of silver nanoparticles with antibiotics has proven to be beneficial this effect against MRSA was determined by disk diffusion method. To assess the synergistic effect, each standard antibiotic disk was impregnated with 30 µL of freshly prepared silver nanoparticles, and then these disks was used in antibacterial activity assays.

3. Results and discussion
A number of approaches are available for the synthesis of silver nanoparticles, e.g., chemical synthesis, radiation-assisted synthesis, electrochemical sonication and biological synthesis. Among these methods, biological synthesis are not only a good way to fabricate benign nano materials, but also reduce the use of substances hazardous to human health and the environment. Non toxic biological synthesis of silver nanoparticles using 5 days old biomass of *Aspergillus flavus* in 9 h was reported by Vigneshwaran et al. Similarly Binupriya et al synthesized silver nanoparticles using 3 days old *R. stolonifer* biomass within 72 h. In this study, we synthesized silver nanoparticles in 20 min using *S. coelicolor* pigment (actinorhodin) by photo-irradiation method. Compared with the above biological methods our synthesis is rapid. Moreover, it is a bio-based synthesis so; it is advantageous over other methods, in being non toxic. To best of our knowledge this is the first report on synthesis of silver nanoparticles using *S. coelicolor* pigment by photo-irradiation.

<table>
<thead>
<tr>
<th>Table 1a – Identification of <em>S. aureus</em> based on morphological, microscopic and biochemical tests.</th>
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</thead>
<tbody>
<tr>
<td>Morphological characters</td>
</tr>
<tr>
<td>1. Colony shape</td>
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<tr>
<td>2. Colony size</td>
</tr>
<tr>
<td>3. Colony color</td>
</tr>
<tr>
<td>Microscopic characters</td>
</tr>
<tr>
<td>1. Gram staining</td>
</tr>
<tr>
<td>2. Motility</td>
</tr>
<tr>
<td>3. Spore staining</td>
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<tr>
<td>Biochemical tests</td>
</tr>
<tr>
<td>1. Catalase</td>
</tr>
<tr>
<td>2. Coagulase</td>
</tr>
<tr>
<td>3. Phosphatase</td>
</tr>
<tr>
<td>4. $\beta$-hemolysis</td>
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</table>
3.1. Synthesis and characterization of silver nanoparticles

The actinorhodin produced by S. coelicolor was used for the synthesis of silver nanoparticles (Fig. 1b). For the synthesis, 15 ml AgNO₃ (10⁻³ M) solution was treated with 1 ml actinorhodin and the solution was exposed to sun light. A color change from colorless to brown took place within a few minutes indicating the formation of silver nanoparticles. The solution mixture also kept in dark (used as control). No change in color was observed indicating no synthesis of silver nanoparticles.

3.1.1. UV-visible spectroscopy
The synthesis of silver nanoparticles was preliminary confirmed by color change caused due to surface plasmon resonance of silver nanoparticles in the visible region.¹¹ The absorbance intensity of the brown color increased steadily as a function of reaction time. The absorption maximum between 400 and 450 nm (Fig. 2a) clearly indicates the formation of silver nanoparticles.

3.1.2. XRD
The crystalline nature of the synthesized nanoparticles was analyzed by X-ray diffraction. Fig. 2b shows a representative pattern of the synthesized nanoparticles after the reduction of AgNO₃. Intense peaks corresponding to (111), (200), (220) and (311) were observed. These peaks can be indexed based on the FCC structure of silver (JCPDS files no. 03-0921), confirming the crystalline nature of the silver nanoparticles.

3.1.3. TEM
A representative TEM image is shown in Fig. 2c. The size of the silver nanoparticles was in the range of 28–50 nm and they are irregular in shape.

3.1.4. FTIR
Fig. 2d shows the FTIR spectra of the purified silver nanoparticles and actinorhodin. The purified nanoparticles exhibited absorption peaks at 1149, 1616, 1645 and 3333 cm⁻¹ due to cyclic C–O–C, C=O and OH functional groups respectively. The peaks obtained were compared with actinorhodin, less intense peaks with slightly shift were observed in the purified silver nanoparticles. From the FTIR spectra it may be inferred

Fig. 3 – Zone of inhibition against MRSA: (a) Only with gentamicin, (a₁) with gentamicin and silver nanoparticles, (b) Only with oxacillin, (b₁) with oxacillin and silver nanoparticles.
that actinorhodin was the reducing agent which is involved in the synthesis of silver nanoparticles.

### 3.2. Antibacterial activity of silver nanoparticles against MRSA

To evaluate antibacterial effect of silver nanoparticles against MRSA we determined the MIC. The MIC of silver nanoparticles against MRSA was estimated (30 µL). The mechanism of the bactericidal effect of silver nanoparticles remains to be elucidated. Several studies have proposed that silver nanoparticles bind to the surface of the cell membrane, disrupting cellular permeability and the respiration functions of the cell. Smaller silver nanoparticles having a large surface area available for interaction have a greater bactericidal effect than larger silver nanoparticles. It is also possible that silver nanoparticles not only interact with the surface of the membrane, but also penetrate inside the bacteria and inactivate DNA replicating ability causing the devastation of the cell.

### 3.3. Synergistic effect of synthesized silver nanoparticles with antibiotics

To study the synergetic effect two antibiotics, gentamicin and oxacillin, with silver nanoparticles were selected against the MRSA isolate. The antimicrobial activity of the antibiotics (gentamicin and oxacillin) increased in the presence of silver nanoparticles (Fig. 3) which may be caused due to interaction of active groups such as, hydroxyl and amide group present in the antibiotic molecules which chelates antibiotic silver nanoparticles interaction. The fold increase in the bactericidal effect was greater for gentamicin than oxacillin when these antibiotics were combined with silver nanoparticles (Table 1b). From the results it is clear that the synthesized silver nanoparticles alone and in combination with antibiotics, exhibited excellent antimicrobial activity against MRSA. Furthermore, as this is bio-based synthesis they become safe, non toxic and alternate antibacterial agent for treatment.

### Conflicts of interest

All authors have none to declare.

### Acknowledgment

Authors acknowledge Prof. A. Venkataraman, Chairman, Department of Materials Science, Gulbarga University, Gulbarga for providing FTIR facility.

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### REFERENCES


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### Table 1b – Zone of inhibition (mm) of different antibiotics (with and without silver nanoparticles) against MRSA.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Silver nanoparticles with gentamicin(a)</th>
<th>Silver nanoparticles with oxacillin(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin (a) 10 mcg/disc</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Oxacillin (b) 1 mcg/disc</td>
<td>10</td>
<td>20</td>
</tr>
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### Table 1c – Results of MIC test of silver nanoparticles against MRSA isolate.

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