Biosynthesis of silver nanoparticles using *citrus sinensis* peel extract and their application as antibacterial agent

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**ABSTRACT**
Silver nanoparticles (Ag-NPs) have attracted huge importance due to their distinctive chemical, biological and physical properties. Silver nanoparticles are widely synthesized by the chemical method, which involves the use of toxic chemicals which affects its applications. The bio-reduction method, in comparison with chemical method is more economic and eco-friendly. In the present work, the bio-based production of Ag-NPs was done by using peel extract of orange (*citrus sinensis*), which played a role of reducing and stabilizing agent. The biosynthesis of silver nanoparticles was optimized by one factor at a time (OFAT) with respect to peel extract concentration, silver nitrate concentration and reaction temperature. The green synthesized silver nanoparticles were characterized by UV-visible spectroscopy, Fourier transforms infrared (FT-IR) spectroscopy, Scanning electron microscopy (SEM) and X-ray diffraction (XRD). Disk diffusion method was used for the study of antibacterial activity of the bio-synthesized silver nanoparticles against the bacteria *Escherichia coli* and *Staphylococcus aureus*. The results showed that at a peel extract concentration of 6%, the temperature of 60°C and silver nitrate concentration of 0.1M, the synthesis of Ag-NPs was effective. The orange peel synthesized Ag-NPs showed effective antibacterial activity against both bacteria. However better activity was observed against bacterium *Staphylococcus aureus*. The results confirmed the synthesis of Ag-NPs using peel extract of *citrus sinensis* and its role as antibacterial agent.

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**INTRODUCTION**
Silver nanoparticles are helpful in today’s situation due to its distinct properties such as optical, magnetic, electrical, shape and size (Skiba *et al.*, 2019). These Ag-NPs have wide applications in the field of electronic components, biosensor materials, complex fibers, aesthetic products and antimicrobial applications (Skiba *et al.*, 2018). Ag-NPs are produced widely by Chemical reduction method under clement environment and large scale production. However, this technique makes use of harmful chemicals which have an unfavorable effect on the surroundings (Yang *et al.*, 2019). This stops the usage of Ag-NPs in healthcare applications. Therefore green production of Ag-NPs has demonstrated to be more beneficial over other methods, as it is economical and environmentally friendly (Gudikandula and Maringanti, 2016). It does not require adverse conditions like usage of harmful chemi-
cals, high pressure and temperature (Bhattarai et al., 2018; Manal et al., 2014). Hence, bio-based synthesis of Ag-NPs has been employed. In green synthesis, the Ag-NPs produced from microbial source are less stable than Ag-NPs produced from the plant source (Manal et al., 2014; Ocsoy et al., 2017). Therefore in the production of Ag-NPs, extract of fruit peel are being utilized. The peel extract plays a role of reducing and stabilizing agent (Dhand et al., 2016). Ag-NPs have shown suppressive effects against microbes (Karatoprak et al., 2017; Sierra et al., 2016). In this study, decayed orange peel extract was used to produce Ag-NPs and produced Ag-NPs were used for the study of antimicrobial activity. The effect of different parameters like concentration of silver nitrate, concentration of peel extract and temperature on the production of Ag-NPs was studied.

MATERIALS AND METHODS

Materials
Silver Nitrate (AgNO₃) used in the synthesis was procured from SRL, India. Media components used were obtained from HiMedia. Cultures of Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) were obtained from the Department of Biotechnology, K.L.E. Technological University, Hubballi, India.

Preparation of peel extract
The orange peels were collected from Hubballi market and were washed with water. It was then air dried. 200 mL of distilled water was added to 100 g of dried orange peels. The mixture was boiled for 10 minutes at 100°C. The filtered peel extract was stored for further use at 4°C.

Green synthesis of silver nanoparticles
For the production of Ag-NPs, 0.1M and 0.01M stock solution of AgNO₃ and orange peel extract were taken. 20 mL of AgNO₃ solution and peel extract were mixed and allowed to react for 1h. The produced Ag-NPs were characterized by UV-Vis Spectrophotometer between the wavelength range 300-600 nm. The production of the Ag-NPs was studied by varying the factors like temperature (Room temperature, 40°C and 60°C), peel extract concentration (2%, 4%, and 6%) and silver nitrate concentration (0.1M and 0.01M). FT-IR, SEM and XRD techniques further characterized the biosynthesized Ag-NPs.

Silver nanoparticles antibacterial activity
Antibacterial activity of Ag-NPs was checked by Agar disk diffusion method. A suspension of the microorganism (Escherichia coli and Staphylococcus aureus) was spread by a glass spreader on nutrient agar. 20μl of an aqueous solution of biosynthesized silver nanoparticles and 20μl positive and negative control (tetracycline and de-ionized water respectively) were added to 5 mm diameter filter paper disks and were located on the inoculated plates. The plates were incubated at 37°C for 24h. The zone of inhibition was measured in millimeters.

RESULTS AND DISCUSSION

UV-vis analysis
The orange peel extract was used to react with 0.1M and 0.01M of AgNO₃ at different orange peel concentration and temperature. Figure 1 showed a color change from yellow color to dark brown for mixtures containing silver nitrate and orange peel extract showing the production of Ag-NPs. It may be due to Vitamin C content of citrus fruit peel. It acts as reducing agent which reduces Ag⁺ of AgNO₃ to Ag⁰ (Durán et al., 2016). The color intensity increased with the incubation period indicating the formation of more number of nanoparticles (Ouay and Stellacli, 2015). The results of UV-vis spectroscopy confirmed the production of Ag-NPs using orange peel extract. It also showed the surface Plasmon band of Ag-NPs between 400-480nm which showed the development of Ag-NPs. The wavelength did not alter much with the change in parameter. But variation in absorption was observed. The increase in absorption indicated the production of numerous nanoparticles (Li et al., 2010). The Plasmon resonance peak varied to longer wavelengths and broadened, as the diameter of the particle enlarged. From Figures 2 and 3, it was observed that the orange peel extract concentration of 6% and temperature of 60°C had maximum effect, whereas 2% of peels extract concentration and room temperature had minimum impact on the biosynthesis of silver nanoparticles.

FT-IR and SEM analysis
To know the role of functional groups in the orange peel in the formation of Ag-NPs, FT-IR analysis was done. The FT-IR results are shown in Figures 4 and 5. The shift in the following peaks at 3374, 1625, 1410, 1058, 872 in orange peel extract indicated its participation in the process of nanoparticles synthesis. The composition of orange peels consisted of pectin, carbohydrates, hemicelluloses and cellulose (Shet et al., 2015b). The reduction of particle to nanoscale is due to the interaction of biological compounds with metal salts through functional groups (Shet et al., 2015b).

SEM was done to analyze the shape and structure of
Figure 1: Biosynthesis of the silver nanoparticles using A) 0.1M AgNO₃, B) 0.01 M AgNO₃ and 6% orange peel extract at 60°C for different time intervals (a) 0 min, (b) 15 min, (c) 30 min, (d) 60 min

Figure 2: UV-visible analysis of the silver nanoparticles synthesized using (a) 0.01M AgNO₃, (b) 0.1M AgNO₃ and 6% orange peel extract at room temperature, 40°C and 60°C

Figure 3: UV-visible analysis of the Silver nanoparticles synthesized using (a) 0.01M AgNO₃, (b) 0.1M AgNO₃ and different concentration of orange peel extract (2%, 4% and 6%) at 60°C
Figure 4: Infrared spectra of the orange peel extract

Figure 5: Infrared spectra of the Ag-NPs produced using 6% orange peel extract and 0.1M AgNO₃ solution for duration of 60 minutes

Figure 6: SEM images of the synthesized Ag-NPs at magnifications (a) 7000x and (b) 10000x

Table 1: Inhibition zone (mm) of the Ag-NPs synthesized using orange peel extract

| Samples                                      | Diameter of Inhibition zone (mm) |
|----------------------------------------------|----------------------------------|
| Deionized water                             | S. aureus: 0                      |
|                                              | E. coli: 0                        |
| Tetracycline                                 | S. aureus: 10.1                   |
|                                              | E. coli: 9.6                      |
| Ag-NP sample synthesized using orange peel and 0.01M AgNO₃ | S. aureus: 8.0                   |
|                                              | E. coli: 7.6                      |
| Ag-NP sample synthesized using orange peel and 0.1M AgNO₃ | S. aureus: 8.2                   |
|                                              | E. coli: 7.9                      |
Figure 7: XRD pattern of the Ag-NPs synthesized using 6% orange peel extract at a temperature of 60°C for the time duration of 1 h

Figure 8: Antibacterial activity of the Ag-NPs synthesized using orange peel extract on (A) Staphylococcus aureus and (B) Escherichia coli. a) –ve control (DI water), b) +ve control (tetracycline), c) Ag-NP sample synthesized using 6% orange peel extract and 0.01M AgNO₃, d) Ag-NP sample synthesized using 6% orange peel extract and 0.1M AgNO₃

The produced Ag-NPs. The results of SEM indicated the presence of Ag-NPs, as shown in Figure 6. It was observed that the Ag-NPs are relatively spherical.

**XRD analysis**

The produced Ag-NPs indicated face-centered cubic crystal structure which was confirmed by XRD pattern as shown in Figure 7. The results of XRD ensured that the peaks at 38.04°, 44.08°, 64.36° and 77.22° attributed to 111, 200, 220, and 311 crystalline structures of the face-centered cubic synthesized Ag-NPs. In addition, the pattern indicated that Ag-NPs were mainly present in the nanocomposites with no contamination peaks.

**Antibacterial activity**

Zone of inhibition was used to study the role antibacterial agent of produced Ag-NPs. The inhibition zone of synthesized Ag-NPs is summarized in Table 1. From Figure 8, it was observed that the negative control (Deionized water) had no inhibition zone and positive control (tetracycline) had clear zone inhibition. There was a clear zone inhibition of silver nanoparticles against Gram-negative (E. coli) and Gram-Positive (S. aureus) bacteria. But more activity was observed against S. aureus. This may be due to the infiltration of Ag-NPs into the cells, causing intracellular loss leading to cell death. E. coli and S. aureus are highly sensitive to silver nanoparticles due to the high lipopolysaccharide and thick peptidoglycan layer of the microorganisms (Shet et al., 2015a).
CONCLUSION

The reported work exhibited an easy, environmental friendly and economical method for Ag-NPs synthesis. Orange peel extract played a role of reducing agent and stabilizing agent. Results showed that the reaction time, concentration of peel extract, concentration of AgNO$_3$ and temperature affected the yield and particle size of the produced silver nanoparticles. The production of Ag-NPs was verified by the analysis of UV-vis spectroscopy, FT-IR, SEM and XRD results. The antibacterial activity of Ag-NPs was determined by disk diffusion method, which showed activity against the bacterial strains E. coli and S. aureus. However better activity was shown against bacterium S. aureus.

Future Scope

The anticancer and antioxidant activity of the synthesized silver nanoparticles can be determined.

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Conflict of interest

The authors declare no conflict of interest.

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