ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: www.ijrps.com

Optimization of Silver Nanoparticles Production using *Aspergillus niger* and Study of Antimicrobial Activity

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Article History:

Received on: 08 Jun 2021
Revised on: 25 Jul 2021
Accepted on: 31 Jul 2021

Keywords:

Silver nanoparticles, Aspergillus niger, Optimized, Antimicrobial property

ABSTRACT

Silver nanoparticles have attracted high attention worldwide for their various applications. The physiochemical parameters such as temperature, media, mixing ratio affect the rate of synthesis of silver nanoparticles and their yield. Thus, optimization of these physiochemical parameters is needed to enhance the production of silver nanoparticles. In this study, silver nanoparticles were synthesized using *Aspergillus niger* culture supernatant. The produced silver nanoparticles were characterized using UV-visible Spectrophotometer at 200 nm to 700 nm, which had a peak at 450 nm, indicates the formation of silver nanoparticles. It was found that Sabouraud Dextrose Broth (SDB) as o ptimum media, 40 ml of supernatant and 10 ml of silver nitrate as optimum mixing ratio and 65 °C as optimum temperature to produce silver nanoparticles. The optimized silver nanoparticles were subjected to antimicrobial activity, and it was found that it is highly effective towards gram-negative bacteria than gram-positive bacteria where the zone of inhibition for *Escherichia coli* was 7 ± 2.7 mm and 5.3 ± 2.1 mm for *Staphylococcus aureus.*

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i4.4872

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INTRODUCTION

Nanoparticles are particles that are nano-sized from 1 to 100 nm (Zomorodian *et al.*, 2016). The nanoparticles can be clearly seen through high efficiency transmission electron microscope (Balakumaran *et al.*, 2016). The smaller the particles, the higher its effectiveness. The size of the particles has an important influence on the application that was synthesized for comparison with its parent compound (Krishnaraj *et al.*, 2012). In the production of nanoparticles, metal nanoparticles take center stage where a variety of metal nanoparticles being produced, such as silver, gold, copper, iron, selenium, lead, silica, titanium, zirconium and platinum (Khan and Jameel, 2016). These nanoparticles are synthesized using physical, chemical, and biological methods. Chemical and physical methods are known to be the usual method of producing nanoparticles, but these methods were expensive, toxic, and hazardous (Ravindrakumar *et al.*, 2020; Zhang *et al.*, 2016). Hence, as an alternative, biological method or known as green synthesis was used to synthesis silver nanoparticles to produces nanoparticles which has a higher surface area, minute in size, stable and high yield (Saadony *et al.*, 2019). The method is also a low cost, fast and easy method to perform. Green synthesis uses living cells or organisms such as fungus, plants, and bacteria as the

producer of nanoparticles (Madakka *et al.*, 2018). Hence, in this project, the *Aspergillus niger* was used to produce silver nanoparticles. *Aspergillus niger* is a fungus, and it was widely used to synthesis silver nanoparticles as it can handle any kind of situations such as high tolerance, bioaccumulation capabilities, agitation, easy inoculation, and easy growth (Jameel *et al.*, 2016). The physiochemical parameters such as temperature, media, mixing ratio not only affect the rate of synthesis of silver nanoparticles but also its yield. It was found that the shape and the size of the nanoparticles can be altered by altering parameters such as pH, temperature, substrate concentration, mixing ratio and reaction time (Balakumaran *et al.*, 2016; Jameel *et al.*, 2016; Khan and Jameel, 2016). Optimum parameters allow higher and efficient production of nanoparticles. Thus, the aim of this project was to optimize the physiochemical parameters such as temperature, media, mixing ratio to synthesis silver nanoparticles via *Aspergillus niger*.

MATERIALS AND METHODS

Preparation of *Aspergillus niger* Culture Plate

Potato Dextrose Agar (Sime Scientific, India) was prepared using the instructions provided by the manufacturers. *Aspergillus niger* master culture plate was obtained from the Manipal International University laboratory. The strain was cultivated on the Potato Dextrose Agar (PDA) plate prepared and was incubated at 28 °C for 7 days (Jameel *et al.*, 2016).

Production of *Aspergillus niger* Biomass

Potato Dextrose Broth (Sime Scientific, India) was prepared using the instructions provided by the manufacturers. *Aspergillus niger* from the culture plate was inoculated in the 150 ml of Potato Dextrose Broth (PDB) and was incubated on a rotatory shaker (Infors HT, Ecotron, Switzerland) at 180 rpm at 28 °C for 7 days to obtain the biomass. After 7 days, the biomass was harvested by filtering it using sterile filter paper (Whatman, China), and the broth obtained was centrifuged at 9000 rpm for 10 minutes to avoid the presence of *Aspergillus niger* in the broth using a centrifuge (Hettich, REF 1406, Germany). The supernatant was used for silver nanoparticle production (Jameel *et al.*, 2016; Koilparambil *et al.*, 2016).

Synthesis of Silver Nanoparticles

The culture supernatant (10 ml) obtained was added to the falcon tube containing 40 ml of silver nitrate ($AgNO_3$) (0.001 M) (Fisher Chemical, UK) and incubated for 48 hours at 28 °C. After 48 hours, a change in colour (yellowish white to brown) was observed, which indicated the synthesis of silver nanoparticles. A control was kept without supernatant did not have any colour changes after incubation. The silver nanoparticles formed was characterized by checking the absorbance at 200 nm to 700 nm using UV Spectrophotometer (Cecil, 2000 Series, England) (Koilparambil *et al.*, 2016). The highest peak was at 450 nm.

Optimization of Silver Nanoparticles Production using Different Parameters

Optimization of Media

The media optimization was done with Potato Dextrose Broth (PDB) and Sabouraud Dextrose Broth (SDB) (Sime Scientific, India). Both the media was prepared according to the instructions provided by the manufacturers and inoculated with *Aspergillus niger*, which was incubated for 7 days on a rotary shaker at 180 rpm at 28 °C. After 7 days, the supernatant was collected by filtering the mycelium with sterile filter paper and centrifuged at 9000 rpm for 10 minutes. Both the supernatant (10 ml) was separately added to 40 ml of silver nitrate in a separate falcon tube and was incubated at 28 °C for 48 hours. Silver nitrate without culture supernatant was incubated alongside as a control. Then the silver nanoparticles produced was characterized by using UV Spectrophotometer at 450 nm (Koilparambil *et al.*, 2016).

Optimization of Mixing Ratio of Culture Supernatant and Silver nitrate

Culture supernatant (PDB) 10 ml with 40 ml of silver nitrate, 20 ml of culture supernatant (PDB) with 30 ml of silver nitrate, 30 ml of culture supernatant (PDB) with 20 ml of silver nitrate and 40 ml of culture supernatant (PDB) with 10 ml of silver nitrate was incubated at 28 °C for 48 hours. A silver nitrate without culture supernatant was incubated alongside as a control. Then the silver nanoparticles produced was characterized by using UV Spectrophotometer at 450 nm (Koilparambil *et al.*, 2016).

Optimization of Temperature

Four different temperature was selected, 28 °C, 37 °C, 48 °C and 68 °C. The culture supernatant (PDB) (10 ml) was mixed with silver nitrate (40 ml) in four falcon tubes and was incubated for 48 hours at selected temperatures. Silver nitrate without culture supernatant was incubated alongside as a control. Then the silver nanoparticles produced was characterized by using UV Spectrophotometer at 450 nm (Koilparambil *et al.*, 2016).

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

Figure 1 shows the synthesis of AgNPs by *Aspergillus niger* supernatant. Fungal supernatant incubated with silver nitrate after 48 hours changed colour from pale yellow (A) to brown (B). This indicated the formation of silver nanoparticles. Meanwhile, the control showed no colour changed with the same incubation condition (C to D). According to Madakka *et al.* (2018), the colour change happens due to the accumulation of silver nanoparticles in the broth.

According to Balakumaran *et al.* (2016), the colour change occurs due to the excitement of surface Plasmon resonances. The silver nanoparticles accumulate at the bottom of the falcon tube, but if the falcon tube is mixed completely, then the whole solution will change to brown. There were no colour changes observed for the control, which was only silver nitrate.

According to Zomorodian *et al.* (2016), the NADH and the NADH dependent nitrate reductase are produced externally by *Aspergillus niger*. This enzyme acts as a reducing agent and capping agent, causing the silver nitrate to be converted to silver ion (Ag+) (Zomorodian *et al.*, 2016; Vishwanatha *et al.*, 2018).

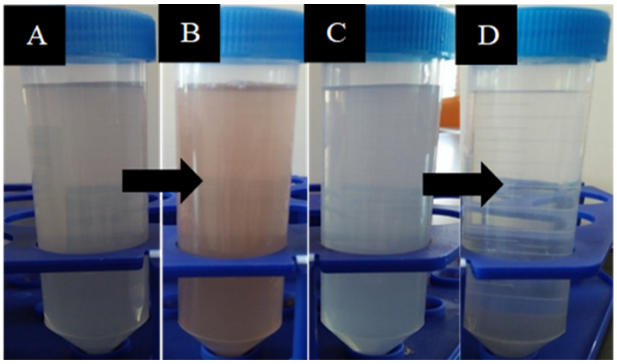

Figure 1: Synthesis of AgNPs by *Aspergillus niger* supernatant after 48 hours of incubation with silver nitrate (A to B) and only silver nitrate as control (C to D)

Characterization of Silver Nanoparticles by UV visible Spectrometry

Figure 2 shows the UV-Vis Spectroscopy absorption spectrum of synthesized AgNPs in the range of 200-700nm. The solution, which changed colour after 48 hours due to the silver nanoparticles production, was characterized using the UV visible spectrophotometer from 200 nm to 700 nm. The highest peak or the lambda maximum was found at 450 nm with an absorbance of 0.573.

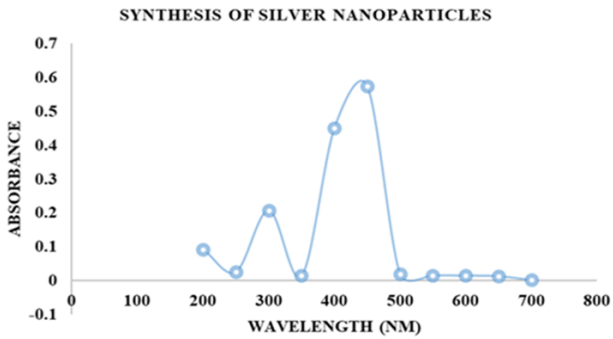

Figure 2: UV-Vis Spectroscopy absorption spectrum of synthesized AgNPs

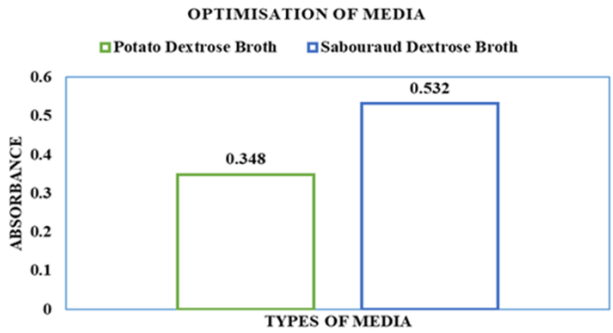

Figure 3: UV-Vis Spectroscopy absorbance of synthesized AgNPs using different media at 450 nm

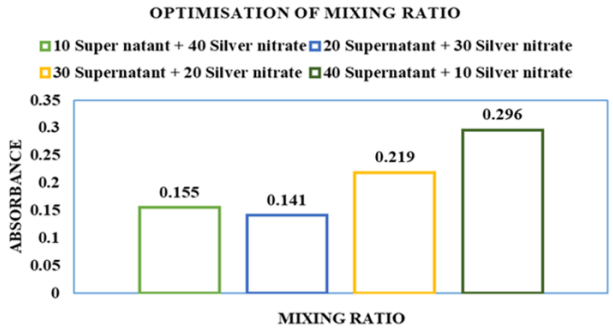

Figure 4: UV-Visible Spectroscopy absorbance of silver nanoparticles produced at different mixing ratios at 450nm

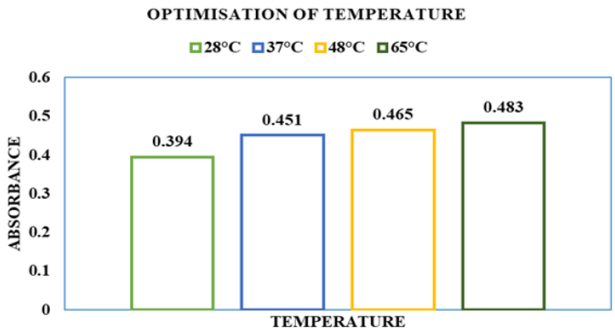

Figure 5: UV-Visible Spectroscopy of silver nanoparticles produced at a different temperature at 450 nm

Table 1: Antimicrobial activity ofoptimized silver nanoparticle

Organism	Zone of Inhibition (mm)		
	Optimized AgNPs	Positive control	Negative control
Escherichia coli	7 ± 2.7	12 ± 1.7	0 ± 0
Staphylococcus aureus	5.3 ± 2.1	12.3 ± 0.6	0 ± 0

The result found was the same with the result published in Madakka *et al.* (2018), where the peak was also found at 450 nm exhibits the formation of silver nanoparticles which is due to the excitement of surface Plasmon resonances. Sagar and Ashok (2012) reported that the highest absorbance was around 440 nm.

Optimization of Parameters

Optimization of Media

Figure 3 shows UV-Vis spectroscopy absorbance of synthesized AgNPs using different media at 450 nm. Potato dextrose broth (PDB) and Sabouraud dextrose broth (SDB) were used for the optimization of media. The result showed that the production of silver nanoparticles was higher in Sabouraud Dextrose Broth (SDB) which was 0.532, compared to Potato Dextrose Broth (PDB) which was 0.348. Among these two media, SDB was found to be the optimum media to produce silver nanoparticles. This is due to the presence of a higher nitrogen source in the media supports the higher production of an enzyme (nitrate reductase), which allowed the higher production of silver nanoparticles when the supernatant was mixed with silver nitrate (Guilger-Casagrande and Lima, 2019). The media also influences the performance of the enzyme (nitrate reductase).

Optimization of Mixing Ratio of Culture Supernatant and Silver Nitrate

Figure 4 shows UV-Visible absorbance readings obtained from different mixing ratios at 450nm. There were four mixing ratios used to produce silver nanoparticles. Among the four, 40 ml of supernatant and 10 ml of silver nitrate were reported to have higher production of silver nanoparticles with an absorbance of 0.296. This was because the higher amount of supernatant provides a higher amount of reductase enzyme, which allows a higher reduction process to produce more silver nanoparticles. The other mixing ratios has lesser silver nanoparticles production due to the less amount of supernatant added where less amount of the enzyme was provided for the reaction.

Hence, in 48 hours, the higher amount of silver nanoparticles was able to be produced only by 40 ml supernatant and 10 ml silver nitrate. This was comparable to the findings by Birla *et al.* (2013) and Shahzad *et al.* (2019), where a higher amount of supernatant produced a higher amount of small-sized silver nanoparticles.

Optimization of Temperature

Figure 5 shows UV-Visible absorbance of silver nanoparticles produced at a different temperature at 450 nm. Four different temperatures were experimented with to produce silver nanoparticles. The temperature used for the experiment were 28 °C, 37 °C, 48 °C and 65 °C. As the temperature increased, the production of silver nanoparticles also increased, which was characterized by UV visible spectrometry. But the experiment revealed that 65 °C has the highest production of silver nanoparticles with an absorbance of 0.483. Hence, 65 °C is declared as the optimum temperature. A similar result was found in Jameel *et al.* (2016), where 60 °C was optimum, and if the temperature goes beyond that, it will cause denaturation of the protein and enzymes needed for the reduction reaction. Temperature lesser than the optimum temperature has lesser silver nanoparticle production due to the incomplete reduction reaction. Enzymes can only work best in the optimum temperature, less than that, it will not work well and beyond that, it may lose its catalytic action (Jameel *et al.*, 2016).

Antimicrobial Testing

Silver nanoparticles was produced using the optimized parameters, and the optimized silver nanoparticles were used to test against *Escherichia coli* and *Staphylococcus aureus* (Table 1). The results showed that gram-negative bacteria produced a larger zone of inhibition compared to gram-positive, where the zone of inhibition of *Escherichia coli* was 7 ± 2.7 mm and 5.3 ± 2.1 mm for *Staphylococcus aureus*. Thus, the result clearly showed that the silver nanoparticles are more effective towards gram-negative bacteria than gram-positive bacteria. According to Madakka *et al.* (2018), Gram-positive has less inhibition than gram-negative due to its cell wall thickness difference. Since the gram-positive has a thicker cell wall, it was not easy for the silver nanoparticles to penetrate the cell wall. But gram-negative has higher inhibition due to its less

thick cell wall. Krishnaraj *et al.* (2012) stated that the silver nanoparticles attack the cell membrane by changing its function and structure. The silver nanoparticles interact with the DNA and the necessary enzymes for the microorganism's metabolism and survival (Madakka *et al.*, 2018; Vishwanatha *et al.*, 2018).

CONCLUSION

In conclusion, it was proven that the *Aspergillus niger* could produce silver nanoparticles that exhibit good antimicrobial properties. The current study proposed that optimum parameters for the biosynthesis of silver nanoparticles using *Aspergillus niger* supernatant were Sabouraud Dextrose Broth (SDB) media, 40 ml of supernatant and 10 ml of silver nitrate mixing ratio and 65 °C as the incubation temperature. The silver nanoparticles antimicrobial activity was highly efficient for gram-negative bacteria than gram-positive bacteria.

ACKNOWLEDGEMENT

We would like to thank the Department of Biotechnology, School of Biotechnology, Manipal International University and Department of Science and Technology, Faculty of Humanities, Management and Science, Universiti Putra Malaysia Bintulu Sarawak Campus, Malaysia, for providing the necessary facility and support of this work.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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