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Research Article **Microbiology**

Biological activity of synthesized silver nanoparticles on *S. aureus* and *P. aeruginosa*

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KEY WORDS

Moringa oleifera, biological synthesis, silver nanoparticles

ABSTRACT

Silver nanoparticles (AgNPs) that were biologically synthesized using *Moringa oleifera* leave extract showed good antibacterial activity against two tested bacterial strains namely: *Pseudomonas aeruginosa* as a model for Gram- negative bacteria *and Staphylococcus aureus* as a model for Gram- positive bacteria. Minimum inhibitory concentration (MIC) of antibacterial silver nanoparticles that were biologically synthesized using *Moringa oleifera* leave extract was tested to estimate the effect of nanoparticles on cell viability, cell integrity, and cell permeability of tested bacteria. The results indicated that there was a great effect of AgNPs on cell viability of bacterial strains tested as there was significance in cell viability between control and treated bacteria with silver nanoparticles. Also, cell permeability of *S. aureus* and *P. aeruginosa* were greatly affected by biologically synthesized silver nanoparticles and statistical analysis revealed that there was significant difference in cell permeability of two tested bacteria between control and treated cells with nanoparticles. However, there were slightly effect of these nanoparticles on cell integrity when the absorbance of DNA was estimated.

Introduction

Many pathogenic bacteria gained resistance to many antibiotics in recent years **(Ibrahim *et al.*, 2020; Shaaban *et al.*, 2015)**. So, many researchers searching for alternative antibacterial agents rather than antibiotics. Recently, they used nanoparticles which have antibacterial activities to overcome these antibiotics resistance **(Moodley *et al.*, 2018)**. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most prominent pathogenic bacteria that affect wounds during healing processes and gained antibiotic resistance rapidly **(Kucińska-Lipka *et al.*, 2015; Shaaban *et al.*, 2021).**

Nanoparticles useful as antimicrobial agent due to their nanoscale size which ranged between 1 and 100 nm **(Rai *et al.*, 2009)**. Nanoparticles could be synthesized using chemical, physical or biological methods but biological method is more favorable than chemical and physical methods due to chemicals that was toxic which involved in reduction, high-energy required during input and high cost downstream processing **(Moodley *et al.*, 2018)**. On the other side, biological methods for synthesis of nanoparticles were ecologically save, less toxic than chemical and physical methods, high yield and downstream processes were low-cost **(Islam *et al.*, 2021; Jadhav *et al.*, 2022)**.

Moringa oleifera are medicinal plant that was used in different folk medicinal purposes. Due to phenolic and flavonoids major constituents in leaves, silver nanoparticles biological synthesis has been done by a chemical interaction between these active constituents and silver nitrate **(Prasad & Elumalai, 2011; Jadhav *et al.*, 2022)**.

So, this research target studying the effect of MIC of biologically synthesized AgNPs that was synthesized using *Moringa oleifera* leaves extract on *S. aureus* and *P. aeruginosa.*

Material and methods

Effect of biologically synthesized AgNPs on cell viability

Kill time analysis of AgNPs that were biologically synthesized at minimum inhibitory concentration against *P. aeruginosa* ATCC 9027 and *S. aureus* ATCC 6538 was done according to **Joray *et al.* (2011)**. Fifty millimeters of nutrient broth medium were inoculated by 5 mL of standard inoculum (1.5×10^8 CFU/ml) and AgNPs at MIC concentrations (1.25 mg/mL) **(Shaaban *et al.*, 2023)** were added. Broth medium inoculated by bacteria was considered as positive control. Then

all cultures were incubated at 150 rpm in shaker at 37°C for 12 h, then the optical densities were measured at time intervals every 2 h at 600 nm using UV-visible spectrophotometer (spectroUVS-2700/ uvs-2800).

Cell integrity

The cell membrane integrity of the bacteria was determined by cellular materials estimation especially DNA due to the effect of antibacterial agents. Minimum inhibitory concentration of biologically synthesized AgNPs suspension were added to 30 mL of the microbial inoculum which its optical density reached 0.5 and the cultures were incubated at 150 rpm in shaking incubator at 37 °C for 12 h. After 2, 4, 6, 8, 10 and 12 hrs of incubation the cultures were centrifuged for 10 min at 4000 rpm and the optical densities supernatants measured at 260 nm using UV-visible spectrophotometer. AgNPs suspension was used as the negative control and the untreated bacterial cultures were used as positive controls **(Du *et al*., 2012).**

Permeability of the cell membrane

The cell membrane permeability was estimated according to **(Kong *et al*., 2008)** using relative electrical conductivity (EC). Tested bacteria that were cultured at 37 °C for 10 h, were centrifuged and the pellets washed with 5 % glucose until its EC became equal to that of 5% glucose then the AgNPs were added at MIC, incubated at 37°C for 7 h and the EC was measured using pH meter (HI-5521) at time intervals every hour (L_2). The equation that used for measuring cell permeability was as follows:

Relative electric conductivity (%) = $(L_2 - L_1) / L_0 \times 100$

Where L_1: was the electrical conductivity of MIC of AgNPs added to 5 % glucose, L_2: was the electrical conductivity of cultures at time intervals, and L_0: was the electrical conductivity of boiled bacteria for 5 min in 5% glucose.

Statistical analyses

Experiments were done in triplicate and statistical analyses were estimated using IBM SPSS version (26). When the p- value was equal to or less than 0.05 the significance was considered.

Results

As shown in Fig. (1) there were great effect of MIC of AgNPs on *S. aureus* and *P. aeruginosa* growth when compared with that of bacterial growth without treatment with AgNPs as there were noticeable significant effect. There was nearly no change in optical densities along 12 h in treated bacterial culture as the optical densities ranged from 0.121 to 0.16 in case of *S. aureus* and from 0.133 to 0.125 in case of *P. aeruginosa*. Whereas, bacterial cell viability of

bacterial culture without treatment (positive control) showed normal cell viability as the optical densities along 12 h ranged from 0.116 to 0.937 in case of *S. aureus* and from 0.163 to 0.733 in case of *P. aeruginosa*.

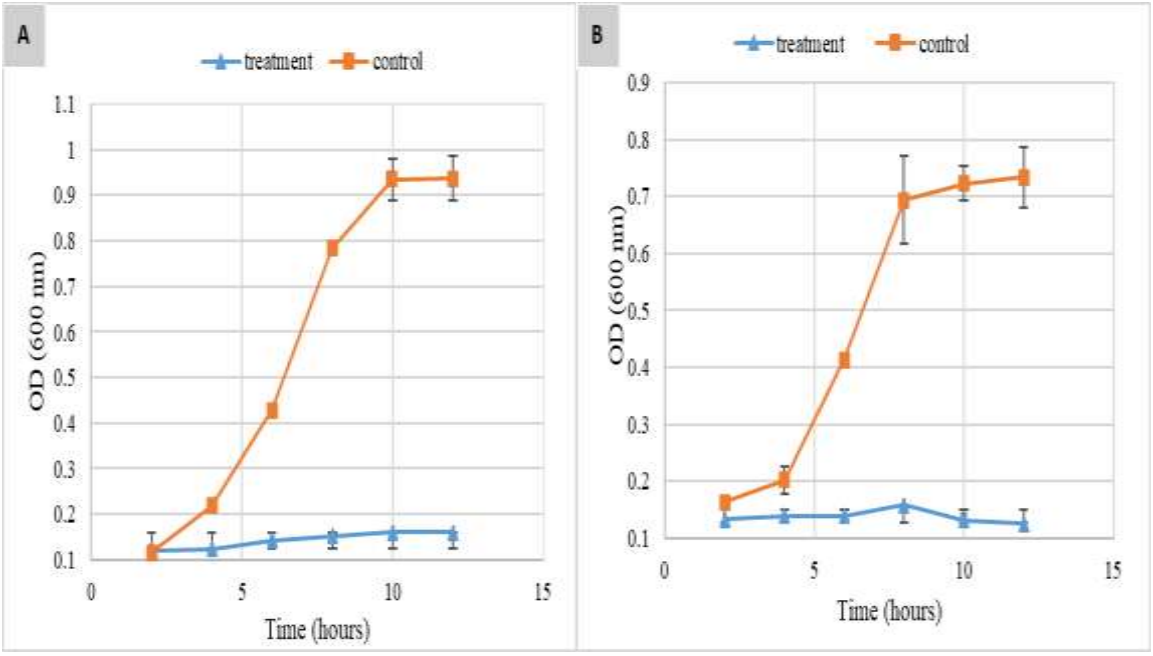

Fig. (1): Biological activity of silver nanoparticles on (A): *S. aureus* and (B): *P. aeruginosa* cell viability.

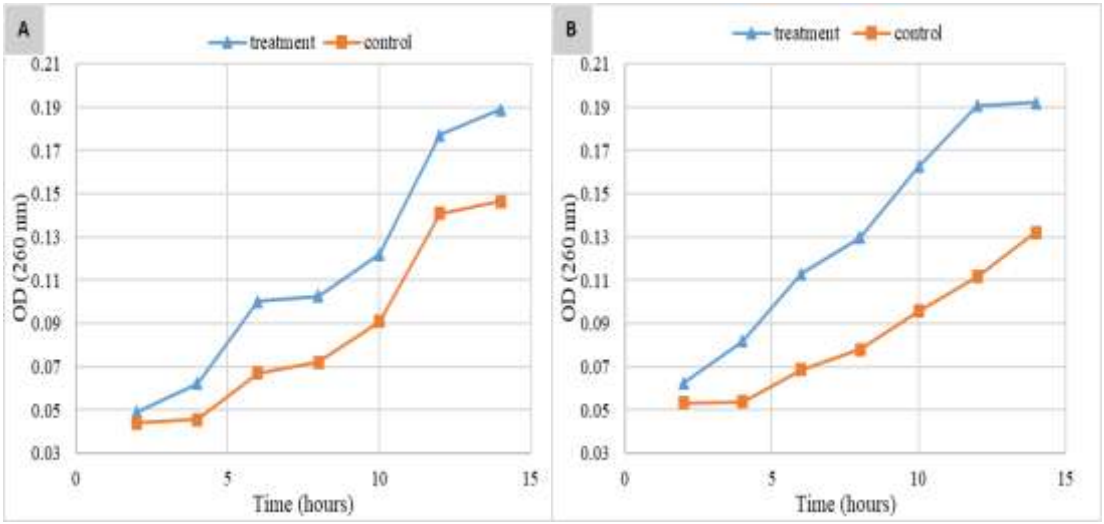

Fig. (2): Biological activity of silver nanoparticles on (A): *S. aureus* and (B): *P. aeruginosa* cell integrity estimated by measuring DNA leakage at 260 nm.

Treatment of two tested bacterial cultures by MIC of AgNPs slightly effect on bacterial cell integrity as shown figure (2A and 2B). After treatment with MIC of AgNPs, *S. aureus* cell membrane integrity affected slightly as the absorbance at 260 nm (DNA) increased from 0.049 to 0.189 after 14 h of incubation and for *P. aeruginosa,* the absorbance at 260 nm increased from 0.050 to 0.192. Whereas, untreated *S. aureus* culture showed slight increase in absorbance at 260 nm when compared with the absorbance of treated culture as the absorbance increased from 0.044 to 0.146 after 14 h of incubation. Also, it was increased from 0.039 to 0.132 in case of *P. aeruginosa.*

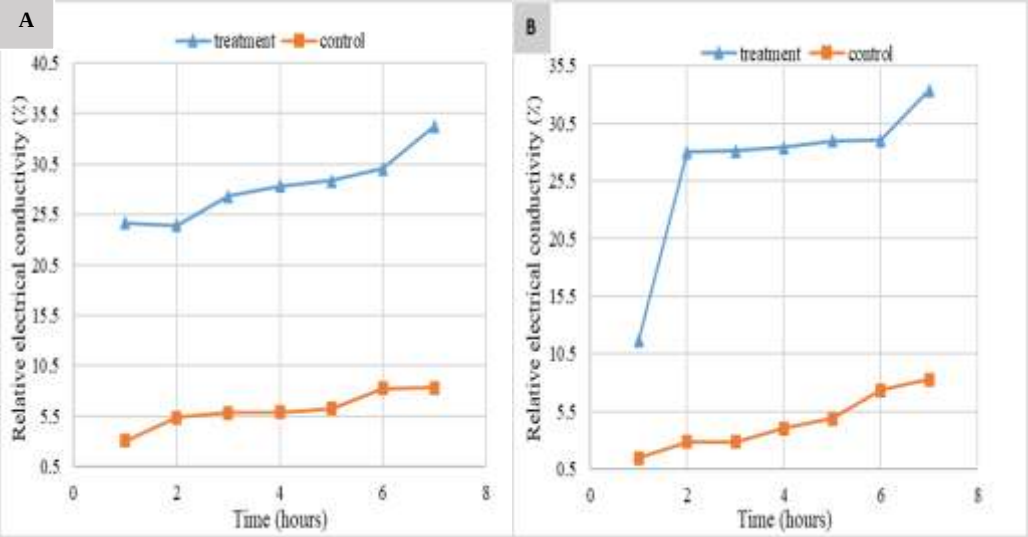

Fig. (3): Silver nanoparticles effect on (A): *S. aureus* and (B): *P. aeruginosa* cell permeability estimated by measuring electrical conductivity.

Treatment of *S. aureus* and *P. aeruginosa* cultures with MIC of AgNPs greatly effect on cell permeability which was estimated by measuring electrical conductivity at time intervals every 1 hour. As shown figure (3A and 3B), there were significant increase in bacterial cell permeability in treated bacterial culture when compared with that of untreated bacterial culture as the percentage electrical conductivity of treated bacterial culture along 7 hours ranged from 24.64 to 34.303 % for *S. aureus* and from11.623 to 33.4 % for *P. aeruginosa*. whereas, that of untreated bacterial culture of *S. aureus* ranged from 3.083 to 8.32 % and from 1.487 to 8.29 % in case of *P. aeruginosa*.

Discussion

Silver nanoparticles that were biologically synthesized using *moringa oleifera* leaves extract showed potential antibacterial activity against *S. aureus and P. aeruginosa* with minimum

inhibitory concentrations 1.25 mg/ml against two tested bacterial strains as reported by **(Shaaban *et al*., 2023).**

In current research, silver nanoparticles that were biologically synthesized showed great effect on cell viability of both *S. aureus* and *P. aeruginosa* as there were no growth estimated due to treatment by AgNPs that were biologically synthesized. This was in turn indicated the excellent effect of silver nanoparticles as an antibacterial agent **(Audtarat *et al*., 2022)**. Also **Ibrahim *et al*. (2021)** and **Jadhav *et al*. (2022)** reported that, the biologically synthesized AgNPs have potential antibacterial activity especially when nanoparticles synthesized using *Moringa oleifera leaves* extract.

Estimation the effect of AgNPs on cell integrity of two tested bacteria resulted in slightly difference in the amount of released materials from bacterial cells between control and treated cells. As reported by **Mikhailova (2020)**, free radicals of silver nanoparticles when contact with bacterial cells affect damaging on the cell membrane affecting cell integrity. Also, the effect of silver nanoparticles on cell integrity of *P. aeruginosa* cells was greater than that of *S. aureus.* This may be due to the lipopolysaccharides that were present in Gram-negative bacteria cell wall which promotes AgNPs adhesion affecting cell integrity **(Pal *et al*., 2007)**.These results were disagreed with **Kota *et al*. (2017)** as they reported that the antibacterial effect of synthesized AgNPs were greater in the case of Gram- negative bacterial than Gram- positive bacterial.

Silver nanoparticles that were biologically synthesized greatly affect cell permeability of both tested bacterial strains. This may be due to attraction between silver ions positive charges and the cell membrane which cause damaging changes in the cell membrane as reported by **Mikhailova (2020).**

Conclusion

Biologically synthesized silver nanoparticles using *Moringa oleifera* leave extract showed excellent effect against two tested bacteria which was confirmed by estimation and detection of the effect of these nanoparticles on cell viability, cell integrity and cell permeability. Future researches should be focused on the exact effect silver nanoparticles against affected bacteria.

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النشاط البيولوجى لجزيئات النانو فضة المُخلقة بيويوجياعلى بكتريا ستافيلوكوكس إريوس و سيدوموناس إيروجينوز

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طبقاً للدراسات السابقة فقد أظهرت جزيئات النانو فضة المُخلقة بيولوجيا بإستخدام مستخلص أوراق نبات المورينجا أوليفرا نشاطاً فعالاً ضد بكتريا ستافيلوكوكس إيريوس و سيدوموناس إيروجينوزا وذلك كطريقة بديلة لمحاولة الإستغناء عن المضادات الحيوية.

وفى هذا البحث أُستخدم التركيز المثبط الادنى لجزيئات النانو فضة المُخلقة بيولوجياً بإستخدام مستخلص أوراق نبات المورينجا أوليفرا لدراسة الفاعلية على منحنى النمو، صلابة الخلايا و نفاذية الخلايا. وأظهرت النتائج تأثيراً واضحاً لجزيئات النانو فضة على منحنى النمو ونفاذية وصلابة الخلايا عندما قورنت الخلايا التى تم معالجتها بجزيئات النانو فضة بتلك الخلايا التى لم يتم معالجتها بجزيئات النانو فضة. وأكدت تلك النتائج أيضاً التأثير الواضح والفعال لجزيئات النانو فضة على منحنى النمو ؛ صلابة الخلايا ونفاذية الخلايا لكل من البكتريا المختبرة.