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Biogenesis of silver nanoparticles from the shoot extract of *Delonix regia* its characterization (UV–Vis spectroscopy and SEM) and evaluation for antimicrobial potential

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Abstract

The current research work explores the production of silver nanoparticles using aqueous extracts of *Delonix regia* (Boj. ex Hook.) Raf. (Angiosperms; Fabaceae) shoots for the bioreduction of Ag metal and its antimicrobial activity. Fourier transform infrared spectroscopy (FTIR), Zeta potential, ultraviolet-visible spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD) have been used to evaluate the produced silver nanoparticles (AgNPs). Both antibacterial and antifungal activity were examined against bacterial and fungal pathogens, *viz*., *Escherichia coli* and *Bacillus subtilis*, and fungal strains, *viz*., *Fusarium oxysporum* and *Aspergillus niger*. The presence of silver nanoparticles was observed by the color change, *i.e*., from pale yellow to dark brown. The zeta potential observed for the produced nanoparticle is -18mv. The SEM and XRD revealed the size of synthesized AgNPs, *i.e*., 35nm and SEM size lies in the range of 40-60 nm. UV-visible absorption spectra were found at wavelength 425 nm. The synthesized nanoparticles are cost-efficient and could be an alternative procedure for the peculiar production of nanoparticles and also act as potential antimicrobial agents.

Keywords: AgNPs; antimicrobial activity; *Delonix regia*; FTIR; SEM; XRD

Introduction

Silver nanoparticles (AgNPs) are gaining popularity in a variety of fields, including medicine, food, industrial applications, and healthcare. They are typically accomplished through a variety of chemical and physical methods such as a specific rod position, laser ablation, and pyrolysis, all of which appear to be extremely dangerous and costly (Singh *et al*., 2010). Biogenic silver nanoparticle (AgNPs) synthesis is efficient, sustainable, cost-effective, and ecofriendly, and has proven to be one of the viable and novel mechanisms for the peculiar production of silver nanoparticles (AgNPs). In recent years, various metals and materials have been involved in the production of nanoparticles from algae, fungi, bacteria, viruses, and several plants (Valli and Geetha, 2016). The determination of biological activity of AgNPs is done under various parameters, which include particle size, shape, and composition, which are used for determining cytotoxicity. Due to the attractive physicochemical properties of silver nanoparticles, they have great potential in the fields of medicine and

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biology (Akter *et al*., 2018). Metallic nanoparticles have exhibited enhanced antioxidant, antimicrobial, and numerous other properties due to their small size and shape, high surface area, and distinct characteristics (Mubarak Ali *et al*., 2011; Dikshit *et al*., 2021).

Delonix regia (Boj. ex Hook.) Raf. (Family Fabaceae), is a large, medium-sized ornamental tree with fern-like, bipinnately compound leaves. It is commonly known as Royal Poinciana, Flamboyant (English), and Gulmohar (Hindi). It is mainly found in Madagascar, Africa, Northern Australia, and India as a semi-deciduous tree (Latif *et al*., 2019). Distinct parts of the *D. regia* plant, like leaves, roots, bark, and flowers, are used for the treatment of various ailments, like fever, bronchitis, anemia, pneumonia, diabetes, and inflammation. It exhibits various physiologically active chemical components such as lupeol, coumarin, carotene, lycopene, sterols, gallic acids, quercetin, etc. *D. regia* is generally used for treating fungal and bacterial infections, inflammation, and liver diseases (Siddiquee *et al*., 2020). Therefore, the aim of this study was to analyze the production of environmentally compatible silver nanoparticles through *D. regia* shoot extract. Different techniques were used for the characterization of silver nanoparticles, such as UV, SEM, XRD, and their antimicrobial activity.

Phytoconstituents of Delonix regia

The plant consists of various pharmacologically active compounds like phenolic compounds that possess many anti-inflammatories, anti-ulcer, anti-microbial, anti-fungal, anti-bacterial, and anti-helmitic properties that are valuable or beneficial in a variety of health conditions (Bhorga and Kamble, 2019).

Phytochemical screening results showed the presence of various bioactive constituents, like leaves that contain alkaloids, carbohydrates, and tannins; bark and shoots that contain terpenoids, flavonoids, tannins, alkaloids, and sterols; and flowers that contain saponins, steroids, carotenoids, and tannins.

Materials and Methods

Hi-media was used to make silver nitrate ($AgNO_3$). Many other reagents, solvents, and materials used are of analytic grade. For the characterization of silver nanoparticles, both milli-Q water and double-distilled water were used.

Collection and identification of plant extract

The shoot of *D. regia* was collected from the campus of Banasthali Vidyapith (Tonk), Rajasthan, India. The authentication number of the reference specimen is BURI-1407/2022, and it was deposited in the herbarium of Banasthali University, Rajasthan, India (BURI).

Preparation of D. regia shoot extract

The collected shoots were rigorously washed with running tap water afterwards using milli-Q water. Before the pulverization process, the shoots were properly dried at room temperature for 10 days so that no moisture content was present.

To prepare the shoot extract, 10 g of finely powdered shoot was mixed with 250 ml of deionized water and boiled at 60 °C for 30 min. Further, the resulting extract was cooled to room temperature before being filtered with Whatman No. 1 filter paper. For subsequent research, the extract which was properly filtered is retained at 4 °C (Das *et al*., 2017).

Preparation of biogenic silver nanoparticles

Following the bioreduction of silver nanoparticles (AgNPs), a 1 mM aqueous solution of AgNPs is produced. A 10 ml *D. regia* shoot extract was mixed with 90 ml of a 1 mM $AgNO_3$ solution and incubated at

28 °C for 24 hours. This bioreduction of AgNO3 can be visually apparent by the change in color pattern, *i.e.*, from pale yellow to dark brown, as depicted in Figure 1. The confirmation of bioreduction was done by the UV visible spectra analysis pathway (Bharathi *et al*., 2018).

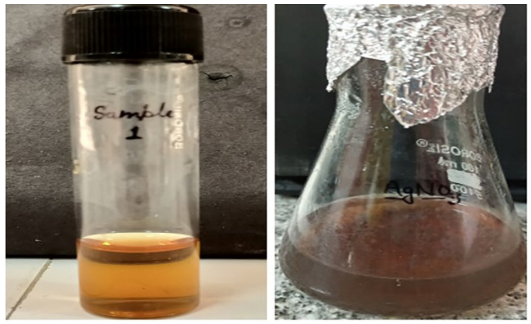


Figure 1. a. *Delonix regia* shoot extract; **b.** Formation of silver nanoparticles

Descriptive characterization of silver nanoparticles (AgNPs)

Ultraviolet- Visible Spectrophotometer

The synthesis of AgNPs was carried out by using a UV-visible spectrophotometer, noticing a distinctive surface plasmon resonance (SPR) peak at a scanning range of 200–800 nm and a speed of 200 nm/min. The dilution of the colored reaction samples was done with deionized water and scanned in ultra-violet visible spectra with a 1 cm quartz cuvette at 25 °C (Chouhan and Guleria, 2020).

X-ray diffraction studies (XRD)

The Bruker Eco D8 Advance X-pert PRO was used to figure out the X-ray diffraction of silver nanoparticles at 40 kV of voltage and a current strength of 20 mA (Mittal *et al*., 2012).

Debye- Scherrer equation was used to calculate X-ray diffraction-

$D = K\lambda/\beta \cos\Theta$

Where, D denotes crystal size

K = shape factor (0.94)

β = full width in radians at half maximum

λ = denotes the X-ray wavelength (1.5418Ǎ)

Particle size analyzer

After 24–48 h of incubation, the particle size was measured using a Zetasizer (Santhoshkumar *et al*., 2011).

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was used to investigate the identification of functional groups on the surface of silver nanoparticles by a shoot extract of *D. regia* within an isolation distance of 4 cm (Reddy *et al*., 2019).

Scanning Electron Microscopy (SEM)

The morphology of *D. regia* stabilized nanoparticles was analyzed by scanning electron microscopy (Ahmed *et al*., 2016).

Antimicrobial assay

The antimicrobial activity of *Delonix regia* shoot extract and its AgNPs was explained by the disc diffusion method. Both Potato Dextrose Agar (PDA) and Nutrient Agar Media (NA) were used for the preparation of fungal and bacterial species. The AgNPs, $AgNO_3$, and antibiotics were loaded into different filter paper discs. The discs were placed on nutrient agar media with bacterial culture and incubated for 12 h at 37 °C, whereas the discs were placed on potato dextrose agar with fungal culture and incubated for 24 hours at 28 °C. After 12 or 24 h, both bacterial and fungal cultures were checked, and the zone of inhibition was recorded (Rathi Sre *et al*., 2015).

Results

Characterization of AgNPs

Ultra- violet visible spectroscopy

After 24 h of observation, the ultraviolet-visible absorption spectra of colloidal silver nanoparticles (AgNPs) were shown. The peak intensity is found near 425 nm, indicating the formation of silver nanoparticles. The agitation of the Surface Plasmon Resonance (SPR) band in the U.V. visible region results in the color change from pale yellow to dark brown (aqueous solution), as depicted in Figure 2.

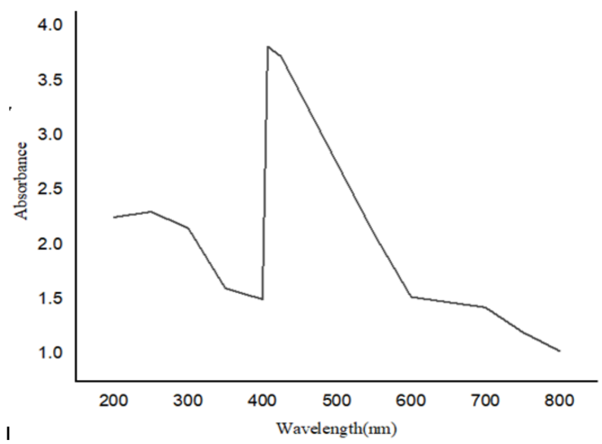


Figure 2. UV visible spectrophotometer of shoot extract of *Delonix regia* (AgNPs)

X-ray diffraction (XRD)

The XRD pattern of crystalline AgNPs is described in Figure 3. Sharp peaks of AgNPs were observed at 38.5°, 39.94°, 44.36°, 64.42°, and 77.44°. The calculation of average sizes of crystallized AgNPs was done using the Debye-Scherrer equation, and the size of AgNPs was observed to be 35.5 nm, which confirms the crystalline nature of synthesized silver nanoparticles (AgNPs).

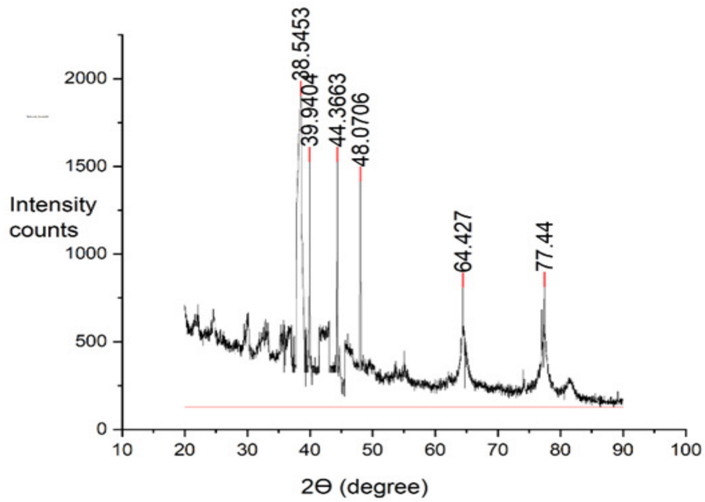


Figure 3. X-ray diffraction pattern of AgNPs using *Delonix regia* shoot extract

<u>Particle size analyzer</u>

The sharp peak of the *D. regia* shoot extract was found to be 88.94 nm. The obtained results revealed that the diameter of AgNPs is 129.6 d (nm), as depicted in Figures 4 and 5.

The zeta potential of *D. regia* shoot extract was investigated and found to be -18.7 mV.

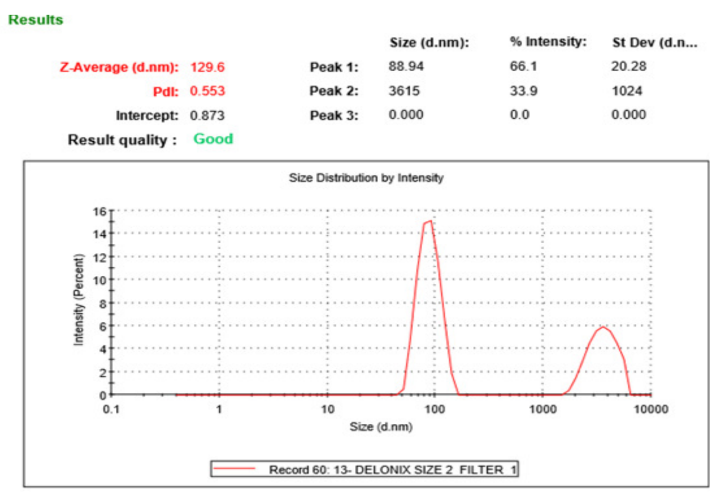


Figure 4. Particle size of silver nanoparticles (AgNPs)using *Delonix regia*

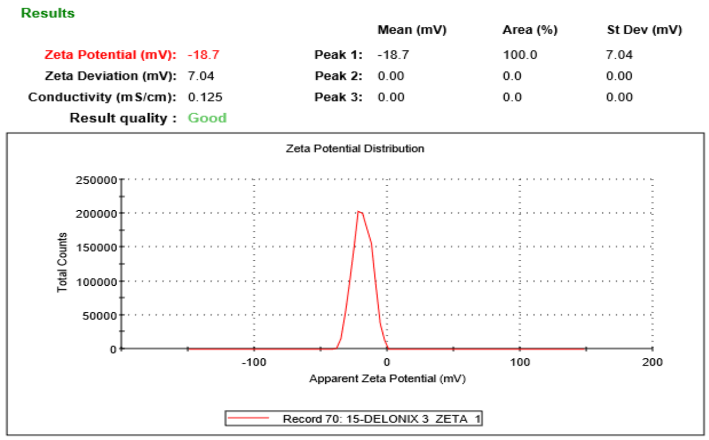


Figure 5. Zeta potential of AgNPs (*Delonix regia* shoot extract)

Fourier- Transform Infrared Spectroscopy (FTIR)

For the identification of the chemical bonds that are present in biogenic AgNPs, the FTIR tool is used. The spectra of *D. regia* shoot extract exhibit various absorption bands at 3367, 2920, 1630, and 1472 cm^{-1}. The band is observed at 3367 cm^{1} due to OH^{-} stretching vibrations. The different bands at 2920, 1630, and 1472 cm^{-1} indicate the existence of aromatic, carbonyl, and amide groups, as well as alkane groups (-C-H stretching). The minor bands at 787, 733, and 683 cm^{-1}, which are attributes of aromatic phenols, indicate the presence of C-H out of plane bend regions as depicted in Figure 6.

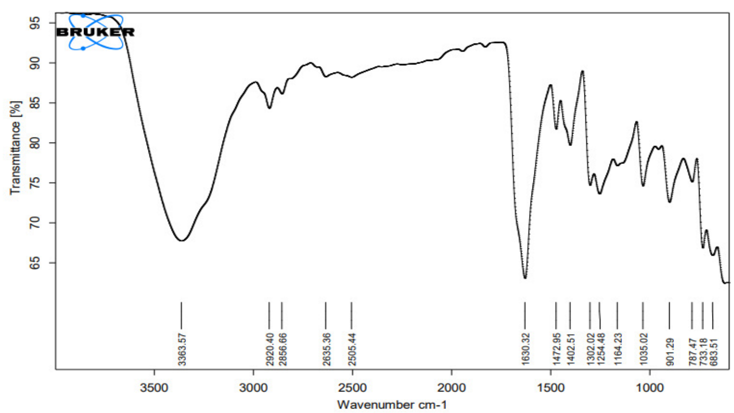


Figure 6. Fourier transform infrared spectroscopy of AgNPs using *Delonix regia* shoot extract

Scanning electron Microscopy (SEM)

The investigation of the topology and size of the prepared samples was done through SEM. The results explained that the crystallized synthesis of silver nanoparticles exists in a rectangular shape, *i.e.*, an average size of 40–60 nm, as depicted in Figure 7.

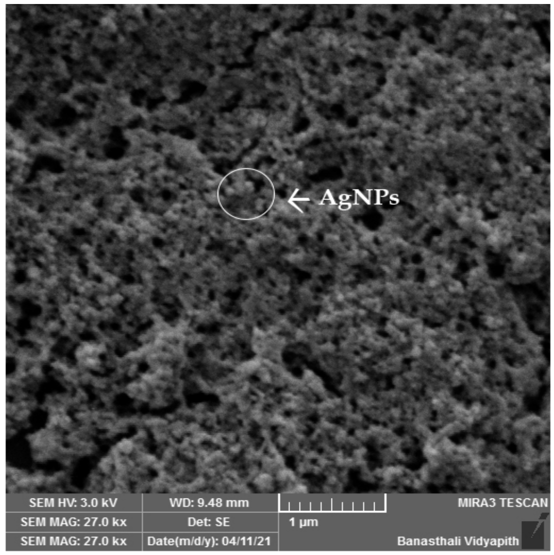
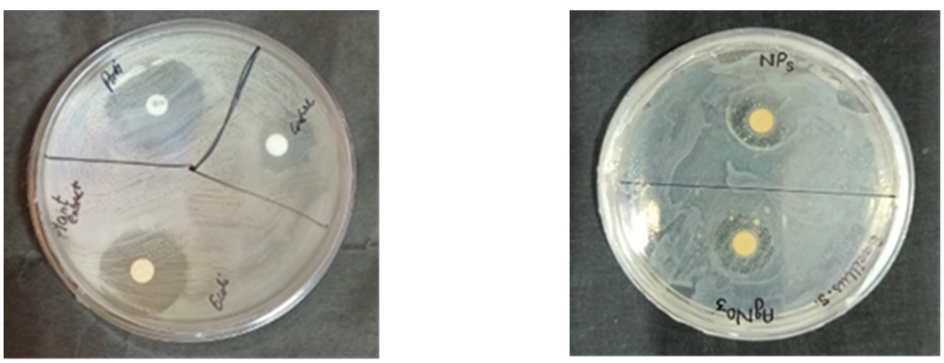


Figure 7. Scanning electron microscopy of synthesized AgNPs using *Delonix regia* shoot extract

Antimicrobial activity

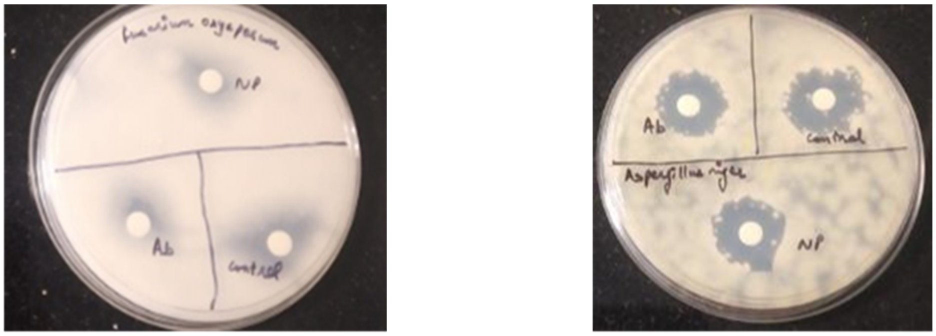
The antibacterial properties were observed against pathogenic bacterial strains, *viz*., *Escherichia coli*, *Bacillus subtilis*, while the antifungal activity was done using fungal strains, *viz*., *Fusarium oxysporum* and *Aspergillus niger*. The disc diffusion method, as shown in Figures 8 and 9, was used for both activities. Thus, the findings indicate that the bacterial pathogen *Escherichia coli* was detected to have a maximum zone of inhibition in comparison to *Bacillus subtilis* (Table 1).

In terms of antifungal activity, *Aspergillus niger* had the greater zone of inhibition when compared to *Fusarium oxysporum* (Table 2).



a. Escherichia coli *b. Bacillus subtilis*

Figure 8. Antibacterial activity of silver nanoparticles using *Delonix regia* shoot extract



a. Fusarium oxysporum *b. Aspergillus niger*

Figure 9. Antifungal activity of shoot extract of *Delonix regia*

Table 1. Antibacterial activity of synthesized silver nanoparticles (AgNPs)

S. No.	Bacterial Species	Zone of inhibition (mm)		
		AgNPs (PE)	Antibiotics (Chloramphenicol)	Control ($AgNO_3$)
1.	*Escherichia coli*	13±0.21^{b}	22±0.35^{c}	12±0.08^{a}
2.	*Bacillus subtilis*	12 ±0.10^{b}	21±0.29^{c}	10±0.09^{a}

Table 2. Antifungal activity of synthesized AgNPs

S. No.	Fungal Species	Zone of inhibition(mm)		
		AgNPs (NP)	Antibiotics (Fluconazole)	Control ($AgNO_3$)
1.	*Aspergillus niger*	14±0.25^{b}	25±0.46^{c}	12± 0.09^{a}
2.	*Fusarium oxysporum*	10 ±0.08^{b}	20± 0.32^{c}	11 ±0.08^{a}

Statistical analysis

The data are shown as averages of three replicates (n = 3). All obtained data were evaluated by IBM SPSS Statistics 20 software. Three-way interactions were executed between the chosen variables. For each output variable, multiple-comparison A Tukey's $p<0.05$ post hoc test was performed to compare the variance of the data. All data are presented as means standard deviation.

Discussion

Scientists are very interested in nanoparticles' significant antimicrobial impact because of the increasing microbial resistance to antibiotics and the development of antimicrobial resistant strains. One of the most encouraging approaches for avoiding microbial resistance is the use of nanoparticles, as they have the capability to fight drug resistance via multiple approaches. Silver nanoparticles (AgNPs) are highly useful in this context as they are effective antibacterial entities due to their enhanced reactivity, resulting from their high surface-to-volume ratio (Almatroudi, 2020). Hence, biosynthesized AgNPs emerge as one of the most viable alternative methods for the curing of microbial infections.

In present study, we have investigated the UV-visible absorption spectra found near the wavelength of 425 nm. The particle size indicated its diameter, *i.e.*, 129.6 nm, and the zeta potential of synthesized silver nanoparticles was investigated to be -18mv. The X-ray diffraction of synthesized silver nanoparticles revealed the intense peak at 38.50°, 39.94°, 44.36°, 64.42° and 77.44° and the average size of crystallized AgNPs was 35 nm. SEM study of silver nanoparticles disclosed that they are rectangular in shape and their size ranges from 40-60 nm. The Fourier Transform-Infrared spectrum of *D. regia* shoot extract revealed different peaks at 3363, 2920,1630 and 1472 cm^{-1} which indicates the presence of bonded OH-group, alkanes, carbonyl and amide bands. The antimicrobial activity showed the maximum zone of inhibition found in the bacterial activity, *viz*., *E. coli* and in fungal activity, the maximum zone of inhibition was found in *A. niger*. The aqueous shoot extract of *D. regia* was known to be an effective reducing and capping agent promoting efficient reduction of AgNPs and production of crystalline, spherical and stable at much lower concentration of plant extracts. Similar observations were noticed by other workers (Abu-Dief *et al*., 2021; Bala *et al*., 2017; Anitha and Sakthivel, 2016).

Conclusions

The synthesis of silver nanoparticles has been found to be environmentally friendly and cost-effective, with numerous applications in fields such as biosensors, cosmetics, preservatives, antimicrobials, and so on. The aqueous shoot extract of *Delonix regia* was known to be an effective bio reducing and capping agent, promoting efficient reduction of silver nanoparticles and the production of crystalline, spherical, and stable plant extracts and silver salts at much lower concentrations. Various techniques, such as XRD, FTIR, and SEM, are used to characterize the biogenic synthesis of AgNPs. According to the findings of this study, plant-mediated synthesis of biogenic AgNPs has a variety of antimicrobial properties that are useful in pharmaceuticals and medical applications.

Authors' Contributions

Both authors came up with the idea and designed the piece. Sharma B carried out the experiment under Alam A's direct supervision. Sharma B performed all statistical analysis and drafted the manuscript. Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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