

Research Article

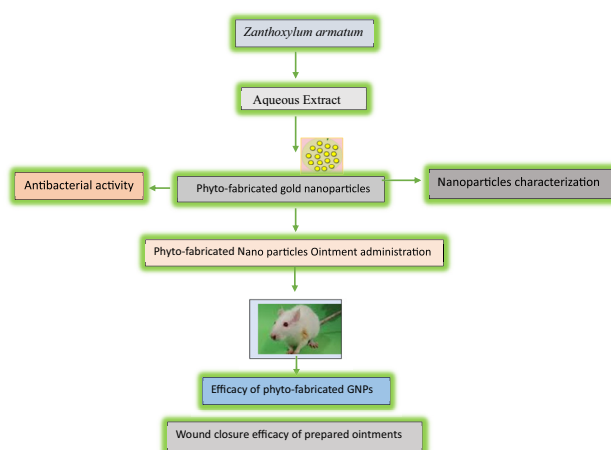
Sumaira Sharif*, Madeeha Shahzad Lodhi*, Iffat Nayila*, Asma Irshad, Mazhar Abbas, Amal Alotaibi, Saima Hameed

Phyto-fabrication and characterization of gold nanoparticles by using Timur (*Zanthoxylum armatum* DC) and their effect on wound healing

<https://doi.org/10.1515/chem-2024-0047>

received April 3, 2024; accepted May 21, 2024

Abstract: Nanotechnology has revolutionized the drug delivery approaches to improve the existing issues of conventional drug delivery systems, especially, the green synthesis of nanoparticles is becoming more suitable for different activities. In this study, phyto-fabricated gold nanoparticles (GNPs) were synthesized using *Zanthoxylum armatum* aqueous extract. GNPs were characterized by different techniques using UV-Vis absorption spectroscopy, X-Ray diffraction (XRD), Fourier transformation infrared spectroscopy (FTIR), Dynamic light scattering (DLS), and scanning electron microscopy (SEM). UV-Vis spectroscopy showed peak at 550 nm. XRD confirmed the crystalline nature of nanoparticles. FTIR showed presence of different functional groups such as C–O, N–H, C=O, C–H, and O–H. SEM and DLS have shown particle size of 30 nm. The antibacterial activity of



Graphical abstract

plant extract and green synthesized GNP were tested against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumonia* which revealed remarkable inhibition of these microorganisms. Burn wound was created on 16 rats and randomized into four groups. Group I was untreated. Group II rats were treated by applying topical ointment cream. Group III rats were treated by applying *Zanthoxylum armatum* cream, and group IV rats were treated by applying GNPs-based cream. Treatment was carried out for 14 days. Wounds treated with GNPs-based cream decreased consistently and almost completely. It can be concluded that GNPs-based cream can be used as an ointment to treat wounds especially burn wounds.

Keywords: *Zanthoxylum armatum*, wound healing, gold-nanoparticles, green synthesis, antibacterial activity

* **Corresponding author: Sumaira Sharif**, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, 1 KM of Defense Road, Postal code: 58810, Lahore, Pakistan, e-mail: sumaira.sharif@imbb.uol.edu.pk

* **Corresponding author: Madeeha Shahzad Lodhi**, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, 1 KM of Defense Road, Postal code: 58810, Lahore, Pakistan, e-mail: madeeha.shahzad@imbb.uol.edu.pk

* **Corresponding author: Iffat Nayila**, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, 1 KM of Defense Road, Postal code: 58810, Lahore, Pakistan; Department of Pharmacy, The University of Lahore, Sargodha Campus, Sargodha, Pakistan, e-mail: iffat.nayila5@gmail.com

Asma Irshad: School of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan

Mazhar Abbas: Department of Basic Sciences (Section Biochemistry), University of Veterinary and Animals Sciences (Jhang Campus), Jhang, 35200, Pakistan

Amal Alotaibi: Department of Basic Science, College of Medicine, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh, 11671, Saudi Arabia

Saima Hameed: Institute for Advanced Study, Shenzhen University, Shenzhen, 518060, P.R. China

1 Introduction

Disinfecting skin from external organisms is one of the most important qualities of a perfect wound dressing because skin shields the body from exogenous chemicals

and organisms. Therefore, poor wound management continues to be one of the clinical issues in healthcare that can result in serious infections and higher rates of morbidity and mortality [1]. Wound treatment facilities are concentrating on the creation of distinctive and cutting-edge methods with significant clinical benefits [2].

The study of nanotechnology has gained interest in a number of disciplines, including medicine, food, agriculture, cosmetics, paints, lubricants, fuel additives, and others. Metal nanoparticles production can be accomplished using a wide variety of chemical and physical methods. Because the chemical methods used to create nanoparticles include harmful and aggressive compounds that serve as capping and reducing agents, they are not thought to be suitable for use in medical applications [3,4]. However, efficient nanomaterials for cutting-edge medical applications have been created through green nanoparticle production using plant extract. Due to their structural characteristics, size, shape, and antioxidant qualities, metal-based nanomaterials are currently attracting increased attention from scientists for nanomedicine applications such as diagnosis and imaging, drug administration, photodynamic treatment, and tissue engineering.

Due to their special characteristics, nanoparticles can be used to treat wound infections. In order to find a more effective medicinal approach, experts have looked into safer green alternatives like plants. Plant-based nanoparticle synthesis is quick, easy, and cost-effective. Additionally, different sizes and forms of these nanoparticles can be created synthetically. Due to their chemical characteristics, optical stability, and simplicity of surface modification, gold nanoparticles (GNPs) have been researched for various medicinal applications such as for use in treating wound healing. Before using GNPs for wound healing, they must have their surfaces modified with other biomolecules [5].

In concordance with collagen, GNPs exhibit dose-dependent skin wound healing abilities. Numerous studies show that using hydrocolloid membranes coated with GNPs greatly slows the rate of wound healing [4]. The anti-oxidative and anti-microbial properties of GNPs were revealed by various investigations into their characteristics, demonstrating a particularly effective aspect in regenerating damaged collagen fibers and enhancing wound healing. GNPs speed up wound healing by boosting anti-inflammatory and anti-angiogenic action [6,7].

Due to its vast application in focused drug administration, imaging, diagnostics, and therapies, as well as their small size, huge surface area, stability, non-cytotoxicity, and adaptable optical, physiological, and chemical properties, GNPs have transformed the world of medicine [8]. GNPs' production mechanism, which uses plant extract,

has a potent antibacterial impact on the particles and facilitates simple salt reduction. This one-step process is appropriate for large-scale manufacturing since it is affordable, rapid, environmentally friendly, and secure for clinical trials [9].

The aim of this study is to prepare green GNPs using *Zanthoxylum armatum* (*Z. armatum*) leaf extract. The efficacy of green GNP-based ointment formulation was checked against burn wound healing in the rat model. The antimicrobial activity of *Zanthoxylum armatum* leaf extract was also compared with green GNPs.

2 Materials and methods

2.1 Collection and preparation of plant extract

Fresh *Z. armatum* was collected from Mirpur, Azad Jammu and Kashmir, identified by Dr. Zaheeruddin Khan from Govt. College University Lahore. It was placed in GC Herbarium center with herbarium no. GC. Herb. Bot-3840. It was washed with distilled water. 20 g leaves were ground in 100 mL of distilled water and filtered through Whatman No. 1 filter paper. The extract was collected and used for the synthesis of GNPs.

2.2 Green synthesis of GNPs using *Z. armatum* leaf extract

Fresh plant extract (5 mL) was mixed with 1 mM HAuCl_4 (95 mL) on the hot plate at 60°C with a magnetic stirrer. After 10–15 min, the solution's color changed from yellow to red wine, indicating the formation of GNPs. The solution was stirred for about 20–30 min to complete the reduction process. The solution was centrifuged at 12,000 rpm, and the obtained nanoparticles were washed with deionized water, dried in the oven, and stored for further use.

2.3 Characterization of phyto-fabricated GNPs

Green synthesized GNPs were fully characterized using several techniques, including UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy (FTIR), Scanning electron microscopy (SEM), X-ray diffraction (XRD), and Dynamic light

scattering (DLS) analysis. The reduction of pure Au^{3+} to Au^0 nanoparticles was analyzed by measurement of the UV-Vis spectrum. FTIR confirmed the functional bio-molecules associated with GNPs. The surface morphology of the prepared sample was evaluated by using SEM. The mean particle size (Z-average-nm) and Polydispersity Index of the prepared nanoparticles were measured by the DLS technique employing Malvern Zeta sizer (Nano ZS). Nanoparticles' crystalline nature was studied using XRD analysis by using Debye-Scherrer equation $D = K\lambda/\beta\cos\theta$ [7].

2.4 Preparation of ointment base

A mixture of 21% w/w of olive oil, 5% w/w of shea butter, 38% w/w of coconut oil, 15% w/w of beeswax, and 21% w/w of avocado oil was melted at a temperature of 40°C and well mixed. At room temperature, the mixture was cooled down. The topical ointment base was used as a negative control without adding *Z. armatum* extract and its synthesized GNP.

2.5 Preparation of ointment by using *Z. armatum* extract and its synthesized nanoparticles

Two types of ointments were prepared by using this ointment base. In the first type of ointment, the plant extract was added to the ointment base as active material, and in the second, green synthesized GNPs were added as the active material. 0.5 g GNPs dried powder was mixed with 40 mL of ointment base cream. The concentration of plant extract used to synthesize 0.5 g GNP was mixed in an ointment base to synthesize another ointment.

2.6 Animals model

Healthy Sprague Dawley albino rats of about equal age and weight (160–210 g) were used for the experiment; animals were housed in polypropylene cages at $25 \pm 2^\circ\text{C}$ within the university animal residence and maintained on pallet feed and water for 2 weeks before the experimentation. Sixteen healthy rats were randomly assigned to four separate groups: group I (control), group II (ointment base), group III (plant extract-based ointment), and group IV (GNPs-based ointment). Hair on the dorsal surface of rats was shaved and then removed with cream. Ketamine (50 mg/kg body weight) was injected into the rats intraperitoneally

and anesthetized. After that, an iron metal was put into boiling water at 100°C for 5 min to make it hot. The partial full-thickness burn (second-degree burn) was induced in an area of 2×2 cm pressing the rod gently on the shaved region [10,11].

2.7 Animal's pre toxicity evaluation

HDF cell lines were obtained from Institute of Molecular Biology and Biotechnology, The University of Lahore. Cell lines were cultured in DMEM and supplemented with 10% FBS and 1% penicillin-streptomycin.

The effect of GNPs on cell viability was measured using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay [12]. Cells were exposed with different concentrations of bio synthesized GNPs and *Z. armatum* plant extract (1, 50, 100, and 200 $\mu\text{g/mL}$) for 24 h. Then, 20 μL /well MTT (5 mg/mL) was added to each well and was incubated at 37°C for 4 h. Finally, 100 μL of DMSO was added to the wells. The absorbance at 570 nm was measured with an ELISA reader [13].

2.8 Experimental design

After the burn wound was implanted in all the rats, the ointments were applied topically except in the negative control group. A simple ointment base was applied to group II, the extract-based ointment was applied to group III, and GNP-based ointment was applied to group IV. No treatment was given to the control group. After applying ointment topically, the sterilize gauze was used to cover the wound area. The bandages were changed and ointment was applied daily. The size of the wound was measured after 2–3 days. Within 14 days, the burn wound was recovered in one group. The size of the wound was measured by placing butter paper on the wound area and marked with a marker according to the shape of the wound.

2.9 Antimicrobial efficacy of phyto-fabricated GNPs against wound-collected microorganisms

The antimicrobial activity of Phyto-fabricated GNPs and *Z. armatum* extract was tested against *S. aureus*, *B. cereus*, *E. coli*, and *K. pneumonia*. The disc diffusion method

evaluated the antimicrobial potential of green synthesized GNPs and *Z. armatum* extract. 100 μL of microorganisms comprised suspension was swabbed uniformly using sterile cotton swabs on agar plates. 6 mm diameter discs were saturated with green synthesized GNPs, *Z. armatum* plant extract, and tetracycline as a positive control. These inoculated plates were then incubated at 37°C for 24 h. The inhibition zones were measured in terms of diameter, i.e., mm. The procedure was done in triplicates [14].

2.10 Statistical analysis

The data were analyzed using one way ANOVA with SPSS/14 computer software (SPSS Inc., Chicago, IL). Results were presented as mean value $\pm$ SD.

3 Results and discussion

3.1 UV-Vis spectra

Figure 1a shows the UV-visible spectrum of GNPs synthesized using *Z. armatum* extract. The spectrum of the GNPs shows the blue shift that comprehends the conversion of Au^+ to Au^0 . The maximum absorbance peak is observed at

the wavelength of about 543 nm with absorption spectra between 510 and 550 nm, which can be regarded because of the d-d transition of Au^+ ions. UV results show that these ions disappeared after the synthesis of GNPs. The disappearance of ions indicated that the cation of Au^+ had been reduced completely.

3.2 FTIR

FTIR spectrum shows a broader peak at $3,440\text{ cm}^{-1}$ of the O-H bond, indicating aromatic, phenol, and alcoholic compounds (Figure 1b). Peaks at $1,650$ and $1,658\text{ cm}^{-1}$ attributed to the presence of amide groups. Some smaller peaks at $1,071$, $1,370$, and $1,412\text{ cm}^{-1}$ confirm the presence of C-O bonds, N-H bonds, and stretching of C-H, respectively. A vast difference has been seen between two peaks present at $1,650$ and $3,440\text{ cm}^{-1}$ that attributed to the contributory part of O-H and C=O groups to reduce the gold(III) ions to gold atoms. Therefore, alcoholic, phenolic, and carboxylic compounds reduced and stabilized GNPs.

3.3 SEM

Figure 1c shows that the average particle size of green synthesized GNPs is almost 30 nm. These GNPs are irregularly spherical, with an average particle size of about

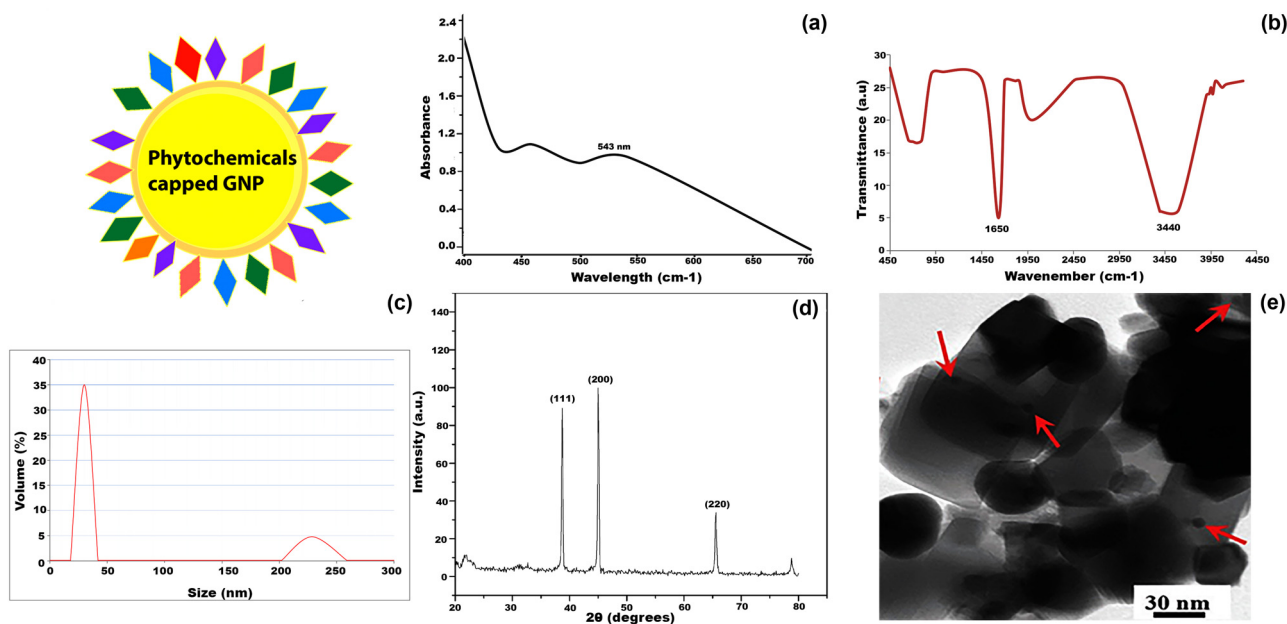


Figure 1: (a) UV-VIS spectrum of green-synthesized GNPs, (b) FTIR spectrum of green-synthesized GNPs, (c) particle size of green-synthesized GNPs, (d) XRD pattern of green-synthesized GNPs, and (e) SEM images of green-synthesized GNPs.

30 nm. The figure also shows some larger GNPs because of the aggregation of small GNPs.

3.4 XRD

The XRD pattern of GNPs synthesized with *Z. armatum* with a concentration of 20% showed Bragg reflection, representing GNP structure. From Figure 1d, the intensity of the peak of (111) present at a diffraction of 38° was much stronger as compared to peaks of (200) and (220) present at a diffraction of 44.2° and 65.5° , respectively. The average mean size of GNPs was considered by implementing the Debye-Scherrer equation by examining the width of (111) Bragg reflection. In the presence of optimum leaf extract, the average diameter from SEM and XRD patterns was 3–30 nm, respectively. The Debye-Scherrer equation is best applicable for monodispersed nanoparticles on a wide range.

3.5 DLS

The polydispersity value of green synthesized GNPs can be seen as 0.437, indicating the perfect distribution of nanoparticles. The non-uniform distribution of nanoparticles may be due to that the nanoparticles synthesized by the green approach give unequal sizes because of the multiple compositions of plants. Based on DLS analysis, the main peak of the GNP curve represents the average size of GNPs, about 30 nm (Figure 1e).

3.6 Efficacy of phyto-fabricated GNPs against wound-collected microorganisms

The antibacterial properties of green synthesized GNPs were examined through MIC and MBC. Table 1 demonstrates the

results of the antibacterial activity of green synthesized GNPs, tetracycline, and *Z. armatum* extract measured using the disc diffusion method. Calculated inhibition zones for microorganisms of *S. aureus*, *B. cereus*, *E. coli*, and *K. pneumonia* were 25.18 ± 0.4 , 24.21 ± 0.4 , 14.23 ± 0.3 , and 13.56 ± 0.2 , respectively.

Green synthesized GNPs demonstrate high antibacterial activity compared to *Z. armatum* extract. The inhibition zones of *Z. armatum* extract for *S. aureus* and *B. cereus* were 15.10 ± 0.2 and 20.21 ± 0.3 , respectively. GNPs have shown prominent inhibition zone against all strains. GNPs showed higher sensitivity towards gram-positive bacteria and *Z. armatum* extract to gram-negative bacteria, calculated by MIC test Figure 2.

The MIC and MBC values of GNPs against microorganism are displayed in Table 2. The MIC values for *E. coli* and *K. pneumonia* were 70.83 and 68.74 $\mu\text{g/mL}$, respectively. The MIC values of GNPs for *S. aureus* and *B. cereus* were 34.61 and 37.98 $\mu\text{g/mL}$, respectively. The phyto-fabrication of GNPs plays a significant role in different features of GNPs. The results confirmed that the extract of *Z. armatum* has antibacterial activity, but green synthesized GNPs have a more improved antibacterial effect (Figure 3).

3.7 Cytotoxicity evaluation

The cytotoxicity potentials of GNPs and *Z. armatum* plant extract were measured in different concentrations (1, 50, 100, and 200 $\mu\text{g/mL}$) against HDF cell line using MTT assay for 24 h (Figure 4). Furthermore, the absorbance rate was measured at 570 nm, which displayed excellent viability on a normal HDF cell line at concentrations up to 100 $\mu\text{g/mL}$ for GNPs. *Z. armatum* plant extract exhibit cell viability of 82.3% with 100 $\mu\text{g/mL}$ while GNPs exhibit more cell viability at the same concentration. In addition, it was observed that GNPs did not cause significant cytotoxicity in the range of used concentrations for MTT assay (Figure 4). This demonstrated that GNPs, provide nontoxic activity on cells.

3.8 Wound closure efficacy of prepared ointments

The outcomes of this study show that the wound healing efficacy of the GNPs-based ointment group significantly improved compared to *Z. armatum* extract-based ointment and ointment base group (Figure 5). At day 14 post-wounding, wounds treated with GNP-based ointment decreased

Table 1: Antibacterial activity of green synthesized GNPs, tetracycline, and *Z. armatum* extract

Bacteria	Green synthesized GNPs	Tetracycline	<i>Z. armatum</i> plant extract
<i>S. aureus</i>	25.18 ± 0.4	31.60 ± 0.2	15.10 ± 0.2
<i>B. cereus</i>	24.21 ± 0.4	27.53 ± 0.2	20.21 ± 0.3
<i>E. coli</i>	14.23 ± 0.3	18.90 ± 0.3	10.9 ± 0.1
<i>K. pneumonia</i>	13.56 ± 0.2	16.44 ± 0.3	11.44 ± 0.3

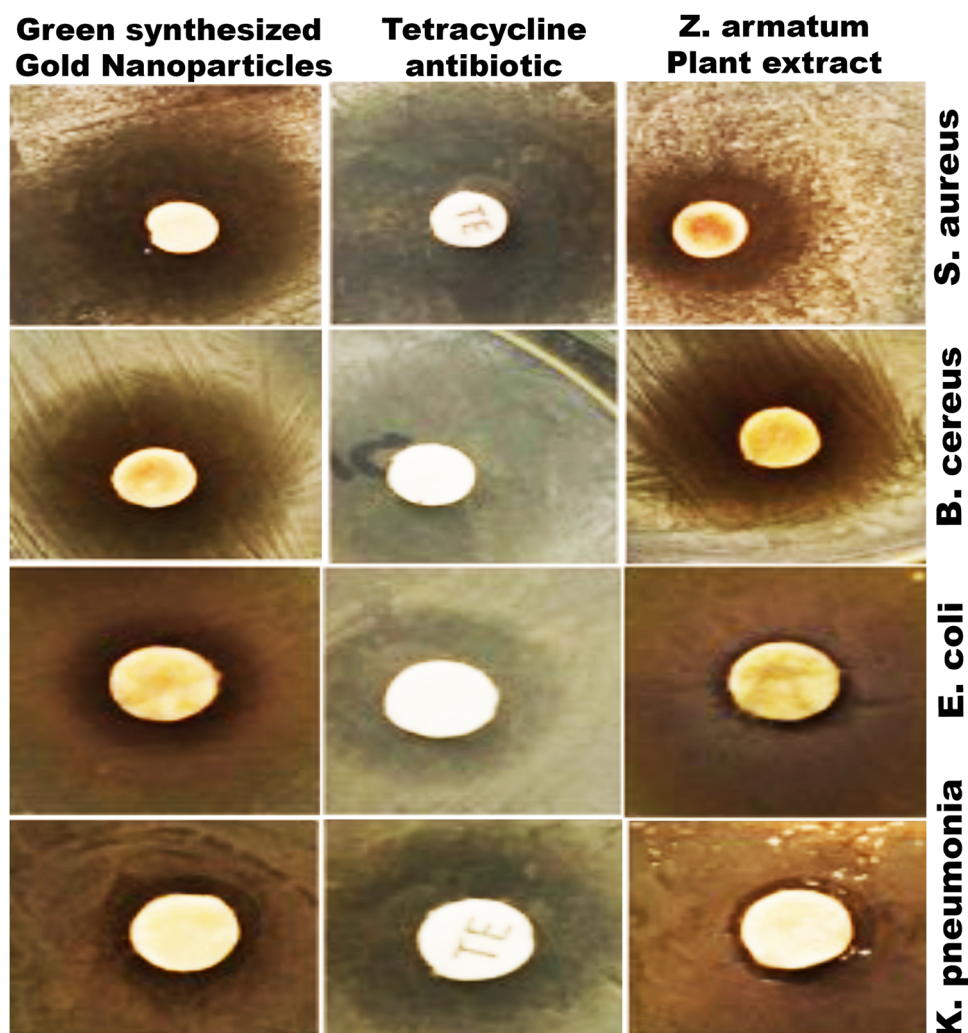


Figure 2: Antibacterial activity of green synthesized GNPs, *Z. armatum*, and tetracycline on *E. coli*, *K. pneumonia*, *S. aureus*, and *B. cereus* calculated using disc diffusion method.

Table 2: MIC values of GNPs against microorganisms

Bacteria	GNPs MIC ($\mu\text{g/mL}$)	GNPs MBC ($\mu\text{g/mL}$)	Tetracycline MIC ($\mu\text{g/mL}$)	Tetracycline MBC ($\mu\text{g/mL}$)
<i>S. aureus</i>	34.61	66	3.45	5
<i>B. cereus</i>	37.98	66	3.71	5
<i>E. coli</i>	70.83	127	4.51	8
<i>K. pneumonia</i>	68.74	127	4.62	8

consistently and almost healed completely. Figure 5 depicts the wound contraction rate, demonstrating the beneficial impact of green synthesized GNPs phyto-fabricated with *Z. armatum*. At day 7 post-wounding, partial wound closure was seen on a wound treated with GNPs-based ointment

(55.5%). At day 12, further advances in wound healing were seen (80.6%), whereas wounds in the negative control group were not healed over the same time (extract-based ointment: 75.6%, ointment base: 70.2%, negative control: 69.2%). At day 14, GNPs showed maximum wound healing activity compared to other groups.

Figure 6 demonstrates the *S. aureus* bacterial growth (log CFU/mL) in different groups of rats. The negative control group shows almost the same number of bacteria on days 3 and 7. The group treated with GNPs-based ointment and extract-based ointment showed a significant reduction ($p < 0.05$) in the total bacteria count after 12 days. No significant differences were observed in the group treated with an ointment base. The reduction in bacterial count by the green synthesized GNPs-based ointment-treated group was more significant ($p < 0.001$).

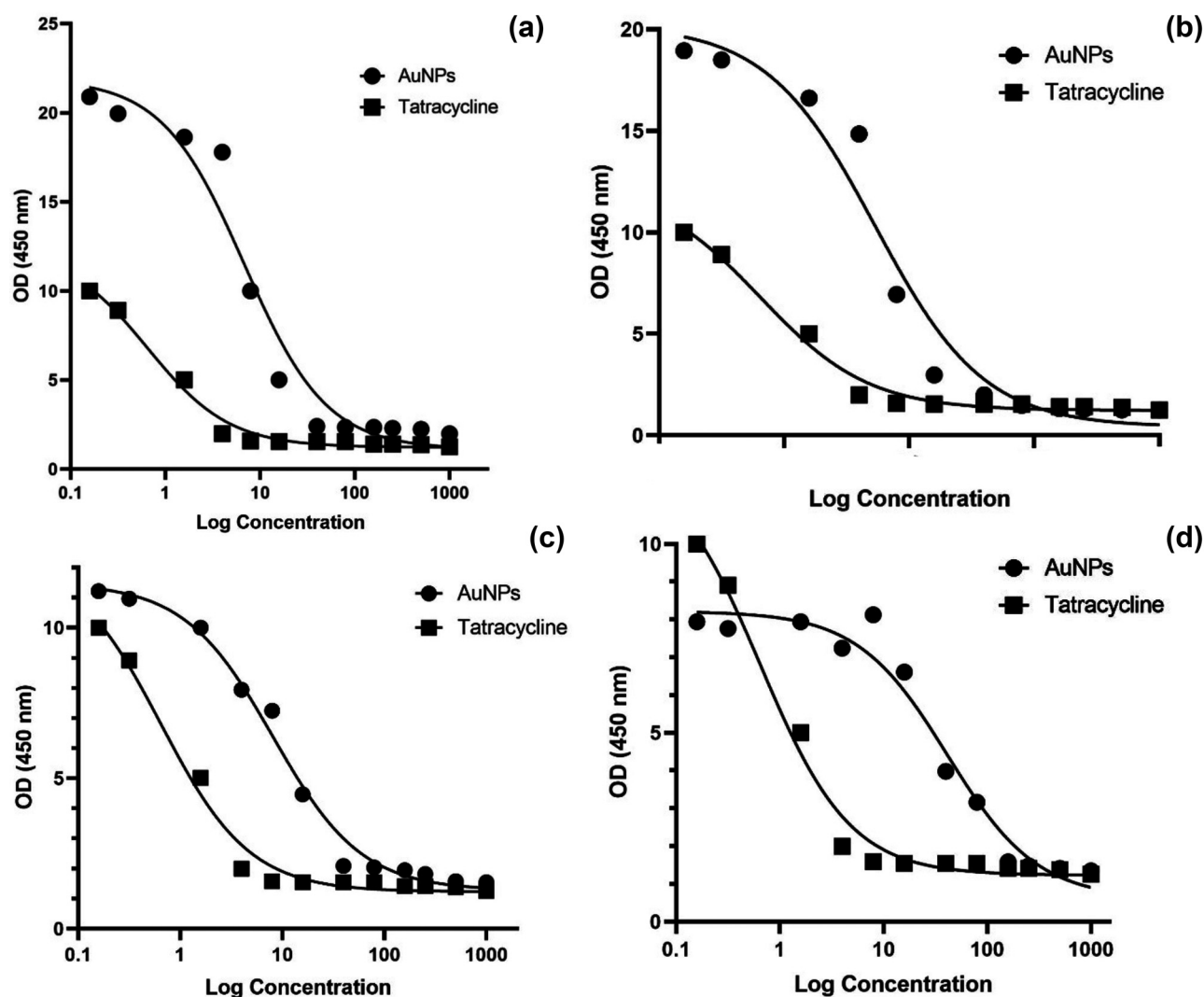


Figure 3: Growth curves of different bacterial strains exposed to green synthesized GNPs and the effective concentration of GNPs: (a) *E. coli*, 70.83 $\mu\text{g/mL}$, (b) *K. pneumoniae*, 68.74 $\mu\text{g/mL}$, (c) *S. aureus*, 34.61 $\mu\text{g/mL}$, and (d) *B. cereus*, 37.98 $\mu\text{g/mL}$.

4 Discussion

Medicinal plants have long been used as external and internal remedies to help heal wounds. They have excellent healing potential as they aid in healing by reducing pain, inflammation, and scarring. *Z. armatum* has been used traditionally, and several studies have shown that it contains phytochemicals with enriched antioxidant, antifungal, anti-inflammatory, and antibacterial properties. Considering the beneficial effects of the selected plant, we objectively evaluated these traditional hypotheses in the present study. Green synthesis of GNPs provides a simple, cost-effective, and eco-friendly alternative to conventional synthesis methods that often involve toxic reducing agents. Green synthesis involves using natural materials, such as plant extracts, as both reducing and stabilizing agents.

In this research project, GNPs were synthesized with *Z. armatum* plant extract, and synthesized GNPs were characterized using FTIR, XRD, SEM, and UV-VIS spectrophotometry. The outcomes demonstrated that the GNPs had a size below 30 nm and verified the successful bio-reduction of gold ions.

These results show that during the production of these GNPs, the *Z. armatum* leaf extract served as a reducing and stabilizing agent. As indicated by FTIR, phenolic chemicals may have been involved, which may have helped to reduce the gold ions. Different researchers extensively assessed GNP cytotoxicity, and GNPs are declared human-friendly metallic nanoparticles. *Z. armatum*-mediated GNPs were also non-cytotoxic to normal fibroblast cells, suggesting their potential as safe agents in therapies. Another study found that the *Z. armatum*-mediated GNPs showed strong antibacterial activity when evaluated. Because of their

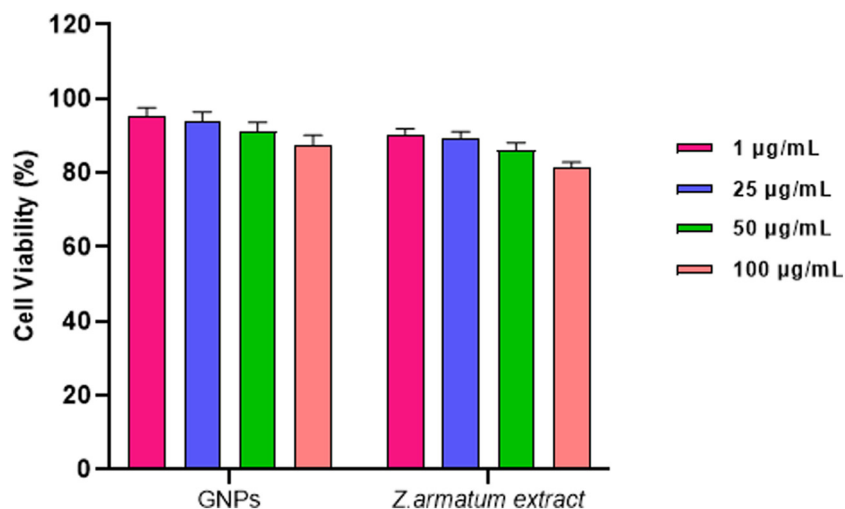


Figure 4: Cell viability of GNPs and *Z. armatum* plant extract after 24 h incubation at different concentrations.

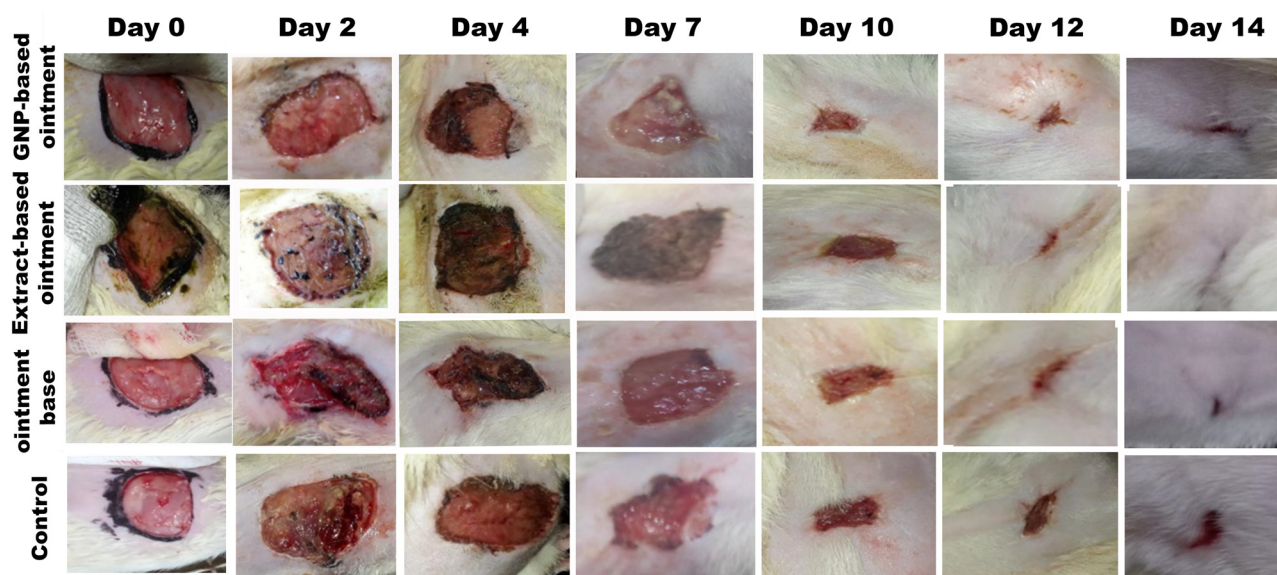


Figure 5: Burn wound of the groups of GNPs-based ointment, *Z. armatum*-based ointment, ointment base, and untreated negative control.

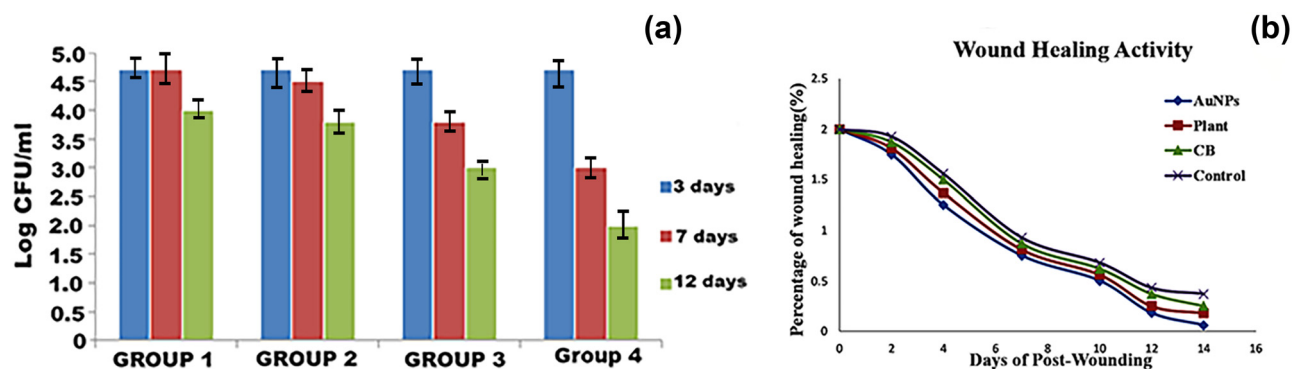


Figure 6: (a) Bacterial count (log CFU/mL) of *S. aureus* in different groups of rats in a burn wound model on different days. (b) Wound healing is displayed as a percentage of wound contraction.

antibacterial, antioxidant, and anti-inflammatory qualities, GNPs have been extensively researched for their potential in wound treatment in terms of wound healing [15]. According to Pivodova et al., there are several possible ways in which GNPs can facilitate wound healing, including via boosting angiogenesis and collagen synthesis [6].

The results of this research project demonstrated that compared to the groups treated with ointment base or plant extract-based ointment, ointment with green synthesized GNPs significantly enhanced wound healing in a burn wound model. Burn injuries treated with cream containing GNPs consistently reduced and nearly disappeared by day 14 post-wounding. At 7 days post-wounding, partial wound closure was observed in wounds treated with GNPs-based cream (55.5%). At day 12, more advanced wound healing was seen (80.6%), in contrast to control groups where wounds took longer to heal (plant: 75.6%, control treated with ointment base: 70.2%, untreated negative control: 69.2%). A cream containing GNPs has also shown significant wound-healing effectiveness. This is partly because GNPs exhibit antibacterial activity that can prevent wound infections and aid in producing extracellular matrix and skin regeneration [16]. Overall, the open wound area was significantly higher in the control groups than in the GNP-based cream-treated rats. The anti-inflammatory, antioxidant, and antibacterial activities of *Z. armatum* may be responsible for this favorable development of skin rejuvenation. GNPs with a high antioxidant capacity are biocompatible, highly reactive, and non-toxic. As a result, it has gained interest as a possible therapeutic target [17].

Consequently, an ointment containing 30 nm-sized GNPs produced by *Z. armatum* could hasten the healing of burn wounds. These encouraging possibilities may enable GNPs to be a practical ingredient in ointments for wound healing. It would take more *in vivo* and *in vitro* research, including clinical trials, to confirm their safety, effectiveness, and possible adverse effects.

5 Conclusion

In the present research, GNPs were generated by the reaction between gold solution and *Zanthoxylum armatum* leaf extract. SEM, UV-Vis, XRD, DLS, and FTIR methods were used to characterize nanoparticles. Based on FTIR spectra, plant functional groups' presence in spectra confirmed GNP synthesis by reducing and capping nanoparticles by plant active groups. SEM and DLS analysis confirmed the size of the synthesized GNPs to be below 30 nm. In the burn wound healing experiments, the application of GNPs-based

ointment improved the burn wound healing significantly, so that they resulted in a decrease in wound area and an increase in the wound contracture. Based on the results, it showed that GNPs had a wide variety of therapeutic and antibacterial properties effective in controlling wound infection and healing burn wounds.

Acknowledgements: Authors wish to thank Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R33), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia for financial support.

Funding information: The research was financially supported by University Researchers Supporting Project number (PNURSP2024R33), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Author contributions: Conceptualization, writing – original draft: S.S. and M.S.L.; preparation and design of the project: I.N.; resources: M.S.L., M.A., and A.A.; review and analysis of the experiments: S.S., A.A., and S.H.; supervision, project administration: S.S., I.N., A.I. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: Authors state no conflict of interest.

Ethical approval: This study was previously approved by the Institution of Molecular Biology and Biotechnology, Bioethical, Biosafety and Biosecurity Committee of Department of Molecular Biology and Biotechnology, University of Lahore with reference number IMBB/UOL/22/12th May 22 and is in accordance with the research guidelines of IMBB, TUOL.

Data availability statement: All data generated or analyzed during this study are included in this published article.

References

- [1] Dubey SP, Lahtinen M, Sillanpaa M. Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of *Rosa rugosa*. *Colloids Surf A: Physicochem Eng Asp*. 2010;364(1):34–41. doi: 10.1016/j.colsurfa.2010.04.023.
- [2] Sharma G, Park J, Sharma AR, Jung JS, Kim H, Chakraborty C, et al. Methoxy poly(ethylene glycol)-poly(lactide) nanoparticles encapsulating quercetin act as an effective anticancer agent by inducing apoptosis in breast cancer. *Pharm Res*. 2015;32(2):723–35. doi: 10.1007/s11095-014-1504-2.
- [3] Haq SI, Nisar M, Zahoor M, Ikram M, Islam NU, Ullah R, et al. Green fabrication of silver nanoparticles using *Melia azedarach* ripened fruit extract, their characterization, and biological properties. *Green Process Synth*. 2023;12(1):20230029. doi: 10.1515/gps-2023-0029.

- [4] Milaneze B, Keijok W, Jairo O, Brunelli P, Janine B, Larissa L, et al. The green synthesis of gold nanoparticle using extract of *Virola oleifera*. BMC Proc 8(4):P29. doi: 10.1186/1753-6561-8-S4-P29.
- [5] Mansoor S, Shahid S, Javed M, Saad M, Iqbal S, Alsaab HO, et al. Green synthesis of a MnO-GO-Ag nanocomposite using leaf extract of *Fagonia arabica* and its antioxidant and anti-inflammatory performance. Nano-Struct Nano-Obj. 2022;29:100835.
- [6] Pivodová V, Franková J, Galandáková A, Ulrichová J. In vitro AuNPs' cytotoxicity and their effect on wound healing. Nanobiomedicine. 2015;2:7–13.
- [7] Noah N. Chapter 6 – Green synthesis: Characterization and application of silver and gold nanoparticles. In: Shukla AK, Iravani S, editors. Green synthesis, characterization and applications of nanoparticles [Internet]. Amsterdam, Netherlands: Elsevier; 2019. p. 111–35. (Micro and Nano Technologies) Available from: <https://www.sciencedirect.com/science/article/pii/B978008102579600006X>.
- [8] Huang X, El-Sayed MA. Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. J Adv Res. 2010;1(1):13–28.
- [9] Chandran K, Song S, Yun SI. Effect of size and shape controlled biogenic synthesis of gold nanoparticles and their mode of interactions against food borne bacterial pathogens. Arab J Chem. 2019;12(8):1994–2006.
- [10] Akhilesh R, Minakshi C, Absar A, Suresh B, Murali S. Synthesis of triangular Au core–Ag shell nanoparticles. Mater Res Bull. 2007;42:1212–20.
- [11] Tomita H, Iwata Y, Ogawa F, Komura K, Shimizu K, Yoshizaki A, et al. P-selectin glycoprotein ligand-1 contributes to wound healing predominantly as a p-selectin ligand and partly as an e-selectin ligand. J Invest Dermatol. 2009;129(8):2059–67.
- [12] Almutary A, Sanderson BS. The MTT and crystal violet assays: Potential confounders in nanoparticle toxicity testing. Int J Toxicol. 2016;35(4):454–62.
- [13] Ibne Shoukani H, Nisa S, Bibi Y, Zia M, Sajjad A, Ishfaq A, et al. Ciprofloxacin loaded PEG coated ZnO nanoparticles with enhanced antibacterial and wound healing effects. Sci Rep. 2014;14:4689.
- [14] Rassaei L, Mika S, Robert W, French F, Richard G, Frank M. Arsenite determination in phosphate media at electroaggregated gold nanoparticle deposits. Electroanalysis. 2008;20:1286–92.
- [15] Rozina AM, Asif S, Klemeš JJ, Mubashir M, Bokhari A, Sultana S, et al. Conversion of the toxic and hazardous *Zanthoxylum armatum* seed oil into methyl ester using green and recyclable silver oxide nanoparticles. Fuel. 2022;310:122296.
- [16] Gan PP, Ng SH, Huang Y, Li SFY. Green synthesis of gold nanoparticles using palm oil mill effluent (POME): a low-cost and eco-friendly viable approach. Bioresour Technol. 2012;113:132–5.
- [17] BarathManikanth S, Kalishwaralal K, Sriram M, Pandian SRK, Youn HS, Eom S, et al. Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. J Nanobiotechnol. 2010;8(1):16.