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Green Synthesis and Evaluation of *Lepidium didymum*-mediated Silver Nanoparticles for *in vitro* Antibacterial Activity and Wound Healing in the Animal Model

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Abstract: Wounds serve as an appropriate medium for the growth of pathogenic bacteria, and bacterial resistance to already available antibiotics demands new and safe approaches in the field of medicine. Silver nanoparticles (AgNPs) exhibited a wide range of applications in biomedicine and emerged as promising nano-antibiotics. The biological preparation of AgNPs by utilizing aqueous plant extract has become an encouraging alternative to traditional chemical methodologies, owing to a viable eco-friendly approach. In the present study, *Lepidium didymum* leaves extract was used for the biosynthesis of AgNPs-LD. The nanoparticles were characterized through UV-Vis spectroscopy, Fourier transforms infrared spectrometry (FTIR), Scanning electron microscopy (SEM), and X-ray diffraction (XRD). The antibacterial activity of AgNPs-LD was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. Further, AgNPs-LD nanoparticles were incorporated into topical gels to evaluate their effectiveness for wound healing in the rat model. UV-visible spectra showed a surface resonance peak around 400 nm correlated with the synthesis of AgNPs; FTIR spectra verified the participation of phytochemicals present in *L. didymum* leaves extract in AgNPs-LD synthesis; and SEM revealed dispersed spherical nanoparticles as well as aggregated clusters. XRD analysis confirmed the crystalline nature, face-centered cubic lattice, and average crystallite size of 21.42 nm. The AgNPs-LD showed promising antibacterial activity against tested strains with a maximum zone of inhibition against *P. aeruginosa* and showed accelerated wound healing capacity comparable to control and standard treatments over the time course of wound healing. The current study concluded that biosynthesized AgNPs-LD nanoparticles are effective as antibacterial agents and are promising novel wound healing products for clinical applications.

Key words: silver nanoparticles, green synthesis, antibacterial efficacy, topical gel, wound healing

1 Introduction

Wounds are tissue injuries resulting in an opening or breaking of the skin caused by physical, chemical, or mechanical damage. They are prone to subsequent bacterial infections inducing inflammation and tissue damage that hinders the healing process eventually presenting significant health problems to patients[1, 2]. Wound healing is a crucial medical issue and a complex process involving regeneration and replacement stages that occur in a chain of events comprised of inflammation, proliferation, and migration of different cell types[3]. Several factors such as infection, contamination, stress, etc. can affect this process, therefore proper wound healing is critical to restoring the disrupted anatomical stability, shortening healing time, minimizing infection risk, and reviving the functional condition of the skin[4, 5]. Various commonly used drugs such as silver sulfadiazine, betaine, and psoralen exhibit toxic effect (side effects) to some extent, which necessitates the search for better ways to promote the wound healing process[6, 7].

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In recent years, nanoparticles have been investigated for their potential as antimicrobial agents and their role in wound healing[8, 9]. Nanoparticles can be defined as materials having one dimension in the nanometer range (1-100 nm)[10]. Among the various metal nanoparticles, AgNPs have been extensively studied for biological applications. Silver metal has inherent antibacterial properties and has been used to treat several diseases such as pleurodesis and skin cauterization[11]. AgNPs have advantages over bulk silver owing to their unusual physicochemical characteristics including nano-scale size, large surface-to-volume ratio, high reactivity, and dramatic increase in the release of silver ions[12].

Several techniques are used for the synthesis of AgNPs. Green synthesis using plant extracts emerged as a simple, cost-effective, safe, reliable, eco-friendly, and sustainable approach[13]. Plant extracts are rich in phytochemicals that work as reducing and capping agents and enable the quick formation of NPs[14]. Although plant extract–mediated synthesis of AgNPs has been explored widely[15], the use of *Lepidium didymum* (*L. didymum*) leaves extract for the synthesis of AgNPs has not been reported previously to the best of the author's knowledge. The *L. didymum*, known as lesser swine cress belongs to the Brassicaceae family used as a home remedy to treat wounds and allergies. The current work is designed to biosynthesize AgNPs using an aqueous leaves extract of *L. didymum*, to characterize AgNPs-LD nanoparticles, to evaluate their antibacterial activity against *S. aureus*, *K. pneumonia*, *P. aeruginosa*, and *E. coli* and to formulate AgNPs-LD topical gel for the investigation of wound healing properties in the rat model.

2 Materials and Methods

2.1 Materials

Silver nitrate ($AgNO_3$) was purchased from Carlo Erba reagents. *L. didymum* leaves were freshly collected from the botanical garden of Bahauddin Zakariya University Multan, Punjab. Carbopol 934® was procured from Avonchem. All the chemicals and reagents used were of analytical grade.

2.2 Biosynthesis of silver nanoparticles using *L. didymum* leaves

L. didymum leaves (5 g) were washed thrice with running tap water and then with distilled water. The washed leaves were boiled in 300 ml of distilled water until the volume was reduced to 100 ml and filtered. The extract was further diluted (Table 1) and used as a reducing and stabilizing agent for nanoparticles synthesis. Different batches of AgNPs-LD nanoparticles were prepared following the composition presented in Table 2. Briefly, silver nitrate solution (1 mM) was added drop wise into *L. didymum* leaves extract in a 100 ml flask and pH was adjusted to 9 by using 1 M NaOH solution. The reaction mixture was continuously stirred and heated (45℃) in the water bath. After a few hours the solution turned from yellow to brownish indicating the formation of AgNPs[16]. In the end, the suspension was centrifuged at 8000 rpm for 20 min. The supernatant was discarded and the pellets were re-dispersed in distilled water. The centrifugation procedure was repeated thrice to remove any phytocompounds or any unbound material from the surface of nanoparticles.

2.3 Characterization of AgNPs-LD

2.3.1 UV-Visible spectroscopy

The optical property of the biologically synthesized Ag-

Table 1 Dilutions of *Lepidium didymum* leaves extract.

Volume of stock solution of plant extract (ml)	Volume of distilled water (ml)	Volume of final solution of extract (ml)	Concentration of final solution of extract (g/100 ml)
10	0	10	5
8	2	10	4
6	4	10	3
4	6	10	2
2	8	10	1

Table 2 Silver nanoparticles with varying concentrations of leaf extract.

AgNPs-LD formulation codes	AgLD1	AgLD2	AgLD3	AgLD4	AgLD5
Volume of 1 mM Silver nitrate solution (mL)	50	50	50	50	50
Volume of extract (ml)	10	10	10	10	10
Concentration of extract (g/100 mL)	5	4	3	2	1

NPs-LD nanoparticles was examined by UV-Visible spectrophotometer (PerkinElmer, Japan) over 300 to 700 nm wavelength[17].

2.3.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of *L. didymum* leaves extracts and synthesized AgNPs-LD nanoparticles were recorded by ATR-FTIR (Bruker IR, Japan) in a transmission range of 4000 cm^{-1} to 400 cm^{-1} [18].

2.3.3 Scanning electron microscopy (SEM)

SEM images of the AgNPs-LD were obtained by scanning electron microscope (Philip model CM 200).

2.3.4 X-ray diffractometry (XRD)

The X-ray diffraction patterns of the AgNPs-LD were recorded using an X-ray diffractometer (JDX 3532; JEOL, Japan) in continuous scan mode over 2θ of 10–80°. The particle size was estimated by using Scherrer's equation[17].

2.4 Antibacterial activity

For the investigation of the antibacterial activity of the AgNPs-LD against *S. aureus*, *E. coli*, *K. pneumonia*, and *P. aeruginosa*, well-diffusion method was applied[19]. Briefly, the stock cultures of the selected bacterial strains were cultured in nutrient broth then the bacterial culture (adjusted to 0.5 McFarland standards) was uniformly spread on Mueller Hinton agar (MHA) plates and 8 mm diameter wells were constructed. Wells were loaded with AgNPs-LD nanoparticles, distilled water (negative control), and the standard disc of tetracycline (positive control). In a separate set of MHA, plates inoculated with the selected bacterial strains antibacterial activity of *L. didymum* leaves extract and standard discs of commonly used antibiotics (imipenem, amikacin, and ciprofloxacin) were evaluated. The MHA plates were incubated at 37℃ for 18 hours and the zone of inhibition was recorded in millimeters[20].

2.5 AgNPs-LD loaded topical gel preparation

For the preparation of AgNPs-LD loaded carbopol topical gel AgLD1 nanoparticles (1% and 1.5%) were dispersed in 20 ml distilled water. 0.5% (w/w) Carbopol® 934 was added slowly into AgNPs-LD suspension with continuous stirring for an hour. Once carbopol was completely hydrated, 2-3 drops of triethanolamine were added to neutralize the dispersion, resulting in the formation of the gel. In the end, glycerol (2% w/w) was added to the gel preparation as humectants[21].

2.6 Characterization of AgNPs-LD topical gel

The AgNPs-LD topical gel was evaluated for physicochemical properties including color, odor, consistency, homogeneity, and pH. The pH of the aqueous solution of gels (1%) was recorded by using pH meter[22].

2.7 Skin irritation-test

The AgNPs-LD topical gel was applied on the back side of shaved healthy rats (n = 6) and signs of erythema/edema were recorded at 6, 12, 24, and 48 hours[23].

2.8 *In vivo* wound healing studies

Adult male Wistar rats weighing 145 grams each were acquired from the animal house of the faculty of Pharmacy, Bahauddin Zakariya University (BZU), Multan, Pakistan. The study protocol was reviewed and approved by the Pharmacy ethical committee, faculty of Pharmacy, BZU, Multan (193/PEC/2021). During and before one week of experiments, animals were housed under controlled temperature (25 ± 1℃) with a light/dark cycle (12/12 hr), and free access to the standard diet and water.

The animals were divided into four groups (n = 6). Lignocaine gel was used to anesthetize the rats. The dorsal side of the rats was shaved and cleaned with 70% ethanol and wounds of 1 cm were produced with the help of a razor. Group A rats were kept untreated as a control. Animals in group B were treated with Quench cream (1% silver sulfadiazine) as standard. Group C rats were dressed with gel formulation of AgNPs-LD 1% and group D rats were dressed with AgNPs-LD 1.5% gel. Formulations were daily applied to the wound. The wound area was photographed and measured on days 0, 3, 7, and 9. The percentage of wound contraction was calculated as follows;

$$\%\ \text{wound contraction} = \frac{W0 - Wd}{W0} \times 100$$

Where, *W0* is the diameter of the wound on day 0 and *Wd* is the diameter of the wound on the day of measurement i.e., days 3, 7, and 9.

2.9 Statistical analysis

The diameter of the wound and % wound healing were recorded as mean values ± standard deviation (SD). Data of percent wound healing were statistically compared by one-way ANOVA followed by the least significant difference (LSD) test applied to identify the intergroup comparisons using the SPSS software program (SPSS Inc. Chicago, IL USA) and p-value < 0.05 attributed as statistically significant[24].

3 Results and Discussion

3.1 Visual analysis

Aqueous extracts of different medicinal plants have been reported for the biogenic synthesis of AgNPs[25, 26]. In the current study, an aqueous extract of *L. didymum* leaves was employed to biosynthesize AgNPs-LD. The synthesis was initiated with the addition of 1 mM $AgNO_3$ solution to the aqueous extracts of *L. didymum* leaves, the color of the extract gradually modified from yellow to brownish, which indicated the formation of silver nanoparticles. The transformation of color in the course of Ag nanoparticle

synthesis is related to the excitation effect of surface plasmon resonance (SPR)[27].

3.2 UV-Vis spectroscopy analysis

UV–visible spectroscopy is a crucial and common technique used to establish the formation of stable metal nanoparticles and to confirm the reduction of Ag ions[17]. The SPR phenomenon arises when metal nanoparticles are irradiated with electromagnetic waves in the visible range. UV–Visible spectroscopy indicated the SPR band around 400 nm suggesting the formation AgNPs (Fig. 1). Formulations prepared by using a low concentration of plant extract (e.g., AgLD5) showed a broader SPR band. However, a sharper and more intense peak was observed in formulations prepared with a high concentration of extract. Broader peaks at lower concentrations suggesting large anisotropic particles[28] might be due to low concentration of functional groups/ biomolecules (such as phenols, alkaloids, sugar) available for capping and stabilization of AgNPs. The increase in the intensity of the peak in AgLD1 might be attributed to the increased number of nanoparticles formed owing to the availability of more biomolecules in leaf extract[26, 29].

3.3 FTIR analysis

FTIR analysis was executed to recognize the major functional groups in the *L. didymum* leaves extract and their potential participation in the reduction, capping, and stabilization of AgNPs-LD nanoparticles. The FTIR spectra of leaves extract and AgNPs-LD are depicted in Fig. 2. The FTIR spectra of *L. didymum* leaves extract showed a band around 3277.6 cm^{-1} indicating O–H stretching vibration of high concentration of alcoholic phytochemicals, a peak at 1577.36 cm^{-1} suggesting amide group, the peak at 1390.98 cm^{-1} owing to -C-O- group (phenolic groups) and peak at 1076.69 cm^{-1} suggesting C-O stretch vibrations[30, 31]. The FTIR spectra of AgNPs-LD nanoparticles also showed a broad band at 3303.25 cm^{-1} and 3339.33 cm^{-1} representing the O-H stretch of alcohols and phenols, respectively. Peaks observed at 2917.75 cm^{-1} and 2916.063 cm^{-1} representing stretching in C-H bonds of aromatic compounds[32]. The bands at 1574.12 cm^{-1} and 1562.23 cm^{-1} indicated characteristic peaks of amide linkage emerging from carbonyl stretch in proteins. The bands at 1101.67 cm^{-1} and 1108.51 cm^{-1} indicated the association of C–N bond in aliphatic amines. The peaks around 872 cm^{-1} are attributed to $C=CH_2$. The major peaks observed in the spectra of leaves extract were also present in the spectra of AgNPs-LD with slight shifts, thus confirming the involvement of various phytochemicals (proteins, alkaloids, phenols) present in leaves extract in reduction, capping and stabilization of AgNPs-LD.

3.4 SEM analysis

The surface morphology of the AgNPs-LD was analyzed using Scanning Electron Microscope. SEM image (Fig. 3) had shown mostly dispersed spherical AgNPs-LD nanoparticles. Aggregates of nanoparticles with no well-defined morphology were also observed. The nanoparticles were held together due to hydrogen bonding and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs[17, 33].

3.5 XRD analysis

The XRD analysis of the synthesized AgNPs-LD showed high-intensity diffraction peaks at 38.73°, 45.02°, 67.64° and 76.88° confirming the crystalline nature of the nanoparticles (Fig. 4). The mentioned peaks correspond to (*hkl*) planes (111), (200), (220) and (311) Bragg reflections indicating face-centered cubic lattice structure[17, 34]. The Full Width at Half Maximum (FWHM) values were used to estimate the average crystallite size using Scherrer's equa-

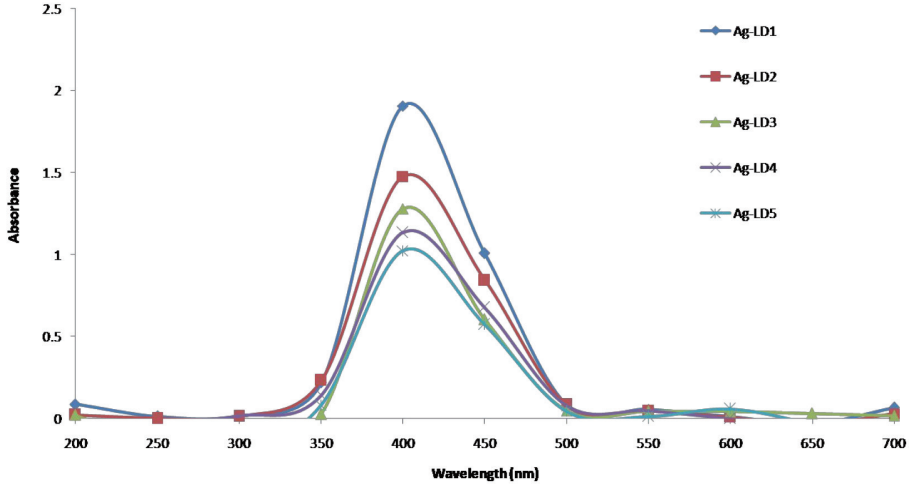

Fig. 1 UV-Vis spectra of AgNPs synthesized by different concentrations of *Lepidium didymum* leaves extract.

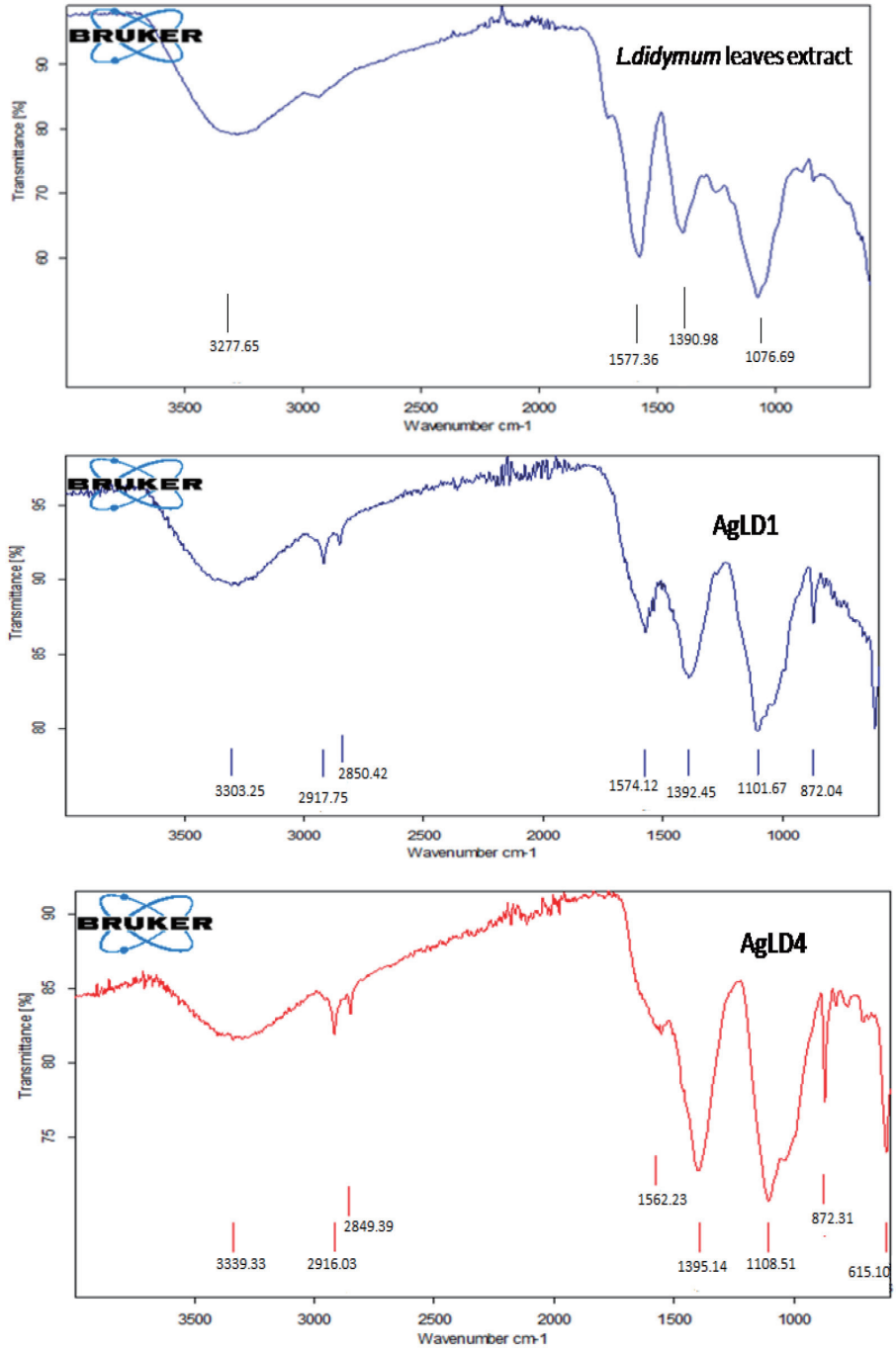

Fig. 2 FTIR spectra of *Lipidium didymum* leaves extract and AgNPs-LD.

tion[35]. The average size of the AgNPs-LD was 21.42 nm.

3.6 Antibacterial activity of AgNPs-LD

Silver nanoparticles can firmly bind with the negatively charged bacterial membrane, which leads to a change in the permeability of the membrane and induces oxidative stress that eventually kills the bacterial cells[36]. Our study showed that AgNPs-LD exhibit significant antibacterial activity against all the tested bacterial strains (Fig. 5) with maximum activity against *P. aeruginosa* and least activity against *K. pneumonia*. The positive control (standard tetracycline disc) has shown no activity against *P. aeruginosa*; 19 mm ZOI against *E. coli* and *S. aureus*; and 17 mm of ZOI against *K. pneumonia*. Distilled water as a negative control was used to determine the effect of solvent in the test solution on the growth of tested strains, no ZOI was observed in all cases indicating that solvent has no role in the antibacterial activity of Ag nanoparticles. The AgNPs-

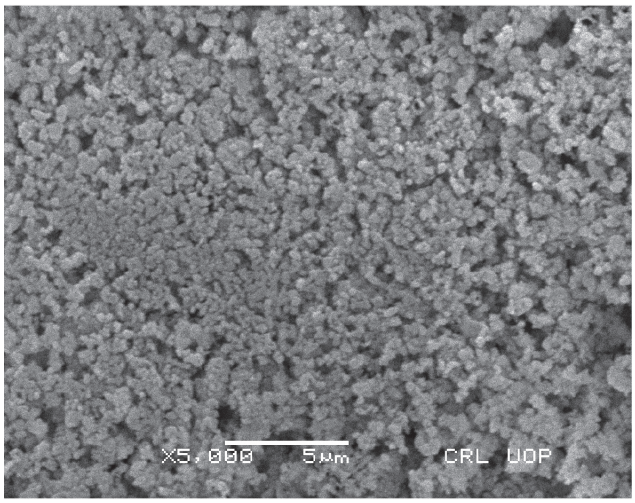

Fig. 3 SEM image of AgNPs-LD nanoparticles.

LD have shown 15 mm to 18 mm ZOI against *P. aeruginosa*, 11 mm to 14 mm ZOI against *E. coli*, 9 mm to 13 mm of ZOI against *S. aureus* and 7 mm to 9 mm of ZOI against *K. pneumonia*. The diameter of ZOI seems to be increased with increasing concentration of *L. didymum* leaf extract used for the preparation of AgNPs-LD nanoparticles in almost all cases might be related to the presence of more surface-bound biomolecules enhancing the antibacterial activity of the AgNPs.

The antibacterial activity of plant leaves extracts and other commonly used antibiotics (imipenem, amikacin, and ciprofloxacin) were also examined. It can be observed in Fig. 6 that *L. didymum* leaves extract has shown the least activity in the case of all tested strains as compared to the biogenically synthesized AgNPs-LD indicating that synergistic interaction of silver ions and bio-molecules present in leaf extract resulted in the formation of stable AgNPs-LD with better antibacterial activity in comparison to LD-leaves extract alone. In the case of *E. coli* leaves extract has shown no activity however the AgNPs-LD have shown large ZOI (14 mm) comparable to that of positive control (19 mm). AgNPs-LD might easily penetrate inside the bacterial cell wall owing to its small size and large exposed surface area allowing possible interaction of Ag with cell wall components and destruction of the cell[37,38].

3.7 Characterization of AgNPs-LD topical gel

The carbopol topical gel base was transparent and colorless while AgNPs-LD topical gels were brownish-black in color due to the incorporation of AgNPs-LD. The pH values of gels were in the range of 6-7 which is suitable for topical application. The homogeneity of all gel compositions was excellent with no lumps.

3.8 Skin irritation test

The AgNPs-LD topical gels had not induced any skin irritation as no signs of erythema and/or edema were recorded in the skin irritation test. Thus, AgNPs-LD topical gels appeared to be biocompatible formulations and can be further evaluated for *in vivo* wound healing studies in animal models[23].

3.9 *In vivo* wound healing

Untreated wounds are susceptible to infections caused by bacteria, particularly *S. aureus*. The broad spectrum antimicrobial activity exhibited by AgNPs has encouraged the development and evaluation of AgNPs-based drug delivery systems for wound healing. AgNPs can treat inflam-

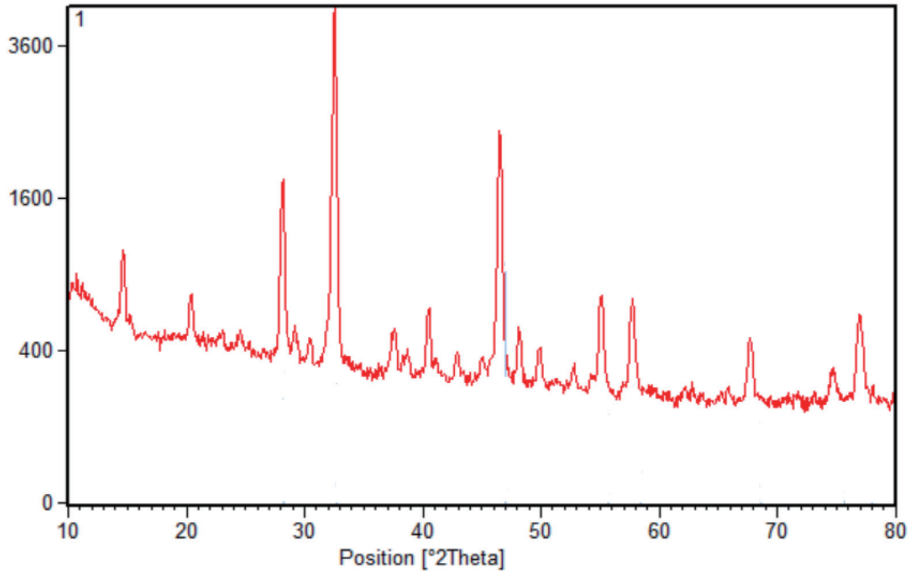

Fig. 4 XRD pattern of AgNPs-LD nanoparticles.

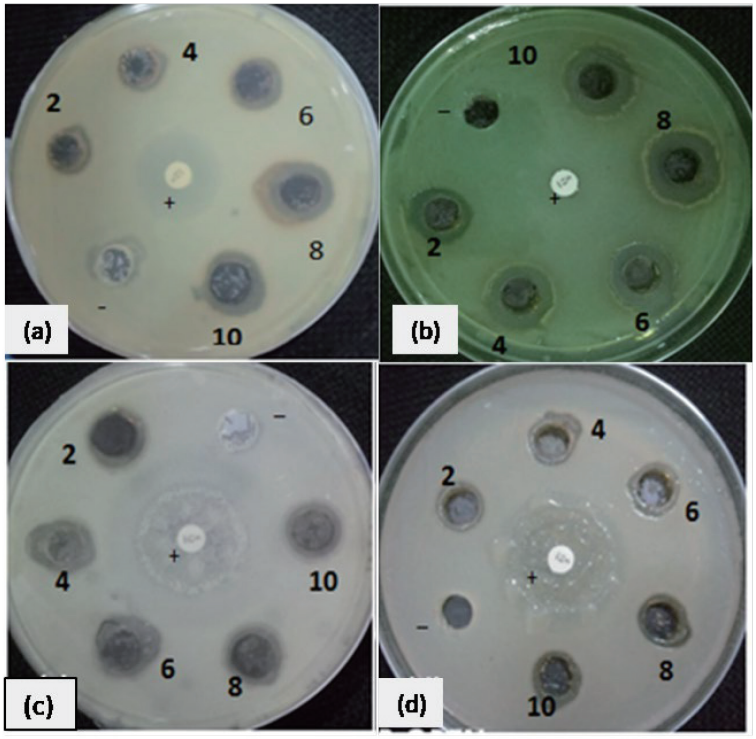

Fig. 5 Antibacterial activity of AgNPs-LD, positive control and negative control against (a) *S. aureus* (b) *P. aeruginosa* (c) *E. coli* (d) *K. pneumoniae*. "2" represent AgLD5, "4" represent AgLD4, "6" represent AgLD3, "8" represent AgLD2 and "10" represent AgLD1.

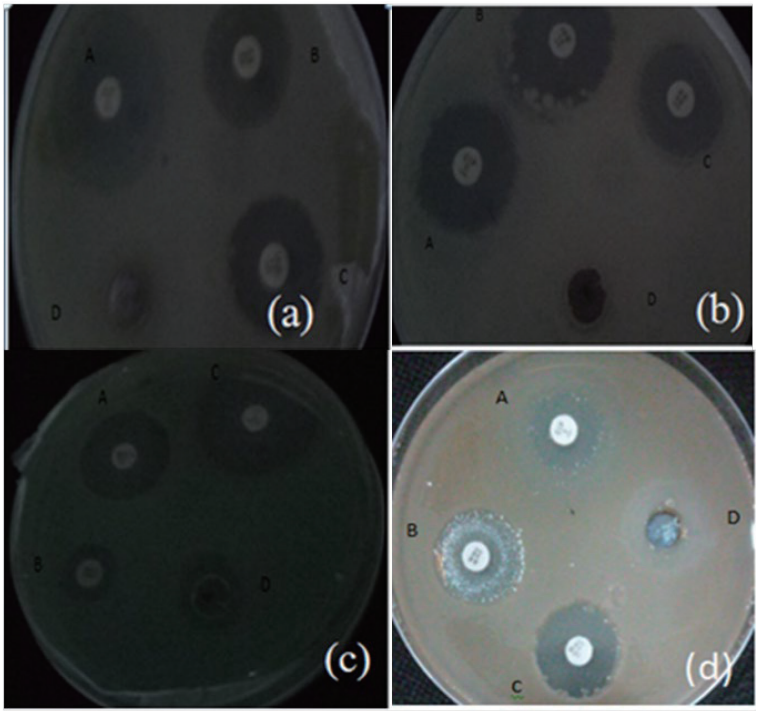

Fig. 6 Antibacterial activity of ciprofloxacin (A), imipenem (B), amikacin (C) and *L. didymum* leaves extract (D) against (a) *S. aureus* (b) *P. aeruginosa* (c) *E. coli* (d) *K. pneumoniae*.

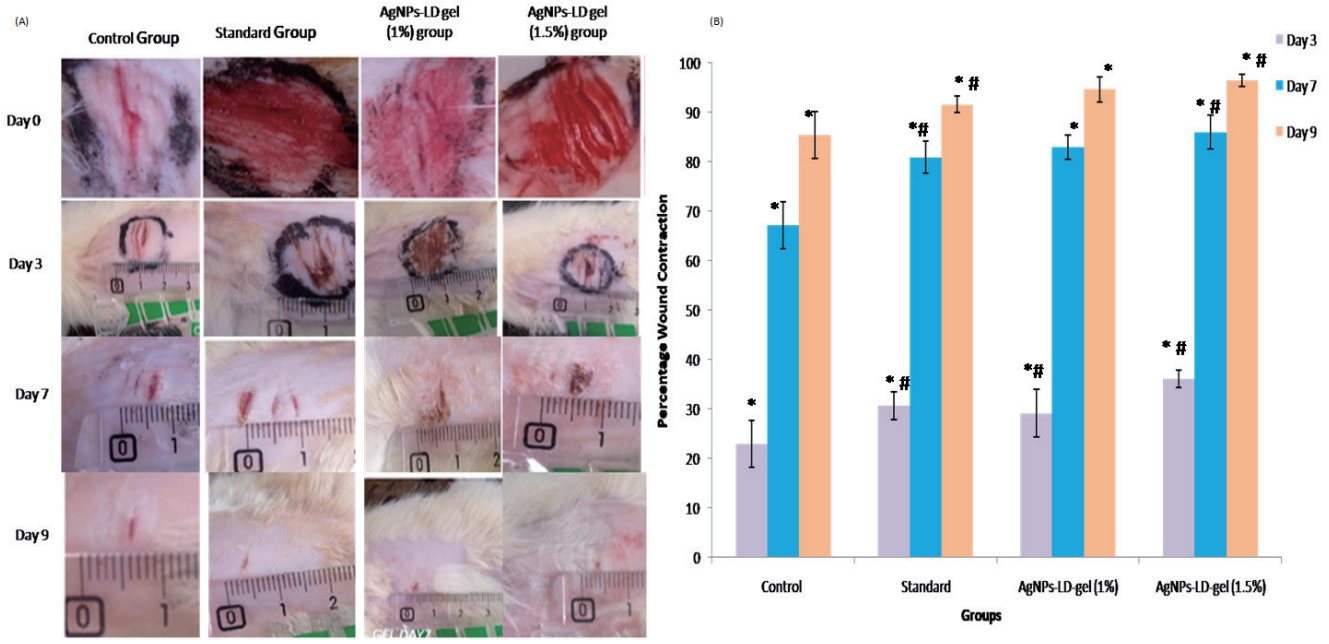

Fig. 7 (A) Photographs of wounds showing average diameter (B) Percent wound contraction on day 3, 7 and 9. Data expressed as mean ± SD. *Group B, C and D vs Group A; # Group 4 vs Group 3 and Group 2. (* and # differences are statistically significant)

mation by modulating cytokine and decreasing the release of inflammatory cytokines and growth factors, and inducing wound healing with decreased scar formation[39, 40]. Therefore, our group has incorporated the AgNPs-LD into gel formulation to evaluate the wound healing potential of the synthesized AgNPs-LD. The percent wound healing of AgNPs-LD gel was compared with Quench cream, a commercially available cream containing 1% silver sulfadiazine used for the treatment of wounds and burns.

The wound diameters were measured and photographed as part of the quantitative examination of wound healing on the 3rd, 7th, and 9th day (Fig. 7A) of treatment. The rate of wound contractions in various groups was calculated, recorded, statistically analyzed, and represented in Fig. 7B. It was observed that the percentage of wound healing was statistically higher in all treatment groups in comparison to the control group which was left untreated. On day 3 AgNPs-LD 1.5% gel group (Group D) revealed statistically significant % wound contraction (36.14% ± 1.76) compared to control (22.94% ± 4.67), standard (30.66% ± 2.72) and AgNPs-LD 1% group (29.15% ± 4.86). The rapid wound healing in animals treated with AgNPs-LD 1.5% gel might be due to the high concentration of silver nanoparticles in the gel. AgNPs-LD 1% gel group (Group C) showed statistically significant % wound contraction as compared to the control group but statistically insignificant percent wound contraction when compared to the standard group. The wound healing percentage was almost the same for both the standard group treated with silver sulfadiazine 1% and AgNPs-LD 1% group treated with a gel containing 1% silver nanoparticles. On day 7, group D revealed a percent wound contraction of 85.93% ± 3.42, while in Group A, B, and C the percent wound contraction was 67.17% ± 4.71, 80.88% ± 3.2 and 82.99% ± 2.45, respectively. It was found that the wound contraction percent for AgNPs-LD 1.5% group was statistically significant in comparison to the control and standard group but insignificant for AgNPs-LD 1% group. On day 9, wounds of AgNPs-LD gel groups were almost completely healed and percent wound contraction was up to 94.65% ± 2.52 for group C and 96.47% ± 1.18 for group D however there was no statistical difference among the two groups. While on the other hand wounds of the control and the standard group had a wound contraction percent of 85.42% ± 4.67 and 91.57% ± 1.68, respectively. The results indicated that AgNPs-LD topical gels have demonstrated enhanced wound healing effects with no scar. Our results are in good correlation to published studies[41, 42], where AgNPs have been incorporated into hydrogels resulting in a gradual decrease in wound size over time. AgNPs are reported to be biocompatible and owing to their anti-inflammatory properties, the topical application of AgNPs in the wound area reduces the release of cytokines and lymphocytes, and also reduces mast cell infiltration, which then promotes wound healing with minimal scarring[43].

4 Conclusion

Aqueous extract of fresh leaves of *L. didymum* can be used as cost-effective, safer, and eco-friendly reducing and capping agents to produce silver nanoparticles. AgNPs-LD showed efficient antibacterial activities against *P. aeruginosa*, *E. coli*, *K. pneumonia*, and *S. aureus*. The wound healing capacity of AgNPs-LD 1.5% gels showed significant wound healing activity compared to commercially available silver sulfadiazine cream, suggesting AgNPs-LD have a beneficial role in the acceleration of the wound healing process.

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Author's Contribution

F. Deeba designed, supervised the study, and proofread the manuscript. S. Parveen executed experimental work and wrote the initial draft. Z. Rashid designed, supervised the project, did statistical analysis, and wrote the final draft. A. Aleem provided technical support for *in vivo* wound healing studies. H. Raza assisted in characterization. All authors approved the final version of the manuscript.

Conflict of Interests

The author declares no conflicts of interest.

References

1) Tavakoli, S.; Klar, A.S. Advanced hydrogels as wound dressings. *Biomolecules* **10**, 1169(2020). doi: 10.3390/biom10081169
2) Sood, A.; Granick, M.S.; Tomaselli, N.L. Wound dressings and comparative effectiveness data. *Adv. Wound Care* **3**, 511-529(2014). doi: 10.1089/wound.2012.0401
3) Broughton, G.; Janis, J.E.; Attinger, C.E. The basic science of wound healing. *Plast. Reconstr. Surg.* **117**, 12S-34S(2006). doi: 10.1097/01.prs.0000225430.42531.c2
4) Zhe, Li.; Milionis, A.; Zheng, Y.; Yee, M.; Codispoti, L. *et al.* Superhydrophobic hemostatic nanofiber composites for fast clotting and minimal adhesion. *Nat. Commun.* **10**, 5562(2019).
5) Farghaly Aly, U.; Abou-Taleb, H.A.; Abdellatif, A.A.; Sameh Tolba, N. Formulation and evaluation of simvastatin polymeric nanoparticles loaded in hydrogel for optimum wound healing purpose. *Drug Des. Devel. Ther.* **13** 1567-1580(2019). doi:10.2147/DDDT.S198413
6) Çevirme, D.; Savluk, Ö.F.; Bas aran, E.K.; Aksoy, R.; Elibol, A. *et al.* Effects of anticoagulant drugs on wound healing process in a rat model: A comparative study. *J. Wound Care* **29**, 44-50(2020). doi: 10.12968/jowc.2020.29.1.44
7) Riepl, M. Compounding pearls – wound care: Drugs and formulations for dermal healing. *Int. J. Pharm. Compd.* **25**, 100-103(2021).
8) Naraginti, S.; Kumari, P.L.; Das, R.K.; Sivakumar, A.; Patil, S.H. *et al.* Amelioration of excision wounds by topical application of green synthesized, formulated silver and gold nanoparticles in albino wistar rats. *Mater. Sci. Eng. C Mater. Biol. Appl.* **62**, 293-300(2016). doi.org/10.1016/j.msec.2016.01.069
9) Shanmuganathan, R.; Karuppusamy, I.; Saravanan, M.; Muthukumar, H.; Ponnuchamy, K. *et al.* Synthesis of silver nanoparticles and their biomedical applications - a comprehensive review. *Curr. Pharm. Des.* **25**, 2650-2660(2019). doi:10.2174/1381612825666190708185506
10) Jeevanandam, J.; Barhoum, A.; Chan, Y.S.; Dufresne, A.; Danquah, M.K. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein J. Nanotechnol.* **9**, 1050-1074(2018). doi: 10.3762/bjnano.9.98
11) Antonangelo, L.; Vargas, F.S.; Teixeira, L.R.; Acencio, M.M.P.; Vaz, M.A.C. *et al.* Pleurodesis induced by talc or silver nitrate: Evaluation of collagen and elastic fibers in pleural remodeling. *Lung* **184**, 105-111(2006). doi: 10.1007/s00408-005-2569-9
12) Lok, C.-N.; Ho, C.-M.; Chen, R.; He, Q.-Y.; Yu, W.-Y. *et al.* Silver nanoparticles: Partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem.* **12**, 527-534(2007). doi:10.1007/s00775-007-0208-z
13) Ahmed, S.; Ahmad, M.; Swami, B.L.; Ikram, S. Plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *J. Adv. Res.* **7**, 17-28(2015). doi.org/10.1016/j.jare.2015.02.007
14) Chandran, S.P.; Chaudhary, M.C.; Pasricha, R.; Ahmad, A.; Sastry, M. Synthesis of gold nanotriangles and silver nanoparticles using aloe vera plant extract. *Biotechnol. Prog.* **22**, 577-583(2006). doi: 10.1021/bp0501423.
15) Nancy, J.; Jain, P.; Rajput, D.; Patil, U.K. Green synthesized plant-based silver nanoparticles: Therapeutic prospective for anticancer and antiviral activity. *Micro and Nano Syst. Lett.* **9**, 5(2021). doi:10.1186/s40486-021-00131-6
16) Mandal, S.; Arumugam, S.K.; Adyanthaya, S.D.; Pasri-

cha, R.; Sastry, M. Use of aqueous foams for the synthesis of gold nanoparticles of variable morphology. *J. Mater. Chem.* **14**, 43-47(2004).
17) Aslam, M.; Fozia, F.; Gul, A.; Ahmad, I.; Ullah, R. *et al.* Phyto-extract-mediated synthesis of silver nanoparticles using aqueous extract of *Sanvitalia procumbens*, and characterization, optimization and photocatalytic degradation of azo dyes orange G and direct blue-15. *Molecules* **26**, 6144(2021). doi:10.3390/molecules26206144
18) Biswas, N.; Thomas, S.; Sarkar, A.; Mukherjee, T.; Kapoor, S. Adsorption of methimazole on silver nanoparticles: FTIR, Raman, and surface-enhanced Raman scattering study aided by density functional theory. *J. Phys. Chem. C* **113**, 7091-7100(2009). doi:10.1021/jp900134n
19) Abbasi, B.A.; Anjum, S.; Hano, C. Differential effects of *in vitro* cultures of *Linum usitatissimum* L.(Flax) on biosynthesis, stability, antibacterial and antileishmanial activities of zinc oxide nanoparticles: A mechanistic approach. *RSC Adv.* **7**, 15931-15943(2017).
20) Rajeshkumar, S.; Malarkodi, C. *In vitro* antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. *Bioinorg. Chem. Appl.* **2014**, 581890(2014). doi.org/10.1155/2014/581890
21) Riaz, A.; Ahmed, N.; Khan, M.I.; Haq, I.-U.; Rehman, A.U. *et al.* Formulation of topical NLCs to target macrophages for cutaneous leishmaniasis. *J. Drug Deliv. Sci. Technol.* **54**, 101232(2019). doi:10.1016/j.jddst.2019.101232
22) Mohamed, M.I. Optimization of chlorphenesin emulgel formulation. *AAPS J.* **6**, 81-87(2004). doi: 10.1208/aapsj060326
23) Mohamad, N.; Amin, M.C.I.M.; Pandey, M.; Ahmad, N.; Rajab, N.F. Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: accelerated burn wound healing in an animal model. *Carbohydr. Polym.* **114**, 312-320(2014). doi:10.1016/j.carbpol.2014.08.025
24) Mousa, A.M.; Almatroudi, A.; Alwashmi, A.S.; Abdulmonem, W.A.; Aljohani, A.S.M. *et al.* Thyme oil alleviates Ova-induced bronchial asthma through modulating Th2 cytokines, IgE, TSLP and ROS. *Biomed. Pharmacother.* **140**, 111726(2021). doi: 10.1016/j.biopha.2021.111726
25) Li, S.; Shen, Y.; Xie, A.; Yu, X.; Qiu, L. *et al.* Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. *Green Chem.* **9**, 852-858(2007).
26) Amin, M.; Anwar, F.; Janjua, M.R.S.A.; Iqbal, M.A.; Rashid, U. Green synthesis of silver nanoparticles through reduction with *Solanum xanthocarpum* L. berry extract: Characterization, antimicrobial and urease inhibitory activities against *Helicobacter pylori*. *Int. J. Mol. Sci.* **13**, 9923-9941(2012). doi:doi:10.3390/ijms13089923
27) Mittal, A.K.; Bhaumik, J.; Kumar, S.; Banerjee, U.C. Biosynthesis of silver nanoparticles: Elucidation of prospective mechanism and therapeutic potential. *J. Colloid. Interface. Sci.* **415**, 39-47(2014). doi:10.1016/j.jcis.2013.10.018
28) Cittrarasu, V.; Balasubramanian, B.; Kaliannan, D.; Park, S.; Maluventhan, V. *et al.* Biological mediated Ag nanoparticles from Barleria longiflora for antimicrobial activity and photocatalytic degradation using methylene blue. *Artif. Cell. Nanomed. B* **47**, 2424-2430(2019). doi: 10.1080/21691401.2019.1626407.
29) Nayak, D.; Ashe, S.; Rauta, P.R.; Kumari, M.; Nayak, B. Bark extract mediated green synthesis of silver nanoparticles: Evaluation of antimicrobial activity and antiproliferative response against osteosarcoma. *Mater. Sci. Eng. C* **58**, 44-52(2016). doi:10.1016/j.msec.2015.08.022
30) Shameli, K.; Ahmad, M.B.; Al-Mulla, E.A.J.; Ibrahim, N.A.; Shabanzadeh, P. *et al.* Green biosynthesis of silver nanoparticles using *Callicarpa maingayi* stem bark extraction. *Molecules* **17**, 8506-8517(2012). doi:10.3390/molecules17078506
31) Abbasi, B.H.; Nazir, M.; Muhammad, W.; Hashmi, S.S.; Abbasi, R. *et al.* Comparative evaluation of the antiproliferative activity against HepG2 liver carcinoma cells of plant-derived silver nanoparticles from basil extracts with contrasting anthocyanin contents. *Biomolecules* **9**, 320(2019). doi: 10.3390/biom9080320
32) Desai, R.M.; Venu, G.; Sanjeev, K.; Jha, P.K. Size distribution of silver nanoparticles: UV-visible spectroscopic assessment. *Nanosci. Nanotechnol. Lett.* **4**, 30-34(2012). doi:10.1166/nnl.2012.1278
33) Elumalai, E.K.; Prasad, T.N.V.K.V.; Hemachandran, J.; Viviyan, S.T. Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities. *J. Pharm. Sci. Res.* **9**. 549-554(2010).
34) Roy, K.; Sarkar, C.K.; Ghosh, C.K. Plant-mediated synthesis of silver nanoparticles using parsley(*Petroselinum crispum*)leaf extract: Spectral analysis of the particles and antibacterial study. *Appl. Nanosci.* **5**, 945-951(2015). doi.org/10.1007/s13204-014-0393-3 K.
35) Jemal, K.; Sandeep, B.V.; Pola, S. Synthesis, characterization, and evaluation of the antibacterial activity of *Allophylus serratus* leaf and leaf derived callus extracts mediated silver nanoparticles. *J. Nanomater.* **2017**, 4213275(2017). doi:10.1155/2017/421327
36) Feng, Q.L.; Wu, J.; Chen, G.Q.; Cui, F.Z.; Kim, T.N. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater. Res.* **52**, 662-668(2000). doi.10.1002/1097-4636(20001215)
37) Aritonang, H.F.; Koleangan, H.; Wuntu, A.D. Synthesis

of silver nanoparticles using aqueous extract of medicinal plants (*Impatiens balsamina* and *Lantana camara*) fresh leaves and analysis of antimicrobial activity. *Int. J. Microbiol.* **2019**, 8642303 (2019). doi: 10.1155/2019/8642303

38) Sofi, M.A.; Sunitha, S.; Sofi, M.A.; Pasha, S.K.K.; Choi, D. An overview of antimicrobial and anticancer potential of silver nanoparticles. *J. King Saud Univ. Sci.* **34**, 101791 (2021).

39) Tian, J.; Wong, K.K.Y.; Ho, C.-M.; Lok, C.-N.; Yu, W.-Y. *et al.* Topical delivery of silver nanoparticles promotes wound healing. *Chem. Med. Chem.* **2**, 129-136 (2007). doi: 10.1002/cmdc.200600171

40) Frankova, J.; Pivodova, V.; Vágnerova, H.; Juranova, J.; Ulrichova, J. Effects of silver nanoparticles on primary cell cultures of fibroblasts and keratinocytes in a wound-healing model. *J. Appl. Biomater Funct. Mater.* **14**, 137-142 (2016). doi: 10.5301/jabfm.5000268

41) Bhubhanil, S.; Talodthaisong, C.; Khongkow, M.; Namdee, K.; Wongchitrat, P. *et al.* Enhanced wound healing properties of guar gum/curcumin-stabilized silver nanoparticle hydrogels. *Sci. Rep.* **11**, 21836 (2021). doi: 10.1038/s41598-021-01262-x

42) Diniz, F.R.; Maia, R.C.A.P.; Rannier, L.; Andrade, L.N.; Chaud, M.V. *et al.* Silver nanoparticles-composing alginate/gelatine hydrogel improves wound healing *in vivo*. *Nanomaterials (Basel)* **10**, 390 (2020). doi: 10.3390/nano10020390

43) Nqakala, Z.B.; Sibuyi, N.R.S.; Fadaka, A.O.; Meyer, M.; Onani, M. *et al.* Advances in nanotechnology towards development of silver nanoparticle-based wound-healing agents. *Int. J. Mol. Sci.* **22**, 11272 (2021). doi:10.3390/ijms222011272