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RESEARCH PAPER

Antibacterial and wound healing activities of biosynthesized AgNPs and ZnNPs from *Sesamum indicum* seeds extract against MDR *Klebsiella variicola* and *Pantoea ananatis*

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ABSTRACT

Objective(s): Wound infection with multidrug resistant bacteria is a challenge of today's medicine. Novel antibacterial agent such as nanoparticles are required to combat such threat. This study aims to find an alternative therapeutic agent to combat the drug resistant bacteria.

Materials and Methods: During period (June to September 2022), 157 clinical samples were collected from different wound infections in different hospitals in Erbil city-Iraq. A total of 83 (52.86%) Gram negative bacteria were identified depending on 16S rRNA (PCR), sequencing and GenBank database. Silver, zinc and Ag–Zn composite nanoparticles from *Sesamum indicum* seeds were biosynthesized. Then, tested against *Klebsiella variicola* and *Pantoea ananatis in vitro* and *in vivo* using albino wistar rat model.

Results: Findings showed that antibacterial effects with inhibition zone of each AgNPs and ZnNPs against *K. variicola* was 15 mm, while, for Ag-Zn composite nanoparticles was 12 mm. Otherwise, inhibition zone of the same NPs against P. ananatis was 14, 22 and 21 mm, respectively. The healing time varied among treatment groups. The healing time for wounds treated with NP-based creams were notably shorter compared to traditional treatments and non-treated group. The healing time of wounds infected with *K. variicola* treated by AgNPs, ZnNPs, Ag-Zn NPs, Vaseline, fusidin, mebo and non-treated groups were 13, 15, 14, 20, 16, 16 and 32 days, and for wounds infected with *P. ananatis* were 14,17, 14, 27, 28, 16 and 33 days, respectively.

Conclusion: These results are promising in terms of nanoparticles' potential ability to be used against pathogenic bacteria.

Keywords: *Antibacterial, Klebsiella variicola, Nanoparticles, Pantoea ananatis, Wound healing*

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INTRODUCTION

Globally, multidrug resistant (MDR) bacterial infections are the primary cause of around 700,000 fatalities. By 2050, this number is predicted to increase to as high as 10 million deaths. Open wounds are the primary entry point for bacteria into the body [1]. Wounds and skin injuries can arise from a variety of causes, including cuts, surgical procedures, burns, gunshot wounds, as well as underlying conditions including diabetes and bedsores. Wound infection is a significant form of nosocomial infection that impacts numerous patients worldwide. The presence of bacteria in the wound has the potential to lead to severe consequences and incur significant medical and financial burdens due to the quick rise of antibiotic-resistant bacteria [2, 3]. The MDR bacteria pose a contemporary medical challenge. These bacteria exhibit the ability to endure extended periods and proliferate even in environments with scarce nutrients. Additionally, they possess the capacity to colonize damaged skin, posing a considerable global threat to public health [4, 5]. There is a demand for a novel class of antimicrobials that are safe, widely effective and easy to apply. These would be essential for treating infected wounds and averting infections associated with multidrug resistance [6, 7].

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Nanotechnology is a rapidly advancing domain focused on creating innovative materials ranging from 1 to 100 nm, with potential applications in anti-infection treatments. Nanoparticles (NPs) have demonstrated harmful effects on bacteria, affirming their suitability for diverse medical uses, such as promoting wound healing. Among these, silver nanoparticles (AgNPs) and zinc nanoparticles (ZnNPs) are the most researched, highly utilized and commercialized nanomaterials for different biomedical purposes, with a notable focus on their effectiveness in wound care [8, 9].

Metal NPs play a vital role in medical, pharmacological, and antibacterial applications. Various strategies, including physical, chemical, green, and biological processes, have been used to synthesize metal NPs. Physical and chemical NPs have been found to be highly expensive, toxic, and can have unwanted adverse effects on humans' health and the environment. Thus, the production of biological and green NPs is favoured for biological and medicinal applications. These products mainly take advantage of organic components like ascorbic acid, H+ ions, and nicotinamide adenine dinucleotide (NAD) in glycolysis as natural reducing and antioxidant agents. This makes them not only cost-effective but also environmentally friendly with minimal side effects on animals and humans [10].

Silver nanoparticles are suitable for assessing wound healing due to their extensive use in medical fields. Numerous scientists have conducted studies on the bactericidal efficacy of AgNPs against both drug-sensitive and multidrug-resistant pathogenic bacteria. These studies have demonstrated that AgNPs are a potent weapon against MDR bacteria and have potential applications in wound healing. In addition, ZnNPs have been documented as a highly stable and non-toxic inorganic antibacterial substance, making them suitable for wound healing applications. Moreover, the Ag–Zn composites exhibit a synergistic effect in inhibiting bacterial growth, which can be attributed to the robust interaction between silver and zinc. Furthermore, NPs have a crucial effect in skin functioning by promoting collagen synthesis, hence facilitating the wound healing process. Additionally, Zn plays a significant role in the process of skin epithelialization [8]. *Sesamum indicum,* an annual plant belonging to the Pedaliaceae family, is widely distributed across tropical regions. Extensive research has unveiled its diverse pharmacological attributes, including anti-inflammatory, antioxidant, antihypertensive, antimicrobial, wound healing and anticancer properties. [11, 12]. One of the promising ways to overcome bacterial resistance to antibiotics is the use of metal nanoparticles and their oxides. This study aimed to assess *in vitro* and *in vivo* antibacterial activity and wound healing of biosynthesis *Sesamum indicum* seeds nanoparticles (AgNPs, ZnNPs and composite Ag–Zn NPs) against the two multidrug resistant Gram negative bacteria; *K. variicola* and *P. ananatis*.

MATERIALS AND METHODS

Isolation and Identification of Gram negative bacteria

A total of 157 clinical samples including gunshot, burn, surgery and ulcers of diabetic and bedsore were collected in different hospitals in Erbil city-Iraq from June to September 2022. All specimens were inoculated directly on blood agar and MacConkey agar, incubated at 37 °C for 24 hr. Gram negative bacteria identified by studying molecular identification using PCR (16S rRNA) gene, and the primers were 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) [13, 14]. A web-based submission tool (https://submit.ncbi.nlm.nih.gov) was used to assist with the submission procedure. Each sequence has been submitted to GenBank and accession number for all isolated bacteria were generated.

Antibacterial sensitivity test

Antibacterial sensitivity test was used to assess the sensitivity of isolated bacteria against 19 antibacterials (Amikacin (AK), Amoxicillin-Clavulanic acid (AMC), Ampicillin (AMP), Ciprofloxacin (CIP), Chloramphenicol (C), Cefotaxime (CTX), Doxycycline (DOX), Gentamicin (CN), Imipenem (IPM), Levofloxacin (LEV), Meropenem (MEM), Nalidixic acid (NA), Nitrofurantoin (F), Norfloxacin (NOR), Rifampin (R), Streptomycin (S), Tetracycline (TE), Tobramycin (TOB) and Trimethoprim (TMP)) by using disc diffusion method (Kirby Bauer) as explained by Clinical and Laboratory Standards Institute (CLSI) [15].

Green biosynthesis of AgNPs and ZnNPs using Sesamum indicum seeds

Collection of Sesamum indicum seeds

Fresh *Sesamum indicum* seeds were bought from local markets in Erbil-Shaqlawa city, and verified the *S. indicum* category in the College of Education, Salahaddin University-Erbil, Iraq.

Preparation of sesame seeds extract

Sesame seeds were washed with distilled

water, dried and grinded. The resulting seed powder (20 gm) was combined with 100 ml of distilled water and boiled at 75 °C for 45 min. The extract of Sesame seeds was filtered twice via Whatman's filter paper, then stored at 4 °C for additional experimentation. The filtrate solution was used as reducing agent for biosynthesis of nanoparticle [16].

Biosynthesis of silver and zinc nanoparticles

For production of AgNPs and ZnNPs, 100 ml of Sesame seeds extract was added dropwise to 400 ml of 1 mM $AgNO_3$ and 1 mM ZnO solutions under stirring, separately. The resulting solutions were stirred for 2 hr at 1000 rpm and at 50 °C. The pH of solutions were controlled between 8-10 by adding sodium hydroxide (NaOH), the solution was incubated for 72 hr at 37 °C. For preparation of NPs powder, the solution was boiled on electrical heater to evaporate of the solvents, the remaining was put in the oven for 2 hr at 200 °C for full drying and sterilization [16, 17]. The combination of Ag-Zn nanoparticles was synthesized by meticulous blending of AgNPs and ZnNPs powders.

Characterization of silver and zinc nanoparticles

Characterization of AgNPs and ZnNPs was performed by double-beam UV-visible spectrophotometer, Fourier Transmission Infrared Spectroscopy (FTIR), X ray diffraction (XRD) measurements. The chemical composition of the prepared NPs was measured by EDX (energy dispersive X-ray spectroscopy) combined with SEM (scanning electron microscopy) [18-20].

Antibacterial activity of silver and zinc nanoparticles

The antibacterial activity of AgNPs, ZnNPs and composite Ag-Zn NPs against MDR *K. variicola* and *P. ananatis* was detected *in vitro* by well diffusion method. Known concentration (0.1%) of 10 mg/ml of synthesized nanoparticles was prepared by dissolving 0.05 gm of each nanoparticle in 5 ml ethanol, the solution was sonicated for 1 hr at 50 °C. The suspension (0.5 McFarland standard $1.5x10^8$ CFU/ml) was inoculated on the Mueller Hinton agar and left till dry. Plates containing wells of 7 mm diameter were filled with 150 µl extract of different NPs, separately. After 24 hr of incubation at 37 °C, the zones of inhibition on the plates were measured in mm [21].

***In vivo* activity of nanoparticles**

Animal care and burn generation

Female Wistar albino rats, with a weight of 200 ± 10 gm, were used for the experimental model. Two rats were housed in an individual cage measuring 40×25×20 cm. Standard care conditions, including a 12-hr light-dark photoperiod, temperature control set at 24 ± 2 °C, and continuous access to food and water, were consistently maintained. The experimental protocol was adhered to ethical guidelines for animal experimentation outlined in Directive 2010/63/EU and was approved by the ethics committee. [22, 23]. Before initiating the study, the rats were allowed a 7-day acclimation period in the laboratory. Totally, 28 rats (n=2/group) were anesthetized using a combination of 2% xylazine and 10% ketamine (10+90 mg/kg body weight) which were administered intramuscularly. Subsequently, professional hair clippers were used to remove the hair. Burn wounds of a specific thickness were created by applying heat to a solid stainless-steel bar (Fig. 1E) on specified 4 cm^2 sections of the animal's dorsal proximal region for a duration of 5 sec (Fig. 1A and B) [24, 25]. To demonstrate the inhibitory effects of the synthesized AgNPs, ZnNPs, and Ag-Zn NPs on antibacterial and wound healing activity, the burned rats were infected with activated pure cultures of *K. variicola* and *P. ananatis*. The activated culture was applied to the wounds of each burned rat (Fig. 1C) and allowed to grow for 24 hr [25-27].

Preparation of creams and wound treatment

The nanoparticle creams were prepared in a concentration of (1% w/w) by dissolving 0.05 gm of each nanoparticle in 2 ml dimethyl sulfoxide (DMSO) within a crucible, then, 5 gm of pure Vaseline wax was added, and heated on the magnetic stirrer hot plate with gentle stirring at 500 rpm for 1 hr to melt the Vaseline and mix it with the nanoparticles, and also to eliminate the DMSO from the mixture. Then, the mixture was cooled

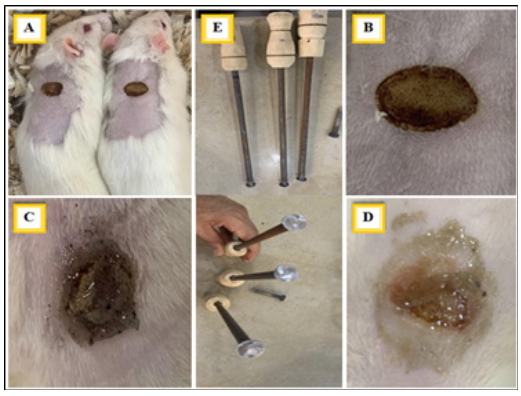


Fig. 1. (A & B) Appearance of different wounds at burning day, (C) infected burned wound with bacteria, (D) treatment of burned wound, (E) used bar for burning rats

to room temperature with stirring to produce a homogeneous cream, which was then sterilized in an autoclave under steam heat at 121°C (15 Ib/in2) for typically 15 min. The sterilized cream was then cooled with continuous stirring (Fig. 2) [8, 25]. The burned rats were randomly divided into four major groups (n=2); test group treated with NPs creams (AgNPs, ZnNPs and composite of Ag-Zn NPs), positive control (pure Vaseline wax), negative control (no treatment) and standard group (Mebo 0.25% and Fusidin 2%). Following 24 hr of infection, the treatment was started, the wound area was swabbed by the mentioned creams in a single dose during 24 hr (Fig. 1D) [25, 28]. Healing times were recorded daily until complete wound closure.

Oral acute toxicity

Single dose toxicity study

To evaluate the toxicity of each nanoparticle in a living system, female Wistar albino rats (weighing 200 ± 10 gm) were chosen for an acute oral toxicity test. The test followed an up-and-down approach as outlined in the OECD Test Guidelines 425 (OECD, 2008). The rats underwent a one-week acclimatization period and were fasted overnight before the dosing [29].

Dose preparation and administration

The required quantity of the studied compounds (mg/kg) relative to body weight was dissolved in ethanol. Subsequently, corn oil was added as a vehicle, and the mixture was gently heated with stirring to achieve homogenization, and evaporation the ethanol (Table 1) [25]. Depending on (OECD 425-27), four rats were weighed, and the limited test dose (2000 mg/kg) relative to body weight was administered orally either in a single dosage or twice within a 24 hr period by gavage via a stomach tube for each nanoparticle. Following dose administration, the animals were individually monitored periodically within the first 24 hr and daily for the next 14 days. Throughout the study, additional observations were conducted on each rat, comparing their condition to the control group, including behavioral alterations and modifications to the eyes, skin and fur [30]. On the 15th day, the rats were anesthetized with a combination of xylazine2% and ketamine10%, sacrificed, exterior surface and abdominal organs were macroscopically investigated [30, 31].

RESULTS

In the present study two different species *K.variicola* and *P. ananatis* for first time were recorded in Iraq depending on molecular study (PCR) using 16S rRNA, sequencing and submitted to NCBI, and the accession numbers were acquired (OQ380694 and OQ422175) respectively.

Antibacterial susceptibility

The present results showed that out of 19 tested antibacterials, *K. variicola* was 100% resistant to 12 antibacterials including Amoxicillin-Clavulanic acid, Ampicillin, Cefotaxime, Chloramphenicol, Doxycycline, Levofloxacin, Meropenem, Nalidixic acid, Nitrofurantoin, Rifampin, Streptomycin, Tobramycin. On the other hand, it was 100% sensitive to Amikacin, Imipenem and Trimethoprim. Moreover, *P. ananatis* was 100% resistant toward Amikacin, Cefotaxime, Chloramphenicol, Doxycycline, Imipenem, Nalidixic acid, Nitrofurantoin, Norfloxacin, Rifampin, Streptomycin, Tobramycin, Trimethoprim, however, it was sensitive to other remaining antibiotics.

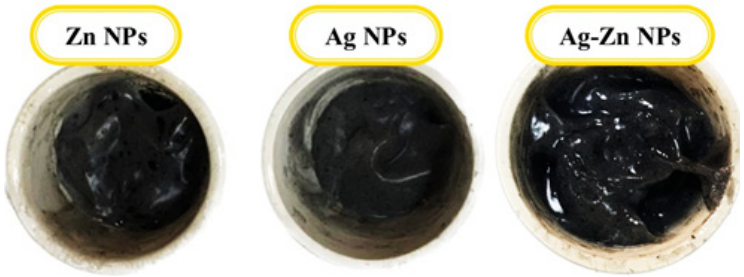


Fig. 2. Prepared nanoparticle creams

Table 1. Dose preparation and administration

Conc. mg/kg	Ethanol (ml)	Vehicle (ml)	No. of doses in 24 hours	No. of used rat	Lethality
2000	2	3	1	1	No
3000	2	3	1	1	No
4000	4	5	2	1	No
5000	4	5	2	1	No

Biosynthesis of nanoparticles

Biological active AgNPs and ZnNPs were successfully produced using *S. indicum* seeds**.** Color change was observed during the initial stages of the process and synthesis of NPs was confirmed, the color of $AgNO_3$ and *S. indicum* seeds was shifted from pale yellow to brown, the color of ZnO and *S. indicum* seeds was shifted from pale yellow to yellowish brown (tan or caramel color) (Fig. 3).

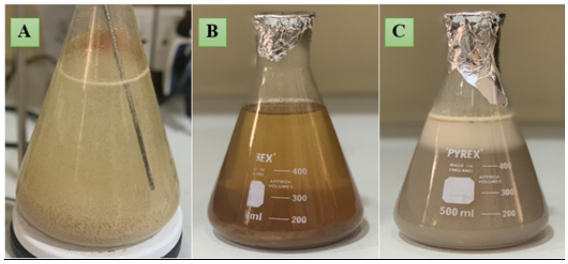


Fig. 3. (A) S. indicum seeds extract, (B) after addition $AgNO_3$, (C) after addition ZnO

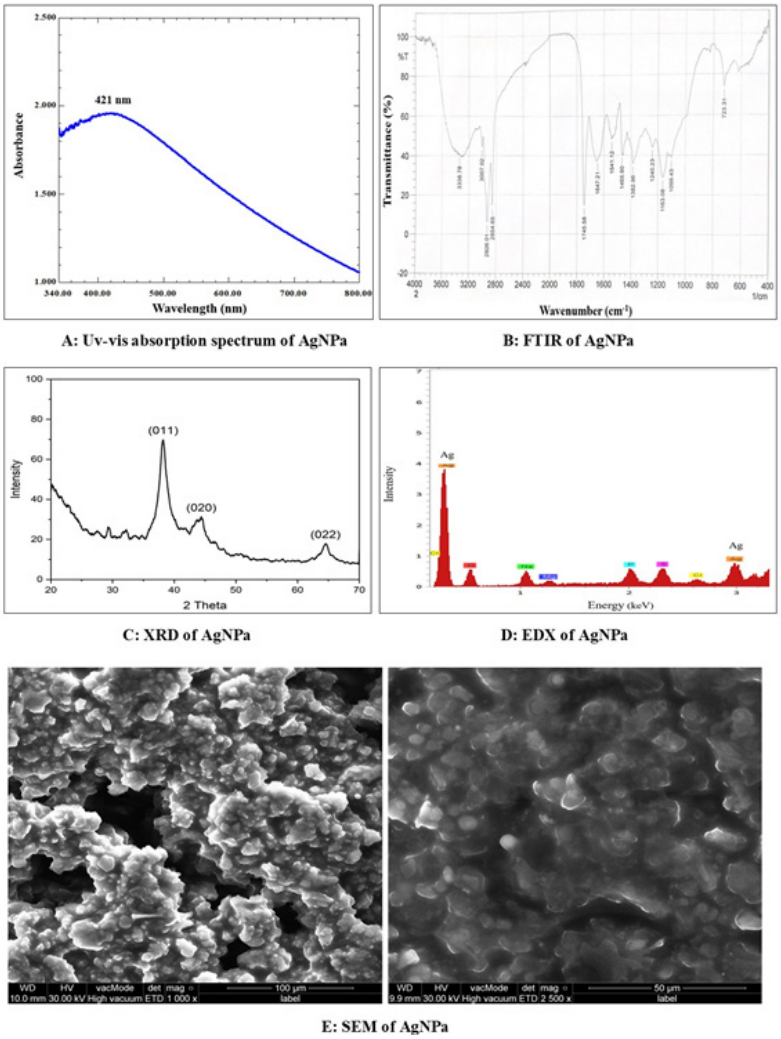
Characterization of AgNPs and ZnNPs from S. indicum seeds

UV-Visible Spectroscopy

The generation of NPs was examined using a UV-Visible spectrophotometer. A strong plasmon absorbance band was observed at 421 nm for AgNPs (Fig 4A) and at 345 nm for ZnNPs (Fig. 5A).

Fourier transmission infrared spectroscopy

The measurements of FTIR showed the spectra between 400 to 4000 cm^{-1} of AgNPs which showed the absorption bands centered at peaks at 723.31, 1647.21, 2854.65, 2926.01 and 3007.02 representing alkene compounds, and at 1099.43 and 1163.08 representing secondary and tertiary



A: Uv-vis absorption spectrum of AgNPa

B: FTIR of AgNPa

C: XRD of AgNPa

D: EDX of AgNPa

E: SEM of AgNPa

Fig. 4. Characterization of AgNPs

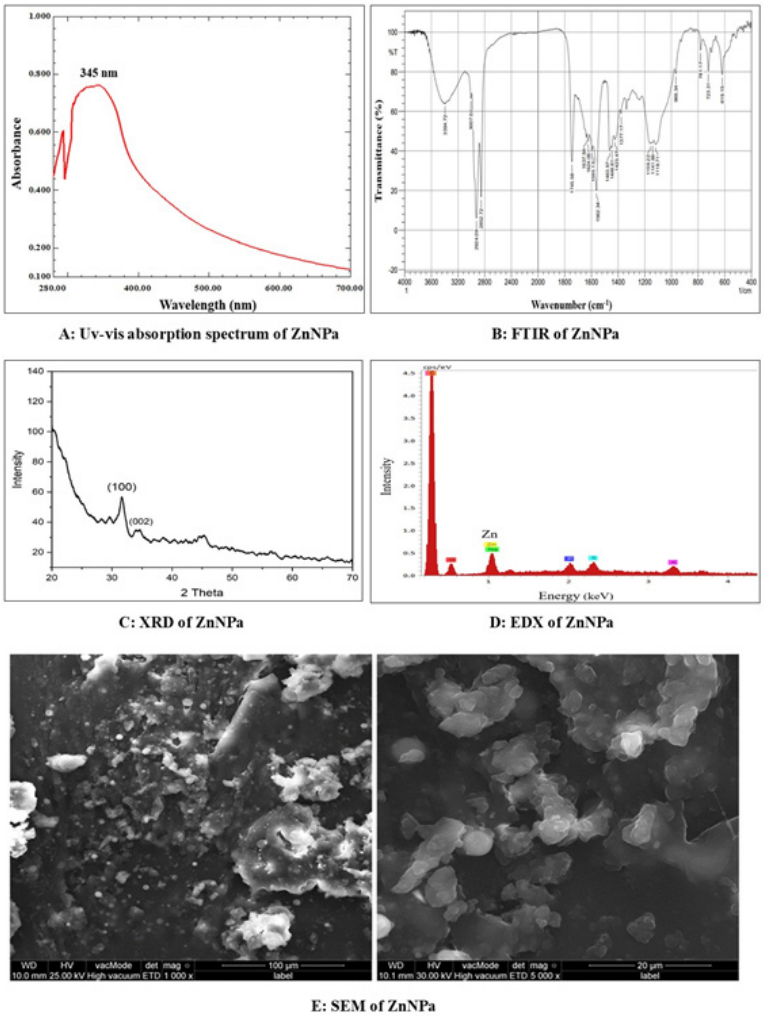


Fig. 5. Characterization of ZnNPs

alcohol respectively. Also, the aminic compound was represented by peak 1240.23, alcohols were represented by peaks 1153.43, phenol was represented by peak 1382.96, peak 1541.12 was nitrogenous compound, while esters compounds were represented by peak 1745.58, and alcohol compounds represented by peaks 3338.76 (Fig. 4B). On the other hand, ZnNPs showed peaks at 619.15 absorption band which indicated the existence of C-Br stretching of the halo compound, band of 723.31, 781.17 and 966.34 revealed C=C bending of the alkene. Similarly, the presence of C-N stretching (secondary amine) and C-H bending (aldehyde) were verified by peaks at 1141.86 and 1377.17 respectively. Presence of C-H bending of alkene revealed by peak 1446.61. At peak 1624.06 imine was observed with C=N stretching, and at peak 1745.58 esters was identified with C=O stretching. Existence of N-O functional group of nitro compound was confirmed by peak at the 1562.34. At the peaks of 2852.72, 2924.09 and 3007.02 alkene were observed with C-H stretching. Aliphatic primary amine was confirmed by N-H stretching at 3394.72 peak (Fig. 5B). All of these vibrations indicated the involvement of several biomolecules in the stability and reduction of NPs.

X-ray differentiation (XRD)

The XRD pattern clearly showed that the Ag and Zn nanoparticles have been produced by the bio-reduction of Ag and Zn ions by Sesame seeds. The XRD spectrum for AgNPs resulted in three intense peaks in the spectrum 38.227, 44.559 and 64.710 assigned to the (011), (020) and (022) planes (Fig. 4C), while, the XRD spectrum for ZnNPs resulted in two intense peaks in the spectrum 31.668 and

34.372 assigned to the (100) and (002) planes the standard XRD (Fig. 5C). X-ray differentiation analysis confirmed that the obtained NPs were pure Ag and Zn monocrystal structures.

EDX spectroscopy

The EDX spectroscopy of AgNPs (Fig. 4D) illustrated the existence of a notable absorption peak at 2 keV, signifying the creation of metallic AgNPs with a crystalline structure. Furthermore, the elemental analysis reveals distinct peak that confirm the existence of Zinc (Fig. 5D).

Scanning electron microscopy (SEM)

The surface morphology of the NPs was investigated via SEM. Images obtained from SEM showed the existence of regularly shaped, spherical, well-separated, and relatively uniform AgNPs and ZnNPs with an average size of 11 nm and 14 nm, respectively (Fig. 4E and 5E).

***In vitro* antibacterial activity of nanoparticles**

The antibacterial activity of AgNPs, ZnNPs and Ag-Zn composite NPs at a concentration of 0.1% was investigated against *K. variicola* and *P. ananatis*. For *K. variicola*, the AgNPs, ZnNPs displayed same degrees of inhibition with zones of 15 mm for each, and Ag-Zn composite NPs displayed 12 mm. Turning to *P. ananatis*, AgNPs, ZnNPs and Ag-Zn composite NPs displayed robust antibacterial effects with zones of 14 mm, 22 mm and 21 mm, respectively as shown in Table 2 and Fig. 6.

Table 2. Inhibition diameters of nanoparticles against isolates under the study

Nanoparticles	Zone of inhibition (mm)	
	K. variicola	*P. ananatis*
AgNPs	15	14
ZnNPs	15	22
Zn-Ag composite NPs	12	21

***In vivo* antibacterial and wound healing activity of NPs**

Healing times were recorded daily until complete wound closure and was varied among the treatment groups. Wounds treated with nanoparticle creams were notably revealed shorter healing time compared to traditional treatments and non-treated group as displayed in Table 3 and Fig. 7.

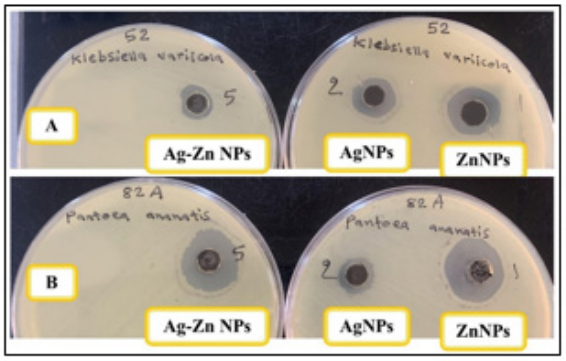


Fig. 6. A) Inhibition zone of NPs against *K. variicola*, B) Inhibition zone of NPs against *P. ananatis*

***In vivo* toxicological study (Acute oral toxicity)**

Throughout the experimental period, no fatalities were reported, and the rats did not exhibit any substantial indications of toxicity. These observations suggest that the tested nanoparticles can be characterized as possibly nontoxic. At the conclusion of the study, the rats were euthanized after being anesthetized. The abdominal organs were tested macroscopically to evaluate any potential changes associated with toxicological effects. Importantly, no notable differences were detected between the test groups and control group, indicating the absence of toxicological concerns related to the administration of AgNPs, ZnNPs and Ag-Zn NPs. This robust evaluation supports the safety profile of the nanoparticles, reinforcing their potential for further biomedical applications.

DISCUSSION

Treating injuries and infections caused by MDR bacteria is an extremely difficult task. Because of this, concentrating efforts to address this problem is essential for improved health care. This study aimed to investigate MDR in wound

Table 3. Healing time of the treated rat wounds under the study

Nanoparticles	Healing time (days)	
	K. variicola	*P. ananatis*
AgNPs	13	14
ZnNPs	15	17
Zn-Ag composite NPs	14	14
Vaseline (Positive Control)	20	27
Fusidin (Standard Control)	16	28
Mebo (Standard Control)	16	16
Non-treated (Negative Control)	32	33

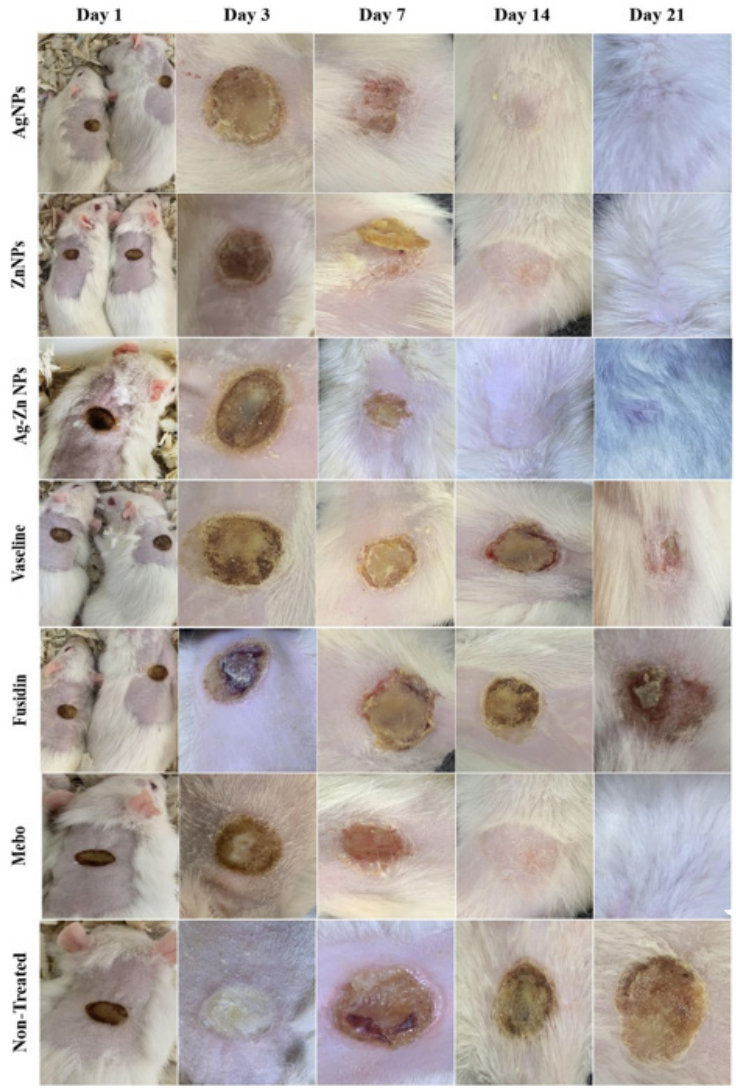


Fig. 7. Wound healing on different days with NPs, positive control, standard control and negative control

isolates obtained from patients who visited several hospitals in Erbil city-Iraq. Out of 157 wound specimens collected during this study, and, depending on molecular study (PCR) using 16S rRNA gene sequence analysis, 83 (52.86%) Gram negative bacteria were identified in different wounds. Chelkeba, Melaku [32] findings aligned with ours concerning the percentage of Gram negative bacteria, as 58.6% of all isolates were Gram negative bacterial present in wound infections.

The discovery of two new records of Gram negative bacteria (*K. variicola* and *P. ananatis*) in Iraq in gunshot wounds assigned as novel and represents a notable development with implications for both clinical medicine and microbiological research. Understanding the characteristics of these bacteria in the context of gunshot wounds is crucial for devising effective treatment strategies and enhancing broader comprehension of wound infections. Identification of these bacteria for the first time, raises questions about their prevalence, pathogenicity and potential impact on wound healing. Similar to the present study, Nakamura-Silva, Macedo [33] reported the first case in Brazil infected by *K. variicola*, at first misidentified as *K. pneumoniae* by the VITEK 2 system, molecular typing through 16S rRNA sequencing revealed 100% identity as *K. variicola*. *K. variicola* is often mistaken for *K. pneumoniae* due to misidentifications using conventional biochemical tests and globally

employed VITEK 2 systems [34]. Furthermore, Kiley, Mende [35] identified only 10 (4%) isolates of *K. variicola* among 237 isolates in human wounds. Moreover, De Baere, Verhelst [36] andLee, Park [37] recorded the first case of bacteremia induced by *P. ananatis* in Belgium and Korea, respectively. They identified the bacterium as *Pantoea spp*. by VITEK 2 system. Then, 16S rRNA gene sequence analysis was conducted using universal primers. Both sequences were compared to the GenBank database using BLAST software (National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov/BLAST/]), and in both cases, the highest match was identified as *P. ananatis* with a similarity exceeding 99%.

Although, unlike to this study, Barrios-Camacho, Aguilar-Vera [38] collected *K. variicola* from various sources include; insects, plants, animals, the environment and a notable proportion from human samples. Moreover, Usuda, Nishio [39] identified that *P. ananatis* as phytopathogen isolated from many ecological niches and plant hosts.

The *K. variicola* is a member of the family *Enterobacteriaceae*, is a bacillus that is Gram negative, facultatively anaerobic and nonmotile. It produces colonies that are circular, convex and mucoid in appearance. It was originally identified as pathogen to plants and animals, but also has been associated with disease in human worldwide [40]. The *P. ananatis,* a bacterium belonging to the *Erwiniaceae* family, Gram-negative, rod-shaped microorganism that can survive in both aerobic and facultatively anaerobic conditions. It commonly can infect a wide range of plants as a phytopathogen. Also, is an opportunistic pathogen for humans and associated with contaminated catheters and penetrating trauma [39].

On the other hand, results of nanoparticles showed that AgNPs, ZnNPs and Ag-Zn nanocomposite were having a potential effect on MDR bacteria and have ability to inhibit growth of bacteria. Various proposed mechanisms suggest how NPs can influence rates of bacterial survival. When NPs bind to the cell membrane of bacteria, critical functions like respiration and permeability are disrupted. Subsequently, these nanoparticles penetrate bacterial cells, inducing the formation of pits in their membranes. Moreover, silver and zinc metal or ions trigger the production of free radicals and reactive oxygen species (ROS), which can harm DNA and denature proteins, ultimately resulting in bacterial death [21].

The management of wounds in medical care is challenging, primarily due to their heightened vulnerability to bacterial infections. Additionally, there is a pressing need for rapid and effective wound healing that minimizes undesirable scarring. This study underscores the potential of certain nanoparticles in treatment creams for enhancing wound healing, particularly complicated by MDR *K. variicola* and *P. ananatis* infections. The findings provide valuable insights into the potential of nanoparticle-based creams in enhancing wound healing in complex infection. Nanoparticles demonstrated promising results, showcasing reduced healing times compared to traditional treatments and controls. Similar findings obtained by El-Banna, Youssef [41], the study showed that wound healing was notably accelerated in the groups treated with nanoparticles compared to other groups, conversely, the control non-treated group had a slower rate of wound healing.

The alignment between the current findings and the study conducted by Kantipudi, Sunkara [8] provides valuable support for the observed wound healing properties of the Ag-Zn composite NPs. Kantipudi, Sunkara [8] investigation concentrating on wound healing using an animal model (albino wistar rats), reported rapid healing within a 10-day period when used Ag-Zn composite nanoparticles. This finding aligns with the current study's results that reported healing time within 13-17 days, suggesting a consistent and robust wound healing effect associated with the use of these composite nanoparticles. The observed variations in healing times may be attributed to the unique properties of each nanoparticle, such as size, composition and their interactions with the wound environment. Nanoparticles may possess antibacterial and regenerative properties that expedite the healing process [42]. Nanoparticles have the dual capability of eliminating microorganisms and promoting skin regeneration. Among different NPs, AgNPs stand out as highly efficient. Their distinctive properties indicate that they can not only prevent wound infections effectively but also enhance the healing of damaged tissues compared to conventional topical treatments [41].

Furthermore, the skin directly exposed to AgNPs and ZnNPs exhibited no observable changes or alterations in the appearance and structural composition. Hence, the tested concentration of AgNPs and ZnNPs is considered safe for application

in topical medical treatments as an alternative antimicrobial therapy, the same results were revealed by Escárcega-González, Garza-Cervantes [43]. Utilizing plant extracts as a reducing and stabilizing agent offers a viable alternative for the production of antimicrobial metal nanoparticles through green synthesis. Presently, the science of pharmacology and toxicology need improved non-toxic therapeutic alternatives that are more potent and exhibit superior antibacterial action against infectious diseases caused by various bacterial strains, particularly MDR in clinical settings [43]. Hence, the biosynthesis of AgNPs, ZnNPs, and Ag-Zn composite NPs, followed by cream generation, is recognized as a highly effective, cost-effective and straightforward method for creating a topical treatment with improved wound healing properties. The combination of Ag and Zn has a synergistic antibacterial activity when compared with other nanoparticles. These findings offer valuable insights for the advancement of novel wound healing medicines. In order to clarify the mechanism of this impact, additional comprehensive experimental evidence will be required.

CONCLUSION

The current study reports the biosynthesis of Ag, Zn and Ag-Zn composite nanoparticles using *S. indicum* seeds. The investigation evaluated their *in vitro* antibacterial properties and *in vivo* excision wound healing activities on Wistar rat models, with a primary emphasis on the formulation of nanoparticle creams. Nanoparticle characterization was conducted through vital instrumental analysis. The results indicated that AgNPs, ZnNPs, and Ag-ZnNPs effectively impede the growth of MDR *K. variicola* and *P. ananatis*. This study highlighted the synergistic effects of nanoparticles as a non-toxic, cost-effective and promising option for topical applications in effective wound healing. Furthermore, all the nanoparticles demonstrated the ability to heal excision wounds within a timeframe of 13-17 days. Additionally, the current study represents a substantial challenge in development of novel nanoparticles for antibacterial drugs and wound creams, offering improved alternative applications. It contributes to an enhanced understanding of the biological impacts induced by these valuable metals, paving the way for a new paradigm in medical research.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Al-Naqshbandi AA, Hassan HA, Chawsheen MA, Qader HHA. Categorization of bacterial pathogens present in infected wounds and their antibiotic resistance profile recovered from patients attending rizgary hospital-erbil. Aro Sci J Koya Uni. 2021;9(2):64-70.
2. Alavi M, Nokhodchi A. Antimicrobial and wound healing activities of electrospun nanofibers based on functionalized carbohydrates and proteins. Cellulose. 2022;29(3):1331-1347.
3. Shariati A, Hosseini SM, Chegini Z, Seifalian A, Arabestani MR. Graphene-based materials for inhibition of wound infection and accelerating wound healing. Biomed Pharmacother. 2023;158:114184.
4. Nobel FA, Islam S, Babu G, Akter S, Jebin RA, Sarker TC, et al. Isolation of multidrug resistance bacteria from the patients with wound infection and their antibiotics susceptibility patterns: A cross-sectional study. Ann med surg. 2022;84:104895.
5. Nițescu B, Pițigoi D, Tălăpan D, Nițescu M, Aramă SȘ, Pavel B, et al. Etiology and multi-drug resistant profile of bacterial infections in severe burn patients, romania 2018–2022. Medicina. 2023;59(6):1143.
6. Al-Naqshbandi AA, Chawsheen MA, Abdulqader HH. Prevalence and antimicrobial susceptibility of bacterial pathogens isolated from urine specimens received in rizgary hospital—erbil. J Infect Public Health. 2019;12(3):330-336.
7. Chen J, Cao Z, Cannon J, Fan Y, Baker Jr JR, Wang SH. Effective treatment of skin wounds co-infected with multidrug-resistant bacteria with a novel nanoemulsion. Microbiol Spectr. 2022;10(2):e02506-02521.
8. Kantipudi S, Sunkara JR, Rallabhandi M, Thonangi CV, Cholla RD, Kollu P, et al. Enhanced wound healing activity of ag–zno composite nps in wistar albino rats. IET Nanobiotechnol. 2018;12(4):473-478.
9. Nqakala ZB, Sibuyi NR, Fadaka AO, Meyer M, Onani MO, Madiehe AM. Advances in nanotechnology towards development of silver nanoparticle-based wound-healing agents. Int J Mol Sci. 2021;22(20):11272.
10. Jomehzadeh N, Koolivand Z, Dahdouh E, Akbari A, Zahedi A, Chamkouri N. Investigating in-vitro antimicrobial activity, biosynthesis, and characterization of silver nanoparticles, zinc oxide nanoparticles, and silver-zinc oxide nanocomposites using pistacia atlantica resin. Mater Today Commun. 2021;27:102457.
11. Manokari M, Latha R, Priyadharshini S, Cokul RM,

Beniwal P, Shekhawat M. Green synthesis of zinc oxide nanoparticles from aqueous extracts of sesamum indicum l. And their characterization. WNOFNS. 2019(23):200-210.
12. Esmaeilzadeh Kenari R, Razavi R. Phenolic profile and antioxidant activity of free/bound phenolic compounds of sesame and properties of encapsulated nanoparticles in different wall materials. Food Sci Nutr. 2022;10(2):525-535.
13. Dos Santos HRM, Argolo CS, Argôlo-Filho RC, Loguercio LL. A 16s rdna pcr-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. BMC Microbiol. 2019;19(1):1-14.
14. Ugbo E, Anyamene C, Moses I, Iroha I, Babalola O, Ukpai E, et al. Prevalence of blatem, blashv, and blactx-m genes among extended spectrum beta-lactamase-producing escherichia coli and klebsiella pneumoniae of clinical origin. Gene Rep. 2020;21:100909.
15. Rabie RA, Khattab F, Badr A, Alkady L. Association between methicillin susceptibility and biofilm production by staphylococcus aureus colonizing atopic dermatitis patients and their impact on disease severity. Microb Infec Dis. 2023.
16. Zafar S, Ashraf A, Ijaz MU, Muzammil S, Siddique MH, Afzal S, et al. Eco-friendly synthesis of antibacterial zinc nanoparticles using sesamum indicum l. Extract. J King Saud Univ Sci. 2020;32(1):1116-1122.
17. Meva FEa, Segnou ML, Ebongue CO, Ntoumba AA, Kedi PBE, Deli V, et al. Spectroscopic synthetic optimizations monitoring of silver nanoparticles formation from megaphrynium macrostachyum leaf extract. Rev Bras Farmacogn. 2016;26:640-646.
18. Ameen F, Altuner EE, Tiri RNE, Gulbagca F, Aygun A, Sen F, et al. Highly active iron (ii) oxide-zinc oxide nanocomposite synthesized thymus vulgaris plant as bioreduction catalyst: Characterization, hydrogen evolution and photocatalytic degradation. Int J Hydrogen Energy. 2023;48(55):21139-21151.
19. Moghaddam FM, Jarahiyan A, Haris MH, Pazoki PY, Aghamiri B. High catalytic performance of cocufe2o4/zif-8 (zn) nanocatalyst for synthesis of new benzimidazole derivatives. J Mol Struct. 2023;1285:135496.
20. Eissa MA, Hashim YZ-Y, Badawi NM. Unlocking the potential of chitosan-based polymeric nanoparticles for the treatment of neurological disorders. Nanomed J. 2024;11(2):107-118.
21. Hamad AH, Chawsheen MA, Al-Naqshbandi AA. Role of laser produced silver nanoparticles in reversing antibiotic resistance in some multidrugresistant pathogenic bacteria. Aro Sci J Koya Uni. 2022;10(1):104-110.
22. Zacharioudaki A, Kostomitsopoulos N. The contribution of veterinarians to the implementation of legislation on the protection of animals used for scientific purposes in greece. J Hellenic Vet Med Soc. 2022;73(2):3913-3920.
23. Marinou KA, Dontas IA. European union legislation for the welfare of animals used for scientific purposes: Areas identified for further discussion. Animals. 2023;13(14):2367.
24. Kim J, Dunham D, Supp D, Sen C, Powell H. Novel burn device for rapid, reproducible burn wound generation. Burns. 2016;42(2):384-391.
25. Samad MK, Hawaiz FE. Synthesis, characterization, antioxidant power and acute toxicity of some new azo-benzamide and azo-imidazolone derivatives with *in vivo* and *in vitro* antimicrobial evaluation. Bioorg Chem. 2019;85:431-444.
26. Zhu M, Liu P, Shi H, Tian Y, Ju X, Jiang S, et al. Balancing antimicrobial activity with biological safety: Bifunctional chitosan derivative for the repair of wounds with gram-positive bacterial infections. J Mater Chem B. 2018;6(23):3884-3893.
27. Rezvani N, Shirvani H, Rostamkhai F. Nano-selenium supplementation upregulate tlr-7, myd88, nf-kb, and traf6 genes in thymus of wistar rats following treatment with cyclosporine a. Nanomed J. 2024.
28. Dwivedi D, Dwivedi M, Malviya S, Singh V. Evaluation of wound healing, anti-microbial and antioxidant potential of pongamia pinnata in wistar rats. J tradit complement. 2017;7(1):79-85.
29. Olela B, Mbaria J, Wachira T, Moriasi G. Acute oral toxicity and anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of piliostigma thonningii (schumach.). Evid Based Complement Alternat Med. 2020;2020.
30. Zarei MH, Lorigooini Z, Khoei HA, Bijad E. Acute oral toxicity assessment of galbanic acid in albino rat according to oecd 425 tg. Toxicol Rep. 2023;11:111-115.
31. Wati H, Muthia R, Kartini K, Setiawan F. Acute toxicity study of the ethanolic extract of eleutherine bulbosa urb in wistar rats. Pharm Educ. 2021;21(2):143-147.
32. Chelkeba L, Melaku T, Mega TA. Gram-negative bacteria isolates and their antibiotic-resistance patterns in patients with wound infection in ethiopia: A systematic review and meta-analysis. Infect Drug Resist. 2021:277-302.
33. Nakamura-Silva R, Macedo LMD, Cerdeira L, Oliveira-Silva M, Silva-Sousa YTC, Pitondo-Silva A. First report of hypermucoviscous klebsiella variicola subsp. Variicola causing primary endodontic infection. Clin Microbiol Infect. 2021;27(2):303-304.
34. Campos TAd, Almeida FMd, Almeida APCd, Nakamura-Silva R, Oliveira-Silva M, Sousa IFAd, et al. Multidrug-resistant (mdr) klebsiella variicola strains isolated in a brazilian hospital belong to new clones. Front Microbiol. 2021;12:604031.
35. Kiley JL, Mende K, Beckius ML, Kaiser SJ, Carson ML, Lu D, et al. Resistance patterns and clinical outcomes of klebsiella pneumoniae and invasive klebsiella variicola in trauma patients. PLoS One. 2021;16(8):e0255636.
36. De Baere T, Verhelst R, Labit C, Verschraegen G, Wauters G, Claeys G, et al. Bacteremic infection with pantoea ananatis. J Clin Microbiol. 2004;42(9):4393-4395.
37. Lee J, Park JH, Kim TS, Park H. A case of bacteremia caused by pantoea ananatis in a patient receiving home parenteral nutrition. Lab Med.12(1):74-78.
38. Barrios-Camacho H, Aguilar-Vera A, Beltran-Rojel M, Aguilar-Vera E, Duran-Bedolla J, Rodriguez-Medina N, et al. Molecular epidemiology of klebsiella variicola obtained from different sources. Sci Rep. 2019;9(1):10610.
39. Usuda Y, Nishio Y, Nonaka G, Hara Y. Microbial production potential of pantoea ananatis: From amino acids to secondary metabolites. Microorganisms. 2022;10(6):1133.
40. Rodríguez-Medina N, Barrios-Camacho H, Duran-Bedolla J, Garza-Ramos U. Klebsiella variicola: An emerging pathogen in humans. Emerg Microbes Infect. 2019;8(1):973-988.
41. El-Banna AH, Youssef FS, Youssef Elzorba H, Soliman AM, Mohamed GG, Ismail SH, et al. Evaluation of the wound healing effect of neomycin-silver nano-composite gel in rats. Int J Immunopathol Pharmacol. 2022;36:03946320221113486.
42. Pormohammad A, Monych NK, Ghosh S, Turner DL, Turner RJ. Nanomaterials in wound healing and infection control.

Antibiotics. 2021;10(5):473.
43. Escárcega-González CE, Garza-Cervantes JA, Vazquez-Rodríguez A, Montelongo-Peralta LZ, Treviño-Gonzalez MT, Díaz Barriga Castro E, et al. *In vivo* antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using acacia rigidula as a reducing and capping agent. Int J Nanomedicine. 2018:2349-2363.