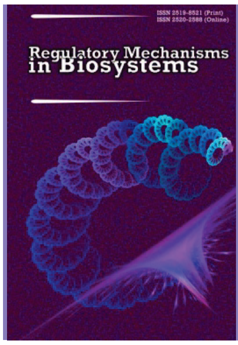

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(4), 821–825
doi: 10.15421/0224117

Effectiveness of silver nanoparticles as antibacterial agents with natural wound healing cream with extracted *Aloe vera* gel

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Article info

Received 07.08.2024
Received in revised form 19.09.2024
Accepted 06.10.2024

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Essa, S. H., Mohammad, S. Q., Kadhum, D. A., & Jalil, I. S. (2024). Effectiveness of silver nanoparticles as antibacterial agents with natural wound healing cream with extracted Aloe vera gel. Regulatory Mechanisms in Biosystems, 15(4), 821–825. doi:10.15421/0224117

The areas of medicine and pharmaceuticals are on the cusp of experiencing a revolution because of the arrival of a plethora of new materials and methods made possible by nanotechnology. A number of subfields within the medical care industry are already reaping the benefits of the opportunities presented by nanotechnology. The potential biological features of silver nanoparticles include antibacterial activity, anti-inflammatory effects, and wound healing effectiveness. Gel is the mucilaginous jelly extracted from the *Aloe vera* plant's parenchyma cells, which might be used in the development of improved dressings for wounds and ulcers. It has recently been drawing attention due to its therapeutic application. This is due to the fact that silver nanoparticles have been shown to have antibacterial activity. This article discusses the function that silver nanoparticles play in the healing process of wounds.

Keywords: AgNp; nanoparticles; wound healing; cream wound healing; *Aloe vera*.

Introduction

Nanotechnology is the science and engineering that goes into the creation, systemization, characterization, and application of materials and devices. Its lowest functional organization is the achievement of a nanoscale in at least one dimension (Saini et al., 2010). Simply based on their size, nanoparticles differ from bulk materials in a number of ways, including chemical reactivity, absorption of energy, and biological mobility (Murthy, 2007). Biosynthesis of AgNp is more friendly and has no toxicity in general, whereas both chemistry and physics synthesis of silver nanoparticles is dangerous (Zhang et al., 2016). The decreasing substances utilized in chemical procedures are very damaging to living creatures. More frequently, biological synthesis is utilized, and it is a substitute for other techniques (Zhang et al., 2020). Biological synthesis processes are straightforward, environmentally friendly, cost-effective, and reliable (Huston et al., 2021). Mixed value, polysaccharides, and biological and irradiation technologies are among the green synthesis methodologies, and have benefits over the traditional ways of chemical agents which are associated with environmental toxicity (Samuel et al., 2022). The leaves of the succulent *Aloe vera* plant contain a gel that retains water. This gel can be used on everything from sunburn to an insect bite to a scratch or a cut because of its hydrating properties.

The gel within the leaves of *Aloe vera* is said to include 12 vitamins, 20 minerals, 18 amino acids, and 200 active chemicals. In particular, *Aloe vera* gel has been used as a functional food resource because of its lack of laxative effects in the manufacture of health beverages. Milk, ice cream, ice cream confections, and other foods also utilise it. Some foods employ *Aloe vera* gel not only as a preservative but also as a flavouring agent (Tottoli et al., 2020; Samuel et al., 2022). The causes of wounds include surgery, trauma, external stimuli (e.g., pressure, burning, and cutting), or pathological illnesses such as diabetes or vascular disorders. These sorts of injuries depend on their source and effects. *Acute or chronic injuries* (Tottoli et al., 2020). Acute injuries are normally repaired in an ordered and suitable manner and the anatomical and functional integrity is restored continuously (Chhabra et al., 2017). Chronic injuries cannot acquire optimum anatomical and functional integrity, on the other hand, the nature, degree and condition of the host and the environment are linked and determined by both disease processes (Hou et al., 2022).

Systemic variables including the patient age, vascular, metabolic and autoimmune illnesses and current medicine treatment might influence the wound healing process (Guo et al., 2022). Wound healing is a collection of very complicated and dynamic events. A certain amount of research has been conducted based on wound type and severity. However, no viable medicines that are extremely stable, cheap and have no side effects can be found in order to repair wounds quickly (Monika et al., 2022). The most promising tool for quick wound healing among all other wound healing substances has been shown to be nanomaterials (Ferreira et al., 2020; Monika et al., 2022). Nanomaterials may be generated in several shapes for tissue regeneration: nanoparticles, nanospheres, nano capsules, nano emulsions, nanocarriers and nano-colloids (Ferreira et al., 2020; Han et al., 2020; Guo et al., 2022; Monika et al., 2022). In wound treatment, there are usually two major types of nanoparticles (NPs): (i) NPs have intrinsic features to support the closure of wounds (Monika & Chandraprabha, 2020); (ii) NPs that are employed as therapy delivery vectors (Jones et al., 2021). The former can be classified into nanoparticles and nanomaterials of metal/metal oxide (Singh et al., 2022). The most investigated compounds are silver, gold and zinc owing to their distinctive characteristics in terms of metal and metal oxide nanoparticles: decreased skin penetration and antibacterial activity (Weller et al., 2020).

The limitations of ordinary silver compounds might be addressed by silver nanoparticles (AgNP), as the surface-to-volume ratio is raised, AgNPs become more powerful at lowered levels, lessening their toxicity (Hubner et al., 2020). Silver pure nanoparticles can control the release of anti-inflammatory cytokine to promote quick injury closure without causing scarring (Ambrogi et al., 2020; Monika & Chandraprabha, 2020; Monika et al., 2022). AgNPs encourage the contractility of wounds by producing myofibroblasts from regular fibroblasting, therefore expediting the healing process (Kumari et al., 2022). In addition, AgNPs stimulate epidermal re-epithelisation through proliferation and relocation of keratinocytes. The principal anti-bacterial action of AgNPs is to create sulfuric connections with bacterial cell membrane proteins or Thiol groups of diverse enzymes, particularly in the respiratory tract. In addition, since DNA includes sulfurous and phosphorous linkages, DNA synthesis during cell division potentially interferes with AgNPs, which inhibits bacterial proliferation (Ambrogi et al., 2020; Kumari et al., 2022).

The mechanism of action of silver NPs can be described as due to their large surface area and the efficient antimicrobial activity of AgNPs. These nanoparticles initially adhere to the cell membrane of bacteria and reinforce the bacterium, interacting with sulfur- and phosphoric group proteins and with DNA (Zhang et al., 2020). Into a low-molecular weight (LM) area, AgNPs that enter bacterial cells, in the center of the bacterium, protect against silver ions and protect the diagnostic DNA from harm. At first, the nanoparticles release silver ions into the bacterial cells in the center, which show their bactericidal activities and increase them. The nanoparticles impair the cell division, causing death of the cell (Ambrogi et al., 2020; Zhang et al., 2020; Kumari et al., 2022). AgNPs suppress inflammation in normal wounds by modulation of cytokines, lowering lymphocyte infiltration and re-epithelization to encourage healing (Orlowski et al., 2020). This research aim to effect of silver nanoparticles wound healing.

Materials and methods

Preparation of culture media. The Muller-Hinton agar medium was created in accordance with the instructions provided by the manufacturing company (Himedia, India), and it was used in the testing of antimicrobial susceptibility. A nutrient broth medium was prepared according to the manufacturing company (Himedia, India). Fresh *Bacillus subtilis* was obtained from the microbiology laboratory in the Faculty of Science of Kufa University and identified by VITEK techniques. Then the *B. subtilis* was allowed to mature on a medium consisting of nutrient broth for one day before being incubated at 37 ºC. In the last step, the fresh adult cells were centrifuged at 6000 xg for 12 minutes in order to remove them, and the supernatant solution was collected in order to prepare the nanoparticles.

Synthesis of silver nanoparticles. The modified chemical reduction approach proposed by Iravani et al. (2014) and Fang et al. (2012), was utilized to manufacture the silver nanoparticles. $AgNO_3$ (0.05 M) in 50 milliliters was heated to boiling point. Then 5 mL of 1% sodium citrate was added. After that, 5 mL of 1% sodium borohydride was injected to the mixture. The solution was mixed vigorously through this process and heated. After the colour had changed, the mixture was cooled to room temperature. Next, the nanoparticles were collected by centrifuging the solution at 12000 xg for 10 minutes, then washed three times by D.W. and one time by ethanol. Finally, the collected NPs was dried at room temperature for one day then stored for future use.

Characterization of synthesis of silver nanoparticles. Visual inspection allowed the detection of the transformation of the colour of the reaction mixture. The fact that it went from being yellow to dark brown indicated that the silver nanoparticles had been made (Rajurkar et al., 2023). Silver ions, denoted by the symbol Ag^+, were changed into silver nanoparticles, denoted by the symbol Ag^0. It was distinguished spectrometerically by using a double beam UV-Vis spectrophotometer (PD-303 UV) at various wavelengths, ranging from 300 to 800 nm. As a point of reference, deionized water is shown along the X-axis of the graph depicting wavelength vs absorbance. The crystalline nature by means of the phase variety and particle size of the AgNPs was detected by using the X-ray diffraction (XRD) with a scanning range between 20^o – 80^o and bond angle two theta. D is the average crystallite size, k is an arithmetic factor. The particle size of the nanoparticles may be calculated using Scherrer's equation, which states that D = k/cos, where D is the average crystallite size (Londoño-Restrepo et al., 2019). Moreover, Field Emission Scanning Electron Microscopy (FESEM) was used to characterize the AgNPs. This is done using a type of electron microscope that images a sample by skimming it by means of a ray of electrons in a raster image design. This ray of electrons interrelates with the atom/molecule that made the sample production signs which consisted of information of the sample superficial structure and conformation (Monshi et al., 2012). The chemical composition of the AgNPs was confirmed by using energy dispersive X-ray (EDS) spectroscopy. In the field of chemical microanalysis, energy dispersive x-ray spectroscopy, often known as EDS or EDX, is a method that is frequently used with scanning electron microscopy (SEM). In order to characterize the elemental composition of the analyzed volume, the EDS method makes use of the detection of x-rays that are generated from the sample while it is being bombarded by an electron beam. Analyses may be performed on features or phases that are as tiny as 1 m or less (Scimeca et al., 2018). The gel was extracted from *Aloe vera* leaves by first extracting its exudates and then scraping its mucilage off with a blunt edged knife. This was done so that the gel could be collected. This mucilage was processed in a blender with a lot of agitation so that it would be consistent. After passing it through a muslin cloth, this solution went through the filter. This uniform solution was used to produce both a cold-extracted gel (CEG) and a hot-extracted gel (HEG). The crude gel was extracted from the extract by adding 3.50 pH hydrochloric acid and then slowly adding 95% alcohol while stirring. Centrifugation was used to separate the gel from the liquid (Hamman, 2008). This medication was prepared by mixing an amount of *Aloe vera* with the same amount of a medical plant (Chrysanthemum). After that, 5:1:1 amount of silver nanoparticles and *Aloe vera* gel was added then mixed vigorously to be ready for use. The antibacterial activity was evaluated against four microorganisms, two-gram positive (G +v) *Staphylococcus aures*, *Klebsiella*, and two-gram negative (G -v) *Escherichia coli* and *Pseudomonas aeruginosa*, which were obtained from the microbiology lab (Hamman, 2008). Nutrient agar was used to cultivate and sustain the bacterial cultures. A cork borer was used to make a hole (five millimetres in diameter). Then, the nutrient agar wells were filled with 0.1 mL of the colloidal AgNPs, the suggested medication, povidone iodide and an ntibacterial tablet. After that all dishes were incubated and left for 24 hrs at 37 ºC. The size of the zone of inhibition (ZI) produced by the Ag nanoparticles after incubation was used to calculate the efficacy of the colloidal solutions against bacteria.

Results and discussion

Characterization of silver nanoparticles (AgNPs). The chemical technique of reduction involves the reduction by the efficient reduction agent in aqueous solution, in the presence of an adequate stabilizer, essential to prevent the development by aggregation of silver particles. During forming silver nanoparticles, the initial concentration of $AgNO_3$, the reduction agent, $AgNO_3$ molar proportions and the stabilizers are impacted by certain characteristics including particle size and aggregation of silver nanoparticles (Ahlawat & Khatkar, 2011). So many ways for the chemical synthesis of the production of silver nanoparticles are recommended; for the production of silver nanoparticles the technique for chemical reduction has been developed, polyol and radiolytic. Chemical reduction method is the best and easiest way of delivering nanoparticles without combination, as it is highly efficient and has a cheap preparation cost. The report of the Commission of the European Parliament (Alves et al., 2023).

UV-visible spectral analysis. It is known that chemically produced nanoparticles of silver contain a surface resonance band that may be detected via UV-visible spectroscopy. This band could be included in the nanoparticles. As seen in Figure 2 for the plasmonic band of solutions containing silver nanoparticles, the absorbance peak frequently linked with nanoparticles is centered at 430 nm.

X-ray diffraction. The environmentally friendly production of AgNPs was given further support by X-ray diffraction (XRD). In Figure 3, we can see that there are four distinct peaks of diffraction occurring at 2 38.2°, 44.4°, 64.7°, and 77.7°, which were indexed with the planes (111), (200), (220) and (311) for the silver. Our results were matched with the Joint Committee on Powder Diffraction Standards (JCPDS) card No. 04–0783 (Table 1). The deviation peaks in our analysis match the silver nanoparticles' Bragg reflections, which is consistent with the findings of prior studies (Alves et al., 2023). Utilizing the Debye-Scherrer equation, D = k/cos, the average nanocrystalline size was determined. Here, D stands for the diameter of the particle size, k is a constant of 1, w is the X-ray source's wavelength (0.1541), fwhm = 0.23, and diffraction angle that corresponds to the structure plane (111). The average crystallite size, as determined by applying the formula devised by Debye-Scherrer to the numbers, is around 18 nm.

a

b

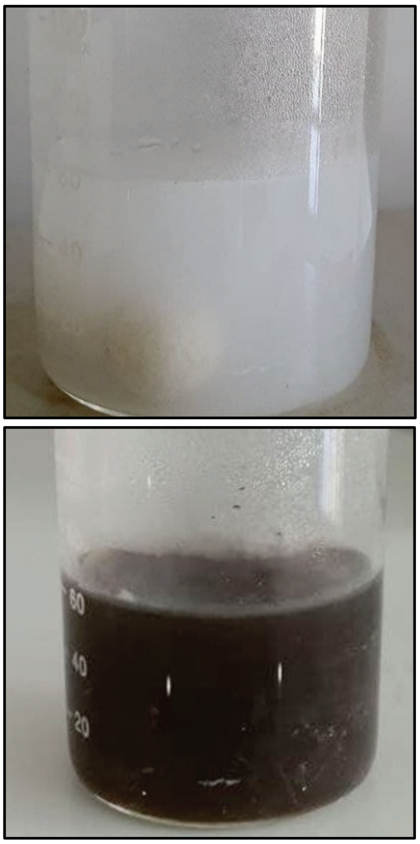

Fig. 1. Color change (*a*) before the production of nanoparticles and (*b*) after the production of nanoparticles

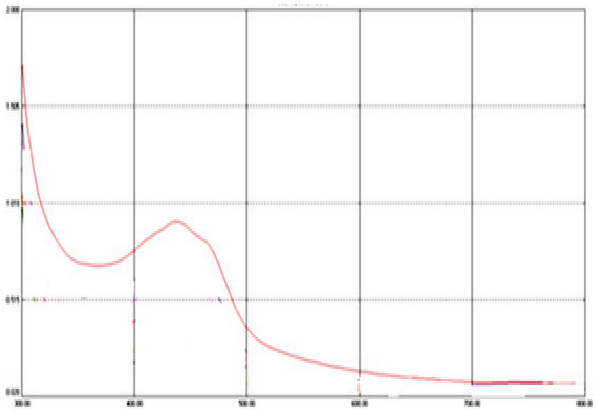

Fig. 2. UV-visible spectra of silver nanoparticles synthesis

Table 1
Diffraction angles of AgNPs, both standard and experimental

Experimental diffraction angle	Standard diffraction angle
38.1	38.117
44.4	44.278
64.7	64.426
77.7	77.472

The sharp peak of (111) with high intensity was detected describing thin film formation on the substrate. The XRD pattern clearly showed that the AgNPs formed by the reduction of Ag^+ ions using chemical reduction are crystalline in nature. Energy Dispersive X-ray Crystallography (EDX) is the method of analysis that was used in the process of chemically characterising or elementally dissecting the nanoparticles. The qualitative and quantitative states of the elements that may be involved in the creation of nanoparticles may be determined using EDX analysis. It was put to use in the demonstration that proved the creation of silver nanoparticles (Szczygłlewska et al., 2023). Figure 4 confirmed the presence of powdered iron at high concentration 81.1%, 11.4% chloride, and 7% oxygen compared to other elements. In other literature synthesis of silver nanoparticles using the medicinal plant *Areva lanata*, the EDX spectrum analysis showing that silver has 64.8% of total element (Choi et al., 2023) while in the synthesis of AgNPs using the *Allmania nodiflora* flower extract the percent of silver element in the EDX spectra was 84% (Chen et al., 2022; Choi et al., 2023).

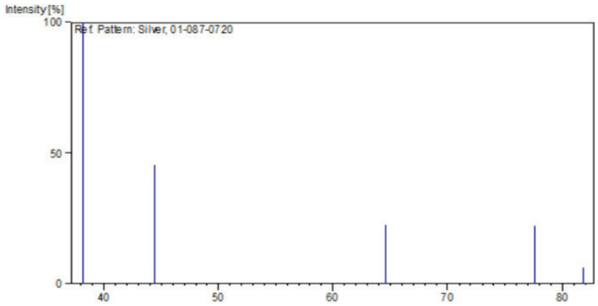

Fig. 3. X-ray diffraction patterns of silver nanoparticles

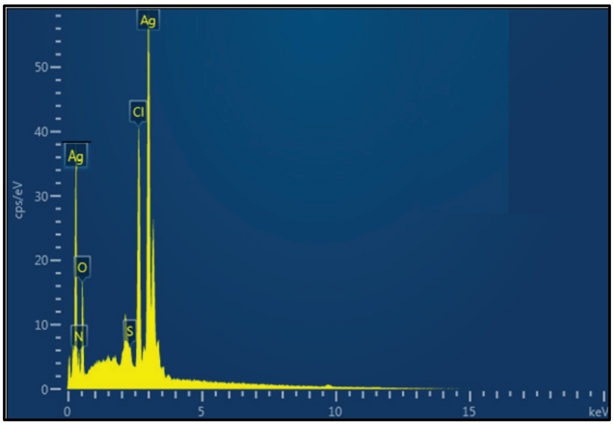

Fig. 4. EDX spectra of IONPs

The field emission scanning electron microscope (FESEM) is yet another method that may be used to determine the size, shape, and dispersion of silver nanoparticles that have been synthesised (Lagashetty & Bhavikatti, 2019). Figure 5 demonstrated that the particles have a spherical form and vary in size from 12 to 20 nm (Lagashetty & Bhavikatti, 2019; Chen et al., 2022; Choi et al., 2022; Sabarees et al., 2022).

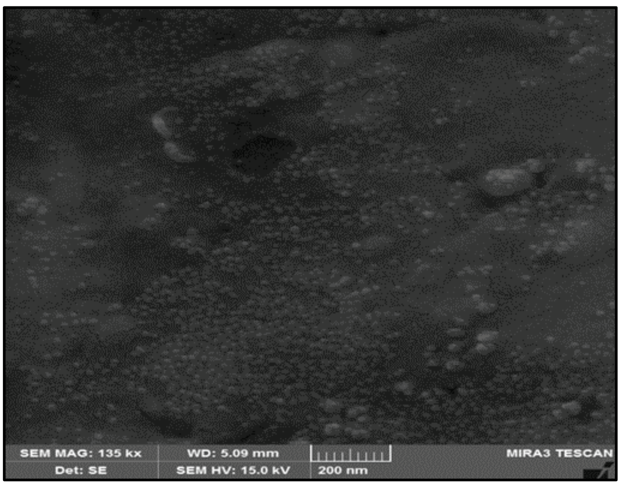

Fig. 5. SEM images of silver nanoparticles

Preparation of suggested wound medication. Herbal preparations are simply one component of alternative medicine and cover a wide variety of treatments. There are presently a wide number of herbal treatments and combinations for wound treatment. These preparations often comprise modest quantities of the plant and a supply material (eg, ointment). The writers have tried to build an easily available list of herbs from the scientific literature. This list is certainly inadequate due to the underrepresentation of herbal therapy in the scientific literature. Very few treatments have been rigorously investigated for their effectiveness and/or toxicity. The authors encourage anyone who is seeking natural therapies to carry out additional investigation.

From ancient times, the antimicrobial properties of silver have been recognized. Silver has been used for the treatment of infections, wound healing and illnesses. In addition, the prophylaxis for ophthalmia neonatorum in babies, was also carried out with silver nanoparticles. Again, recent technological advancements have been made in the realm of silver antibacterial impact, particularly from the beginning of nanotechnology. Nanoparticles have recovered their lost state, therefore providing quicker wound healing and antibiotic resistance effectiveness. As inorganic nature is neither dangerous nor safe, these silver nanoparticles have long been in use and are equipped for killing roughly 650 infectious microorganisms (Bruna et al., 2021; Sharma et al., 2022).

Conclusion

In conclusion, it is essential for the overall health and wellbeing of patients that the process of wound healing be both efficient and comprehensive. In recent years, a significant amount of headway has been made in the quest to understand the cellular and molecular processes that underlie the process of wound healing. Medications such as topical antibacterial medicines are still significant in the therapeutic treatment of wounds and ulcers in the modern day. In addition, the use of nanotechnology and the incorporation of knowledge of cellular and subcellular activities that take place during the normal process of healing has the potential to result in improved future therapeutic treatments. The use of nanotechnology presents significant prospects for the advancement of wound care. The nanoscale paves the path for the creation of innovative materials that may be used in cutting-edge medical technologies. Nanoparticles of silver have exceptional biological capabilities, including anti-inflammatory and antiviral activity, as well as antibacterial qualities with decreased bacterial resistance. These properties may be found in silver nanoparticles. When it comes to the conservative treatment of wounds and burns, silver nanoparticle dressings have recently emerged as the new gold standard. It has not yet been determined whether or not this technology can live up to its full potential. Silver nanoparticles have been shown to have remarkable wound-healing qualities, but the processes behind these effects are not yet fully known. It will be important for future study to learn more about these mechanisms in vivo.

The authors report that they have no conflict of interest.

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