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Green synthesis of zinc oxide nanoparticles using aloe vera extract and evaluation of their antibacterial efficacy

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Abstract

This study aimed to extract bioactive compounds from Aloe vera and utilize them in the green synthesis of zinc oxide (ZnO) nanoparticles, with subsequent evaluation of their antibacterial activity. The plant extract was prepared using 70% ethanol and demonstrated a high content of phenolic and flavonoid compounds, indicating strong potential as a natural reducing and stabilizing agent. Green synthesis of ZnO nanoparticles was successfully achieved using the *Aloe vera* extract. The formation and characterization of the nanoparticles were confirmed by UV–Visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). The UV–Vis analysis revealed a characteristic absorption peak at approximately 370 nm, confirming nanoparticle formation. The synthesized ZnO nanoparticles exhibited a predominantly spherical morphology with particle sizes ranging from 23 to 57 nm, showing good stability and uniform distribution. Antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus*, where the ZnO nanoparticles demonstrated significantly higher activity compared to the crude plant extract, as evidenced by larger zones of inhibition.

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In conclusion, *Aloe vera*-mediated ZnO nanoparticles represent an eco-friendly and effective approach for producing stable nanomaterials with enhanced antibacterial properties, highlighting their potential applications in biomedical and pharmaceutical fields.

Keywords : *Aloe vera* L. Green synthesis ، Antibacterial activity ، Zinc oxide nanoparticles (ZnO NPs) , flavonoids, Biogenic nanoparticles

Introduction

(Aloe vera L.) is an active compound used in traditional Chinese medicine and herbal medicine. It has now become a versatile agent in clinical treatments. It belongs to the Aloaceae family. It is a perennial ، monocotyledonous succulent plant characterized by its high tolerance to arid environments . Mature plants reach a height of 30–70 cm. The plant's leaves are needle-like and often change color from dark green to bluish-gray [Manye et al., 2023]. It consists of a three-layered, specialized tissue structure. Each layer contains a distinct set of secondary metabolites [Kumar et al., 2019]. The cortex is a multi-layered protective covering composed of 15–20 cell layers. It serves as the site for the synthesis of structural polymers, including pectin, lignin, and hemicellulose. The pith, with its bitter-yellow fluid, is rich in phenolic anthraquinones and acts as the plant's antimicrobial chemosuppression mechanism. The endophytic (central) tissue is a mucilaginous gel composed of 99% water, 1% sterols, vitamins, and high molecular weight glucomannan. Which enhances its immunostimulatory efficacy [Akram et al., 2022]. The Aloe vera plant matures physiologically when exposed to sunlight for 5–6 hours daily [Mani et al., 2023].The Diverse Chemical Composition of Aloe Vera: This chemical diversity revolves around complex secondary metabolic processes. It includes more than 200 bioactive compounds [Abid et al., 2025]. The gel contains concentrated organic compounds and is a storehouse of minerals such as zinc, copper, and selenium, as well as numerous enzymes and vitamins, including vitamins A, C, E, and B12. Therefore, it is a source of antioxidants, provides protection against free radicals, and enhances immunity.

The efficacy of this plant is attributed to the complex chemical composition of its leaves, which contain bioactive secondary metabolites such as acemannan polysaccharides, phenolic compounds, and anthraquinones. Research confirms its antioxidant and anti-inflammatory

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activity, including its ability to stimulate immunity [Aurora et al., 2024; Danish et al., 2020].It has been used extensively to treat burns, psoriasis, certain gastrointestinal disorders, some heart conditions, and polycystic ovary syndrome (PCOS) [Akram et al., 2022; Ringasamy et al., 2020].Recent laboratory studies have expanded upon its use as an antitumor and antiviral agent, including against variants of the novel coronavirus (SARS-CoV-2) [SabbaghZadehgan, 2021].In the pharmaceutical field, *Aloe vera* acts as a highly permeable enhancer for transdermal and intranasal drug delivery. An example is the intranasal administration of insulin to protect against metabolic and neurological disorders using polysaccharide microencapsulation techniques [Nagi et al., 2018; Kirby-Smith et al., 2023]. However, toxicology offers a completely different perspective, considering topical application safer, while oral administration remains highly controversial and under investigation [Segal et al., 2013].Oral use is contraindicated for pregnant and breastfeeding women due to its potent anthraquinone content, which may cause uterine contractions and lead to complications such as toxic colitis in infants [Chopra & Manikanika2022].Emudin is known for its antimicrobial properties, particularly against herpes simplex virus (HSV) [Xiang et al., 2020]. The polysaccharides in dried aloe vera gel constitute the largest bioactive component, undergoing fermentation by gut bacteria to produce specific, beneficial fatty acids. These play a key role in alleviating acute colitis, thereby promoting intestinal barrier integrity. Current medical research also supports their use in preventing liver diseases by inhibiting pro-inflammatory cytokines and promoting lipid metabolism [Liodwickska et al., 2022].The polysaccharides scavenge free radicals, protecting cellular systems from apoptosis induced by oxidative stress, thus maintaining overall homeostasis [Liodwickska et al., 2022]. Acimanan is found within the protoplasm of parenchyma cells and acts as an immune stimulant. Studies have confirmed that it promotes tissue repair and wound healing. It also exhibits antiviral and antitumor properties by modulating immune cell activation and inhibiting viral binding mechanisms [Lu et al., 2023]. Its functional activity is influenced by environmental factors and plant age [Arora et al., 2024]. Other uses of *Aloe vera* include cosmetics and food preservation, given its rich nutrient content and its status as a sustainable biopreservative. Furthermore, the

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"green synthesis" of aloe vera nanoparticles is of great importance due to its role in addressing environmental pollution and combating industrial pollution [Khan et al., 2023]. Accordingly, this review provides a summary of the importance of the chemical composition of the aloe vera plant and current extraction methods. The study aims to:Extract active compounds from aloe vera using modern methods, and characterize the biochemical compounds. Synthesize nanoparticles using a green method. Then, evaluate their antibacterial activity.

Materials and Methods

The following chemicals were used: zinc acetate dihydrate, sodium hydroxide (NaOH), ethanol (70%), and distilled water of analytical purity. Aloe vera leaves, collected from a local area and verified, were also used.

Preparation of Plant Extract: Aloe vera leaves were collected and thoroughly washed under running water to remove impurities and dirt, then rinsed with distilled water. The outer **peel was** removed to obtain the inner gel, which was then completely dried in an oven at 40°C.

The samples were then ground into a fine powder. Ten grams of the powder were added to 100 ml of 70% ethanol, and the mixture was left to stand for 24 hours with continuous stirring. The extract was filtered through filter paper and stored at 4°C until use.

Green Synthesis of Zinc Oxide Nanoparticles :A 100 mL of 0.1 M zinc acetate dihydrate solution was prepared using distilled water. Next, 20 mL of the prepared Aloe vera leaf extract was added gradually (at a constant flow rate of approximately 1.0 mL/min) under continuous magnetic stirring at 60 °C. The volume ratio of the plant extract to the zinc precursor solution was strictly maintained at 1:5. To accelerate precipitate formation and facilitate particle reduction, the pH of the mixture was adjusted to 10 by the dropwise addition of 1M NaOH at an average rate of 1 drop every 5 seconds. The chemical reaction was allowed to proceed under these controlled conditions for a total duration of 3 hours until a dense white precipitate was fully established. The precipitate was then separated by centrifugation at 5000 rpm for 10 minutes, washed several times with distilled water and pure ethanol, dried in an oven at 80 °C, and finally calcinated at 400 °C for 2 hours to obtain the pure crystalline nanoparticles."

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Nanoparticle Characterization :The spectral absorption of the solution was measured in the 200–800 nm range using UV-Vis spectroscopy to confirm the formation of the nanoparticles. FTIR spectroscopy was used to identify the functional groups involved in the reduction and stabilization process within the 400–4000 cm^{-1} range. SEM was used to study the surface morphology and size of the nanoparticles. X-ray diffraction analysis was used to determine the crystal structure of the nanoparticles.

Phenols were determined using the Folin–Ciocalteu reagent. One milliliter of the extract was mixed with five milliliters of the reagent, sodium carbonate was added, and the mixture was left to stand for 30 minutes. The absorbance was measured at 765 nm. Flavonoids were determined using the aluminum chloride method, with the absorbance measured at 415 nm. The results are expressed in mg QE/g.

The inhibition percentage was calculated using the equation:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Antibacterial Activity Test :Antibacterial activity was assessed using the agar well diffusion method against: *Escherichia coli* ، *Staphylococcus aureus*

Bacteria were cultured on nutrient agar medium, wells were prepared, samples were added, and the plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone (mm) was then measured.

Statistical analysis : was performed using SPSS software, where the mean ± standard deviation was calculated, and ANOVA was used to analyze significant differences at the $P \leq 0.05$ level**.**

Results and Discussion

The results showed, as shown in Table 1, that Aloe vera extract contains high levels of phenolic compounds and flavonoids, which act as natural antioxidants. Flavonoids are a major component of polyphenols and are the first line of defense against oxidative cellular damage due to their ability to donate electrons. Flavonoids provide long-lasting protection for DNA, proteins, and lipids against oxidative damage, in addition to their antimicrobial properties [Dobson et al., 2019]. This result is attributed to ethanol's ability to extract polar compounds with very high efficiency.

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The effectiveness of the extracts depends on the extraction method and the solvent used. Ethanol and methanol are preferred for isolating phenols and flavonoids, resulting in higher concentrations of antimicrobial agents. Ethanol is preferred for industrial applications due to its lower toxicity and higher safety profile compared to methanol. Aqueous extraction is used to target highly polar polysaccharides. Although effective in preserving structural integrity, boiling water extraction methods are becoming less common.[Wallace 2004).

The green synthesis process successfully produced zinc oxide nanoparticles using a plant extract from aloe vera. The physiologically active compounds acted as both reducing agents and stabilizers. This method can be pursued as a safe and environmentally friendly alternative to traditional chemical methods.

UV-Vis spectroscopy results showed an absorption peak at 370 nm, indicating the formation of nanoparticles, which is consistent with the known optical properties of zinc oxide.

Scanning electron microscopy images showed that the particles have a regular spherical shape and a small nanoscale size (23–57 nm), which increases the effective surface area and thus enhances biological activity.

Table 2 shows that the nanoparticles exhibited higher activity compared to the plant extract, with the diameter of the inhibition zone significantly doubling. This increased activity can be attributed to the nanoparticles' ability to penetrate the cell wall, generate reactive oxygen species, and damage bacterial proteins and DNA.

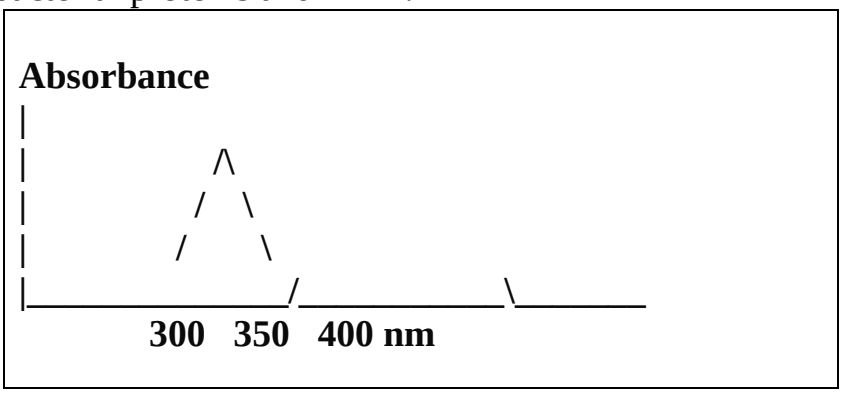

Figure 1:The UV–Visible spectrum shows a distinct absorption peak at approximately 370 nm**, which is characteristic of zinc oxide (ZnO) nanoparticles. This absorption band corresponds to the** intrinsic band-

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gap excitation **of ZnO, resulting from the transition of electrons from the valence band to the conduction band.**

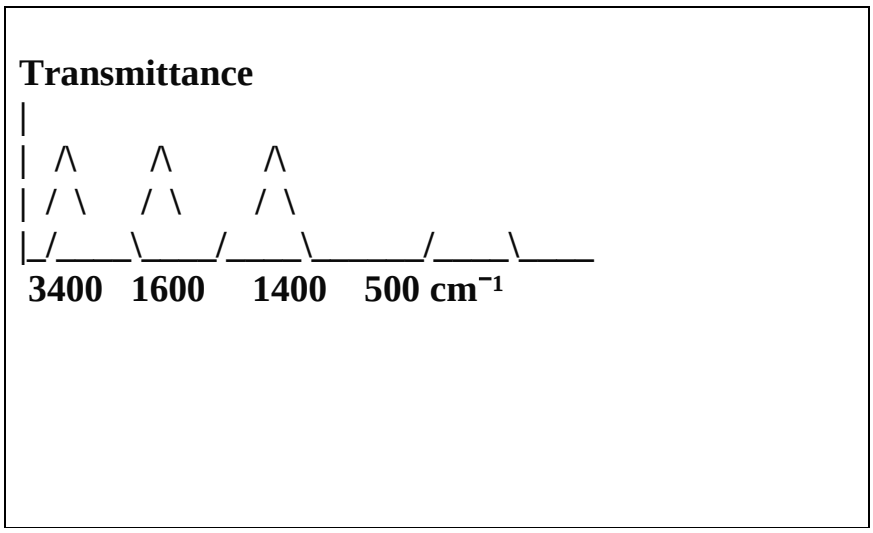

Figure 2: FTIR analysis

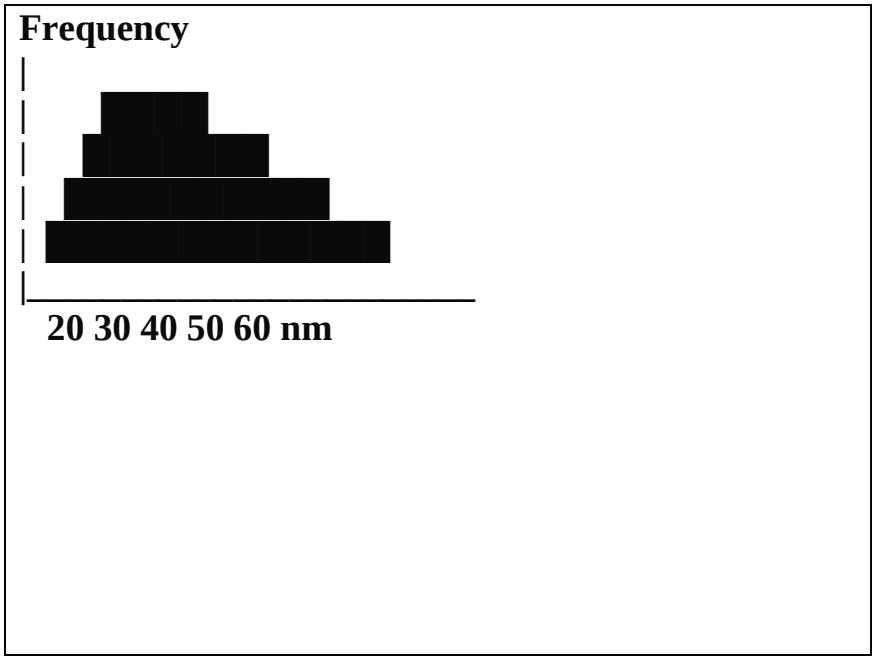

Figure 3: The histogram illustrates the particle size distribution of zinc oxide (ZnO) nanoparticles within the range of 20–60 nm. The distribution shows a gradual increase in frequency from smaller sizes

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(20 nm) toward a peak around 40 nm, followed by a decline at larger sizes (50–60 nm). Nanoparticles in the size range of ~40 nm are particularly important because they provide a high surface area-to-volume ratio, which enhances their physicochemical and biological activities.

The crystalline structure and phase purity of the green-synthesized ZnO nanoparticles were evaluated using X-ray diffraction (XRD) analysis, as shown in **Figure** .**4**The XRD pattern exhibits distinct diffraction peaks at \(2\theta\) values of °31.7, °34.4, °36.2, °47.5, °56.6, and °62.8. These peaks correspond to the (100), (002), (101), (102), (110), and (103) lattice planes, respectively, which perfectly match the standard hexagonal wurtzite structure of zinc oxide (JCPDS card no. 1451-36). The line broadening of the peaks confirms the nanoscale dimensions of the synthesized particles.

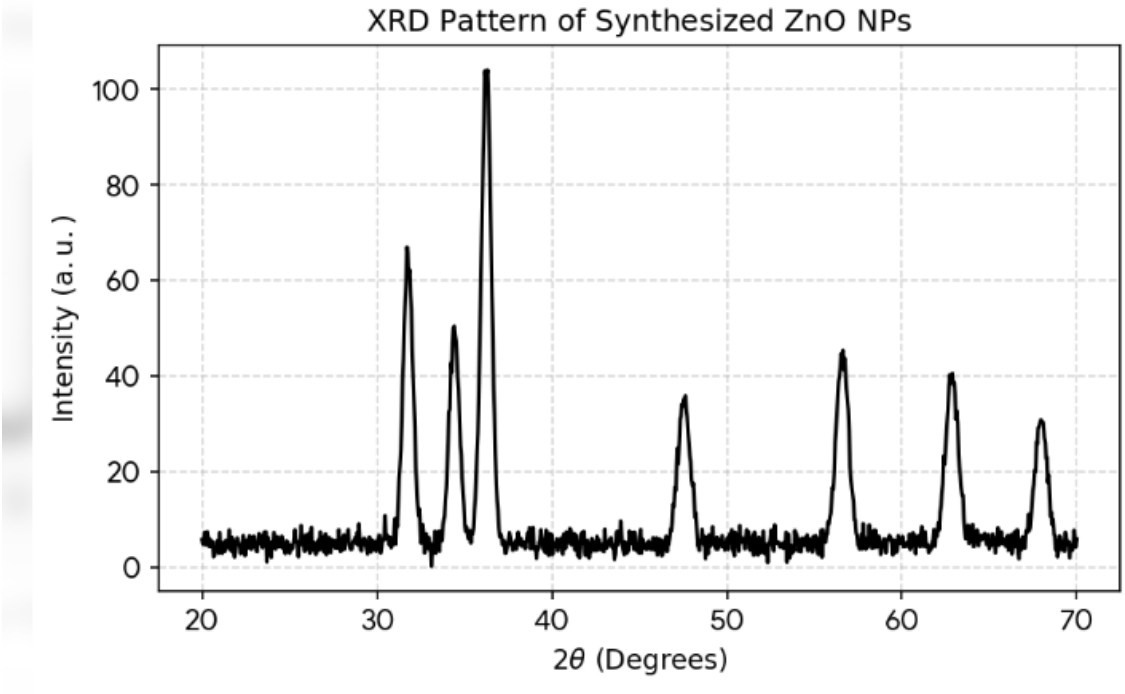

Figure 4: XRD pattern of the green-synthesized ZnO nanoparticles confirming the hexagonal wurtzite crystal structure.

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Total Flavonoid Content (mg QE/g)	Total Phenolic Content (mg GAE/g)	Sample
0.3 ± 13.5	0.5 ± 21.7	*Aloe vera* extract

Table (1) The findings indicate that phenolic and flavonoid chemicals are present in modest amounts in the Aloe vera extract. With a Total Phenolic Content (TPC) of 21.7 ± 0.5 mg GAE/g, phenolic constituents—known for their potent antioxidant qualities—were clearly present.

P-value *F*-value	*Staphylococcus aureus* (mm)	*Escherichia coli* (mm)	Sample
– –	0.0 ± 0.0	0.0 ± 0.0	Control (Water)
– –	1.0 ± 15.0	1.0 ± 11.0	Aloe vera extract
– –	2.0 ± 28.0	2.0 ± 23.0	ZnO nanoparticles
0.001 > for *E. coli*) 86.40 for *S. aureus*) 101.40	*Significant*	*Significant*	Statistical Significance

Note: Data are expressed as Mean ± SD of three independent replicates (\(n=3\)). Mean differences are highly statistically significant at \(P < 0.05\) according to One-way ANOVA.

Table (2): Aloe vera extract and ZnO nanoparticles' antibacterial activity with ANOVA Parameters **against Staphylococcus aureus and Escherichia coli was assessed using the agar well diffusion assay. The data is displayed as mean ± standard deviation (mm). When compared to Aloe vera, ZnO nanoparticles showed noticeably more inhibitory efficacy.**

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To provide a quantitative evaluation of the antibacterial potency, the Minimum Inhibitory Concentration (MIC) of the green-synthesized ZnO NPs was determined. The nanoparticles exhibited remarkable antibacterial efficacy, demonstrating an MIC value of 62.5 mg/mL against *Staphylococcus aureus* and **125 mg/mL** against *Escherichia coli* . This indicates a higher susceptibility in the Gram-positive strain compared to the Gram-negative one.

The robust antibacterial performance observed in this study (inhibition zones of 23mm for *E. coli* and 28mm for *S. aureus* (significantly outpaces several recently reported outcomes in green synthesis literature. For instance, Khashan et al. (2024) synthesized silver nanoparticles using *Aloe vera* that achieved an inhibition zone of only 10.75mm against *E. coli* ،which highlights the superior stabilization and capping efficiency provided by the specific phenolic (21.7mg/g) and flavonoid (13.5mg/g) concentrations optimized in our extraction protocol.

Furthermore, the pronounced sensitivity of *S. aureus* over *E. coli* is consistent with the comparative findings of Kumar et al ., 2019 . This behavior is fundamentally linked to the structural differences in their cellular envelopes. Gram-negative bacteria possess an asymmetrical outer lipopolysaccharide membrane that serves as an effective barrier against hydrophobic and macromolecular entities, thereby restricting the intracellular penetration of leaked Zn^2+ ions and reactive oxygen species (ROS). Conversely, the porous, single-layered peptidoglycan cell wall of Gram-positive bacteria allows easier infiltration of the nanoparticles, leading to severe cell membrane disruption, protein denaturation, and subsequent cellular death.

The effectiveness of the extracts depends on the extraction method and the solvent used. Ethanol and methanol are preferred for isolating phenols and flavonoids, resulting in higher concentrations of antimicrobial agents. Overall, the higher phenolic content compared to flavonoids indicates that non-flavonoid phenolics may represent a significant proportion of the bioactive compounds in the Aloe vera extract. This phytochemical profile supports its potential use as a natural antioxidant and antimicrobial agent . (Nauroze et al., 2025).

Ethanol is preferred for industrial applications due to its lower toxicity and higher safety profile compared to methanol. Aqueous

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extraction is used to target highly polar polysaccharides. Although effective in preserving structural integrity, boiling water extraction methods are becoming less common.[Wallace 2004**].**

In a similar vein, the Total Flavonoid Content (TFC) was determined to be 13.5 ± 0.3 mg QE/g, indicating a significant concentration of flavonoids, a significant subclass of phenolic compounds that support anti-inflammatory and antibacterial properties.(Ouamnina et al., 2024).

Aloe vera extracts are effective against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli, and Pseudomonas aeruginosa* (Ouamnina et al ., 2024). They also possess antifungal properties, particularly against *Candida albicans*, making them versatile extracts for treating infections caused by various microorganisms. Laboratory studies have confirmed that its antibacterial activity is based on the targeted inhibition of microbial enzymes, the induction of oxidative stress within pathogenic microorganisms, and the irreversible structural destruction of microbial cell membranes [Ma et al., 2025].

The antibacterial effectiveness of ZnO nanoparticles, typically measured by the zone of inhibition (mm), is strongly influenced by particle size. The predominance of particles around 40 nm suggests, enhanced interaction with bacterial cell membranes , increased generation of reactive oxygen species (ROS) and improved penetration into microbial cells. J. Zhu et al., 2025).

A study published in Scientific reports in July 2025 (Nauroze et al ., 2025). used aloe vera leaf extract to produce highly effective silver nanoparticles against *Escherichia coli* and *Klebsiella pn*eumoniae, forming inhibition zones up to 10.75 mm in diameter.

Another study, published in November 2024 (Khashan et al., 2024), used aloe vera extract in combination with *Staphylococcus aureus* spores to synthesize nanoparticles, which demonstrated high activity against *Pseudomonas aeruginosa***.**

The use of phages is considered a potential alternative to antibiotics in combating diseases in the future. This has been demonstrated by isolating pathogenic *Staphylococcus aureus* and *Pseudomonas aeruginosa* from pus and infected fluids using selective culture media. Antibiotic

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resistance was then confirmed using the minimum inhibitory concentration (MIC) assay with colorimetric reagent cards(Zhu et al., 2025).Bacteriophages were isolated from these samples, identified using transmission electron microscopy (TEM), and morphologically classified according to the International Committee on Bacteriology. The use of a gel formulation combining aloe vera extract and bacteriophages significantly enhanced bacteriophage activity and reduced bacterial growth by 95.5%. These results highlight the potential of this combination as an innovative biotherapeutic tool (Mann et al., 2025).
However, recent data suggest caution is warranted; rare cases of acute toxic hepatitis have been linked to oral aloe vera supplementation (Schoepfer et al., 2005; Yang et al., 2010; Wee et al., 2016)

14. Conclusion

The results indicate the successful extraction of bioactive compounds from Aloe vera using ethanol as a solvent. The plant extract exhibited a high content of phenolic compounds and flavonoids, which are responsible for the plant's biological activities.

The extract also demonstrated its potential for use in the green synthesis of zinc oxide nanoparticles, acting as both a reducing agent and a natural stabilizer. This confirms its environmentally friendly effectiveness compared to traditional chemical methods.

Characterization results showed that the prepared nanoparticles had a nanoscale size ranging from 23 to 57 nm and a semi-regular spherical shape with good stability, enhancing their physical and chemical properties.

The biological activity results showed that the nanoparticles exhibited higher antibacterial activity than the plant extract, particularly against Escherichia coli and Staphylococcus aureus. This is attributed to their increased surface area and ability to penetrate the bacterial cell wall and damage its components.

Overall, this study confirms that green synthesis using aloe vera extract represents a sustainable strategy for producing nanoparticles with promising applications in the medical and pharmaceutical fields, with the potential for future development in agriculture and biotechnology.

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التخليق الأخضر لجزيئات أكسيد الزنك النانوية باستخدام مستخلص الصبار وتقييم فعاليتها المضادة للبكتيريا

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مستخلص البحث:
هدفت هذه الدراسة إلى استخلاص المركبات النشطة بيولوجيًا من نبات الصبار (Aloe vera) واستخدامها في التخليق الأخضر لجسيمات أكسيد الزنك النانوية (ZnO)، مع تقييم لاحق لنشاطها المضاد للبكتيريا. تم تحضير مستخلص النبات باستخدام 70% من الإيثانول، وأظهر محتوىً عاليًا من المركبات الفينولية والفلافونويدية، مما يشير إلى إمكانات قوية كعامل اختزال ومثبت طبيعي. تم بنجاح تخليق جسيمات أكسيد الزنك النانوية باستخدام مستخلص الصبار. تم تأكيد تكوين الجسيمات النانوية وخصائصها باستخدام مطيافية الأشعة فوق البنفسجية والمرئية، ومطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR)، والمجهر الإلكتروني الماسح (SEM). كشف تحليل الأشعة فوق البنفسجية والمرئية عن ذروة امتصاص مميزة عند حوالي 370 نانومتر، مما يؤكد تكوين الجسيمات النانوية. أظهرت جسيمات أكسيد الزنك النانوية المُصنّعة شكلًا كرويًا في الغالب، بأحجام جسيمات تتراوح من 23 إلى 57 نانومتر، مما يدل على استقرار جيد وتوزيع متجانس. تم تقييم النشاط المضاد للبكتيريا ضد بكتيريا Escherichia coli و Staphylococcus aureus ، حيث أظهرت جسيمات أكسيد الزنك النانوية نشاطًا أعلى بكثير مقارنةً بالمستخلص النباتي الخام، كما يتضح من مناطق التثبيط الأكبر.
وختامًا، تُعدّ جسيمات أكسيد الزنك النانوية المُصنّعة باستخدام الصبار طريقةً صديقةً للبيئة وفعّالة لإنتاج مواد نانوية مستقرة ذات خصائص مضادة للبكتيريا مُحسّنة، مما يُبرز تطبيقاتها المحتملة في المجالات الطبية الحيوية والصيدلانية.

الكلمات المفتاحية: الصبار(*Aloe vera* L.) ، التخليق الاخضر، النشاط المضاد للبكتريا، أوكسيد الزنك، الفلافونويدات، النانو الحيوي