

Analgesic Activity of Gold Nanoparticles Biosynthesis and Analgesic Activity of Gold Nanoparticles (AuNPs) From Leaf Extract of *Ziziphus zizyphus* for Pain Management

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ABSTRACT

Aim/Background: The growing need for novel methods of preparing nanoparticles is evident in recent scientific pursuits. Environmental concerns about the security of current production processes are growing along with interest in this subject. Current nanoparticle production processes are frequently costly and include the use of environmentally detrimental substances. This work investigates the production of Gold Nanoparticles (AuNPs) by using *Ziziphus zizyphus* leaf extract. This method provides a cost-effective, highly efficient, and environmentally safe alternative for producing nanoparticles. Additionally, this work examines the efficacy of prepared AuNPs in alleviating pain. **Materials and Methods:** Our approach involved using biologically active chemicals as a natural reducing agent present in an aqueous leaf extract. The synthesized AuNPs were analyzed using a range of analytical techniques, such as X-ray Diffraction (XRD), Thermogravimetric Analysis (TGA), Atomic Force Microscopy (AFM), UV-visible spectroscopy (UV-vis), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Energy Dispersive X-ray analysis (EDX). **Results:** UV-vis spectrum showed the formation of SPR band with optical absorption around 527 nm. TEM, DLS, Zeta potential results exhibited the formation of stable AuNPs with an average hydrodynamic diameter of approximately 54 ± 0.8 nm. The TGA analysis validated the effectiveness of this biosynthetic method by showing that the plant extract's biomolecules coated and protected the AuNPs. Analgesic studies revealed that the AuNPs synthesized in this method demonstrated significantly greater ability to reduce pain at a comparable dosage of 200 mg/kg when compared to aspirin. The increased pain-relieving effectiveness reported in the AuNPs, compared to the *Ziziphus* extract alone, is probably because of a synergistic interaction between the AuNPs and the secondary metabolites found in the plant extract. **Conclusion:** The present investigation on pain management showed that the combined effect of the AuNPs and *Ziziphus zizyphus* leaf extract is more effective in pain management compared to the individual use of the extract and aspirin medication.

Keywords: Analgesic Activity, Biocompatibility, Gold Nanoparticles, Green Synthesis, *Ziziphus zizyphus*.

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INTRODUCTION

Due to the distinct optical, catalytic and magnetic properties of the metallic nanoparticles, they have gained significant attention in the last few years. Nanoparticles parameters, including their structural morphology, shape, size and homogeneity have a significant impact on their physical characteristics, as indicated in multiple studies.¹ Scientists have investigated many techniques, such as biological, physical and chemical methods, to produce these nanoparticles.²⁻⁴

Procedures used to synthesise metal nanoparticles frequently generate noxious byproducts that adhere to the surfaces of the nanoparticles and cause potential health hazards. A toxic quaternary ammonium surfactant, i.e., cetyltrimethylammonium bromide, is an example of a regularly utilized substance in the production of gold nanorods. On the other hand, using natural plant extracts to produce nanoparticles offers a safer alternative that is environmentally beneficial. Some examples of such plants are *Daucus carota*, *Coriandrum sativum*, *Solanum lycopersicum* and *Bischofia javanica*. Additional plant materials include leaves of *Hibiscus cannabinus*, peels of *Cymbopogon flexuosus* (lemongrass) and *Citrus unshiu*, flowers of *Bacopa monnieri* and *Moringa oleifera*,⁵ along with extracts from *Aloe Vera* leaves⁶ and *Ananas comosus*.⁷⁻¹² Various review articles have also been published, providing comprehensive information on the



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production of several types of nanoparticles using diverse plant extracts.¹³⁻²²

AuNPs are highly adaptable nanomaterials that have a wide array of uses, such as cancer treatment via hyperthermia,²³ improvement in Surface-Enhanced Raman Spectroscopy (SERS),²⁴ and the absorption of Infrared Rays (IR) in optical applications.²⁵ Various methods have been devised to synthesise AuNPs with different shapes and sizes.²⁶ Wet chemical synthesis methods have effectively generated isotropic structures, such as rods, wires, plates and teardrop forms.²⁷ Conversely, the use of microorganisms, plants, or their extracts for biological production offers a more ecologically sound and less complex alternative to traditional chemical approaches.

Research has shown that inorganic nanoparticles, such as AuNPs, can interact with microorganisms, potentially exhibiting antibacterial and antifungal effects.²⁸⁻³¹ AuNPs have been shown to demonstrate significant antibacterial properties,^{32,33} possibly attributed to their cytotoxicity against diverse microorganisms. These effects are believed to result from interactions with functional groups on the surfaces of bacterial cells, leading to cell death or inactivation. The antibiotic activity of AuNPs has been recognised as either the surface coating of the nanoparticles or leftovers from the fabrication process rather than the core of the nanoparticles themselves.³⁴ Different perspectives exist in the scientific discourse on the toxicity of nanoparticles. Several studies show that AuNPs are usually non-toxic for living things, no matter what size they are or what coatings are used, like citrate, cysteine, biotin, or sugars.³⁵⁻³⁷ Alternatively, several studies suggest that the harmfulness of 2 nm cationic AuNPs depends on the amount used, whereas negatively charged nanoparticles of the same size were discovered to be harmless at comparable levels.^{35,38}

The synthesis of nanoparticles by using plant extracts is a prominent environmentally safe method, as most plants demonstrate biocompatibility and guarantee safe handling.³⁹ This study involves the production of AuNPs using *Ziziphus zizyphus* leaf extract, with an emphasis on investigating the analgesic effects of the resulting mixture. To the best of the researchers' knowledge, no prior studies have documented the use of this plant for the synthesis of AuNPs or its analgesic properties. The formed AuNPs were analyzed by techniques such as FTIR, UV-vis and TEM. The results of the analgesic activity demonstrate that the combination of plant extract and AuNPs provides superior pain-relieving effects compared to the individual effects of aspirin and the plant extract alone.

MATERIALS AND METHODS

Materials

Chloroauric acid (HAuCl_4) and other chemicals were obtained from Sigma-Aldrich, Shanghai, China. Before starting the research,

all the glassware used was thoroughly cleaned with distilled water to assure cleanliness and prevent any contamination.

Experimental Methods

Synthesis of AuNPs

To achieve the reduction of Au^{3+} ions to Au^0 , a plant extract was prepared using *Ziziphus zizyphus*. Specifically, 10 g of leaves were washed thoroughly and placed in a 200 mL Erlenmeyer flask containing 100 mL of sterile double-distilled water. The mixture was heated for 5 min. For the conventional experimental setup, 5 mL of the prepared plant extract was combined with 45 mL of a 1 mM HAuCl_4 solution in water. The temporal progression of the reduction of AuCl_4^- was monitored using UV-visible absorption spectroscopy.

Purification of AuNPs

After synthesis, the AuNPs were centrifuged using a tabletop Eppendorf centrifuge at 14,000 rpm for 20 min, under ambient conditions. This step aimed to remove large particle aggregates. After some time, the transparent top layer was meticulously isolated and subjected to additional refinement using PD-10 desalting columns from Sigma-Aldrich in Shanghai, China. The eluates, with a total volume of around 3.5 mL, were subsequently subjected to dialysis in a 10 mM PBS (pH 7.0) using dialysis bags with 20 kDa molecular weight cut-off from Spectrum Labs. This dialysis procedure included a buffer exchange after 2 hr and 15-18 hr prolonged incubation.

Characterization

UV-visible spectroscopy

To study the optimal concentration levels of the plant extracts UV-vis spectroscopy was used. The experiment was conducted at regular intervals, ensuring consistent concentrations of both the aqueous Gold Chloride (AuCl_3) solution and plant extract. The formation of AuNPs was indicated by a distinct ruby-red colour change. Confirmation of nanoparticle synthesis was achieved by analyzing the absorbance peak range of the colloidal AuNPs, which spanned from 200 nm to 800 nm using a PerkinElmer Lambda 25 spectrometer (Shanghai, China). The change in colour was observed within 30 sec after combining the AuCl_3 with the plant extract and the synthesis of the nanoparticles was finished within 3 min from the beginning of the reaction. The spectroscopic evaluations were conducted on freshly synthesised samples at room temperature. Quartz cuvettes with a 1 cm optical path were utilised for this purpose.

X-ray Diffraction (XRD)

A MAXima_X XRD-7000 system (Sigma-Aldrich, Shanghai, China) is used to conduct XRD analysis. The system functioned at a 20 mA of current and 40 kV of operating voltage, utilising a Cu K- α radiation source with a wavelength of 1.54 Å.⁴⁰ The system

covered a range of 20 values, from 30° to 75°. To get this data, colloidal AuNPs were first exposed to centrifugation at a force of 10,000 times the acceleration due to gravity for duration of 15 min at room temperature. The resultant pellets were rinsed with deionized water for three times, with 5 mL of deionized water for each rinse. Afterwards, the samples were allowed to freeze-dry at a temperature of -54°C in a vacuum environment with controlled pressure in order to prepare them for XRD examination.

Thermogravimetric Analysis (TGA)

The samples were exposed to TGA using a PerkinElmer Diamond TG/DTA STA 6000 system (PerkinElmer, Shanghai, China). The analysis was conducted throughout the temperature ranges from room temperature to 900°C. The heating was regulated at a rate of 10°C per min while maintaining an oxygen flow of 20 mL/min. The samples, previously freeze-dried at -54°C under controlled pressure and vacuum conditions, were then placed in a clean pan attached to a highly accurate balance. The weights of the samples were precisely recorded both at the beginning and during the experiment. In order to completely remove all moisture, each sample was preheated at 100°C for 15 min. Afterwards the temperature gradually rose with the predetermined rate.

Transmission Electron Microscopy (TEM)

The TEM analysis was conducted using a FEI Titan (from FEI Company, Shanghai, China) functioning at 300 kV. The microscope was outfitted with a Gatan Tridiem GIF-863 post-column filter. For sample preparation, the samples were diluted in distilled water to a final concentration of 0.05 mg/mL. Subsequently, the suspension was deposited onto Lacey carbon grids (300 mesh, SPI Supplies, 3330C-CF) and left to dry naturally before microscopic examination.

Atomic Force Microscopy (AFM)

To conduct AFM experiments, the nanoparticle samples were diluted to 0.05 mg/mL concentration in distilled water and subsequently spread them evenly onto a zinc substrate. The surface properties of the nanoparticles were analysed by scanning a 1×1 µm region using a set point of 10 nm and a scan rate of 1 µm/s. The image acquisition and analysis were carried out using the Bruker Dimension 3100 AFM system, which was equipped with Nanoscope 5 software, both manufactured by Bruker in Shanghai, China.

Dynamic Light Scattering and Zeta Potential

We used a Brookhaven Zeta-PAL zeta potential analyser (Shanghai, China) to discover the AuNPs hydrodynamic diameter. Each sample, including a concentration of 50 µg/mL in deionized water, underwent DLS. The analysis consisted of 10 runs per sample, with each run lasting 30 sec. Additionally, each sample measurement was repeated thrice. The experimental

parameters were set to a temperature of 25°C, an applied voltage of 5 V, electric field strength of 13.89 V/cm and a refractive index of 1.330. The average surface energy was determined using the Smoluchowski coagulation equation, using a wavelength of 659 nm and 7 automated attenuation settings. The data shown here is a composite derived from three distinct synthesis batches, each of which underwent ten replicates for measurement.

Analgesic activity

Formalin test

Albino male Wistar rats, which weighed between 150-200 g, were housed in plexiglass enclosures and had unrestricted access to water and food. The testing was conducted at the midpoint of a 12-hr light/12-hr dark cycle, and each rat was tested only once. A 0.9% saline solution (w/v) was used to prepare the *Ziziphus zizyphus* mediated AuNPs at doses of 60, 70, 80 and 90 mg/kg, as well as morphine sulfate at doses of 5 and 10 mg/kg. The solution was administered intraperitoneally (i.p.) in a volume spanning from 1.5 to 2 mL. There was no active medication administered to the control group; only the vehicle was administered in the same volume. The formalin test, which was initially described by Dubuisson and Dennis (1977), was employed to assess the antinociceptive effects of the treatments. The rats were confined to a conventional observation chamber for 1 hr prior to the formalin test. A 50 µL injection of 2.5% formalin was administered to the dorsal surface of the left hind paw. Following the formalin injection, the rats were monitored for a total of 60 min and the duration of time spent licking the injected paw was used as an indicator of pain response. The initial 5 min following injection were considered the early phase of the response, while the period from 15 to 60 min was classified as the late phase. 15 min prior to the formalin injection, the medications were administered.

Hot Plate Method

Swiss albino mice were confined in colony cages, with humidity levels ranging from 50-55% and a temperature of 25±2°C. With a controlled light/dark cycle of 12 hr, each cage contained not more than 1 or 2 mice. Standard animal feed was administered to the mice. The rodents were acclimatized to the laboratory environment prior to the experiments. The hot plate method was employed to evaluate the analgesic activity in accordance with the established protocols. The highly sensitive paws of mice exhibit responses such as paw withdrawal and leaping in response to heat. As part of the experiment, mice were positioned on an Eddy's hot plate that was maintained at 54±2°C. To prevent thermal injury, the maximum exposure duration was limited to 15 sec. Responses and reaction times were documented using a stopwatch. Diclofenac sodium was employed as the positive control. AuNPs were administered intraperitoneally at concentrations of 100, 200 and 300 mg/kg.

RESULTS AND DISCUSSION

This work aimed to reproduce the conventional fabrication procedure of AuNPs, producing nanoparticles comparable in size to those previously reported in our investigations. Turkevich and Frens were responsible for developing the original method. Their approach entailed the reduction of a solution of gold hydrochlorate by employing sodium tris citrate at a temperature of 100°C.⁴¹ Nevertheless, our methodology presents a simpler and ecologically conscious (single step) technique for producing AuNPs. We utilised Image J software (version IF1.46r) to analyse the nanoparticles, specifically focusing on particle enumeration and size distribution utilising TEM images. Our research discovered that almost 90% of the nanoparticles observed were spherical and displayed monodispersity. This is a notable advantage of our environmentally friendly synthesis method, which eliminates the need for harsh and costly chemicals typically used in conventional procedures.

Upon combining the *Ziziphus zizyphus* extract with an aqueous solution of HAuCl_4 , we promptly noticed a significant colour change, to a vibrant ruby-red. The observed alteration in colour signifies the synthesis of AuNPs, which exhibit a distinct ruby-red shade when their size is below 30 nm, owing to localised SPR.^{42,43} In this situation, a phytochemical-driven process is the suggested mechanism for nanoparticle formation. The plant extract, which contains a variety of powerful reducing agents such as antioxidants, enzymes and phenolic compounds, facilitates the reduction of gold cations to AuNPs.⁴⁴⁻⁴⁷ The reduction process converts HAuCl_4 into elemental gold (Au^0) in its zero-oxidation state. Subsequently, the phytochemicals stabilise these particles, leading to the isotropic AuNPs formation.

The fabrication of AuNPs was evident as the solution's colour changed noticeably, shifting from a pale yellow to a vibrant ruby-red. The alteration in hue was followed by a conspicuous plasmon resonance peak within the range of 525-540 nm, typically peaking between 527-535 nm in the UV-visible spectrum (Figure 1). The existence of this peak is a distinctive attribute of spherical AuNPs, particularly those with a diameter between 30 and 50 nm.^{48,49} AuNPs were analysed using DLS, which yielded an average hydrodynamic diameter of approximately 54 ± 0.8 nm (Figure 2A). The polydispersity index, which quantifies the range of particle sizes, was found to be approximately 0.321%. This value signifies a distribution that is moderately uniform, consistent with past research results.^{50,51} The presence of consistent particle size or shape fluctuations is frequently associated with the medium's lack of uniformity.

Zeta potential measurements are widely acknowledged as a crucial indicator of colloidal particle stability. The values represent the total electrical charge that the surface functional groups of the particles have generated. A zeta potential value exceeding +25 mV or falling below -25 mV generally indicates a stable colloidal state for nanoparticles.^{50,51} The surface energy of the AuNPs in our investigation was found to be -41.2 ± 0.2 mV (Figure 2B). These findings indicate that, when AuNPs are placed in a buffer solution, they form a stable colloid that shows excellent dispersion and no noticeable aggregation for duration of 6 months.

By examining TEM pictures, it was noted that the particles formed were mainly composed of spherical, polycrystalline AuNPs. In addition, the samples contained several anisotropic forms, such as hexagonal and triangular platelets, as well as truncated single nanosheets, as depicted in Figure 3. The truncation geometries, which are frequently observed in disc-shaped nanostructures, have been previously documented in chemically produced

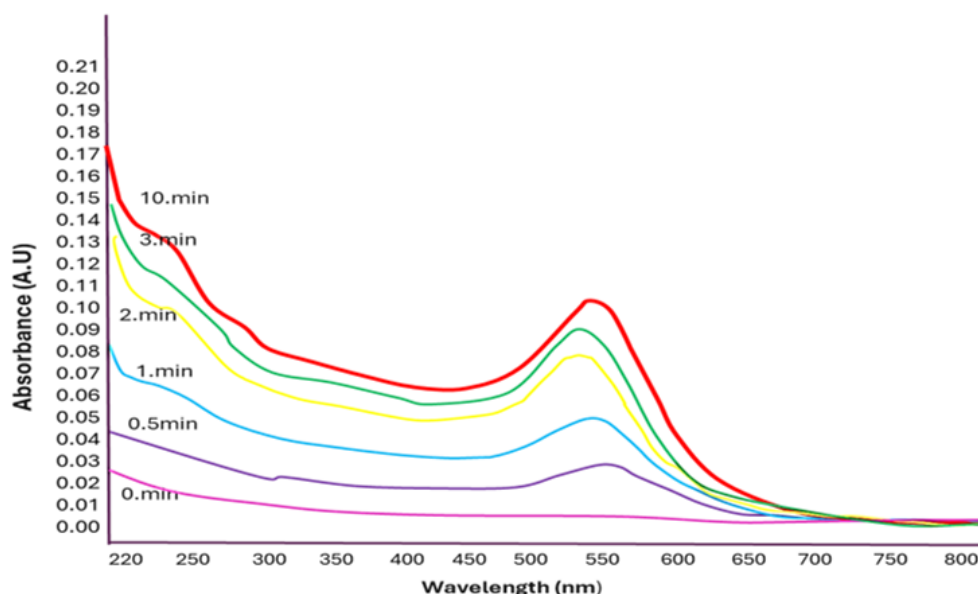


Figure 1: UV-visible spectra of AuNPs synthesized from ziziphus extract.

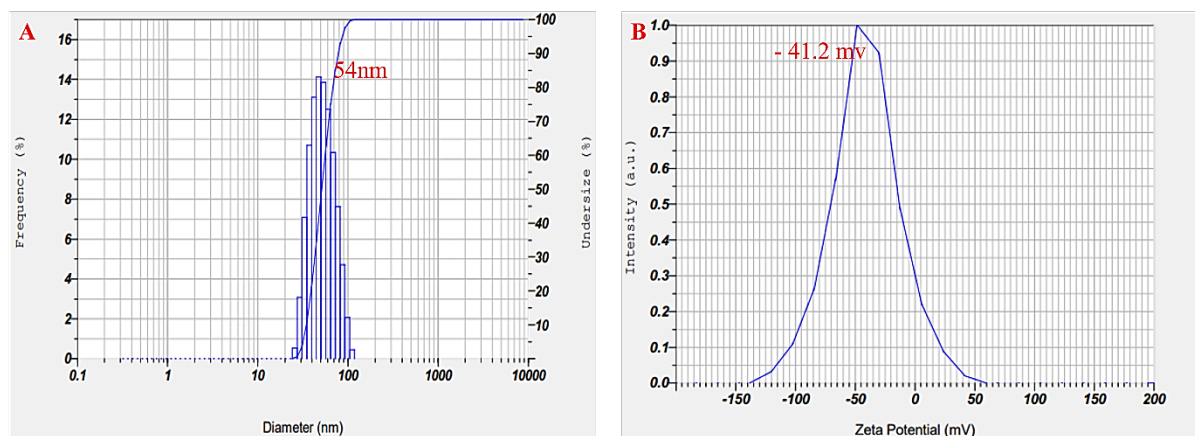


Figure 2: DLS (A) and Zeta potential (B) of AuNPs synthesized from ziziphus extract.

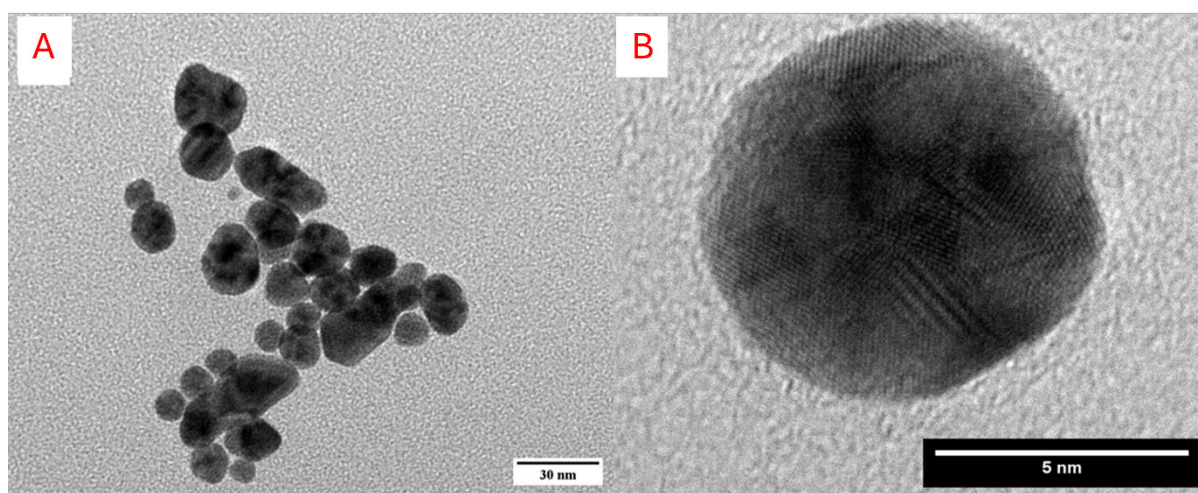


Figure 3: Unstained TEM image showing the presence of small AuNPs with an average diameter of 10-15 nm

AuNPs,^{52,53} and silver nano-triangles.^{54,55} Image analysis using Image-J software determined that around 10% of the overall nanoparticle population consisted of triangular and hexagonal AuNPs. The TEM pictures revealed the existence of AuNPs, some as small as 3 nm in size, as depicted in Figure 3. Further examination using higher-magnification TEM confirmed the presence of their lattice structure, as seen in the inset of Figure 3. The SEM-EDX examination confirmed that the nanoparticles were primarily made of gold, definitively excluding the possibility of contamination. Furthermore, AFM investigation revealed that these particles had a uniform size and were distributed in a confined range, as illustrated in Figure 4.

AFM was used to calculate the dimensions and dispersion of the produced AuNPs. The scanning was conducted over a $1 \times 1 \mu\text{M}$ region using a tapping mode, enabling the generation of 2D as depicted in Figure 4. The photos displayed a uniform distribution pattern of the AuNPs, with most of the particles having sizes varying from 40 to 50 nm and a spherical shape. This observation is consistent with the results obtained from DLS and TEM investigations.

Additional information on the structure was obtained through XRD research, as depicted in Figure 5. The XRD analysis indicated four distinct diffraction peaks in the $(20^\circ\text{--}80^\circ)$ 2θ region. The peaks observed at 38.1° (111), 44.5° (200), 64.7° (220) and 77.8° (311) correspond to the different facets of the face-centred cubic (FCC) crystal lattice of gold. This pattern is consistent with previous research conducted on similar gold nanostructures.⁵⁶ Furthermore, the recorded highest values align with the lattice planes and FCC architectures of AuNPs produced using alternative environmentally friendly techniques, as reported in previous scientific literature.^{22,57-59}

TGA, shown in Figure 6, was used to measure the amount of biologically active chemical residues attached to the AuNPs. These residues, primarily phenolic compounds and small proteins from the plant extract, were likely adhered to the nanoparticle surface. The implementation of meticulous purification processes played a crucial role in eliminating contaminants from the sample. TGA results revealed a degradation of approximately 37% of the organic constituents linked to the AuNPs. This observation suggests that the physiologically active chemicals present in

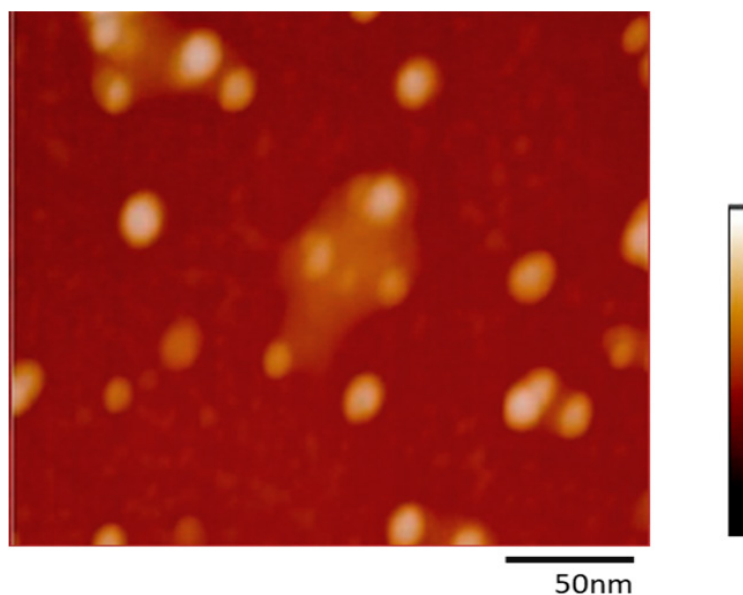


Figure 4: An atomic force microscopy two-dimensional image of generated particles.

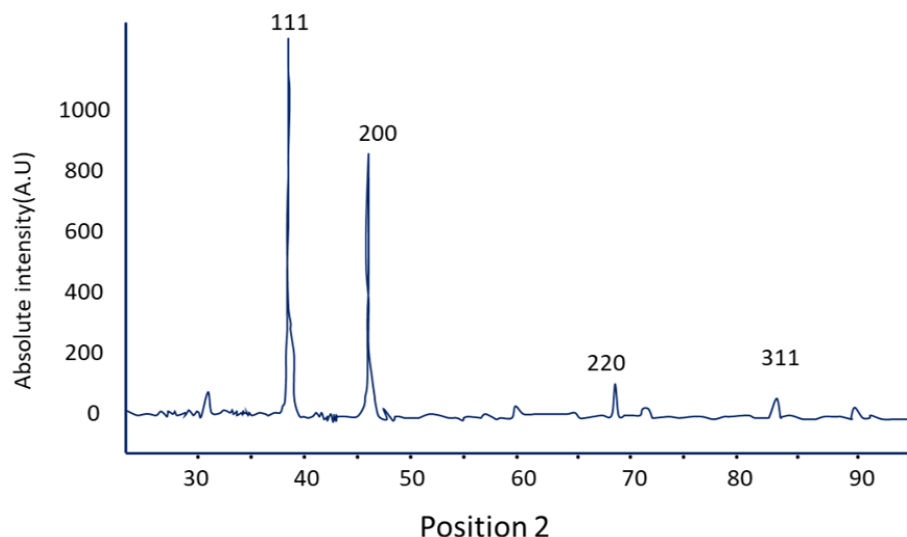


Figure 5: X-ray diffraction of crystalline AuNPs characterized by four peaks corresponding to standard bragg reflections.

the plant extract efficiently adhered to the AuNPs surface. Furthermore, it is possible that this coating process is linked to the observed change in the Raman spectral spectrum, specifically within the wavelength range of $\lambda_{\max}$ 527 and 535 nm. Changes in the Raman spectra frequently suggest alterations in the chemical bond lengths and the symmetry of the nanoparticles.⁶⁰⁻⁶²

Analgesic activity

Formalin test

Figure 7 illustrates the impact of the AuNPs on the formalin test. The statistical analysis indicates that all test groups exhibited significant differences from the control group in both the early and late phases of the test ($p \leq 0.001$). A dose-dependent inhibition of pain was observed in both phases of formalin-induced

nociception when the AuNPs was administered intraperitoneally at concentrations ranging from 60 to 90 mg/kg. It is important to note that the AuNPs antinociceptive effect at 80 and 90 mg/kg in the early phase was greater than that of morphine at 5 mg/kg but was less than that observed with morphine at 10 mg/kg ($p \leq 0.001$). Similarly, the AuNPs exhibited higher antinociceptive activity than morphine at 5 mg/kg and lower than morphine at 10 mg/kg during the late phase ($p \leq 0.001$). In either the early or late portions of the pain response, there was no significant difference between the responses to the AuNPs at 80 and 90 mg/kg ($p \leq 0.05$).

Hot plate method

Swiss albino mice were subjected to the hot plate method to assess the analgesic potential of the aqueous leaf extract of *Ziziphus*

zizyphus and its AuNPs (Figure 8). The leaf extract and AuNPs were administered at various intervals (0, 30, 60 and 90 min) and the mean latency time (in sec) was recorded. A mean latency time of 2.37 ± 0.11 sec was observed in the control group (Group I) treated with saline 30 min post-administration. Conversely, Group II, which received the standard analgesic diclofenac sodium, demonstrated a statistically significant increase in mean latency time, which was 7.11 ± 0.22 sec. This indicating considerable analgesic activity. In comparison to the control, the leaf extract and its AuNPs exhibited substantial, dose-dependent analgesic effects. The maximum mean latency time for AuNPs was 5.98 ± 0.41 sec at a dose of 300 mg/kg, followed by 5.29 ± 0.29 sec at 200 mg/kg and 4.44 ± 0.20 sec at 100 mg/kg, all of which

were recorded 30 min after drug administration. In comparison to the conventional diclofenac sodium, the AuNPs provided analgesia at doses of 100, 200 and 300 mg/kg, respectively, at 62.45%, 74.40% and 84.11%.

Compared to the saline control, the aqueous leaf extract and its AuNPs exhibited substantial analgesic effects 60 min after administration. The AuNPs at 300 mg/kg and 200 mg/kg exhibited latency times of 7.71 ± 0.39 sec and 6.77 ± 0.30 sec, respectively, which corresponded to 80.16% and 75.90% of the analgesic effect of diclofenac sodium. Diclofenac sodium exhibited a maximum latency time of 8.92 ± 0.32 sec during the study. Paw motion latency time exhibited a comparable pattern 90 min following drug administration. The synthesized AuNPs displayed a more

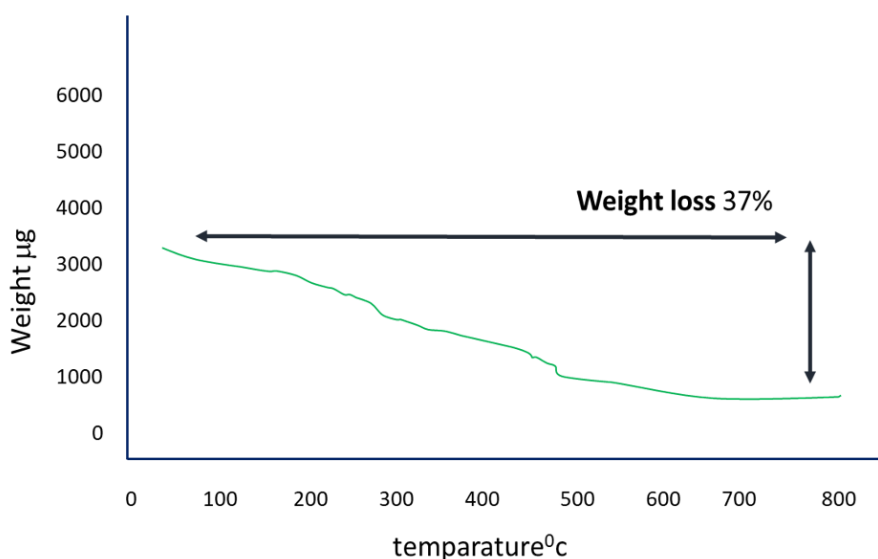


Figure 6: Thermogravimetric analysis of AuNPs.

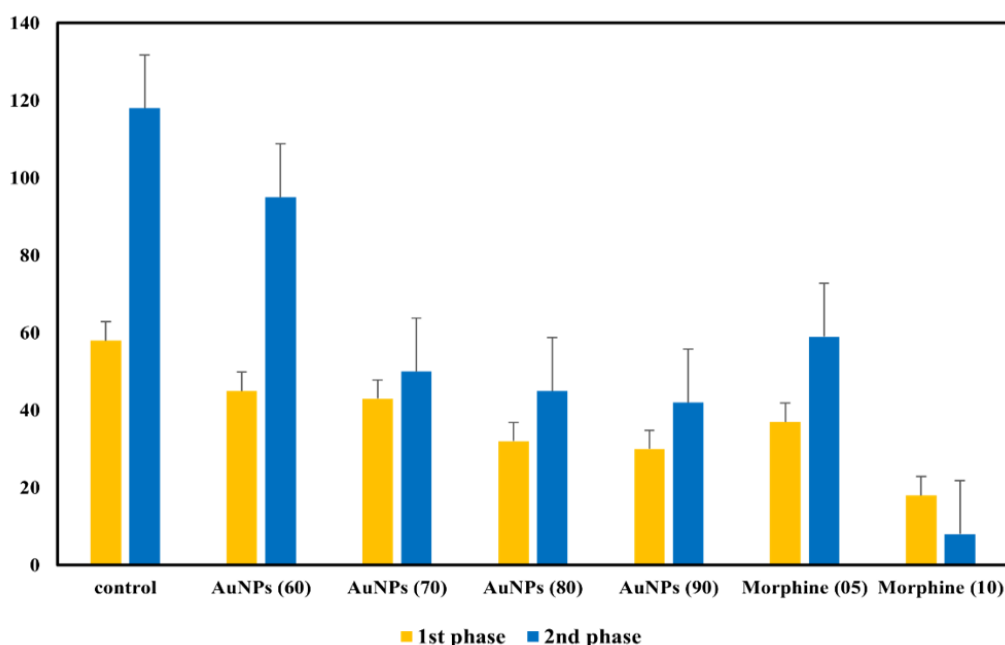


Figure 7: Effect of AuNPs and Morphine on the scores of 1st phase (A) and 2nd phase (B) of the formalin test.

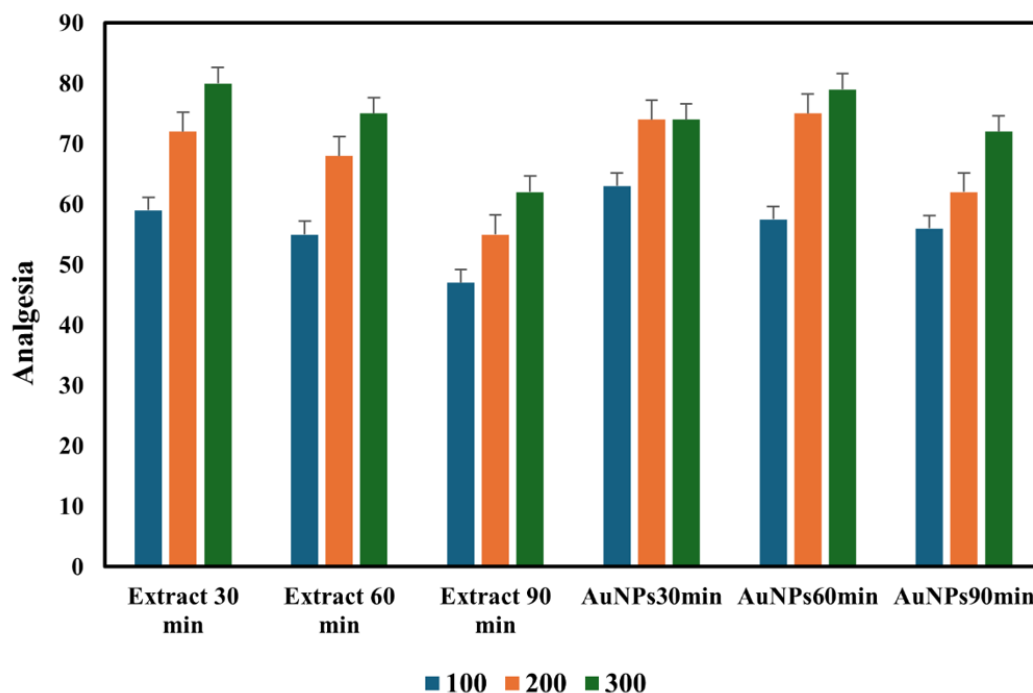


Figure 8: Effect of AuNPs on latency time of mice exposed to hot plate method.

pronounced analgesic effect compared to the aqueous leaf extract alone. Analgesic, diuretic and anti-inflammatory properties have been reported in numerous plants that contain flavonoids. In this study, the leaf extract of *Ziziphus zizyphus*, which is abundant in flavonoids and phenols, exhibited a robust analgesic effect.

CONCLUSION

This work introduces a method that is effective, quick and reproducible method for synthesizing AuNPs in an environmentally benign way, using of *Ziziphus zizyphus* leaf extract. UV-vis spectrum showed the formation of SPR band with optical absorption around 527 nm. TEM, DLS, Zeta potential results exhibited the formation of stable AuNPs with an average hydrodynamic diameter of approximately 54 ± 0.8 nm. Additionally, analgesic studie revealed that the AuNPs synthesised in this method demonstrated significantly greater ability to reduce pain at a comparable dosage of 200 mg/kg, when compared to aspirin, a widely recognised pain reliever. The increased pain-relieving effectiveness reported in the AuNPs, compared to the *Ziziphus* extract alone, is probably because of a synergistic interaction between the AuNPs and the secondary metabolites found in the plant. The data clearly demonstrate that the AuNPs synthesised using this environmentally friendly technique has considerable promise for application in pain control strategies.

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CONFLICT OF INTEREST

All the authors have declared that no conflict of interest related to this work.

ABBREVIATIONS

AuNPs: Gold nanoparticles; **HAuCl₄:** Chloroauric acid; **XRD:** X-ray Diffraction; **TGA:** Thermogravimetric Analysis; **AFM:** Atomic Force Microscopy; **UV-vis:** UV-visible spectroscopy; **SEM:** Scanning Electron Microscopy; **TEM:** Transmission Electron Microscopy; **EDX:** Energy Dispersive X-ray analysis; **FCC:** Face-centred cubic.

SUMMARY

This work introduces a rapid, efficient, and reproducible method for synthesizing AuNPs using *Ziziphus zizyphus* leaf extract, without the need for expensive reducing agents. The synthesized AuNPs were analyzed using a range of analytical techniques, such as X-ray Diffraction (XRD), Thermogravimetric Analysis (TGA), UV-visible spectroscopy (UV-vis), Transmission Electron Microscopy (TEM), and Energy Dispersive X-ray analysis (EDX), which confirmed the formation of stable polyphenol capped AuNPs with an average hydrodynamic diameter of approximately 54 ± 0.8 nm. Additionally, the present work showed the effectiveness of the prepared AuNPs in pain management. AuNPs synthesized via this method demonstrated significantly greater pain-relieving ability compared to aspirin. The enhanced pain-relieving effect is likely due to a synergistic interaction between the AuNPs and the secondary metabolites in the plant. The findings suggest that

AuNPs produced using this eco-friendly method have substantial potential for pain management applications.

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