RESEARCH ARTICLE

Characterization of Bimetallic (Zinc and Gold) Nanoparticles of *Cryptostegia grandiflora* Plant Extract and Their Biological Screening

RS Jadhav[1*], AT Inamdar[2], SK Mohite[2], RM Gavali[3]

[1]*Department of Pharmaceutical Chemistry, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth, Deemed to be University, Maharashtra, India.*
[2]*Department of Pharmaceutical Chemistry, KE'S, Rajrambapu College of Pharmacy, Kasegaon, Maharashtra, India.*
[3]*Department of Quality Assurance, KE'S, Rajrambapu College of Pharmacy, Kasegaon Maharashtra, India.*

Received: 20th February, 2024; Revised: 27th March, 2024; Accepted: 19th June, 2024; Available Online: 25th September, 2024

ABSTRACT

The goal of this learning was to explore the uses of *Cryptostegia grandiflora* plant extract in the green formulation of bimetallic zinc-gold nanoparticles along with pharmacological evaluation. Green synthesis and other techniques are more advantageous than chemical and physical procedures because they produce nanoparticles in an environmentally responsible and economically viable manner. An ethanolic extract of *C. grandiflora* was obtained through the use of Soxhlet extraction. Additionally, an ethanolic extract of *C. grandiflora* and components from the extract were identified using GC-MS research after mixing plant extract (*C. grandiflora*) with zinc acetate and chloroauric acid solutions and letting it sit for a while. UV-visible spectroscopy was employed to analyze the formation of nanoparticles, while FTIR was used for their characterization.

Keywords: Bimetallic nanoparticles, FTIR analysis, Soxhlet extraction, Chemical and physical processes, Biological screening.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.28

How to cite this article: Jadhav RS, Inamdar AT, Mohite SK, Gavali RM. Characterization of Bimetallic (Zinc and Gold) Nanoparticles of *Cryptostegia grandiflora* Plant Extract and Their Biological Screening. International Journal of Drug Delivery Technology. 2024;14(3):1450-1455.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The better features of bimetallic nanoparticles (BNPs) over monometallic nanoparticles (NPs) make them more appealing from a scientific and technological perspective. Due to their synergistic effect, BNPs—formed from two different metals—demonstrate improved electrical, optical, magnetic, catalytic, thermal, plasmonic, and biological properties. Since BNPs are made without the need for costly or dangerous chemicals or harsh synthesis conditions, the green synthesis of BNPs is receiving more and more attention. Furthermore, the production of biocompatible BNPs through plant-mediated synthesis has the benefit of being capped by secondary metabolites that are therapeutically significant. Out of all the BNP kinds, gold (Au) is the most physiologically produced bimetallic nanostructure that has been documented to, yet significant research has focused on Au–Ag nano-systems. However, in our study, we have developed even more impactful BNPs, such as zinc oxide and gold bimetallic nanoparticles (ZnO+Au BNPs).[1]

Applications of BNP[2]

- The combination of zinc and gold at the nanoscale leverages the strengths of both metals, leading to multifunctional nanoparticles.
- When it comes to catalyzing different organic reactions, such as hydrogenation and oxidation reactions, they are more efficient than single-metal nanoparticles in terms of activity and selectivity.
- The strong antibacterial qualities of Zn/Au NPs make them valuable for preventing infections in medical equipment, coatings, and wound dressings.
- Biosensors that detect diseases, environmental pollutants, and biomolecules can be equipped with Zn/Au NPs. These sensors' sensitivity and specificity are enhanced by their large surface area and functionalization potential.
- Recent progress in BNP synthesis has broadened their range of applications, particularly in the biomedicine area. Among the various therapeutic uses, The ability of BNPs to inhibit the growth of both Gram+ve and Gram-Ve bacteria highlights their versatility as a novel antimicrobial agent.

Advantages of Bimetallic nanoparticles[3]

- Synergistic effects between two metals can result in the creation of new active sites and an overall improvement in catalytic activity.
- Better selectivity than single-metal nanoparticles is offered

**Author for Correspondence: ranjitjadhav705@gmail.com*

by BNPs, which can be customized for certain processes.
- Combinations of two metals can improve the stability of the catalyst, minimizing sintering and deterioration throughout the reaction.
- Certain BNPs have enhanced magnetic characteristics that improve MRI contrast, which is helpful for biomedical imaging.
- Combining two metals can result in a more potent antibacterial effect that is beneficial for both environmental and medical applications.

Disadvantages of Bimetallic nanoparticles[4]

- It can be difficult to precisely manage the structure, size, shape, and content of bimetallic nanoparticles.
- Expensive and resource-intensive high-purity precursors and specialized equipment are frequently needed for the synthesis procedures.
- Bimetallic nanoparticles may be more prone to break down in specific situations, such as hot environments or the presence of strong chemicals.
- Specific metal combinations may have shorter lifespans and be less effective due to increased susceptibility to oxidation or corrosion.
- The cost of producing bimetallic nanoparticles increases with the value of some metals, such as platinum or gold.

MATERIAL AND METHOD

Tables show the supplies and tools needed to create bimetallic nanoparticles. Soxhlet extraction was used to create an ethanolic extract of *Cryptostegia grandiflora*. Furthermore, components from an ethanolic extract of *C. grandiflora* were identified using GC-MS research, and the extract was prepared with the use of a microwave. The progression of the development of nanoparticles was tracked by means of UV-vis spectroscopy. The nanoparticles were characterized using SEM and FTIR after the incubation of plant extract (*C. grandiflora*) with zinc acetate.

Production (BNPs- Zinc+Gold)

The easiest method includes the blending of 2 metals as starting materials, such as Zn and Au. A stabilizing/ reducing agent is introduced into the mixture, which aids the metal ions reduction to atoms and supports stabilizing the aggregation process. This approach is known as co-reduction. The 5 mL of gold nanoparticles **(**AuNPs) (Liquid form) is mixed with 5 mL zinc nanoparticles (ZnNPs) (Liquid form) and sonicated the mixture with the help of a sonicator at 370c. After that the, 1-mL of polyglutamic acid (PLA) was added to the nanoparticles mixture to stabilize the particles. After sonication, the mixture was heated at 100 to 200°C to get bimetallic nanoparticles.

Impact of Different Conditions on Nanoparticle Synthesis

Significant advancements in theoretical understanding and practical application of pH followed by the start of the pH concept. Today, pH plays a crucial role in numerous chemical processes, and as a result, the pH meter has become one of the most commonly used analytical instruments in laboratories. The size, shape, stability and yield of synthesis of nanoparticles were affected by some conditions such as pH, concentration, Temperature, incubation time, etc.

Characterization of Nanoparticles[5-7]

The synthesized bimetallic (zinc and gold) nanoparticles (BNPs) were characterized to assess their bio-reduction using fourier transform infrared spectroscopy, UV-visible spectroscopy, and analysis of morphology and size distribution.

UV Visible Spectroscopy

Examining the optical properties of particles is most commonly done using a widely utilized technique known as UV-visible spectroscopy. The color changes observed signaled the production of nanoparticles. The formation of BNP (zinc and gold) by means of the ethanolic extract of *C. grandiflora* was monitored in real-time with UV-visible spectroscopy (Systronics ET2705) over a wavelength range of 200 to 800 nm, with the maximum absorbance being recorded.

Fourier Transforms Infrared Spectroscopy (FTIR)

The synthesized bimetallic (Zn@Au) nanoparticles and extract of *C. grandiflora were* subjected to FTIR spectrometric analysis. Using the JASCO FTIR-4600 equipped with attenuated total reflectance (ATR) technology from Japan, the spectra were recorded over a wavenumber range of 4000 to 600 cm^{-1}, with a scanning rate of 25 scans per spectrum.

Particle Size Analysis

The majority of a nanoparticle's qualities depend on its size. In actuality, nanoparticles' novel features do not take hold until the nanometer size range is achieved. The total surface area exposed per unit mass is called the specific surface area. The connection between particle size and surface area is inverse. If the particles are assumed to be spherical and have a narrow size distribution, the specific surface area can be used to estimate the average particle size.

Zeta Potential Measurement

Zeta potential, which is connected to the surface charge of the nanoparticle. A nanoparticle with a zeta potential ranging from -10 and +10 mV is generally regarded as nearly neutral. Conversely, a zeta potential above +30 or below -30 mV suggests that the nanoparticle possesses strong cationic or anionic characteristics. The zeta potential can influence how effectively nanoparticles traverse membranes, given that most biological membranes are negatively charged. Cationic nanoparticles, in particular, tend to have higher toxicity because they can more readily disrupt cell membranes.

Pharmacological Evaluation

Antibacterial assay[8-10]

- *Preparation of nutrient broth slants and sub-culturing of microorganisms*

To prepare the nutrient agar medium, agar 1-g, beef extract 500 mg, NaCl 250 mg, and peptone 500 mg were dissolved

using distilled water 50 mL. The solution was boiled and then poured into test tubes, which were covered with cotton. Succeeding sterilization in an autoclave at 15 psi (121°C) for 15 minutes, the tubes were located at an angle for 30 minutes to let them solidify. Under aseptic conditions, pure cultures of *Bacillus subtilis* ATCC 6051 were streaked onto the slanted agar surfaces and placed in an incubator at a temperature 37°C for time 24 hours.

- *Formulation: Culture media for antibacterial sensitivity test*

Nutrient agar media (pH 7.2) was prepared by dissolving agar 20 g of, beef extract 10 g, peptone 10 g, NaCl 5 g in boiling distilled water having quantity 1000 mL and mixed well. Once the solution was autoclaved at 15 psi (121°C) for 15 minutes, it was allowed to cool to around 45°C. Subsequently, the solution was transferred into sterile petri dishes in a sterile environment, with about 20 to 25 mL of media per plate. The plates were left at room temperature to allow the media to solidify.

- *Inoculation of the bacterial suspension onto the culture media*

The standardized inoculum, calibrated to ensure complete coverage on the petri plate, was picked up with a sterile, non-toxic cotton swab. This swab was then used to evenly streak the agar surface of the plate, making three passes while rotating the plate at a 60° angle between streaks. After streaking, the plate was left to dry for 5 to 15 minutes with the lid on. Then bore was punched on the prepared plates by using a sterile well (8 mm).

Using a sterile micropipette, 100 µL of the synthesized nanoparticle solution and 100 µL of the standard drug cefixime-USP were each loaded into the respective wells under aseptic conditions. Plates were initially held at RT for 30 minutes, following this, the plates were incubated at 37°C for 24 hours. The diameters of the inhibition zones were then measured in millimeters using a ruler. Data were analyzed statistically, with descriptive statistics mean with ± standard deviation. The %inhibition across different groups was compared using an independent samples t-test, with worth calculated at $p < 0.05$.

Anticancer Activity[11-13]

Trypan blue assay - Experimental procedure

The number of live cells in a cell solution is determined using the dye exclusion test. It is based on the concept that, as opposed to living cells, which maintain their cell walls intact, trypan blue, eosin, and propidium are examples of particular dyes. To determine if cells have absorbed or rejected the dye, a cell solution is visually examined after a dye is added.

In the following methods, viable cells are characterized by a transparent cytoplasm, whereas non-viable cells exhibit a blue cytoplasm. Initially, 50 µL of HeLa CCl2 cell lines were collected into a microcentrifuge tube. Each tube received 50 µL of all the nanoparticle samples at a concentration of 100 µL after three minutes of incubation. These samples were created by combining DMSO with pH 7.4 of phosphate buffer and incubating them for three minutes in an incubator. After an additional three minutes in the incubator, the samples were analyzed on a Neubauer slide to assess the total count of viable and non-viable cells.

MTT assay-experimental procedure

Cells were cultured at a concentration of 1×10^4 cells/mL for 24 hours at 37°C with 5% CO_2. After this period, 100 µL of the cell suspension was dispensed into each well of a 96-well microplate, along with 100 µL of LM series samples at concentrations of 10, 30, and 100 µg/mL. Control wells contained DMSO (0.2% in PBS) to evaluate cell viability. Each condition was tested in triplicate. The plates were incubated for 24 hours at 37°C with 5% CO_2. Following incubation, the medium was removed, and 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well. The plates were then incubated at 37°C for 4 hours to allow for the formation of formazan crystals. Afterward, the medium was completely removed, and 200 µL of DMSO was added to dissolve the crystals. The plates were covered with aluminum foil and incubated at 37°C for 10 minutes. Absorbance at 550 nm was then measured for each sample to assess cell viability.

RESULT AND DISCUSSION

Bimetallic (Zinc and Gold) Nanoparticles (BNPs)

Determination and synthesis of bimetallic (Zinc and Gold) nanoparticles. Bimetallic synthesis can be accomplished most simply using co-reduction as shown in Figure 1. Two metal precursors are combined in this process. One way to stabilize the aggregation and decrease the metal ion to atoms is to add a reducing or stabilizing chemical to that combination.

UV-visible spectra of bimetallic (Zinc and Gold) nanoparticles

Table 1 and Figure 2 display the ultraviolet-visible spectroscopy absorption spectra of zinc & gold Zn@Au nanoparticles obtained in water. A peak of exciton can be observed at 268 and 278 nm. The peak in the dotted curve at 268–278 nm.

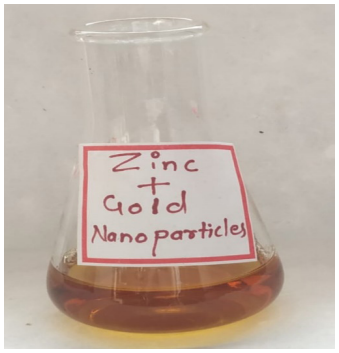

Figure 1: Synthesis of bimetallic (Zinc and Gold) nanoparticle

Table 1: Effect of on absorption intensity at 268 and 278 nm of bimetallic nanoparticle

Wavelength	*Absorption*
268	1.459
278	0.526

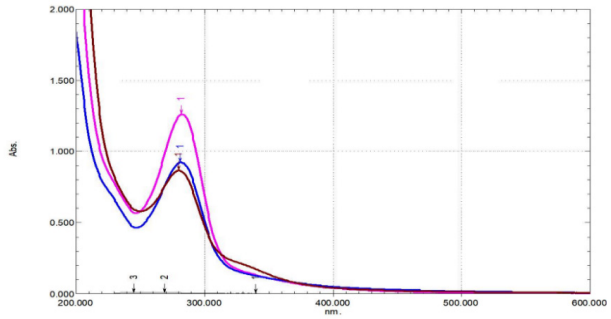

Figure 2: UV-visible absorption spectra of the synthesis of bimetallic nanoparticles

Table 2: FTIR interpretation of BNPs

Functional group	*IR frequency peak (cm^{-1})*	
	Standard	*Detected*
C-C	Below 900	779.71
C-O	900–1200	1103.46
C=C aliphatic	1200–1400	1279.91, 1364.61
C=C aromatic	1450–1600	1450.83,1520.56
C=O	1500–1700	1612.34, 1737.67
O-H	3200–3400	3368.44

FTIR analysis of BNPs

Table 2 and Figure 3 represent the result obtained through FTIR analysis of bimetallic nanoparticles.

Particle Size Analysis of BNPs

The particle size of prepared bimetallic nanoparticles was measured using a particle size analyzer and light scattering-based laser diffraction (HORIBA SZ100) (Figure 4). The average particle size is 112.6 nm.

Zeta potential analysis of BNPs

The formulated BNPs have a zeta potential of -10.1 mv (Figure 5).

Pharmacological Evaluation

Antimicrobial screening

The antibacterial profile of bimetallic was evaluated by measuring the zone of inhibition against gram +ve (*Bacillus subtilis*, ATCC 6051) bacterial strain *via* the well diffusion method, as shown in Figure 6. The bimetallic compound demonstrated strong antibacterial activity in comparison to the standard streptomycin, as illustrated in Table 3.

Anticancer Activity

MTT assay method

Results are depicted in Table 4, shown in Figures 7, 8 and 9 at the different concentrations and with sample code G+Z (AuNPs & ZnNPs). After analyzing and comparing, more inhibition was observed against the HeLa CCL2 cell line. On the basis of the percentage of inhibition, we can conclude that the sample G+Z(AuNPs & ZnNPs) shows good anticancer activity.

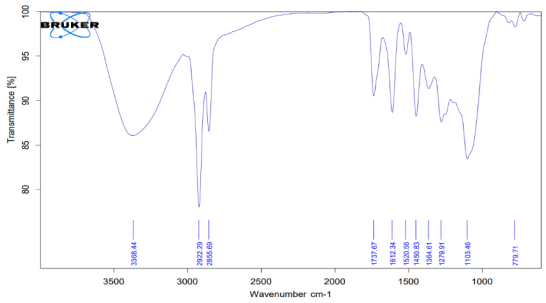

Figure 3: FTIR analysis of BNPs

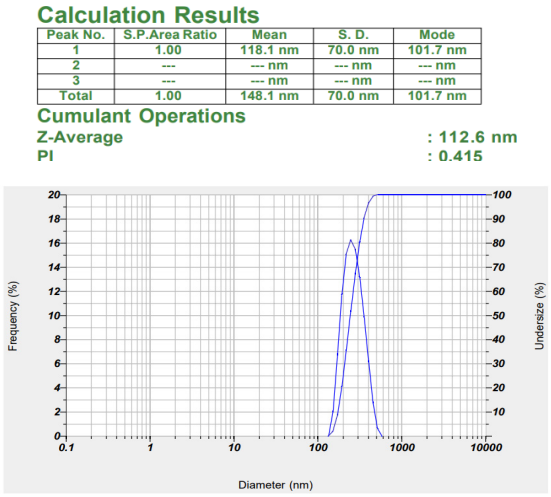

Figure 4: Particle size analysis of BNPs

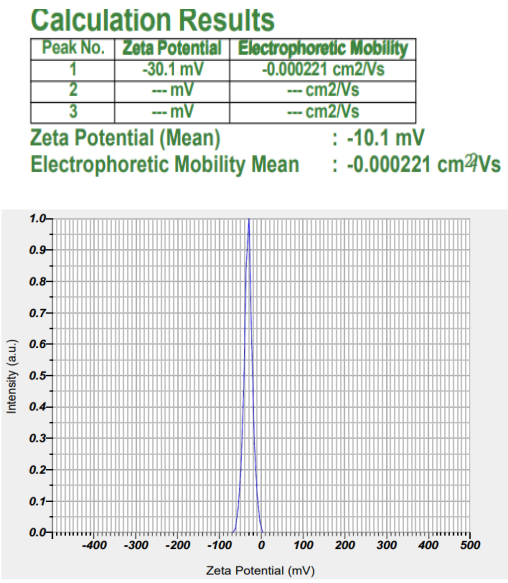

Figure 5: Zeta potential graph of bimetallic nanoparticles

Table 3: Antimicrobial activity of BNPs

S. No.	*Sample*	*Zone Ddiameter (mm)*
1.	Control	0
2.	Standard (Streptomycin)	30
3.	Bimetallic	12

Table 4: Impact of the compound on the HeLa CCL2 cell line by means of MTT assay

S No	*Sample*	*Conc. (µg/mL)*	*OD*			*Mean*	*%Inhibition (%)*	*%Viability (%)*	*IC_{50} (µg/mL)*
1.	Control		1.307			--	--	--	--
2.	Std. (5,Fluror-acil)	250	0.839	0.738	0.739	0.744	41.45	58.54	321.15
		500	0.882	0.647	0.628	0.632	50.95	45.04	
		750	0.470	0.450	0.437	0.433	67.64	31.35	
		1000	0.279	0.278	0.279	0.278	78.72	21.27	
3	G+Z NPS	250	1.224	1.209	1.219	1.213	6.19	82.81	922.15
		500	1.098	1.065	1.013	1.060	19.89	71.11	
		750	0.871	0.861	0.872	0.862	33.43	56.57	
		1000	0.551	0.560	0.541	0.531	52.84	52.16	

Figure 6: Antibacterial activity of synthesized BNPs

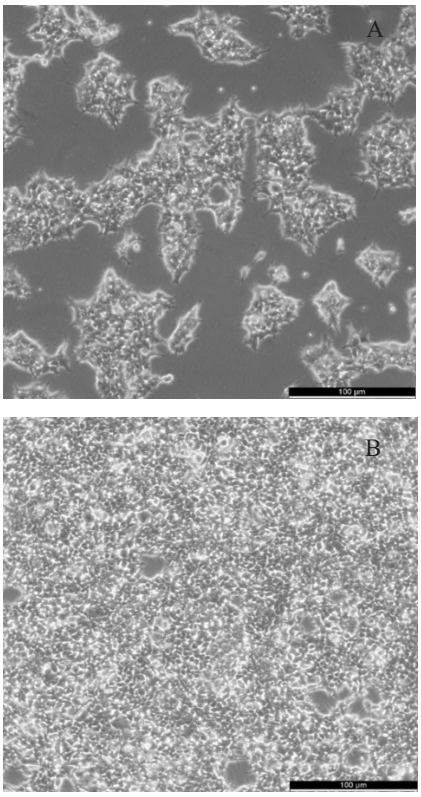

Figure 7: A: Standard B: Control

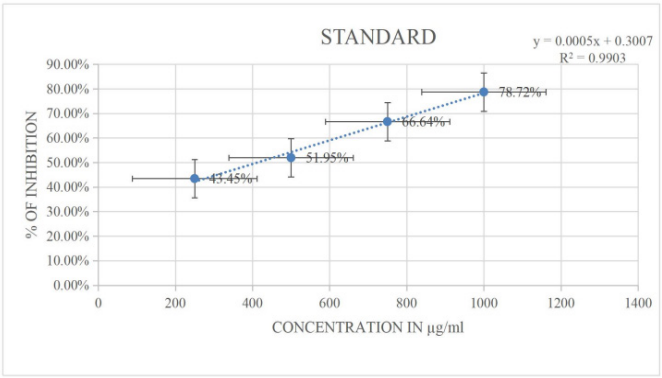

Figure 8: Graphical representation: Anticancer activity of standard by MTT assay

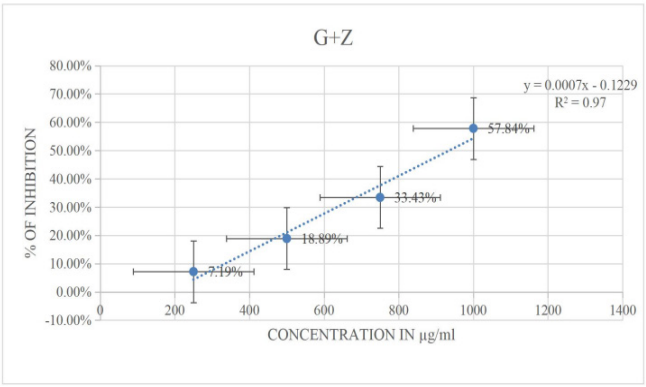

Figure 9: Graphical representation: Anticancer activity of bimetallic compound by MTT assay

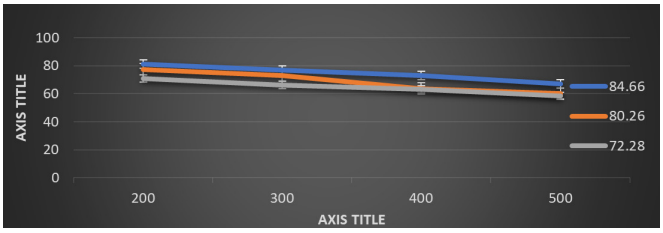

Figure 10: Graphical representation: Anticancer activity by trypan-blue method

Table 5: Compound effect against Hela CCL2 cell line by using trypan-blue method

Sample	*Percentage of viability (%)*				
Conc. (µg/mL)	100	200	300	400	500
C. grandiflora	84.66	81.11	76.64	72.85	67.02
BNPs	80.26	77.49	73.20	63.61	60.12
Std.(5-FLU)	72.28	70.79	66.28	63.02	58.58

Trypan-Blue Method

Using the trypan blue method, the anticancer activity of the *C. grandiflora* extract and the prepared bimetallic nanoparticles was evaluated. It is reported that the inhibition rate of conventional medicine by performing anticancer activity is 5-fluorouracil and BNPs demonstrate substantial activity when compared to the drug and BNP samples (Table 5 and Figure 10). In cell lines, BNPs show better percent viability than the *C. grandiflora* extract. In Hela CCL2 cell line *C. grandiflora* shows 67.02% viability and BNPs show 60.12% viability.

The samples were evaluated for anticancer activity, and among those tested, fewer live cells were observed in the breast cancer cell line compared to the standard drug 5FU. Based on the percentage of live and dead cells, it can be concluded that the samples exhibit significant anticancer effects on cell lines of breast cancer.

CONCLUSION

Zinc and gold nanoparticles made using green synthesis were demonstrated to have enhanced pharmacological characteristics. It could be applied as a preventative measure against bacteria.

REFERENCES

1. Anjum S, Nawaz K, Ahmad B, Hano C, Abbasi BH. Green synthesis of biocompatible core–shell (Au–Ag) and hybrid (Au–ZnO and Ag–ZnO) bimetallic nanoparticles and evaluation of their potential antibacterial, antidiabetic, antiglycation and anticancer activities. RSC advances. 2022;12(37):23845-23859. https://doi.org/10.1039/D2RA03196E.
2. Mali NN, Reenu Y, Jyotiram S, Vinod G. On Review–Brief *Cryptostegia grandiflora* Linn Roxb. 2022;9(4):110-114. https://doi.org/10.32628/IJSRST229413.
3. Agarwal P, Fatima A, Singh PP. Herbal medicine scenario in India and European countries. Journal of Pharmacognosy and Phytochemistry. 2012;1(4):88-93.
4. Mali N, Javalglkar A, Kale S. Phytochemical Evaluation of *Cryptostegia grandiflora* Linn RoxB Extract. Journal of Pharmaceutical Research; 2018; 7(5);82-87. http://dx.doi.org/10.5281/zenodo.1254251.
5. Ahmad S, Patil K, Koli G, Rahman BA, Barde L, Deshpande M, Tare H. Design, Development and Characterization of Econazole loaded Nanoparticles for Topical Application. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):358-362. DOI: 10.25258/ijpqa.14.2.20.
6. Khandelwal KR. Practical Pharmacognosy Techniques and Experimental. 24th edition; Nirali Prakashan; 2014;13-23.
7. Abdulbaqi, M.R. and Abdulelah, F.M. (2019). Application and Evaluation The Effect of Metallic Nanoparticles on Metronidazole Performance as a Novel Technology. International Journal of Pharmaceutical Quality Assurance 10(3): 97-104. DOI: 10.25258/ijpqa.10.3.30.
8. Mukherjee PK, Gunasekhran R, Subburaju T, Dhanbal SP, Duraiswamy B, Vijayan P, Suresh B. Studies on the antibacterial potential of *Cryptostegia grandiflora* R. Br.(Asclepiadaceae) extract. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 1999;13(1):70-72. https://doi.org/10.1002/(SICI)1099-1573.
9. Rivera DE, Ocampo YC, Castro JP, Caro D, Franco LA. Antibacterial activity of Physalis angulata L., Merremia umbellata L., and *Cryptostegia grandiflora* Roxb. Ex R. Br.-medicinal plants of the Colombian Northern Coast. Oriental Pharmacy and Experimental Medicine. 2015;15:95-102. https://doi.org/10.1007/s13596-014-0176-0.
10. Singh B, Sharma RA, Vyas GK, Sharma P. Estimation of phytoconstituents from *Cryptostegia grandiflora* (Roxb.) R. Br. in vivo and in vitro. II. Antimicrobial screening. Journal of Medicinal Plants Research. 2011;5(9):1598-605.
11. Shah R, Rosso K, Nathanson SD. Pathogenesis, prevention, diagnosis and treatment of breast cancer. World journal of clinical oncology. 2014;5(3):283-293. https://doi.org/10.5306%2Fwjco.v5.i3.283.
12. Kumar VP, Harikrishnan N. Anastrozole and Paclitaxel Loaded Nanocrystals: Evaluation of Anticancer Activity. International Journal of Drug Delivery Technology. 2024;14(2):973-977. DOI: 10.25258/ijddt.14.2.56
13. Kendre NS, Wakte P. Bioactivity Guided Fractionation and Elucidation of Anticancer Properties of Madhuca longifolia Leaf Extracts. International Journal of Drug Delivery Technology. 2022;12(4):1869-1876. DOI: 10.25258/ijddt.12.4.64.