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Development And Optimization Of Gold Nanoparticle Synthesis From Bauhinia Purpurea Extracts

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Abstract:

Objective:

This study shows how to easily make gold nanoparticles (AuNPs) using Bauhinia Purpurea extracts a simple and green approach.

Method:

This method offers a biocompatible, economical, and environmentally friendly method without hazardous chemicals. A central composite design (CCD) was used to find the best way to make nanoparticles, adjusting the quantity of Bauhinia Purpurea Extract quantity of Hypochloro aureate and the incubation time to see how they all worked together. Samples of the mixture were taken every hour to track the development of nanoparticles using the UV–Vis spectrophotometer. The reaction mixture gradually turned from dark green to mulberry hue within the first 0.5 to 3 Hr, signaling the formation of AuNPs.

Results:

We found that using 5.5 ml of Extract, 5.5 ml Hypochloro aureate and a 1.25 h incubation period produced the most AuNPs. Optimized formulation further tests, including UV–Vis spectroscopy, DLS, XRD, FTIR, SEM, and DSC, showed that these nanoparticles were generally spherical, with an average size of 31.96 nm. The sample also had a good PDI of 0.293, and the powder was mostly made up of a highly crystalline Au–3C gold phase (70.8%). Antiarthritic studies revealed that the synthesized AuNPs exhibited significant inhibitory activity against standard diclofenac sodium.

Conclusion:

This research shows that Bauhinia Purpurea extract can be used to create AuNPs in an environmentally friendly way. These AuNPs show promise for fighting arthritis. The statistical optimization model revealed that 5.5ml of HAucl4, 5.5 ml of extract and 1.25 Hr time is optimum condition to synthesize a higher yield of AuNPs.

Keywords: ***Gold*** *Nanoparticles, Bauhinia Purpurea extract, Bio-synthesis, Characterization, Central composite design, Optimization.*

INTRODUCTION:

Nanotechnology has emerged as a encouraging avenue for treating illness, particularly in the development of therapeutic agents. Among these, gold nanoparticles (Au-NPs) have become the focus of rapid research due to their unique physicochemical properties and their potential role in disease treatment. The present study investigates the green synthesis of gold nanoparticles using Bauhinia Purpurea (B. Purpurea) extracts and evaluates their effectiveness in arthritis treatment. The synthesis method leverages the medicinal properties of B. purpurea to develop a biocompatible and eco-friendly alternative to conventional nanoparticle production techniques.

BAUHINIA PURPUREA: A MEDICINAL PLANT FOR GREEN SYNTHESIS:

B. purpurea namely as butterfly tree small to medium-sized deciduous tree known for its ornamental and medicinal properties. The tree, growing up to 10–12 meters, produces obcordate-shaped leaves, bright

pink or white flowers, and seedpods that dry on the tree before explosively dispersing seeds. This plant is widely grown in full sun with well-drained soil and has been used in traditional medicine for its anti-inflammatory and antioxidant properties. Studies indicate that B. purpurea contains alkaloids, flavonoids, tannins, glycosides, and saponins, which play a crucial role in stabilizing and reducing gold nanoparticles during the green synthesis process.

BIO SYNTHESIS OF GOLD NANOPARTICLES USING B.PURPUREA EXTRACT:

The use of B.Purpurea extract for nanoparticle synthesis has gained traction due to its sustainability, cost-effectiveness, and avoidance of toxic chemicals. Several studies have demonstrated that plant-derived phytochemicals act as reducer and stabilizer in Au-NP preparation. For instance: Alas A. Alijabali (2018) prepared Au-NPs using Ziziphus Ziziphus extract, highlighting that phytochemicals effectively reduce and stabilize nanoparticles. Paz Elia (2013) demonstrated plant extracts from Salvia officinalis, Lippia citriodora, Pelargonium graveolens, and Punica granatum compounded extracts to generate stable Au-NPs. Jayanta S. Boruch et al. (2021) showed that the methanolic leaf extract of Moringa oleifera enhanced the bioactivity of green-synthesized Au-NPs through neuroactive modulation. Sulan Akhar et al. (2022) found that Hibiscus-based Au-NPs exhibited superior inhibitory effects compared to Au-NPs synthesized using curcumin.

The efficacy of Au-NPs synthesized using B. purpurea is expected to be similar, given the presence of phytochemicals that facilitate reduction and stabilization. Krishnaveni Marimuthu et al. (2014) confirmed the presence of key bioactive compounds in B. purpurea, which may contribute to the preparation of bioactive.

PHYSICOCHEMICAL ACTIONS OF GOLD NANOPARTICLES:

The resources of Au-NPs significantly ecofriendly synthesis method and reaction. Studies suggest that Particle size and morphology are affected by the polarity index of the reaction medium (Mohamed Hassan Hussain et al., 2020). The shape and size distribution of Au-NPs determine their biological activity and cellular uptake. Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) confirm particle size and morphology (Jayanta S. Boruch et al., 2021). Thermogravimetric analysis (TGA) reveals that plant biomolecules cap and stabilize the nanoparticles (Alas A. Alijabali et al., 2018). Fourier Transform Infrared Spectroscopy (FTIR) and UV-Vis Spectroscopy are used to confirm the presence of Au-NPs and identify functional groups accountable for reduction and stabilization.

Synthesis of nanoparticles using plant and plant leaves has more advantages than that of the microbial based method because in the plant-based synthesis technique, there is no complex process of maintaining the microorganism's cell culture [14,15]. In plant mediated synthesis of metal nanoparticles the reaction rate, morphologies, and particle size growth could be maintained by varying the reaction conditions like pH, plant extract concentration, temperature, and the mixing ratio of the reactants[15]. Fundamentally, bio-mediated metal nanoparticle synthesis is carried out in extracellular or intracellular media. In the intracellular media, the reaction between the metal and biomaterials occurs inside the plant; on the other hand, extracellular synthesis takes place in vitro. Many research findings show that the extracellular production of nanoparticles by plant extracts has been better compared to the intracellular method of synthesis since it reduces the extraction and purification procedures[16,17]

Luis Alberto Contreras[19] Alvarez et al., 2019 found a way to create AuNPs using Coffea Arabica proving that the plant extract acted as a reducing agent as well as stabilizer and functionalizer for the synthesized nanostructures. This is also confirmed that factorial design employed nanoparticles with Coffea Arabica extract allowed for a controlled and reproducible synthesis.

AU-NPS FOR ARTHRITIS TREATMENT:

Arthritis is a chronic inflammatory condition characterized by joint swelling, pain, and oxidative stress. Several studies have explored the use of Au-NPs for arthritis treatment: Maliha Uroos et al. (2021) demonstrated the antiarthritic activity of Manilkara zapota-derived Au-NPs, which showed enhanced analgesic and anti-inflammatory effects compared to crude plant extracts. Anupama Singh et al. (2022)

found that synthesized G-AgNPs significantly reduced paw swelling in arthritis-induced rat models and alleviated oxidative stress by restoring antioxidant enzyme activity. Sunilkumar et al. (2019) observed that Bauhinia purpurea bark extract exhibited dose-dependent antiarthritic activity, reducing inflammatory cytokine levels (Tumour Necrosis Factor-α, Interleukin-1β, Interleukin-6) while increasing anti-inflammatory cytokines (Interleukin-10).

These findings suggest that B. purpurea-derived Au-NPs may provide a novel, biocompatible, and efficient treatment option for arthritis, with enhanced bioavailability and targeted therapeutic effects. The biocompatibility and toxicity of gold nanoparticles are critical factors in their clinical application. Z.A. Zakaria et al. (2011) conducted toxicity studies on B. purpurea aqueous extracts, reporting an LD50 of >2 g/kg with no observed toxic effects. In addition, Sunilkumar et al. (2019) found that B. purpurea bark extract was safe at different dosages, further supporting its potential for therapeutic applications. The use of green-synthesized Au-NPs minimizes toxicity concerns associated with chemically synthesized nanoparticles, enhancing their suitability for biomedical use.

This study highlights the potential of B. Purpurea-derived gold nanoparticles for arthritis treatment. The green synthesis method provides an eco-friendly and sustainable approach to nanoparticle production, leveraging the medicinal properties of B. purpurea for enhanced therapeutic efficacy. The synthesized Au-NPs exhibit promising anti-inflammatory, antioxidant, and antiarthritic properties, making them a potential alternative to conventional arthritis treatments. Future studies should focus on in vivo evaluations, long-term toxicity assessment of B. purpurea-based Au-NPs for human use. This research contributes to the growing body of evidence supporting the use of plant-based nanotechnology in modern medicine and highlights a promising avenue for arthritis treatment.

METHODOLOGY:

MATERIALS:

HERBARIUM AND VALIDATION:

The shell bark of B. Purpurea was collected from Cheramadevi, Tirunelveli District, Tamil Nadu, India, and authenticated by Dr. Mutheeswaran S., Department of Botany, St. Xavier's College. Gold Chloride purchased from Trichy Research Institute of Biotechnology Pvt Ltd, Tamilnadu.

EXTRACTION OF BAUHINIA PURPUREA EXTRACT:

Powdered stem bark was grinding the herbal material, mixing it with the solvent, and then straining the liquid to recover the solid residues. Extracts were dried and used for characterization.

SYNTHESIS OF AUNPS:

Synthesis of Gold nanoparticles carried out using Hypo Chloroauric acid and Plant extract (B.Purpurea). 0.1 M Hypo Chloroauric acid in double distilled water. Hypo Chloroauric acid and Plant extract (B.Purpurea) were mixed together in ratios of 5:5, 6:4, 7:3, 8:2 and 9:1. In this different ratio concentration 5:5 ratio concentration were selected for the bulk preparation because it shows the higher production than other ratios. The reaction mixture was heated below the boiling point and continuously stirred at 800 rpm using magnetic stirrer. The mixture turned into Mulberry colour within 1 hr. The whole reaction was carried out in the dark. The obtained suspension was centrifuged at 15,000 rpm for 15 min. The pellet containing Gold nanoparticles was washed 3–4 times with deionized water to remove impurities. The precipitated nanoparticles were lyophilized. Lyophilized nanoparticles were stored in a cool, dry, and dark place and further their characterization was carried out.

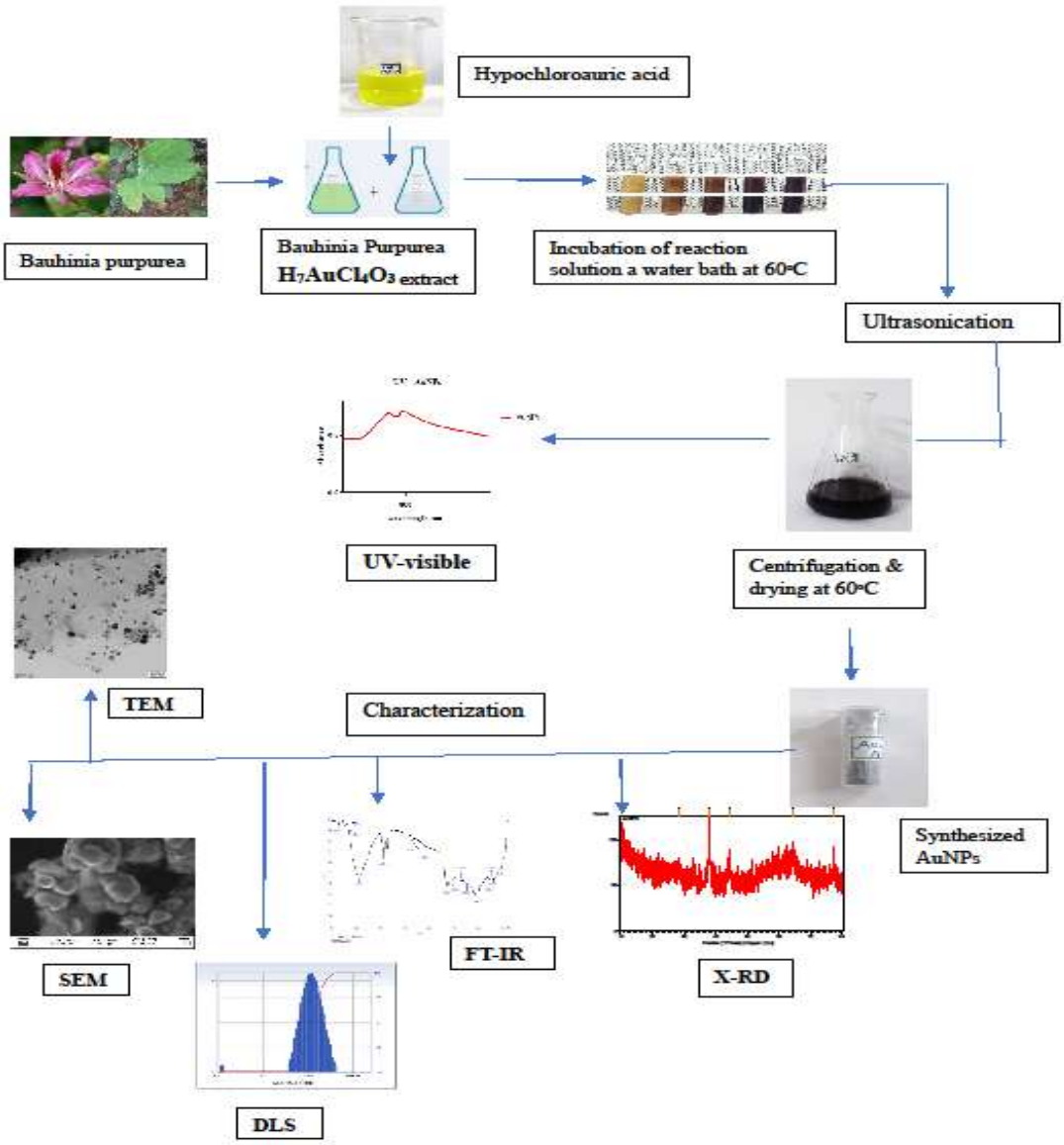

Figure 1. The experimental framework of synthesis and characterization of AuNPs

Optimization of gold nanoparticles using central composite design:

The effects of hypochloroauric acid, incubation time, Bauhinia Purpurea extract quantity on gold nanoparticle formation were studied using two level factorial design. Preliminary experiments were conducted for each individual parameter (OVAT), as detailed in table 1, a central composite design (CCD) is used to study and identify the best interaction effect of experimental process conditions. The goal was to develop a model system that accurately predicts the maximum yield of gold nanoparticles. Additionally, a system model was created to best represent the interaction between parameters with a minimal number of experimental runs. Statistical optimization was used to achieve high yield. The design of experiments (DOE) and data analysis were conducted using design expert software (version 13.0.0) the DOE focused on three parameters: aqueous concentration of HAUCl4, incubation time, extract quantity

each with two levels. Based on central composite design 18 experimental runs were recommended. The response yield was determined by estimating the quantity by gram.

Table1. Independent variables and levels used in the FCCD for the optimization of AuNPs biosynthesis:

Variables	Coding	Unit	Levels	
			-1	+1
Extract	A	ml	2	9
HAucl4	B	ml	2	9
Incubation time	C	Hr	0.5	3

CHARACTERIZATION OF AU-NPS:

UV-Visible Spectroscopy: The UV–visible spectroscopy is a commonly used techniques. Light wavelengths in the 300–800 nm are generally used for characterizing various metal nanoparticles in the size range of 2 to 100 nm. Spectrophotometric absorption measurements in the wavelength ranges of 400–450 nm and 500–550 nm are used in characterizing the silver and gold nanoparticles. The visual indication of the color exchange and the formation of Mulberry colour indicated the formation of the AuNPs. The formation of the AuNPs was confirmed by scanning the absorption maxima of the AuNPs colloid between 190 and 1100 nm on a PerkinElmer Lambda 25 spectrometer. The color change was observed 0.5 min after the mixing of the plant extract and gold chloride solution. The nanoparticle formation was completed within 3 min of the reaction initiation. The spectroscopic analyses were carried out on a freshly prepared sample at ambient room temperature using quartz cuvettes with an optical path length of 1 cm.

FTIR Analysis: The functional groups of the absorption bands of the specimens (AuNPs B.Purpurea studied by Fourier transform infrared spectroscopy (FTIR: Nicolet 6700 with FTIR spectrometer, spectral range 400 – 4000 cm^{-1}). For data analysis and explanation, the characteristics bands of each spectrum were labelled and highlighted between 4000 and 400 $cm^{-1.}$

XRD: X-ray diffraction measurements were taken on a MAXima_X XRD-7000 Alagappa University operating at a voltage of 40 kV and a 20-mA electrical current with a Cu-Kα (λ = 1.54 Å) radiation source in the region of 2θ from 30° to 75°. Colloidal AuNPs were centrifuged at 10,000× g for 15 min at ambient temperature. Pellets were washed with DD water three times with 5 mL each, and the sample was freeze-dried (−54 °C under vacuum and pressure) prior to the analysis.

SEM and TEM: SEM analysis was performed to determine their microscopic characters the morphology and the geometry of the AuNPs were investigated by a Nova Nano lab 200 scanning electron microscope. Transmission Electron Microscopy Model: Morgagni 268FFL at 80KV was used at 80kv ub bright field imaging mode for detailed morphology and structure of the prepared AuNPs.

Dynamic Light Scattering (DLS): The hydrodynamic diameter of gold nanoparticles was determined using a Zeta-PAL (zeta potential analyzer). All AuNPs samples (50 µg/mL) were suspended in deionized water. Ten runs with a 30 s duration each were set for each measurement. Each measurement was repeated three times under the following conditions: 25 °C, electric field 13.89 V/cm, refractive index 1.330, and voltage 5 V. The mean zeta potential was calculated using the Smoluchowski coagulation equation at a 659 nm wavelength with (seven) automatic attenuation settings. Data were reported from three independent syntheses; each set of measurements had 10 replicates.

ANTIARTHRITIC ACTIVITY

The BSA (Bovine Serum Albumin) Denaturation Method and Egg Albumin Denaturation Method are widely used in vitro models to evaluate the antiarthritic potential of compounds, including gold nanoparticles (Au-NPs). These methods simulate protein denaturation, a process that occurs in

inflammatory diseases like arthritis, and assess the ability of a substance to inhibit this denaturation, which is indicative of its anti-inflammatory properties.

PROTEIN DENATURATION ASSAY (INVITRO ANTIARTHRITIC STUDY):

Anti-arthritic activity of the AuNPs was determined using the method with minor modifications. The reaction mixture consisted of the 100 µl AuNPs extracts (final concentrations 100, 250, 500 µg/mL) and 100 µl of 5 % aqueous solution of bovine serum albumin (BSA); pH was adjusted using 1N HCl. The test samples were kept for incubation at 37 °C for 20 min and heated to 57 °C for 3 min. The mixture was allowed to cool for 10 min after which absorbance was measured at 280 nm. The blank comprise of the sample and distilled water was used as the negative control. The positive control was diclofenac sodium (final concentration 100, 250, 500 µg/mL). Percentage inhibition was calculated using the formula:

%Stabilization=100–[A1 sample /standard/A0 control×100]

Where A1 was the absorbance in the presence of the sample/standard (Diclofenac sodium) and A0 was the absorbance of the control reaction.

RESULTS AND DISCUSSION:

COLOR FORMATION OF SYNTHESIS OF GOLD NANOPARTICLES:

UV-VISIBLE SPECTROSCOPY:

The formation of AuNPs was evident from the change in solution color from dark green to mulberry (Figure 2) as well as from the presence of the typical plasmon peak in the range of 525–540 nm with a peak maximum in the range of approximately 527–535 nm in the UV-vis spectrum. Monitoring the reaction kinetics using UV-vis spectroscopy confirmed the completion of the reaction after 3 min as evident from the stability of the plasmonic peak, with no significant change beyond this time, as shown in figure 3.

Figure 2. Synthesis and Optimization of Gold Nanoparticles from Bauhinia Purpurea Extracts

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Figure 3. Synthesis of Gold nanoparticles and characterization of nanoparticles using UV-Vis Sprectoroscopy

STATISTICAL ANALYSIS TO OPTIMIZE THE SYNTHESIS OF GOLD NANOPARTICLES

To find the ideal conditions for turning hypochloroauric acid to gold nanoparticles, we experimented with different combinations of incubation time, hypochloroauric acid concentration, plant extract concentration using two –level factorial design. A central composite design was used to carry out the study and identify the best combination of experimental process conditions to synthesis and develop a model system for achieving the highest AUNps yield. The design also helped to develop a system model that accurately reflects the interactions between the parameters with the minimal number of experimental runs. Table 2 displays the RSM-CCD for three parameters with the yield of AUNps as the outcome. The highest yield 567was recorded at the the center point (run 11). The lowest yield, 256 was recorded at run 1.

Table 2. The RSM-CCD of three parameters and AuNPs 'Yield'.

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	Extract	HAucl4	Incubation Time	Yield
		ml	ml	Hr	Lamda Max
1	1	2	2	0.5	256
14	2	5	5	2.51134	564
2	3	9	2	0.5	387
8	4	9	9	2	545
19	5	5.5	5.5	1.25	566
20	6	5.5	5.5	1.25	565
12	7	5.5	11.3863	1.25	386
4	8	9	9	0.5	547
10	9	11.3863	5.5	1.25	378
3	10	2	9	0.5	398
15	11	5	5	1.25	567
6	12	9	2	2	347
13	13	5.5	5.5	-0.0113446	562
9	14	-0.386275	5.5	1.25	392

17	15	5.5	5.5	1.25	564
5	16	2	2	2	374
11	17	5.5	-0.386275	1.25	373
18	18	4.5	4.5	1.0	568
16	19	5.5	5.5	1.25	568
7	20	2	9	2	374

A model was built to predict AUNPs yield (Y) based on the concentration of Bauhinia Purpurea Extract concentration, HAuCl4 concentration, Incubation time. The model shown in Table 3, was analysed using ANOVA to assess its fit to the experimental data. A "Prob> F" value of less than 0.05 generally indicates a significant model. The model's extremely low p-value (<0.0001) demonstrates its high significance, meaning it is very ublikely the results are due to chance. The analysis revealed the concentration of Extract, HAucl4 and Incubation time along with the interactions between Extract and HAucl4 (AB) and Extract and Incubation time (AC), as well as the squared effects of Extract concentration and HAucl4 concentration (A^2 and B^2) all had a significant impact on AuNPS yield. An F-test confirmed the model's significance (F-value = 103.57), suggesting only a 0.01% chance the results are random. The lack –of-fit F-value (2.37) was small, indicating the model's good fit. Finally , the adeq precision of 32.329, well above the desired 4, shows a strong signal and further supports the model's reliability. This model helps explore the design space. Furthermore, the variability and data fit statistics of a predicted with a real response were checked by the coefficient of determination (R2). AN R2 value close to 1 means the model does a good job of predicting the results we saw in the experiments. Ideally, this value should fall between 0 and 1, and the closer it is to 1, the better the fit. The predicted R2 of 0.9528 is in reasonable agreement with the Adjusted R^2 of 0.9798, and the variation is due to independent variables in the synthesis of AuNPs.

In this study, a 3D surface response plot was used to visualize the regression equation from the experimental model. This plot helps to examine how each factor interacts and identify the best conditions for maximizing AuNPs yield in biosynthesis. The plot shows three independent variables and the fourth dependant variable represents the response of the experiment.

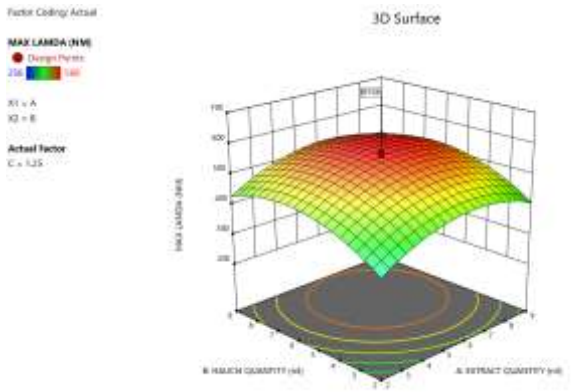

Figure 4. Plot of HAucl4 Concentration, Extract and Incubation time

Figure 4,5,6, shows the interaction effects of the concentration of Extract, HAucl4 of the solution and incubation time on the yield of AuNPs. According to Fig.4, as the Concentration of HAucl4 increases from 5 to 9 ml and the Extract concentration increases from 2 to 9, the yield of AuNPs increases, whereas the HAucl4 gradually turns 2, and the yield of AuNPs starts decreasing. The U-shape 3D-response surface also suggests an optimization condition in the biosynthesis of AuNPs with a decisive effect on HAucl4

concentration. HAucl4 concentration instantly has recognizable effects during the Au + reduction to the gold particles. The formation of AuNPs are accelerated by increasing the extract concentration. This suggests that the higher concentration of Gold along with the availability of bioactive reducing agents (enzymes and proteins), facilitates the reduction reaction and allows it to reach the optimal equilibrium for AuNPs synthesis more rapidly20.

Table 3. The analysis of variance (ANOVA) and fit statistics of the quadratic model of AuNPs Yield:

Source	Sum of Squares	Df	Mean Square	f-value	p-value	
Model	1.671E+05	4	41771.33	15.58	< 0.0001	significant
A-EXTRACT QUANTITY	11742.39	1	11742.39	4.38	0.0538	
B-HAUCl4 QUANTITY	19941.73	1	19941.73	7.44	0.0156	
C-INCUBATION TIME	3611.21	1	3611.21	187.25	<0.0001	
A^2	71797.62	1	71797.62	26.78	0.0001	
B^2	75828.29	1	75828.29	28.29	< 0.0001	
C2	150.81	1	150.81	7.820	0.0189	
Residual	40212.89	15	2680.86			
Lack of Fit	40201.55	10	4020.16	1773.60	< 0.0001	significant
R2	0.9894					
Adjusted R2	0.9284					
Predicted R2	0.9023					
Adeq Precision						

MODEL VALIDATION USING RESIDUALS:

A residual study evaluated the model's validity and how close a model approximation is to the real experimental data. Figure 5 presents the main tools important for diagnosing the model validation model. The residuals vs. predicted plot, as well as the residuals vs. experimental run, showed a random scatter pattern. The residuals are evenly spread across both positive and negative values, ranging from -3 to + 3. Therefore, the model generated is adequate for analyzing and evaluating the optimization experiment.

Moreover, experimental model validation was made by analyzing the predicted values and the experimental values of AuNPs yield. A graph from Fig. 6 shows a linear relationship between the predicted versus experimental results of AuNPs yield, and the correlation graph indicates that a predicted model fits well with the actual results with an error of R = 0. 989.

The model was successfully created. It reveals a connection between the variables that influence AuNPs yield production. All trials were repeated three times to ensure the accuracy and reliability of the CCD model of RSM and to better understand the response. An experiment was conducted to analyze the optimization results and confirm the developed model based on the optimal conditions for AuNPs synthesis. The quadratic model predicted a AuNPs yield of 568 unit at the optimum conditions Extract = 5.5, HAucl4 concentration = 5.5ml, and incubation time = 1.25 h). Experimental verification was done using triplicate at the same optimal conditions. Accordingly, AuNPs yield of 568 unit was obtained from experimental work.

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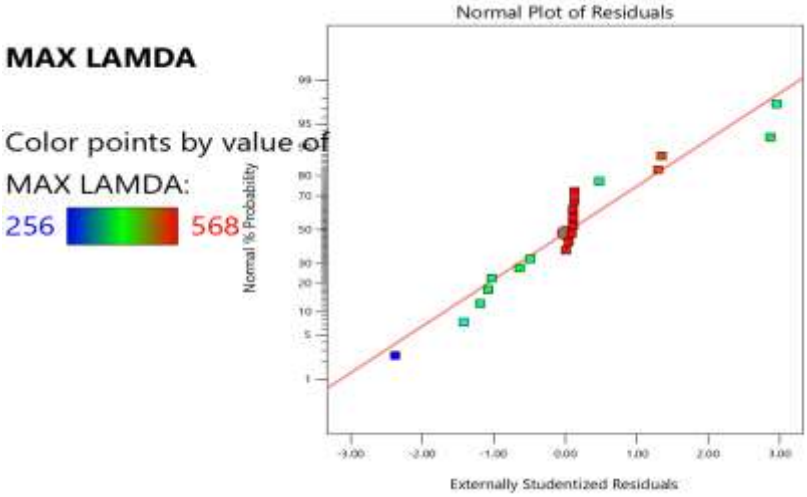

Fig 5. Normal Plot of Residual versus the experimental run

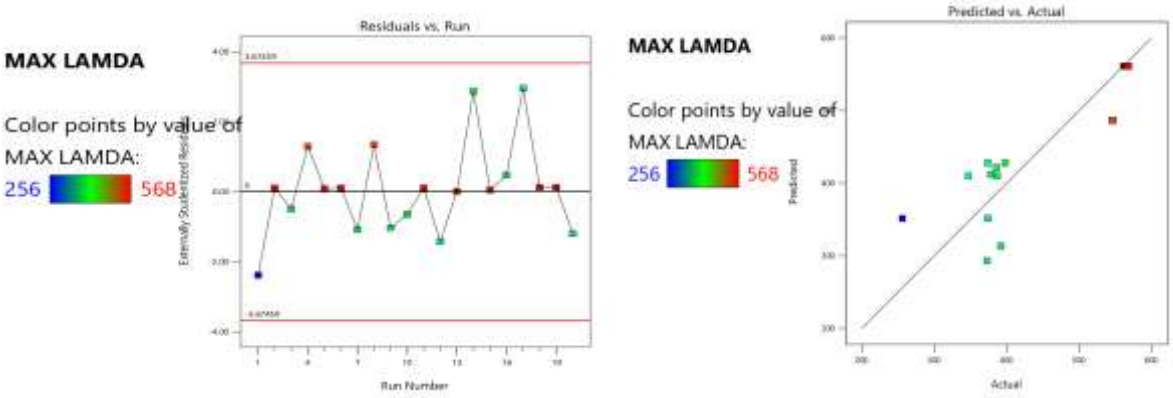

Fig 6. The comparison plot of experimental and predicted values of gold nanoparticles yield.

SYNTHESIS GOLD NANOPARTICLES CHARACTERIZATION:

FTIR ANALYSIS:

FTIR spectroscopy technique is very useful for the identification of chemical bonding of the organic materials when conjugated with metallic nanoparticles. FTIR indicates the presence of possible biomolecules that could be responsible for reduction and capping of AuNPs when prepared with using B.Purpurea. The broad bands at about 3202 and 3315 cm−1 are due to O-H stretching bond, for AuNPs . The peaks observed at approximately 2920 and 2850 cm−1 are due to the C-H stretching mode. In the spectrum of AuNPs-B.Purpurea. (Figure 7)

XRD ANALYSIS:

The XRD patterns would likely show these distinct peaks at the 2θ values mentioned above. The identification of these specific planes (such as 111, 200, 220, and 311) confirms that the synthesized Au-NPs exhibit the FCC crystalline structure typical of bulk gold. This indicates that the gold nanoparticles are of high purity and have a consistent crystalline orientation, which enhances their biomedical properties, including drug delivery and catalysis. The results also suggest that the plant extract of Bauhinia purpurea AuNPs plays a role in controlling the size and shape of the nanoparticles. Sharpness and intensity of the peaks might indicate good crystallinity, which could be attributed appearance of bioactive compounds in the plant extract. These compounds may act as capping agents during the synthesis process, preventing aggregation and promoting the formation of well-defined, stable nanoparticles. (Figure 7

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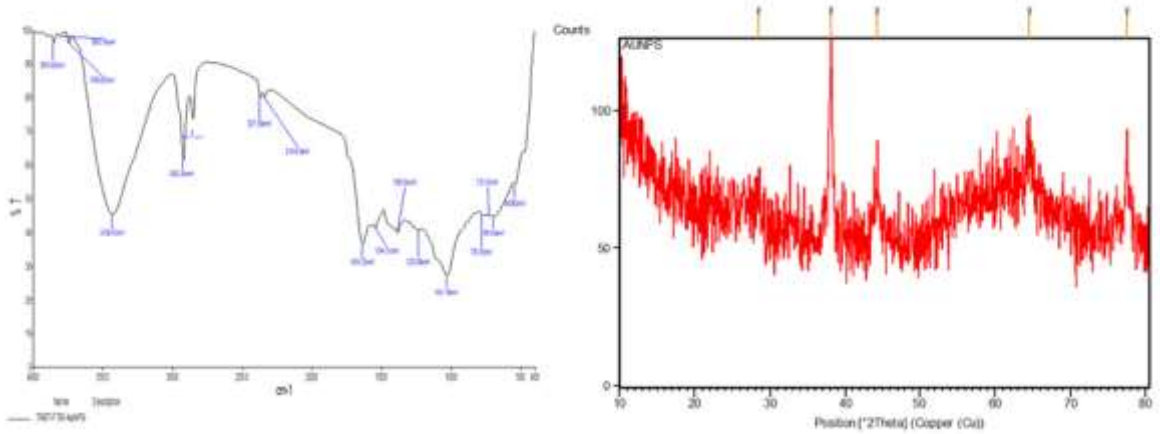

Figure 7: Characterization of gold nanoparticles using FTIR and

SEM AND TEM ANALYSIS:

The SEM images would likely show that the Au-NPs have a spherical or quasi-spherical shape, which is typical for nanoparticles synthesized via a green method. This spherical shape suggests that Bauhinia purpurea extract acts as a stabilizing agent during the synthesis process. The nanoparticles might show some degree of aggregation, depending on the interaction between particles and stabilizing agents from the plant extract. However, the size of the particles is generally expected to fall within the range of 10–50 nm, which is typical for gold nanoparticles synthesized by plant-based methods. The SEM images can also reveal any roughness or smoothness on the nanoparticle surface, which could indicate the presence of organic molecules from the plant extract interacting with the gold surface. (Figure 8)

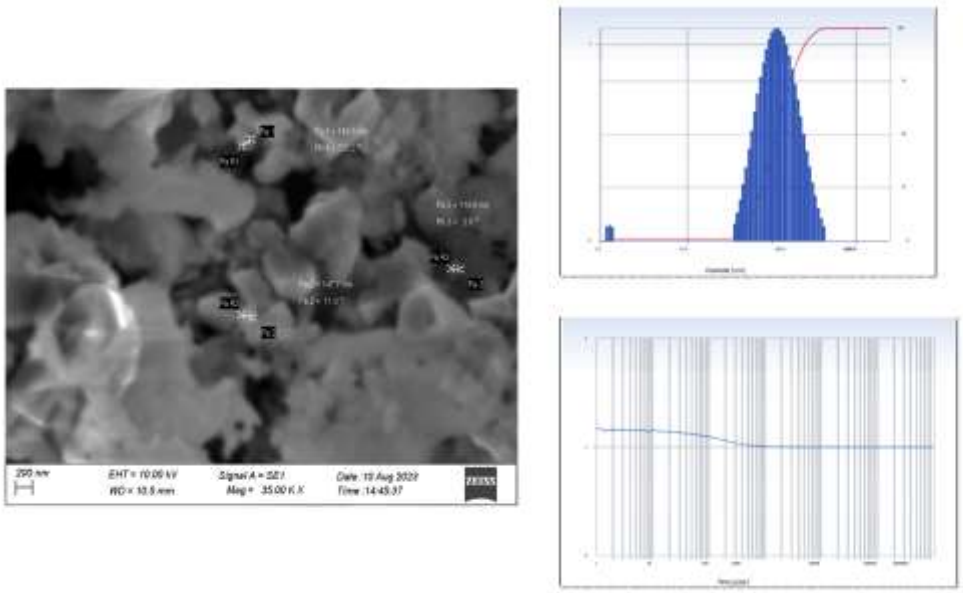

Figure 8. Characterization of Gold nanoparticles using Scanning Electron Microscopy and Dynamic Light Scattering

TEM would confirm the spherical nature of the Au-NPs and provide a more precise measurement of their size, typically in the 10–30 nm range, depending on the synthesis conditions. TEM would show that the nanoparticles are relatively uniform in size, with minimal agglomeration. This suggests that the B. purpurea extract provides effective stabilization of the nanoparticles. TEM would reveal the crystalline structure of the Au-NPs, with distinct lattice patterns, confirming the high quality of the gold nanoparticles. This would also support the claim that the nanoparticles were successfully reduced and stabilized by the plant extract.

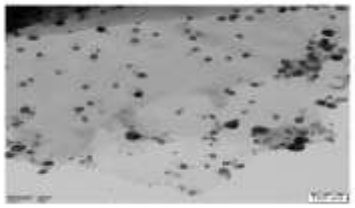

Figure 9. Characterization of Gold nanoparticles using Transmission Electron Microscopy

DLS ANALYSIS:

Au-NPs exhibited a hydrodynamic diameter of 56.8 ± 0.3 nm, Poly Dispersity Index (PDI) and particle size distribution as shown in experiments with AuNPs. The DLS results would give the average particle size in solution, which may differ slightly from the values obtained by SEM or TEM due to the solvent environment. The hydrodynamic size would likely range between 20–50 nm, indicating that the nanoparticles are well-dispersed in solution. DLS would provide a narrow size distribution curve if the synthesis was efficient and the nanoparticles PDI value A broad distribution could indicate the presence of a range of particle sizes, possibly due to incomplete stabilization or aggregation. DLS can also provide zeta potential measurements, which give an indication of the stability of the nanoparticles in suspension. A high zeta potential (either positive or negative) would suggest good colloidal stability, meaning the Au-NPs are unlikely to aggregate over time.

INVITRO ANTIARTHRITIC ACTIVITY:

The results of the antiarthritic study reveals that percentage inhibition of synthesized gold nanoparticles increases with increase in concentration and the higher percentage inhibition was found to be 76.88% at 250 µg/ mL concentration. Hence, proving that the synthesized AuNPs inhibits protein denaturation there by inhibiting auto antigen production the main cause for the onset of inflammation leading to arthritis. The IC_{50} value was above 100 µg/mL and the gold nanoparticles exhibited good anti-arthritic activity. Fig 10 show the antiarthritic activity of Bauhinia Purpurea AuNPS

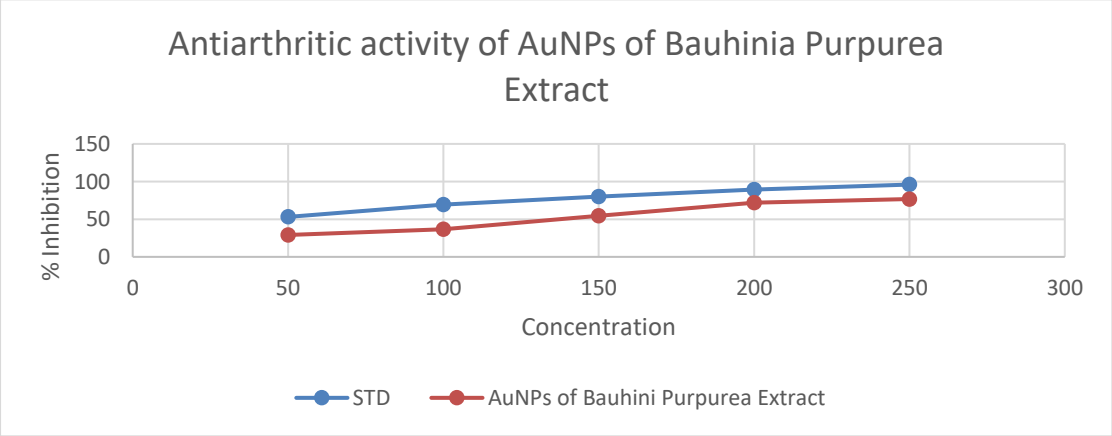

Fig 10. Invitro antiarthritic study of B.Purpurea AuNPs

DISCUSSION:

This study successfully synthesized gold nanoparticles (Au-NPs) through a green, eco-friendly, and cost-effective method, utilizing the extract of Bauhinia purpurea (B. Purpurea). The synthesis process aligns with current trends in nanotechnology, where plant-based methods are increasingly favored over conventional chemical or physical methods due to their environmentally sustainable nature. By using plant extracts, particularly B. purpurea, this study highlights the potential of bio-based synthesis for the generation of nanoparticles with both high efficacy and minimal environmental impact.

The method of nanoparticle synthesis using herbal extracts is rooted in the action of phytochemicals, such as flavonoids, polyphenols, and other hydrocarbons, which serve as both reducer and stabilizing agents. In this study, B. purpurea extract was demonstrated to play a vital role in the formation of Au-NPs. Phytochemicals in the extract interact with gold salts (usually gold chloride or gold nitrate) to reduce gold ions (Au^{3+}) into elemental gold (Au^{0}), leading to the creation of nanoparticles. This biosynthesis approach is advantageous as it avoids the use of toxic chemicals typically involved in nanoparticle production, making it an eco-friendly alternative.

UV-Visible spectroscopy confirmed the successful formation of Au-NPs by showing a characteristic surface plasmon resonance (SPR) peak in the **range of 525–540 nm**, a typical feature of gold nanoparticles. The appearance of the SPR band in the **527–535 nm** range further supports the creation of well-dispersed Au-NPs. This spectral data confirms that the nanoparticles are uniform in size and well-dispersed, which is essential for their functional efficacy, especially in biomedical applications.

The XRD analysis indicated that the Au-NPs were crystalline, which is significant because the crystalline structure of nanoparticles often dictates their stability and reactivity. Specific diffraction peaks in the XRD pattern were observed at 2θ values of 38.2°(111), 44.4°(200), 64.5°(220), and 77.5°(311) corresponding to the planes of the face-centered cubic (FCC) structure of gold. These sustained results are previously published and confirm that the synthesized Au-NPs are indeed gold in their elemental form, without any unwanted phases or impurities.

In addition, the SEM and TEM results provided a clear picture of the nanoparticle morphology, showing spherical and quasi-spherical shapes, with some degree of aggregation. The average size of the nanoparticles to be in the 20–30 nm range, which is ideal for enhancing biological interactions while minimizing toxicity. Such a size range is useful for biomedical usage such as drug delivery and targeted therapy, where particles need to be small enough to circulate in the bloodstream but large enough to carry therapeutic agents effectively.

The results of these assays demonstrated that the Au-NPs synthesized using B. purpurea extract effectively inhibited the denaturation of BSA and egg albumin, suggesting that the nanoparticles possess significant anti-inflammatory activity. This antiarthritic effect can be attributed to the surface functional groups on the nanoparticles, which might interact with inflammatory proteins or enzymes, modulating the inflammatory response.

The phytochemicals in B. purpurea extract, which also act as reducing agents during nanoparticle synthesis, may contribute to the biological activity of the Au-NPs. These phytochemicals, namely flavonoids and polyphenols, have inherent antioxidant and anti-inflammatory resources, and their presence on the nanoparticle surface might enhance the interaction of the nanoparticles with inflammatory markers, providing added therapeutic effects.

Moreover, the biocompatibility of the Au-NPs, as shown by FTIR results showing the presence of O-H, C=O, and other functional groups, supports their potential for safe use in therapeutic applications. The FTIR analysis suggests that the nanoparticles are likely stabilized by these bioactive compounds, reducing the risk of adverse effects in living

Arthritis, particularly rheumatism (RA), is a long-term inflammatory condition that affects millions of people worldwide. Current treatment options, including analgesics (NSAIA). and slow-acting antirheumatic drugs (SAARDs), often have significant side effects and limitations. The development of alternative treatments, such as the use of nanoparticles, offers promising solutions to overcome these challenges.

The antiarthritic potential of Au-NPs, as demonstrated in this study, provides an avenue for the evolving of new therapeutic agents for arthritis action. Au-NPs, with their ability to modulate inflammation at the molecular level, could serve as a drug delivery vehicle or as standalone therapeutic agents. Their unique surface properties and small size make them excellent candidates for targeted drug delivery, allowing for the precise delivery of anti-inflammatory drugs to affected areas, thus reducing side effects and improving therapeutic outcomes.

CONCLUSION:

Biosynthesis of AuNPs using plant extracts has appeared as a novel approach due to the broad availability of plants extracts and the biodegradability of active metabolite components. In the present study a robust, simple, cheap, and eco-friendly biosynthesis of AuNPs using an Bauhinia Purpurea extracts was successfully achieved via the reduction of HAucl4. For the first time, the process conditions for the biosynthesis of AuNPs using an Bauhinia Purpurea extract optimized using the response surface methodology. After performing DOE based on central composite design (CCD), the statistical optimization model revealed that 5.5ml of HAucl4, 5.5 ml of extract and 1.25 Hr time is optimum condition to synthesize a higher yield of AuNPs.

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CONFLICTS OF INTEREST

All authors declare that they have no financial interest or relationship that influence the work presented in this research paper

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