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SYNTHESIS, CHARACTERIZATION AND OPTIMIZATION OF GOLD NANOPARTICLES USING FRUITS OF *LUFFA CYLINDRICA*

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ABSTRACT

Gold nanoparticles are under immense study owing to their ease in synthesis, stability against oxidation, and biocompatibility. They also display unique optical properties. Nanoparticles are synthesized by different physical and chemical methods; however, they have a perilous impact on the environment due to their use of hazardous chemicals. Synthesis of gold nanoparticles by plant method is cost-effective, environment-friendly, easy to implement, and easy to large-scale manufacture. The phytochemicals in plant extract act as reducing and stabilizing agents to produce nanoparticles. This study depicts an optimized synthesis of gold nanoparticles using *Luffa cylindrica* fruit extract. *Luffa cylindrica* are known to possess properties such as analgesic, antirheumatic, hemostatic, and show pharmacological activities such as anti-inflammatory, immunomodulatory, hepatoprotective, etc. The gold nanoparticles were synthesized by treating an aqueous solution of *Luffa cylindrica* plant extract with the chloroauric acid solution. Further, the synthesized gold nanoparticles were optimized by parameters such as the volume of extract, incubation time, temperature, and concentration of chloroauric acid. The nanoparticles were characterized using UV-Vis spectroscopic analysis while particle size was determined. The Z-average particle size was found to be 92.18.

KEYWORDS: Green synthesis, gold nanoparticles, *Luffa cylindrica*, Uv-visible spectrum, particle size, zeta potential, aqueous extract.

INTRODUCTION

Nanotechnology is a brisk, upcoming area in material science research that deals with controlling and manipulating materials at the nanoscale (10^{-9}). It is also acknowledged as

dwarf technology since the word “nano” in Greek refers to “very small”. It focuses on synthesizing biologically compatible nanomaterials and nanoparticles. Since the last few decades, an overlapping of nanosized particles, molecular engineering, and biotechnology led to the emergence of a new field of nanobiotechnology. Its emphasis on the synthesis, control, and application of materials at the nanometric scale. The varied properties of nanoparticles are a function of their size and shape rather than the constant properties of their bulk counterparts. The difference in the properties can be attributed to their large surface area making them highly reactive.[1,2,3,4]

Nanoparticles are materials at the nanoscale, that are defined as a tiny object that behaves as a whole unit. The extent of application of metallic nanoparticles is non-descriptive as they have profound roles in optics, electronics, biomedicine, tissue engineering, food industry, and nanomedicine. They have distinctive properties concerning their shape, size, and structure of the particles that are in turn dependent on the method of synthesis.[5,6]

Numerous physical and chemical methods such as evaporation–condensation, liquid-phase synthesis, photoreduction, electrolysis, pyrolysis, ion sputtering, reverse micelle, chemical reduction, hydrothermal, sol gel and certain chemicals are used for the production of nanoparticles.[7,8] However, these methods are not only expensive but utilization of chemicals as in sodium citrate leads to the formation of toxic compounds which gets adsorbed on the surfaces, affecting human and environmental health.[9,10,11] Therefore, to minimize the demerits of the following methods, an eco-friendly alternative was developed which involved the usage of plant extracts.

Plant extracts are equipped with various secondary metabolites like flavonoids, alkaloids, terpenoids, phenolics, amino acids, and steroids which acts as reducing, stabilizing, and capping agent. Plant-mediated synthesis is a simple, economical, ecologically friendly method that eliminates the use of cell cultures and possesses negligible harm to humans as this method utilizes water as a reducing agent and nullifies the exposures to hazardous chemicals.[1,12,13,14]

Gold nanoparticles are also called colloidal gold which are small spheres of gold.[15] In comparison with other metallic nanoparticles such as iron, zinc, silver, copper, manganese, gold nanoparticles (AuNPs) has an inert core due to which they are relatively non-toxic and are convenient for leukaemia therapy, contrast imaging, and phytodynamic therapies.[16] The

ease of synthesis, high stability against oxidation, unique optical properties lead its use as catalyst, biosensor, with applications in biomedicine including DNA labelling and drug delivery, cell imaging, and immunostaining. Besides, AuNPs are used in monitoring and controlling environmental pollution, due to CO oxidation, water gas shift reaction, and selective oxidation of hydrocarbons along with the detection of heavy metals.[17] AuNPs also have a profound role in diagnostic, and therapeutic nanomedicine also possess activities such as anticancer, antimicrobial, anti-inflammation as stated in the literature.[18,9]

Luffa cylindrica is an abundantly popular fast-growing climber that is native to India. The fruits are oblong or cylindrical with a smooth appearance and develop a brown colour on maturation.[19] The synonyms of the plant are smooth gourd, luffa, loofah, turiya, turai, tori, etc. The plant not only possess antirheumatic, analgesic, and haemostatic properties but also have pharmacological activities like CNS depressant, Immunomodulatory, Anti-tumour, Anti-HIV, Anti-Inflammatory, and is hepatoprotective along with *In-Vitro* Antioxidant. The saturated fats and calories are low, and the plant possess dietary fibre, vitamin C, Riboflavin, Zinc, Thiamine, Iron and Magnesium.[20]

This paper emphases on green synthesis of AuNPs using plant extracts from the fruit of *Luffa cylindrica* and optimization of the synthesized gold nanoparticles by varying parameters such as the volume of extract (in mL), incubation time (in hrs), temperature (℃), the concentration of chloroauric acid (in mM) along with characterization via UV-vis spectroscopy, and particle size determination.

MATERIAL AND METHODS

PLANT COLLECTION AND AUTHENTICATION

The entire plant was obtained from a local market of Thane region, Mumbai, India. The plant specimen was authenticated and identified as *Luffa cylindrica* (L.) M.Roem. (synonyms: *Luffa aegyptiaca* Mill) belonging to family Cucurbitaceae, by Blatter Herbarium, St. Xavier's College, Mumbai, India. The sample matched with the Blatter Herbarium specimen number Shah-4952 by G.L.Shah.

PREPARATION OF AQUEOUS FRUIT EXTRACT

Fresh fruits of *L.cylindrica* were washed thoroughly with distilled water, minced and boiled for 30 mins in distilled water under continuous agitation (1000rpm) at 95 °C employing mechanical stirrer (Model RQ-129 Remi Industry IND.LTD). The extract formed was filtered

via muslin cloth to remove the undesirable plant parts. The filtrate acquired was centrifuged at 10000 rpm for 15 mins through Cooling Centrifuge (Model C24B41 Remi Industry IND.LTD). The resultant supernatant liquid was filtered through Whatman No.1 filter paper (pore size 25µm) to collect a clear solution, which was refrigerated (4°C) for further experiment.

CHEMICALS

Chloroauric acid ($AuCl_4$) was purchased from Sigma Aldrich. A strength of 1mM was prepared in an amber-coloured flask by the addition of 34mg $AuCl_4$ to 100ml of distilled water.

SYNTHESIS OF GOLD NANOPARTICLES

To 50 ml of Chloroauric acid solution of 1mM strength, 5ml of fruit extract was added and stirred continuously. This reaction mixture was then heated at 60 °C under continuous agitation in a water-shaker bath (Model ICCM 1133, Remi Industry IND.LTD) for 45 mins. This resulted in a colour change of the mixture from faint yellow to a dark violet/purple solution (***Figure 1***) which indicates the formation of AuNPs, and the stability was monitored using UV-Visible Spectroscopy (Model UV-1800, Shimadzu)

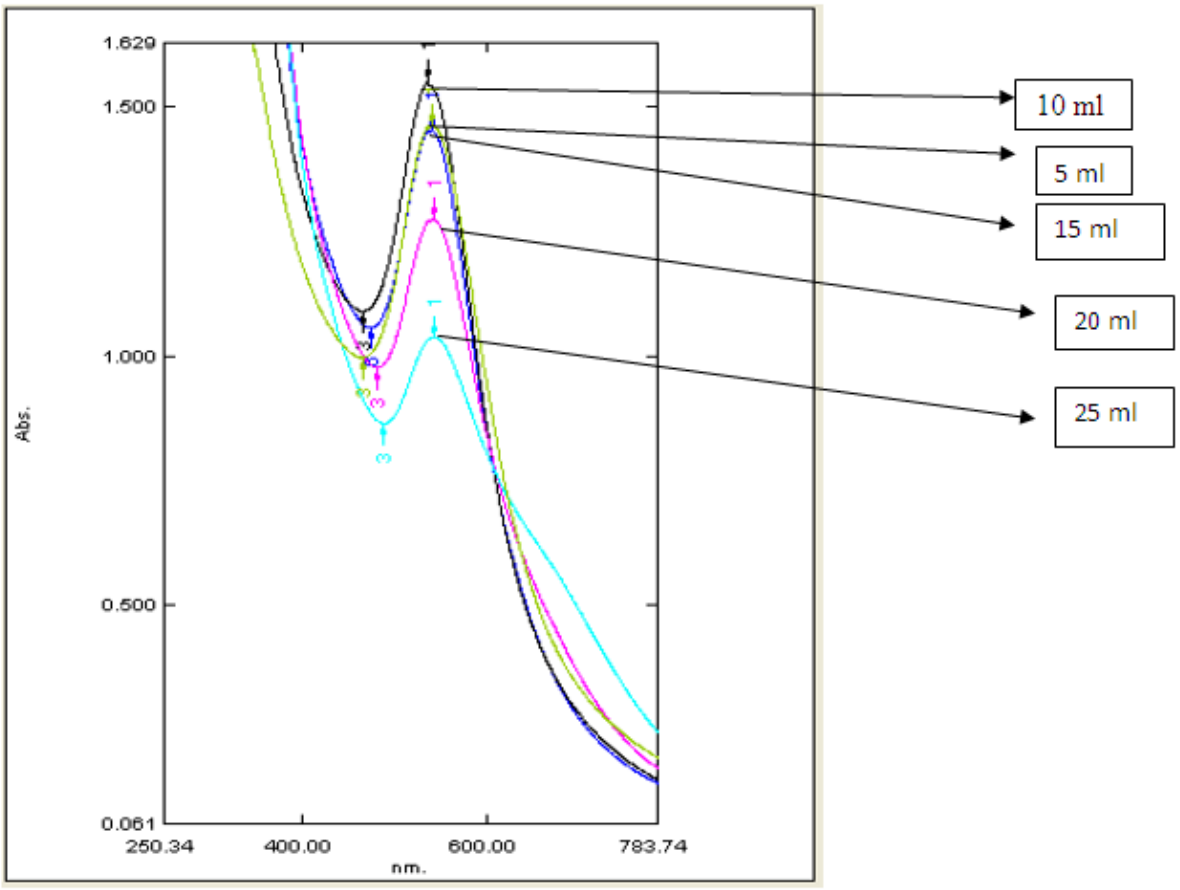
Figure 1: Colour Change indicating the formation of gold nanoparticles.

OPTIMIZATION OF THE AUNPS

The prepared AuNPs were optimized by utilization of varying parameters such as volume of extract (ml), incubation time (hrs), temperature (℃) and concentration of chloroauric acid (mM). The λ_{max} and pH of all batches of optimized gold nanoparticles are shown in Error! Reference source not found..

A) Volume of fruit extract (ml)

Different batches of gold nanoparticles (Batch 1-5) were synthesized by adding 1mM Chloroauric acid solution to different concentrations of *Luffa cylindrical* extract i.e. 5ml, 10ml, 15ml, 20ml, 25ml to 50ml while keeping the other conditions such as time and temperature constant (60℃ and 45 min). λ_{max} and pH of each of the synthesized gold nanoparticles were observed using a UV-Visible spectrophotometer (Graph 1).

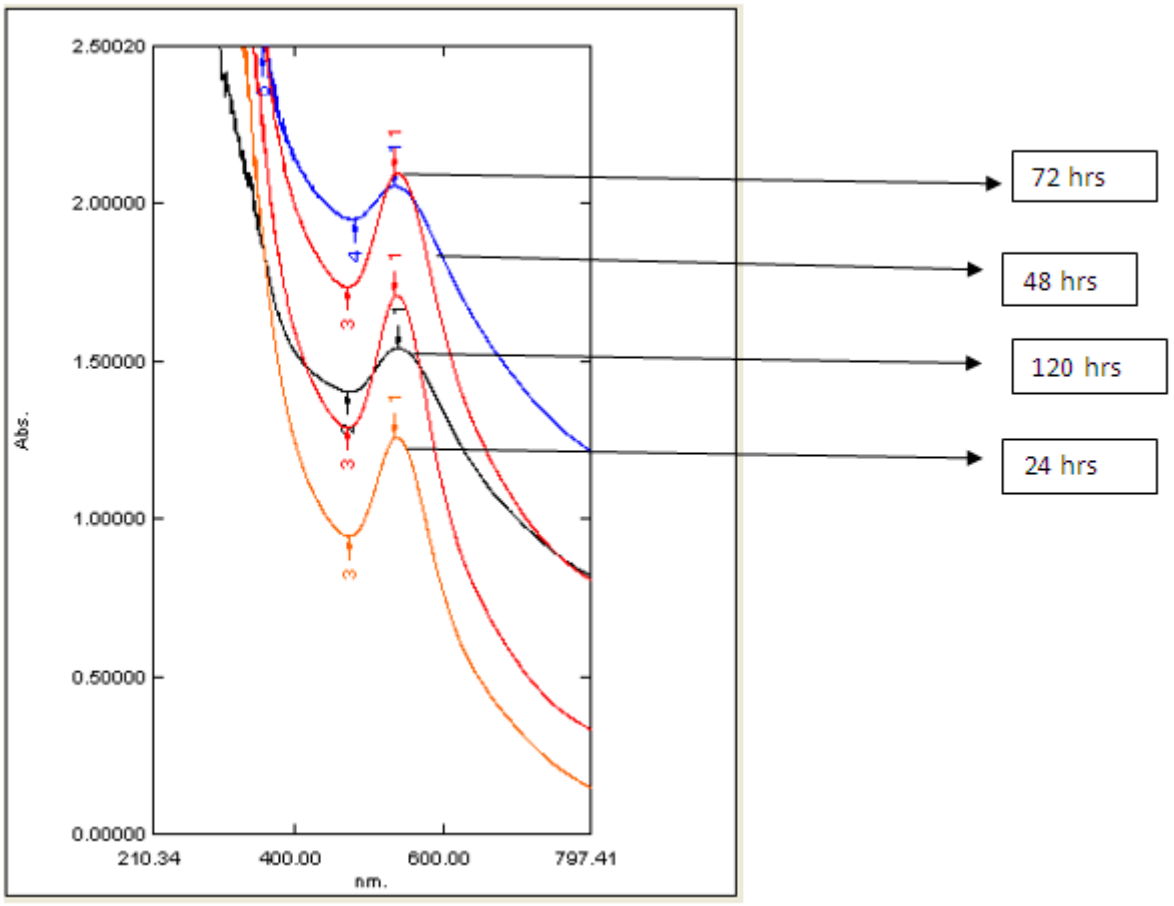


Graph 1: Comparative study of UV spectra of gold nanoparticles synthesized using varying volume of *Luffa cylindrica* extract 5ml, 10ml, 15ml, 20ml, 25 ml.

B) Incubation time (hrs)

To optimize reaction time for synthesis of gold nanoparticles, two batches of gold nanoparticles (Batch 6 & 7) were synthesized at room temperature and the reaction was monitored as a function of time from 0 min to 120 Hrs. Batch 6 was prepared with 1mM Chloroauric acid solution added to 10mL of Luffa extract at room temperature and Batch 7 was prepared by adding 1mM Chloroauric acid solution to 25 mL of Luffa extract under the same conditions. λ_{max} and pH of each of the synthesized gold nanoparticles were observed as

a function of time (i.e. 0, 24th, 48th. 72nd Hours). The UV spectra of the two batches were compared (Graph 2)

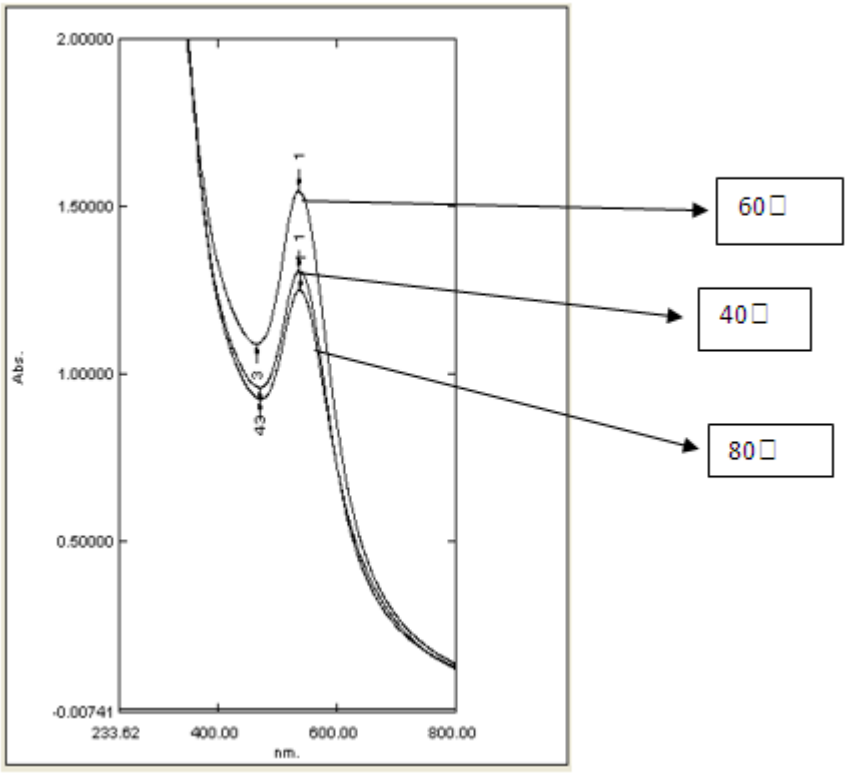


Graph-2: Comparative Study of Batch-6 synthesized using 10ml of *Luffa cylindrica* extract and monitored after 24,48,72,120 hrs.

C) Temperature ℃

To optimize the influence of temperatures on the biosynthesis of gold nanoparticles, three batches of gold nanoparticles (Batch 8,9,10) were synthesized at three different temperatures namely 40℃, 60℃, and 80℃ till the completion of the reaction. The reaction mixture was then monitored as a function of temperature with respect to time. In the 8^{th} (a and b) batch, 1mM Chloroauric acid solution was added to 10 ml and 25 ml of *Luffa cylindrical* extract and the nanoparticles were synthesized at 40℃. The 9^{th} batch (a and b) batch was prepared by adding 1mM chloroauric acid solution to 10 ml and 25 ml of *Luffa cylindrical* extract and the nanoparticles were synthesized at 60℃. Similarly, the 10^{th} (a and b) batch was synthesized by adding 1mM chloroauric acid solution to 10 ml and 25 ml of *Luffa cylindrica* extract and the nanoparticles were synthesized at 80℃. λ_{max} of each of the synthesized gold nanoparticles

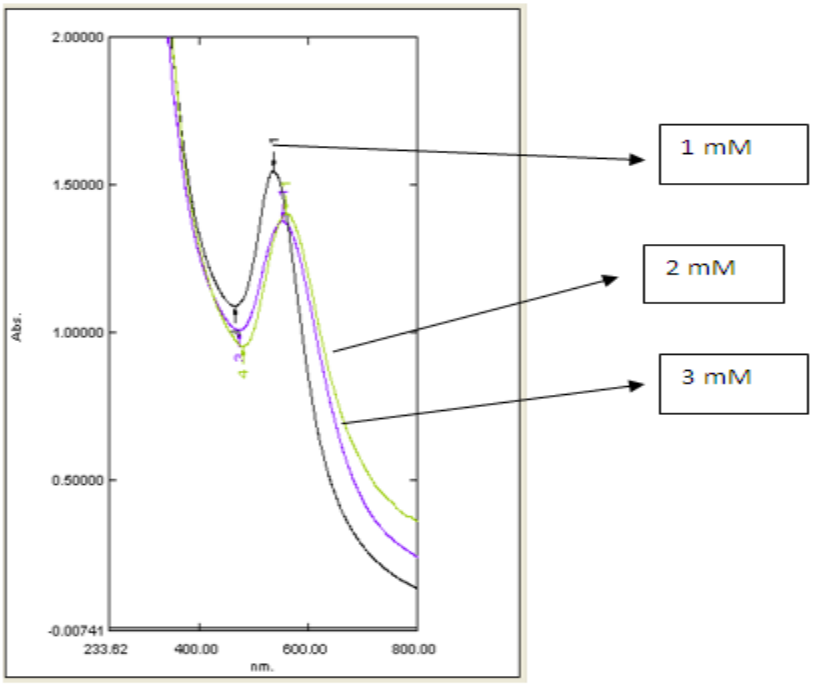
was observed as a function of temperature and the UV Spectra of these temperatures were compared (Graph 3)



Graph-3: Comparison of gold nanoparticles synthesized using 10mL extract of Luffa cylindrica fruit extract at varying temperature of 40℃, 60℃ and 80℃.

D) Concentration of chloroauric acid (mM)

To optimize the influence of concentration of Chloroauric acid on the biosynthesis of AuNPs three batches of gold nanoparticles (Batch 13,14,15) were synthesized using three different concentrations of strength 1mM, 2mM, 3mM using 10 ml of *Luffa cylindrical* extract at 60℃ for 45 mins in water shaker bath without altering the λ_{max} and pH of each of the synthesized gold nanoparticles. The UV-Visible Spectra were compared (Graph 4), and the reaction mixture was monitored as a function of the concentration of chloroauric acid solution such as 1mM, 2mM, and 3mM.



Graph-4: Comparative Study of gold nanoparticles synthesized using varying concentration of chloroauric acid and 10 ml of *Luffa cylindrica* extract.

Table 1: λ_{max}, pH and conditions for optimization of gold nanoparticles of *Luffa cylindrical.*

1.	Parameters						
A)	Volume of Extract		Batch	λ_{max}	pH	Temperature (°C)	Time
	5mL		1	540	3.6	60	45min
	10mL		2	535	3.67	60	45min
	15mL		3	539.4	3.82	60	45min
	20mL		4	541.5	4.11	60	45min
	25mL		5	524	3.97	60	45min
B)	Incubation Time (Hrs)						
	10 mL (Vol of Extract)	0^{th} Hr	6	535	3.62	RT	24 hrs
		24^{th} Hrs		535	3.52	RT	48 hrs
		48^{th} Hrs		535	3.99	RT	72 hrs
		120 Hrs		538.5	3.95	RT	120hrs
	25 mL (Vol of Extract)	0^{th} Hr	7	535	4.01	RT	24 hrs
		24^{th} Hrs		535	3.90	RT	48 hrs
		48^{th} Hrs		538	3.96	RT	72 hrs
		120 Hrs		539	3.97	RT	120hrs
C)	Temperature (°C)						
	10 mL (Vol of Extract)	40°C	8(a)	538	-	40°C	1.5hrs
		60°C	9(a)	535	-	60°C	45min
		80°C	10(a)	535	-	80°C	30min
	25 mL (Vol of Extract)	40°C	8(b)	534	-	40°C	1.5hrs
		60°C	9(b)	542	-	60°C	45min
		80°C	10(b)	534	-	80°C	30min
D)	Volume of Chloroauric acid (mM)						

	10 mL (Vol of Extract)	1mM	13	535	3.68	60℃	45min
		2mM	14	553	3.92	60℃	45min
		3mM	15	559	3.04	60℃	45min

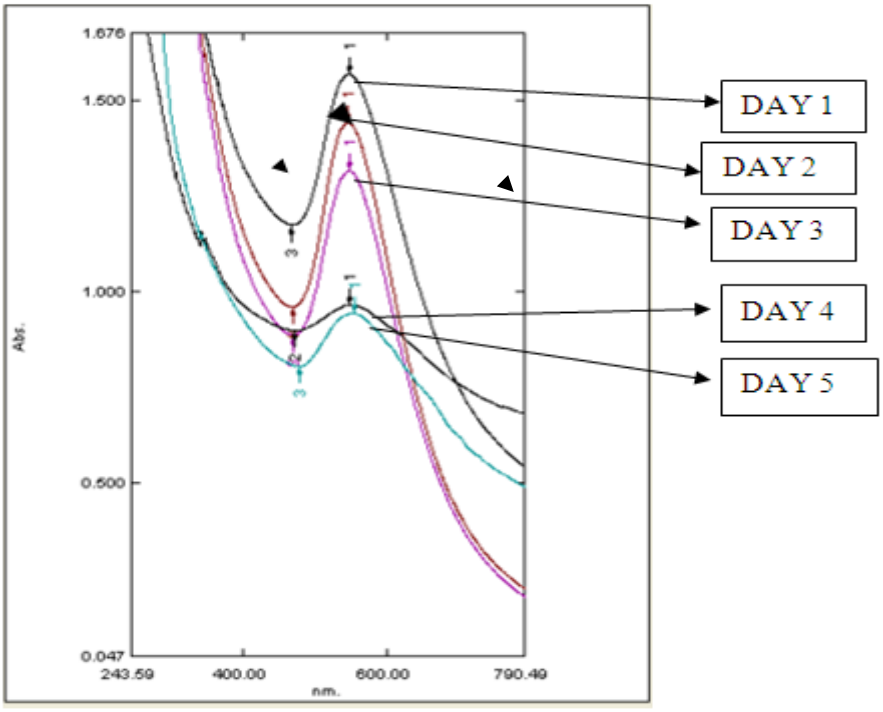
CHARACTERIZATION OF AUNPS

UV-VISIBLE SPECTROPHOTOMETER

The stability of the synthesized nanoparticles was monitored using UV Visible Spectrophotometer between the wavelength 200-800 nm using Shimadzu spectrophotometer for a period of one week (***Table 1***). The sample was prepared by using small aliquot of sample diluted by water. The nanoparticles were found to be stable throughout the week as seen through the Spectral data. The UV Spectra peaks were initially found to be narrow and sharp but as the days were progressed the peaks broadened indicating an increase in the size of nanoparticles (Graph 5).

Table 1: λ_{max} and pH of synthesized gold nanoparticles over a period of 5 days.

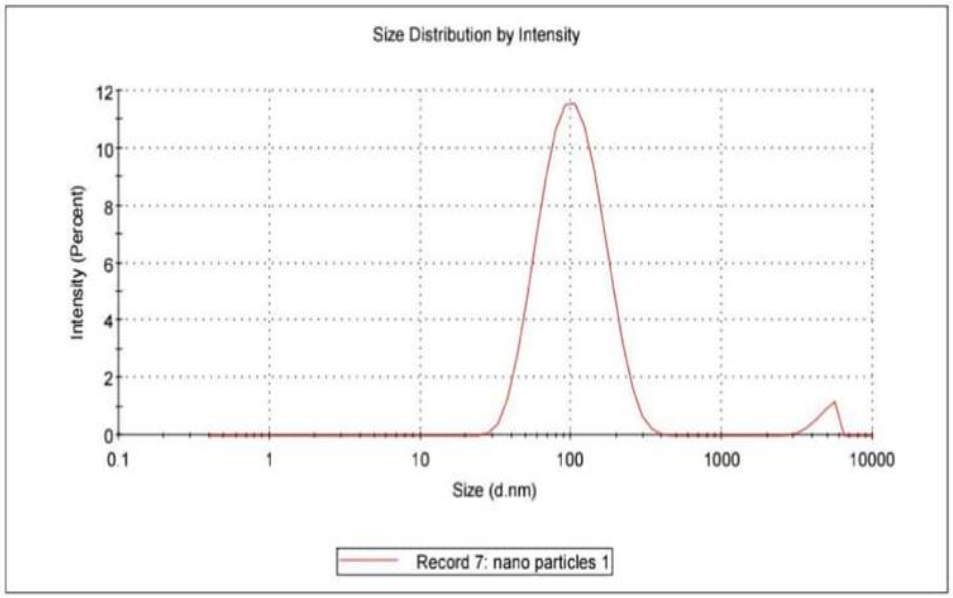
Days	pH	$\lambda_{. max}$	Absorbance
DAY 1	3.68	547 nm	1.315
DAY 2	3.79	545 nm	1.44
DAY 3	3.58	547nm	1.568
DAY 4	3.52	554 nm	0.942
DAY 5	3.74	547 nm	0.966



Graph-5: Comparative study of stability of the synthesized gold nanoparticles using 5ml fruit extract over a period of 5 days.

PARTICLE SIZE DETERMINATION

The size and dispersal nature of AuNPs were determined by dynamic light scattering (DLS) Zetasizer Version 7.12. The sample was dispersed in water. The Z-average particle size was found to be 92.18 d.nm (Graph 6) The percentage intensity and the standard deviation was found to be 97.1% and 52.35 respectively.



Graph-6-Particles size distribution of synthesized gold nanoparticles using 10ml fruit extract of *Luffa Cylindrica.*

RESULTS AND DISCUSSION

Gold nanoparticles were synthesized using fruit extract of *Luffa cylindrica* with 1mM chloroauric acid at 60℃, within 45mins. The physiological change from faint yellow to dark violet or purple, with the presence of a typical peak having $\lambda_{.max}$ of 547 nm in the UV-Spectra indicated the formation of AuNPs. This was evident that the extract had the potential to reduce Au ions into Au nanoparticles. The stability of the prepared AuNPs was monitored for a week using UV-Visible Spectrophotometer and pH meter. Through Spectral data, it was clear that the nanoparticles were stable throughout the week. The absorption maxima (λ_{max}) of the sample on all the five days was found to be in the range 544-547nm. The synthesized gold nanoparticles were found to be stable at an acidic pH of 3.52 on all the five days.

Gold nanoparticles were optimized using parameter of volume of extract. Results stated that for AuNPs synthesized using 10ml and 25ml of volume of extract (i.e. batch 2 & 5), lowest λ.

max values of 535nm and 524nm were obtained, respectively. Also, the peak for 10ml extract had the highest intensity indicating the highest number of gold nanoparticles synthesized. Hence, 10ml and 25ml of the volume of extract were found to be the optimum quantity of fruit extract required for the biosynthesis of AuNPs.

For Incubation time, it was observed that as the incubation time increased, the intensity of the bluish violet colour was found to increase. This increase in colour intensity could be as a result of an increase in the number of gold nanoparticles with time. Mono dispersed particles of almost spherical shape were observed with increase in the incubation time, indicating that room temperature is not an ideal temperature for synthesizing AuNPs. UV-Visible Spectroscopic Analysis for both the batches, at 120th hour, still showed a peak between 500-600nm indicating the presence of gold nanoparticles in the reaction mixture. Also, from the spectra, it became clear that Batch 6 (using 10ml of the volume of extract) showed narrow and high-intensity peaks when compared to Batch 7 (using 25 ml of the volume of extract) after UV analysis on successive days. Hence, 10mL was found to be the optimum quantity of fruit extract required for the biosynthesis of gold nanoparticles.

The temperature was the next parameter optimized in which, it was observed that, as the temperature was increased, the gold nanoparticles were synthesized at a faster rate. The formation of gold nanoparticles was confirmed through their respective UV spectra. UV-Visible Spectroscopic Analysis for all the batches synthesized at 40℃, 60℃, and 80℃ gave the peak between 500-600nm indicating the formation of gold nanoparticles. However, from the comparative study, it became evident that batch 8(a – using 10ml of the volume of extract) and (b – using 25ml of the volume of extract) synthesized at 60℃ showed narrow and high-intensity peak when compared to batch 9 and batch 10 at 40℃ and 80℃ respectively after UV analysis. Hence, 60℃ was found to be the optimum temperature for the synthesis of gold nanoparticles. Also, it became evident that batch 11(a) and batch 12(a) synthesized (using 10ml of the volume of extract) showed narrow and high-intensity peaks when compared to batch 11(b) and batch 12(b) (using 25 ml of the volume of extract) after UV analysis. Hence, 10ml of the volume *of Luffa cylindrica* extract was found to be the optimum quantity required for the biosynthesis of gold nanoparticles at 60℃.

As for the concentration of Chloroauric acid, the synthesized gold nanoparticles at 60℃ using 10ml extract showed a shift towards higher wavelength λ_{max} (535nm to 559nm) indicating an

increase in particle size. However, UV-Visible Spectroscopic Analysis for all three batches, still showed a peak between 500-600nm indicating the presence of gold nanoparticles in the reaction mixture. From the comparative study, it became evident that batch 13 (using 1mM concentration of chloroauric acid) showed narrow and high-intensity peaks when compared to batch 14 (using 2mM concentration of chloroauric acid) and batch 15 (using 3mM concentration of chloroauric acid) after UV analysis. Hence, 1mM concentration of chloroauric acid was found to be the optimum concentration for the synthesis of gold nanoparticles using 10ml of the volume of extract at 60℃.

CONCLUSION

The focal point of this study was to synthesize, optimize, and characterize the newly synthesized gold from the aqueous extract of *Luffa cylindrica*, which demonstrated the ability to reduce Au ions into Au nanoparticles. The characterization of synthesized gold nanoparticles via Ultraviolet spectroscopy confirmed the formation of AuNPs from with a typical peak having a λ_{max} of 547nm in the UV-Spectra. The Z-average particle size was found to be 92.18 d.nm. The percentage intensity and the standard deviation were recognized as 97.1% and 52.35, accordingly. Through optimization, the ideal parameters for synthesizing gold nanoparticles from *Luffa cylindrica* were found as temperature 60℃, a time period of 45mins, the concentration of chloroauric acid required to be 1mM, and the volume of *Luffa cylindrica* fruit extract to be used as 10ml.

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