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RESEARCH ARTICLE

Green Synthesis of Gold Nanoparticles using *Colchicum autumnale*and its characterization.

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Abstract

Recent interest in the development of new and novel strategies for the generation of gold nanoparticles stems from their potential applications in diversified fields.Biosynthesis of nanoparticles is now established as an emerging area of nanoscience research. A bottom-up 'green' and rapid synthetic route using *Colchicum autumnale* leaf broth as reducing and stabilizing agent produced gold nanoparticles at ambient conditions. The nanoparticles were characterized by UV–vis spectrophotometer, FTIR, Field Emission Scanning Electron Microscope, X-ray diffraction and DLS. FTIR spectra indicates that the reduction of Au^{3+} to Au^{0} was mediated by alkaloids present in the plant leaf broth. The size of synthesized nanoparticles are in the range of 70-120nm, which has been confirmed by DLS and FE-SEM.The size of nanoparticles decreases with increase in broth concentration. The morphology was irregular spherical to polygonal and crystallized in face centered cubic symmetry.

Introduction:-

Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. There have been impressive developments in the field of nanotechnology in the recent past. Currently there is a growing need to develop environmentally benign nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol.

Green route synthesis of nanoparticles has been achieved using environmentally acceptable plant extracts as reducing and capping agents. Plants and microbes are currently used for metal nanoparticle synthesis. The use of plants for synthesis of nanoparticles is rapid, low cost, ecofriendly process. Gold has a long history of use .Red colloidal gold has been used as medicine for revitalization in China and India [(1)].Gold nanoparticles (GNPs) have attracted the attention of many researchers interested in the field due to their biological applications like cancer therapy and imaging [(2,3)].In photodynamic therapy, when the light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up killing the tumor cells.

The use of gold nanoparticles dates back to the 16th century, for both medical and staining purposes. Gardea-Torresdey et al. [(4, 5)].demonstrated gold and silver nanoparticle synthesis within alfalfa plants from solid media. Gold nanoparticles were formed when lemongrass (*Cymbopogonflexuosus*) leaf extract was reacted with aqueous $AuCl^{4-}$ ions [(6)]. Sastry et al. reported the biosynthesis of nanoparticles using plant leaf extracts and their potential application. They studied bioreduction of chloroaurate ions and silver ions by extracts of geranium[(7)]and neem leaf[(8)].Thereafter, gold nanoparticles have found application in analytical methods such as colorimetric techniques for the determination of heavy metal ions in aqueous solutions[(9)]. Gold nanoparticlesare also used in the field of sensors [(10, 11)]. In biology, gold nanoparticles are used for the development of biosensors, DNA labels [(12, 13)] and in medicine [(14)]. However, spherical gold nanoparticles have been used to generate functional electrical coatings [(15)]. The plasmon resonance absorption of colloidal gold particles has been exploited in a proposed DNA detection

method [16].The optical electronic properties of gold nanoparticles are being explored for use in high technology applications such as sensory probes, electronic conductors, therapeutic agents, organic photovoltaic. It is interesting to find the application of gold nanoparticles in plastics, coatings, nanofibers and textiles, since they serve as antifungal, antibacterial and antimicrobial agent. Gold nanoparticles are quite dense, thus allowing them to be used as probes for transmission electron microscopy.

Colchicum autumnale also known as autumn crocus and meadow saffron is the genus of flowering plants in the iris family. It resembles the true crocuses, but blooms in autumn. Colchicine ($C_{22}H_{25}NO_6$), a tropolone alkaloid responsible for its anticancer activity by showing antimitotic activity and used for dispersion of tumors and other neoplastic diseases is naturally produced in the plant. All parts of the plant contain toxins. The greatest concentration of toxins is found in the seeds and the bulb (corm) (Cooper& Johnson, 1984; Frohne&Pfander, 1983). Colchicine is present in the flowers (0.1 to 0.8% in fresh flowers; up to1.8% in dried flowers), in the seeds (0.2 to 0.8%) in the bulb (corm) (0.4 to 0.6%). The leaves contain very low amounts of colchicine (Gessner&Orzechowski, 1972).The other toxins present, which are closely related to colchicine, include: desacetylmethylcolchicine ($CHNO_5$), desacetylthiocolchicine ($CH_{23}NO_4S$), colchicoside, demethyldesacetylcolchicine, colchicine amide(Figure 3) .Colchicine is approved by the US FDA for the treatment of gout and familial Mediterranean fever. It is also used in plant breeding to produce polyploid strains and known to affect chromosomes and cell division [17].

In our work, we have investigated leaves of *Colchicum autumnale* plant to develop their gold nanoparticles. The morphology was studied and chemical characterization was done. In green synthesis, it is believed that natural material extract act as reducing agent for the generation of metal nanoparticles

Experimental:-

Plant material:-

*Colchicum autumnale*Linn. leaves.

Chemical:

Chloroauric acid or Gold (III) chloride trihydrate,$HAuCl_4$ (~50%) was used as a precursor obtained from Sigma Aldrich. Freshly prepared gold solution was used to carry out the proposed experiment.

Instrument:

Shimadzu-2450 UV-vis spectrophotometer,Zetasizer Nano ZS (Malvern instruments, UK), Field Emission Scanning Electron Microscope (FESEM), Fourier Transform Infrared Spectroscopy (Thermo-Scientific-NICOLET 6700), Bruker-AXS D8 Advance (X-ray powder diffraction).

Procedure:-

Preparation of leaf broth

10 gm *Colchicum autumnale* leaves were washed thoroughly with running tap water and then with Millipore water. These leaves were then air dried and cut into small pieces and dispersed in 100mL millipore water which was subjected to heat at 100°C for 20 minutes. This solution was filtered and centrifuged at 4000rpm for 15 minutes. The supernatant was obtained and stored at room temperature.

Synthesis of gold nanoparticles

0.001M gold solution was prepared.50mL of this solution was added to 50mL millipore water to obtain 5×10^{-4}M gold solution for further experiments.

A concentration variation study was done wherein, four samples were prepared each containing same volume of gold solution($5x10^{-4}$ M), but different volume of leaf broth. Sample A containing the least amount and sample D the most. (Please refer to table no.1)

Characterization of Gold nanoparticles (GNPs)

UV-vis spectrophotometer

The kinetics of GNP formation was monitored using Shimadzu-2450 UV-vis spectrophotometer. Ultraviolet spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample. Nanoparticles have optical properties that are sensitive to size, shape, concentration agglomeration state and refractive index near the nanoparticle surface, which makes UV-vis spectroscopy a valuable tool for identifying, characterizing and studying these materials. Scattering from a sample is typically very sensitive to the aggregation state of the sample.UV-vis spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticles solution.The wavelength was fixed between 200-800nm for initial scanning.

Zeta potential and Size distribution studies:-
Zeta potential of the GNPs was monitored using Zetasizer Nano ZS (Malvern instruments, UK) with a 663nm red laser and was capable of both particle size analysis (using DLS-Dynamic Light Scattering as the basic principle of operation) and Zeta Potential measurement (using Doppler Electrophoresis as the basic principle of operation).For the analysis, the nanoparticle sample of desired concentration was flushed through a disposable folded capillary cell (DTS 1060). Nanoparticles with zeta potential values greater than +25mV or less than -25mVtypically have higher degrees of stability.

FE-SEM:-
Field Emission Scanning Electron Microscope (FESEM) is the microscope that works with electrons instead of light. These electrons are liberated by a field emission source. The object is scanned by electrons according to a zigzag pattern. It is used to visualize very small topographic details on the surface or entire or fractioned objects. In order to be observed with a SEM, objects are first made conductive for current. This is done by coating them with an extremely thin layer (1.5-3.0 nm) of gold.

FT-IR:-
Fourier Transform Infrared Spectroscopy (Thermo-Scientific-NICOLET 6700) is a technique in which, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. It can be used to determine the functional group of various components present in the sample.

XRD analysis:-
X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of crystalline material and can provide information on unit cell dimensions. Bruker-AXS D8 Advance was used to analyze the sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's law ($n\lambda=2d\sin\theta$).This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material.

Results and discussion

Color change of the solution from colorless to light pink, purple and wine red was observed (Figure- 2). There was a gradual increase in the intensity of color with increasing time. We anticipate the reduction of $Au^{3+}$to Au^{0} is favored by increase in the concentration of leaf broth anddifferent alkaloids present in the leaf broth act as the reducing agent.

UV-Vis and kinetic studies of GNPs:-
Formation of gold nanoparticles can be visually witnessed by the color change. The formation of various nanoparticles from their different salts give characteristic peaks at different wavelengths that can be monitored using UV-vis spectrophotometry. A progressive increase in the characteristic peak with increase in the reaction time and concentration of leaf broth is a clear indication of nanoparticle formation.Concentration variation study of *Colchicum autumnale* leaf broth was carried out with 5×10^{-4}M Gold (III) Chloride ($HAuCl_4$) showing that under UV-vis spectrophotometry the peak for different concentrations range between 540-570nm which corresponds to the average particle size ranging from 70-120nm (Refer to table-1) As the volume of leaf broth increases, the wavelength of absorption shifts towards the blue wavelength which suggests decrease in the particle size (Figure 4)

Particle size distribution and zeta potential study:-
Dynamic Light Scattering (DLS) is the basic principle of operation for Zetasizer Nano ZS.The undisputable advantage of DLS method is the feasibility to investigate the dispersity in situ with data averaging over a large ensemble of particles contained in the examined volume. It is also suitable for studying the slow kinetic processes at specific times .It measures the rate of intensity fluctuation and then uses this to calculate the size of particles. The Z-average (d.nm) value of GNPs was found to be 94.36nm (Figure 5). The size of particles are in the range of 50-

200nm.It was also observed that the solution with higher concentration of leaf broth shows small particle size. This might happen because, when the concentration of leaf broth is less, i.e., solution is dilute, the extraneous particles (solvent molecules) must be interfering with the results, yielding erroneous data. The average particle size of all the four concentrations justify the anticipated behavior of GNPs. The solution with minimum volume of leaf broth and that with maximum volume of broth show maximum and minimum size respectively. The nanoparticles seem stable after 24 hours and give identical readings. This method measures how fast a particle moves in a liquid when an electric field is applied. The zeta potential of a sample determines whether the particles within a liquid tend to flocculate or not. Nanoparticles with zeta potential values greater than +25mV or less than -25mV typically have high degrees of stability. Zeta potential of GNPs was -21.7mV.We anticipate that these particles might form agglomerates. (Figure 6)

A typical trend was observed in our research where particle size decreases with increase in the volume of broth, however, the polydispersity index shows a reverse trend (Table no. 2, Graph 1, 2) .Polydispersity or heterogeneity index maximum value is arbitrarily limited to 1.Increase in the PDI is attributed to the formation of various sized particles formed as a result of high concentration of broth reducing the gold ions.Also, since the particles are in various phases of nucleation they lead to varying size of particles in the solution. A PDI value of 1 indicates that the sample has a very broad size distribution and may contain large particles or aggregates that could be slowly sedimenting.

FESEM:-

FESEM images were obtained using Hitachi FESEM, Model S-4800.The images confirm that the average particle size lies between 60-100nm, having irregularly spherical to polygonal shape. Nanoparticles show polydisperse distribution.FESEM images support UV-vis and particle size distribution results (Figure 7).

FTIR:-

A comparative analysis was done between the peaks of crude leaf broth and GNPs to identify the possible biomolecules responsible for reduction of Au^{3+} ions. The aim was to detect the shift of prominent peaks between the two. The similarity between the spectra suggests that they possess same compounds. Chloroauric acid solution, after complete reduction of the metal ions and formation of gold nanoparticles, was centrifuged at 5,000 rpm for 30 minutes; the pellet obtained was further centrifuged thrice to isolate the metal nanoparticles from free proteins or other compounds present in the solution prior to FTIR analysis. The characteristic peaks of the primary amine group (N-H) were observed at 3263.2 cm^{-1} and 3247.1 cm^{-1} and that of alkane (C-H) were perceived clearly at 2930.7 cm^{-1} and 2931.3 cm^{-1} for GNPs and *Colchicum autumnale* leaf broth, respectively. Broad peaks around 3300 cm^{-1} taken from the leaf broth confirm the existence of both –NH and –OH groups. The typical peak at 1783.3 cm^{-1} in leaf broth spectra disappeared in the GNP spectra which clearly suggests that C=O is expended in the nanoparticle synthesis. Some peaks in GNP spectra were observed close to 600 cm^{-1}, signifying the presence of R–CH group [18, 19], (Table 3, Figure 8)

XRD:-

The structure of biologically synthesized GNPs were analyzed by XRD measurements. Bragg's reflections at 2θ=38.20, 44.39, 64.59, 77.57 can be indexed to the (111), (200), (220) and (311) orientations, respectively, confirmed the presence of gold nanoparticles (GNPs). Bragg's reflection of face centered cubic structure of metallic gold (Joint Committee on Powder Diffraction Standards No.-04-0784), reveals that the synthesized GNPs are composed of pure crystalline gold. (Figure 9)

Figures:-

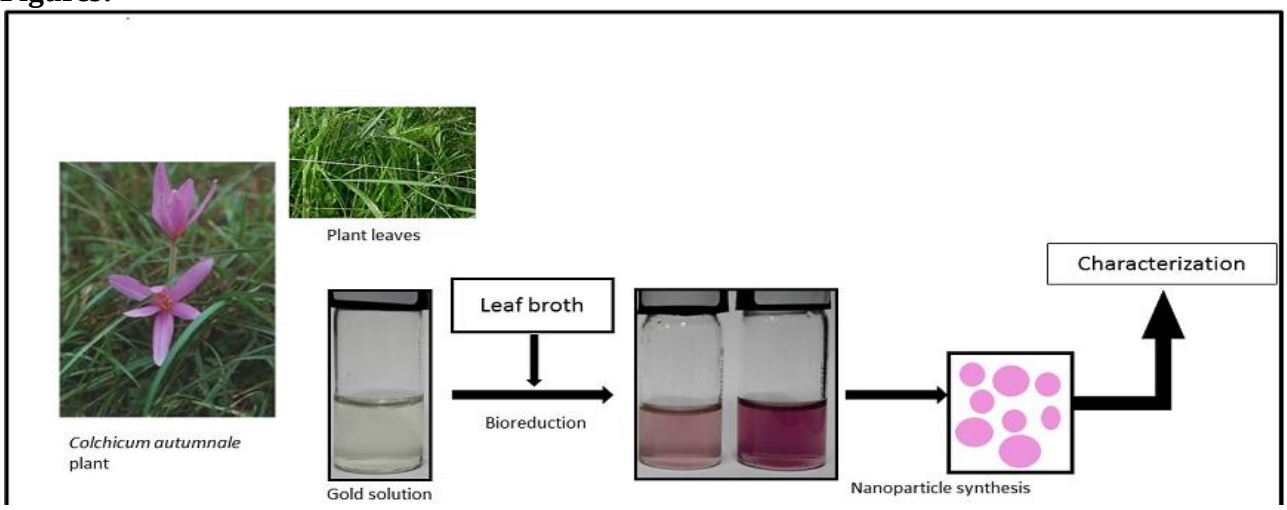


Figure 1: Schematic diagram of nanoparticle synthesis and characterization.

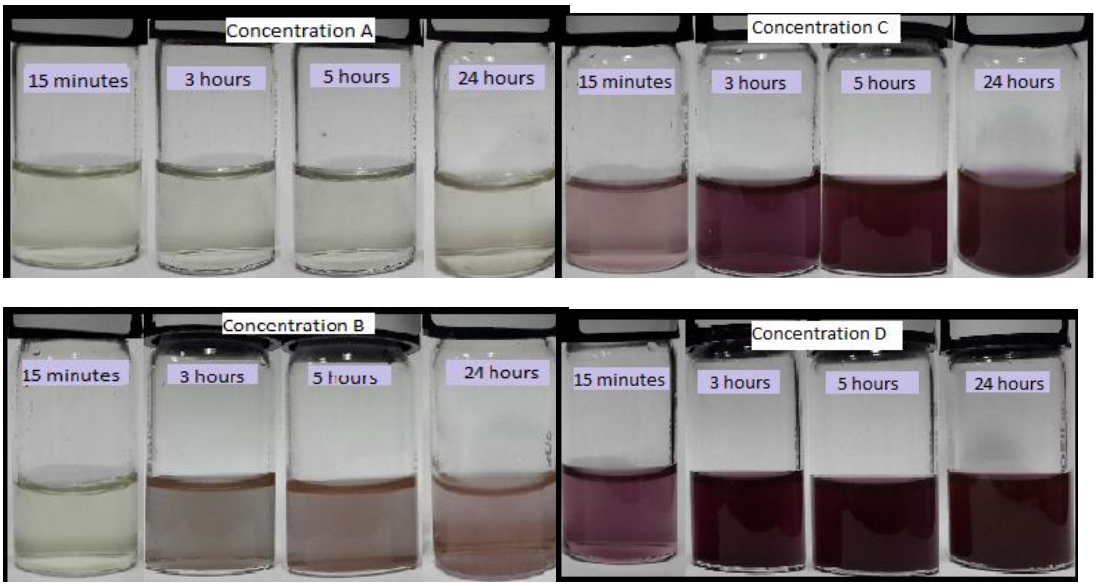
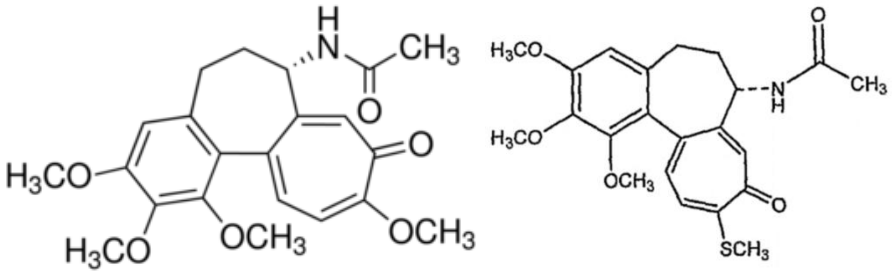


Figure 2: Color change depicting formation of gold nanoparticles for various concentrations of leaf broth.



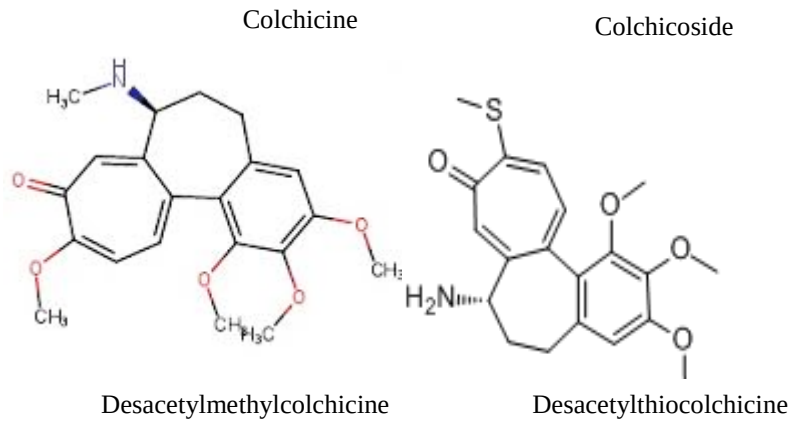


Figure 3:Colchicine and related alkaloids.

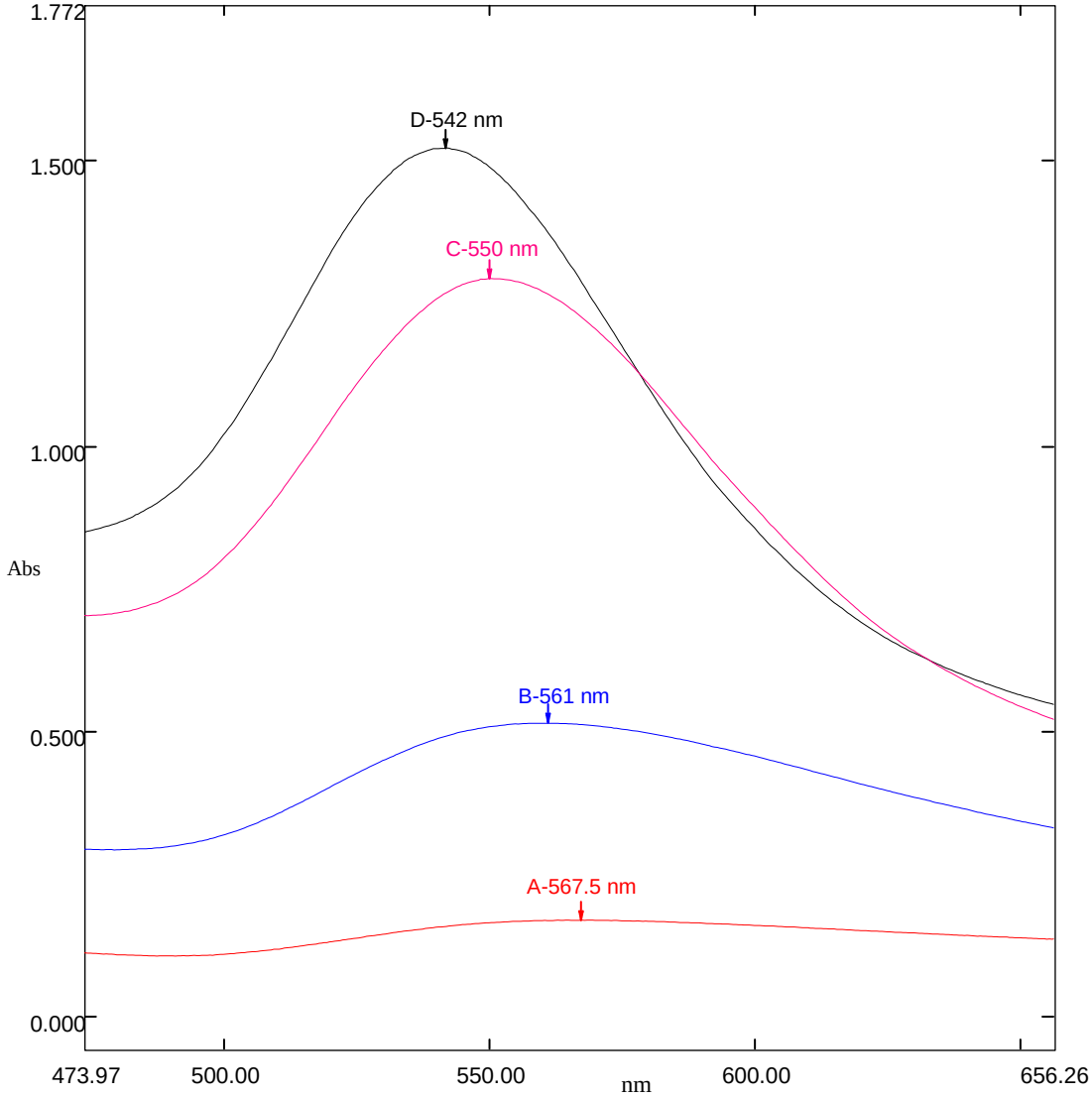


Figure 4: UV-vis spectra results of four different concentrations showing peaks for gold nanoparticle (Table -1)

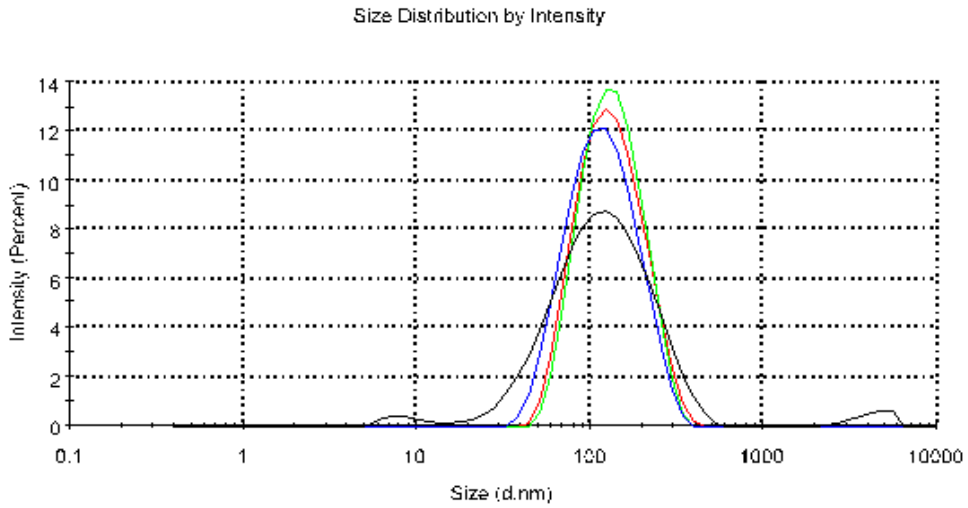


Figure 5: Size distribution results for various concentrations.

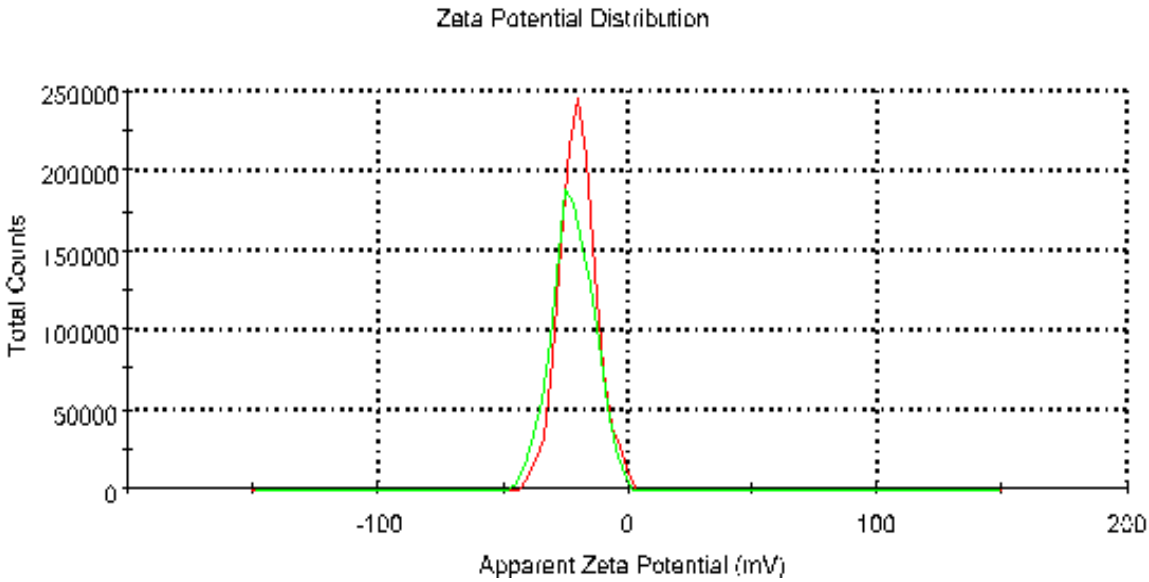


Figure 6: Zeta Potential distribution of gold nanoparticle solution

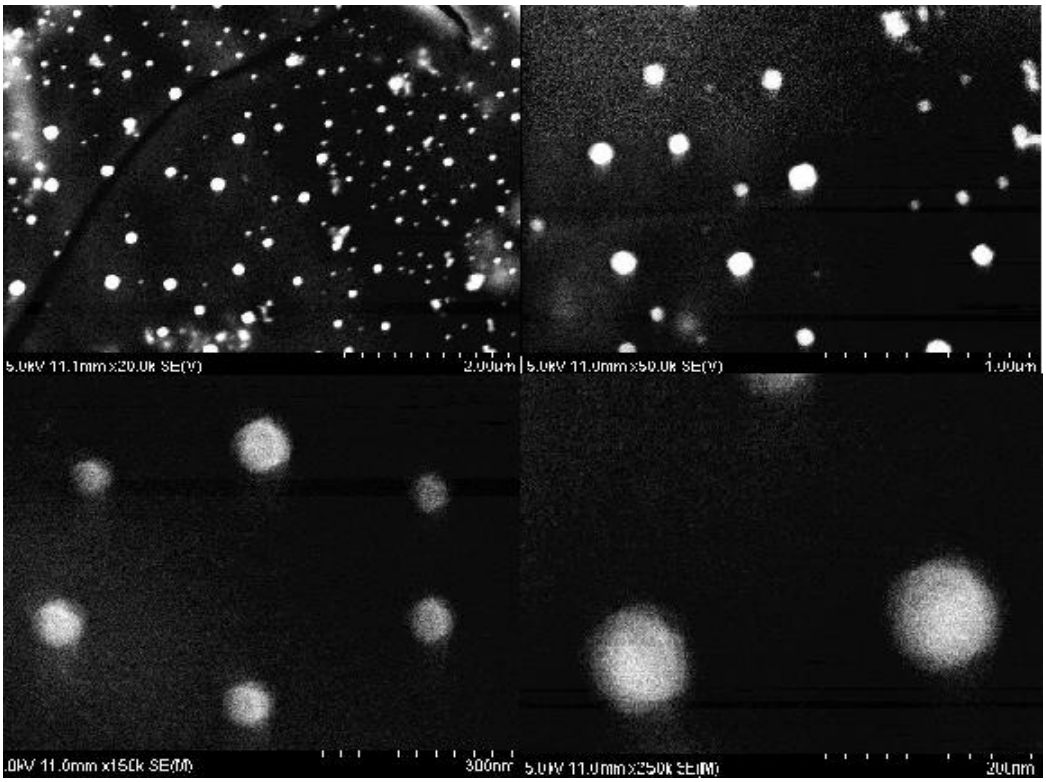


Figure 7: SEM images of gold nanoparticles

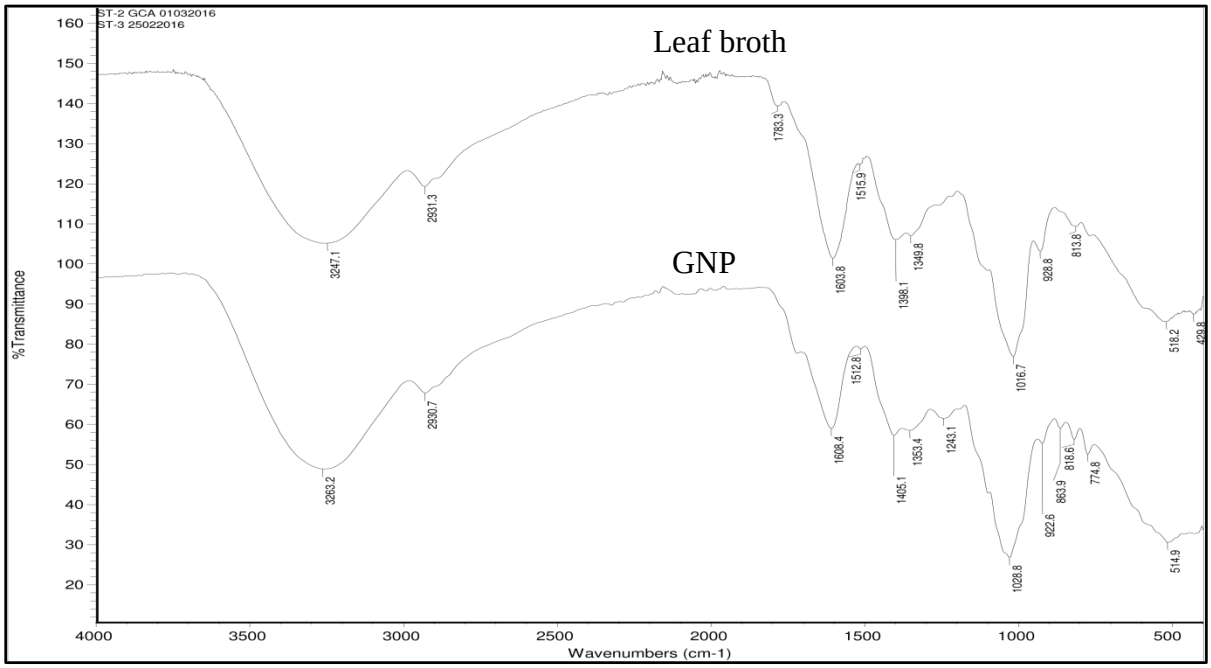


Figure 8: FT-IR spectral analysis

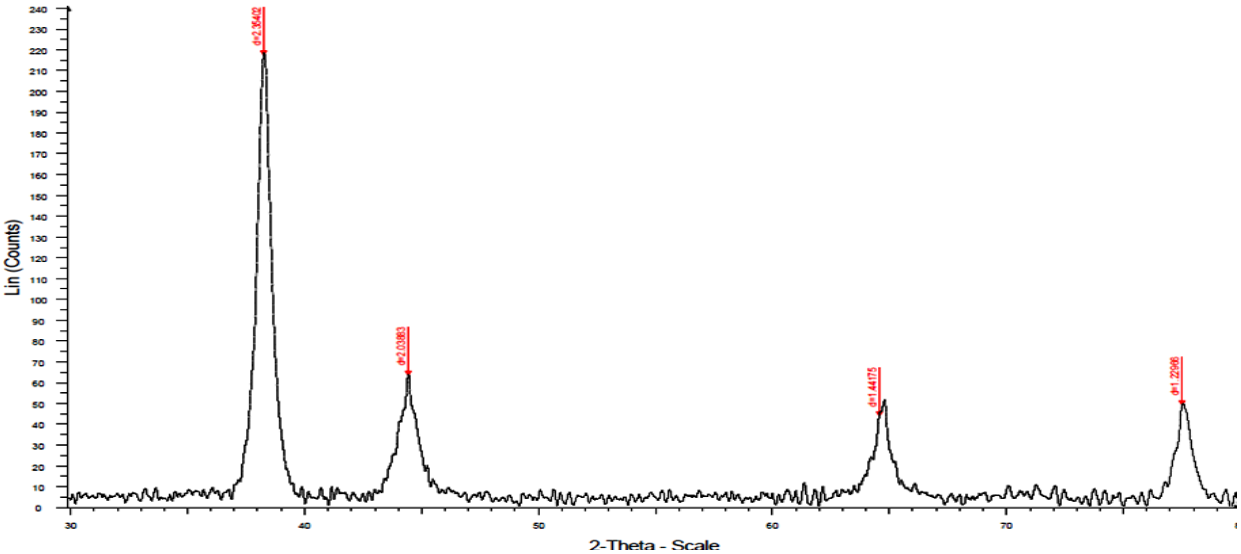
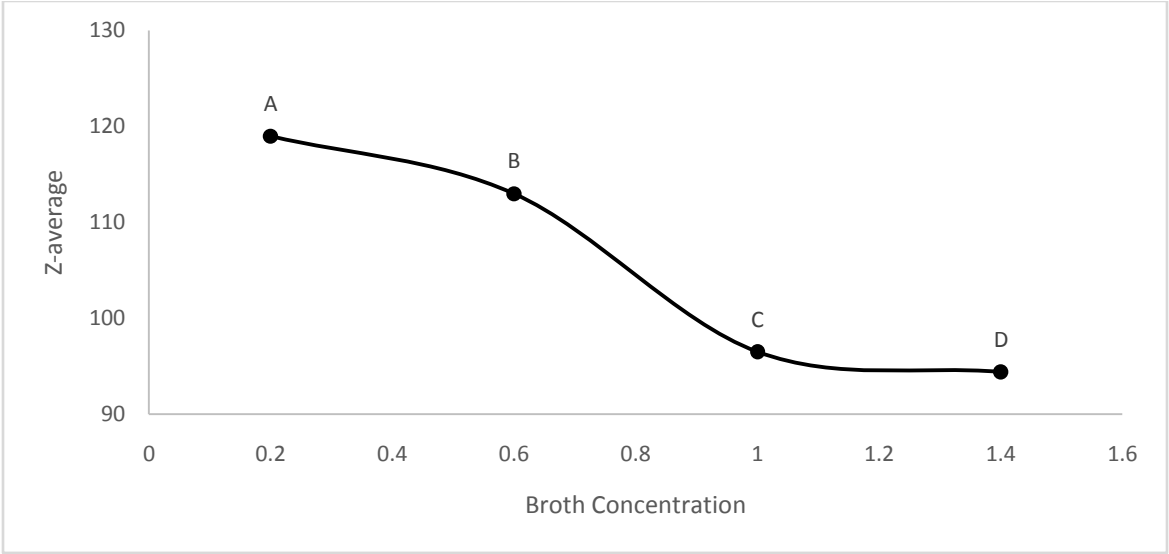
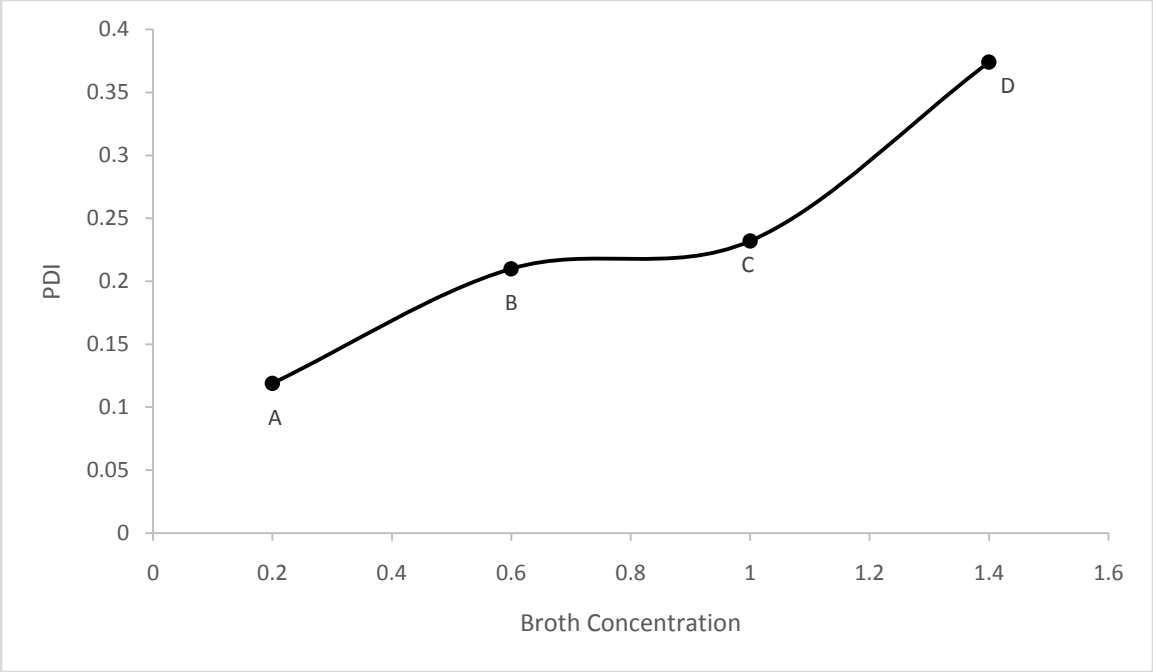


Figure 9: XRD pattern of synthesized GNPs

Graphs:-



Graph 1: Comparative study of particle size for different Broth concentrations after 24 hours



Graph 2: Comparative study of Polydispersity for different Broth concentrations

Tables:-

Table 1. Comparative study – Four concentrations

Sample	Volume of gold solution (5×10^{-4}M)(mL)	Volume of plant leaf broth(mL)	Volume of millipore water(mL)	Total volume of solution (mL)	λ_{max}=SPR band
A	10	0.2	1.8	12	567.5
B	10	0.6	1.4	12	561
C	10	1.0	1.0	12	550
D	10	1.4	0.6	12	542

Table 2. Comparative study – Z-average versus Polydispersity index (PDI)

Sample	Z-average(d.nm)	PDI
A	119	0.119
B	113	0.210
C	96.5	0.232
D	94.4	0.374

Table 3: Summary of prominent peak shifts between FT-IR spectra of Leaf broth and GNPs.

Peak position in extract(cm^{-1})	Peak position in GNP solution(cm^{-1})	Shift in position(cm^{-1})	Type of chemical bond
3247.1	3263.2	+ 16.1	1°,2° amines, amides(N-H)
2931.3	2930.7	- 0.6	Carboxylic acids, alkanes (O-H ,C-H)
1783.3	No peak observed	-	Acid halide (C=O)
1603.8	1608.4	+ 4.6	Cyclic alkene (C=C)
1515.9	1512.8	- 3.1	Aromatics (C-C stretch, in ring)
1398.1	1405.1	+ 7	Sulphate (S=O)
1349.8	1353.4	+ 3.6	Nitro compounds (N-O symmetric stretch)
1016.7	1028.8	+ 12.1	Amine(C-N stretch)
928.8	922.6	- 6.2	Carboxylic acid (O-H bend)

Conclusions:-

In this study, the gold nanoparticles were synthesized using green route, i.e., leaf extract of *Colchicumautumnale.* The alkaloids of this plant served as capping and stabilizing agent for the nanoparticles. Green route for nanoparticle synthesis was chosen because it is rapid, economic and environmentally benign. The average particle size was in the range of 70-120nm which has been confirmed by SEM images. These images affirm the results of DLS showing similar particle size and polydispersity. DLS results show a typical trend wherein the particle size reduces with increase in the concentration of leaf broth. Morphology of GNPs was irregular spherical to polygonal. XRD spectra indicate the face centered cubic symmetry of GNPs.Our work seeks potential application in the further development of cellular imaging and cancer research because gold is reported to serve essential role in cellular imaging, biosensors and cancer therapy.

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Notes:-

Names of the companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the Aditya Birla Science and Technology Company Private Limited or Aditya Birla Group over others not mentioned.

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