Ind. J. Pharm. Edu. Res., 2023; 57(2s):s198-s212.
https://www.ijper.org

Review Article

A Recent Review on Synthesis, Characterization and Activities of Gold Nanoparticles Using Plant Extracts

Sheeja Koliyote*, Jessy Shaji

Department of Pharmaceutics, Principal K.M. Kundnani College of Pharmacy, Mumbai, Maharashtra, INDIA.

ABSTRACT

Despite advances in synthetic medications, medicinal herbs have empowered mankind with a wide range of efficacious medicines to lessen or eliminate illnesses and suffering from diseases. Since the last decade there has been increasing enthusiasm for the synthesis of gold nanoparticles particularly from herbal extract because the method is a one pot process, environment friendly, safe, convenient and can be produced in large scale. Aqueous extract of several parts of herbs have been utilised, like flower, fruit peel, fruit juice, leaf, stem, seed, rhizome for synthesizing gold nanoparticles. Plants contain phytoconstituents such as alkaloids, flavonoids, polysaccharides, phenols, terpenoids and proteins. These secondary metabolites present, serve as capping and reducing agents in plants, increasing the rate of nanoparticle reduction and stabilisation. Change in colour of yellow coloured auric chloride solution used in the synthesis to ruby red or deep purple or magenta confirms the formation of gold nanoparticles. Various techniques such as UV-visible spectroscopy, scanning electron microscopy, fourier transform infrared spectroscopy, transmission electron microscopy, atomic force microscopy, dynamic light scattering, powder X-ray diffraction, energy dispersive spectroscopy and zeta potential are used to characterize nanoparticles of various sizes, shapes, and surface areas. This review's goal is to present an overview of recent developments in herbal extract-assisted gold nanoparticle synthesis, characterization and applications.

Keywords: Plant extract, Gold nanoparticles, Synthesis, Characterization, Activity.

Correspondence:
Sheeja G Koliyote
Department of Pharmaceutics, Principal K.M. Kundnani College of Pharmacy, Cuffe Parade, Colaba, Mumbai-400005, Maharashtra, INDIA.
Email: sheejakoliyote@gmail.com

Received: 26-01-2022;
Revised: 24-06-2022;
Accepted: 20-01-2023.

INTRODUCTION

Nature is a store house of different plants containing active bio-molecules which are potential source of drugs against health and diseases. Reviewing the history of mankind, it has been seen that plants are integral part of world civilization and medicinal system for thousands of years. Utilization of plants for treatment of various ailments have been practiced all over the world namely from countries like China, Japan, Egypt, Brazil and India.[1] In fact plants are still a popular medicinal alternative for 80 percent of people in underdeveloped nations due to their easy availability, cost-effectiveness, biocompatibility, natural origin, and acceptability. Aspirin, artimesinin, serpentine, colchicine, digoxin, ephedrine, morphine, physostigmine, Z guggulsterone, pilocarpine, reserpine, taxol, tubocurarine, paclitaxel, and vinblastine are some of the medications said to be derived from plants.[2] Hence, the future medicine therefore will walk miles holding the hands of medicinal herbs, therefore research and development in this arena is required using upcoming technologies.

Gold is a noble metal that has long been associated with power and wealth. Egyptians employed gold metal solubilized water for mental and spiritual purification in the 18th century. Gold was used to treat ailments like smallpox, skin ulcers, syphilis, and measles in many ancient societies (Egypt, India, and China). Medical devices such as pacemakers and stents, which are used to treat cardiac problems, are now made of gold. Middle ear gold implants and dental restoration using gold alloys has been reported.[3]

Gold Nanoparticles (GNPs) are one of the prominent metal nanoparticles with a wide variety of applications in various fields of science and technology. GNPs are employed in many fields like cancer therapeutics, bioimaging, diagnosis, drug delivery, optoelectronics, biomedical, catalysis, super conductors, biosensing.[4] GNP have been synthesized using various methods as shown in Figure 1.[5] Physical method is hazardous, capital intensive and is inefficient in materials and energy use.[6] Chemical method involves use of toxic and harmful chemicals that get adsorbed on the surface and are accountable for various biological hazards.[7] It employs highly concentrated reducing agents and stabilizers that can endanger the environment and public health.[8]

DOI: 10.5530/ijper.57.2s.24

Microbial method involves elaborate maintenance of cell cultures, difficulty of scaling up, biohazardous on production, low speed of synthesis.[9]

Amongst all these methods plant mediated synthesis of GNPs as shown in Figure 2 is gaining more importance owing to (a) easy synthesis (b) rapid rate of synthesis (c) biocompatibility (d) non-hazardous (e) higher potential for reduction (f) water is used as solvent (g) zero contamination (h) does not need special conditions like temperature, pressure, hazardous chemicals and (i) functions as a reducing and capping agent at the same time.[10] As a result, the study of gold nanoparticles synthesis utilising plant extract has generated a lot of interest. GNPs derived from plant extract are employed in biomedicine for a variety of applications, including leukaemia therapy, biomolecular immobilisation and biosensor creation. GNPs have also been used as anti-angiogenesis, anti-malaria and anti-arthritic medicines. Metal nanoparticles (as in bhasmas) in combination with herbs may function as effective treatments, according to ayurvedic principles.[11] Active compounds present in herbs serves as reducing agent while forming GNPs, stabilize it and in combination they can possess a better therapeutic potential. The concept of herbal nanoparticles for drug delivery may excite certain possible research groups in the future, resulting in potentially eye-catching findings.

Different plant parts used for formulation of GNP

From Table 1 it can be seen that different plant parts have been used for the synthesis of GNP right from flower, root, leaves to onion which is a modified stem belonging to the families Thymelaeaceae, Liliaceae, Araliaceae and Malvaceae.[12-15] For synthesizing GNPs the concentration of chloroauric acid used for each plant has been 1mM using different volumes ranging from 300ul for *H. rosa-sinensis* fresh leaves to 95mL used for *G. glauca* flower. The concentration of plant extract used also has been different for each plant from 5% of powdered stem bark of *G. glauca* to 25% of *A. cepa* but the reaction took place at varying temperatures showing surface plasma resonance between 500nm to 600 nm. Synthesis of GNPs depends on a number of factors, including the concentration of plant extract, concentration of chloroauric acid, duration of the reaction, temperature of the solution and pH of the solution as shown in Figure 2. It is thus possible to generate nanoparticles of the desired size and shape by determining the corelation between these variables in a regulated way. For instance, an increase in the amount of auric chloride and extract often results in an increased size and irregular shape of the GNPs due to agglomeration.

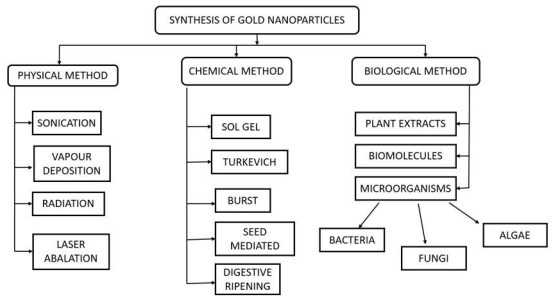

Figure 1: Various methods of making gold nanoparticle.

Various colours of GNP obtained using different parts of plant

Gold Chloride Solution (GCS) or auric chloride or tetrachloroauric acid is freely soluble in water giving a yellow-coloured solution. When GCS is mixed with the plant extract which is mostly brown in colour, results in formation of GNP having a distinct colour. This visual change in colour of the mixture is the first indication of formation of GNPs which is very distinct for a particular plant extract. Every plant contains different phytochemicals which give a particular-coloured GNP. The surface of GNP exhibits an unique surface plasmon resonance (SPR) phenomenon, which results in substantial extinction of the wavelength of radiated light. The collective oscillation of free conduction electrons within the metal after interaction with the associated electromagnetic field confers this distinct activity associated to GNPs optical property. Excitation of SPR vibrations causes colour to manifest in red, magenta, purple, violet, or pink-ruby red in the range of 500-600nm wavelength confirming the production of GNPs.

The appearance of different coloured solution as shown in Figures 3 a-e such as: cherry yellowish red obtained using *A. cepa* stem,[13] ruby pink obtained using *H. rosa-sinensis* leaf,[15] ruby red obtained using *A. maurorum* flower,[16] dark purple obtained using *R. lanceolata* fruit,[17] magenta pink obtained using *L. angustifolia* leaf,[18] is due to activation of SPR vibration, confirms the production of gold nanoparticles. It should also be noted that most of the GNP generated are 25 nm in size and spherical in shape. It has been observed that many plant extract yielded violet coloured GNP as shown in Table 2.[17-24] The presence of phenolic substances such as flavonoids and tannins among the vast spectrum of phytochemicals present in the below mentioned plants can be ascribed to the violet/ purple colour of the GNP.

Characterization of synthesized gold nanoparticles

UV-visible spectroscopy (UV-vis), Dynamic Light Scattering (DLS), Zeta potential, Transmission Electron Microscopy (TEM), energy dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), powder X-Ray Diffraction (XRD), and Atomic Force Microscopy (AFM) are being used to characterize nanoparticles of various shapes, sizes and surface areas.[25] From Figure 4 it is evident that AFM and Zeta which was performed 30% and 20% of the time are the least preferred test amongst all the tests.

Table 1: Bioinspired Gold Nanoparticle Synthesis Using Extracts of Different Parts of Plant.

Plant Part	Name /Family	Concentration of Plant	Concentration of Auric Chloride	λ_{max}(nm)	Method	References
Flower	*Gnidia glauca*	5%	1 Mm GCS	540	40°C 20 min	12
Modified stem	*Allium cepa*	25%	0.2 mL extract +50mL 1 Mm GCS	540	Rt	13
Root powder	*Panax ginseng*	10% sterile water boil for 30 min filter	5 mL extract+25 mL 1mM GCS heat to 80°C 1hr 10 min	560	80°C	14
Leaves	*Hibiscus rosa-sinensis*	8% fresh cut leaves D/W microwave for 3 min	8 mL extract +300ul GCS 1 mM at 420W 90sec	520	Microwave	15

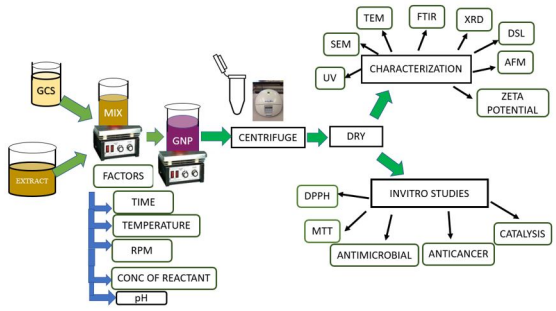

Figure 2: Simple illustration for the production, characterization and *in vitro* studies done on green synthesized gold nanoparticles.

UV-VIS-spectroscopy analysis of GNP synthesized using different plant extracts

Usually, UV–vis-spectroscopic analysis is utilised to characterize noble metallic nanoparticles, also including gold. Due to the SPR phenomenon, GNPs have a substantial absorption in the visible region, with a maximum absorption in the range of 500–600 nm. The absorption peak of *W. trilobata* leaf extract mediated GNP was reported to be located at 533nm as shown in Figure 5a. A ratio of 1:9 of this extract and GCS was used at room temperature and the colour change to deep purple was obtained in 30 min.[26] Abootorabi *et al.* revealed the reduction of gold ions into GNP in a size controlled manner by 2 antioxidant rich herbs of barberry and saffron stigma extract using one step green synthesis method.[27] Almost 24 hr of reaction time was required at the temperature of 50°C and pH 7.5 to yield SPR band at 520 nm as shown in Figure 5b. Dudhane *et al.* employed *T. arjuna* leaf extract for the environmentally friendly extracellular fabrication of metallic gold nanoparticles.[28] The colour of the synthesised nanoparticles was ruby red, with a maximum peak at 523nm as shown in Figure 5c. In another study, GNPs synthesized from flavanoids present in the whole plant extract of *C. asiatica* showed absorption peak at 542nm as shown in Figure 5d. The synthesis was investigated at three temperatures: 40°C, 55°C, and 70°C.[29] In comparison to GNPs synthesised at high temperatures, which had sharp and narrow SPR peaks with increased sphericity, GNPs synthesised at low temperatures were polydispersed, anisotropic, and big, as evidenced by a wider SPR peak.

Because of the SPR phenomenon, substantial absorption in the visible region was found for all synthesised GNP, with a maximum in the range of 500–600 nm. This is due to a collective oscillation of free conduction electrons mediated by an interacting electromagnetic field with the metal nanoparticles in consideration.

FTIR analysis of GNP synthesized using different plant extracts

The absorption peaks of FTIR spectroscopy correspond to the frequency of vibrations between the bonds of the atoms in the material, resulting in a fingerprint of a sample. The FTIR technique is used to identify the biomolecules that are likely to be involved in the reduction and production of GNP.

It can be observed from Table 3 and Figures 6 a-d, that the new bonds formed in the FTIR of plant extracts mediated GNPs were due to hydroxyl, primary amine, amide, aromatic, carboxylic acids and carbonyl functional groups present in the extract.[30,31] The FTIR spectrum of GNP synthesised from various plant extracts revealed the presence of reducing agents such as polysaccharides, terpenoids, polyphenols, sugars, alkaloids, phenolic and proteins, which are secondary plant metabolites consisting of these functional groups that react with metal ions and reduce it to produce GNP. These phytoconstituents also have the ability of capping the nanoparticles that have been produced, providing them stability and biocompatibility.

SEM analysis of GNP synthesized using different plant extracts

Morphological studies of the synthesized GNP, for example, SEM, TEM and AFM are done using microscopic techniques. The external morphology (texture), chemical constitution, and orientation of the materials that make up the sample are all revealed by SEM. In most situations, a two-dimensional representation displaying spatial differences in these attributes is generated.

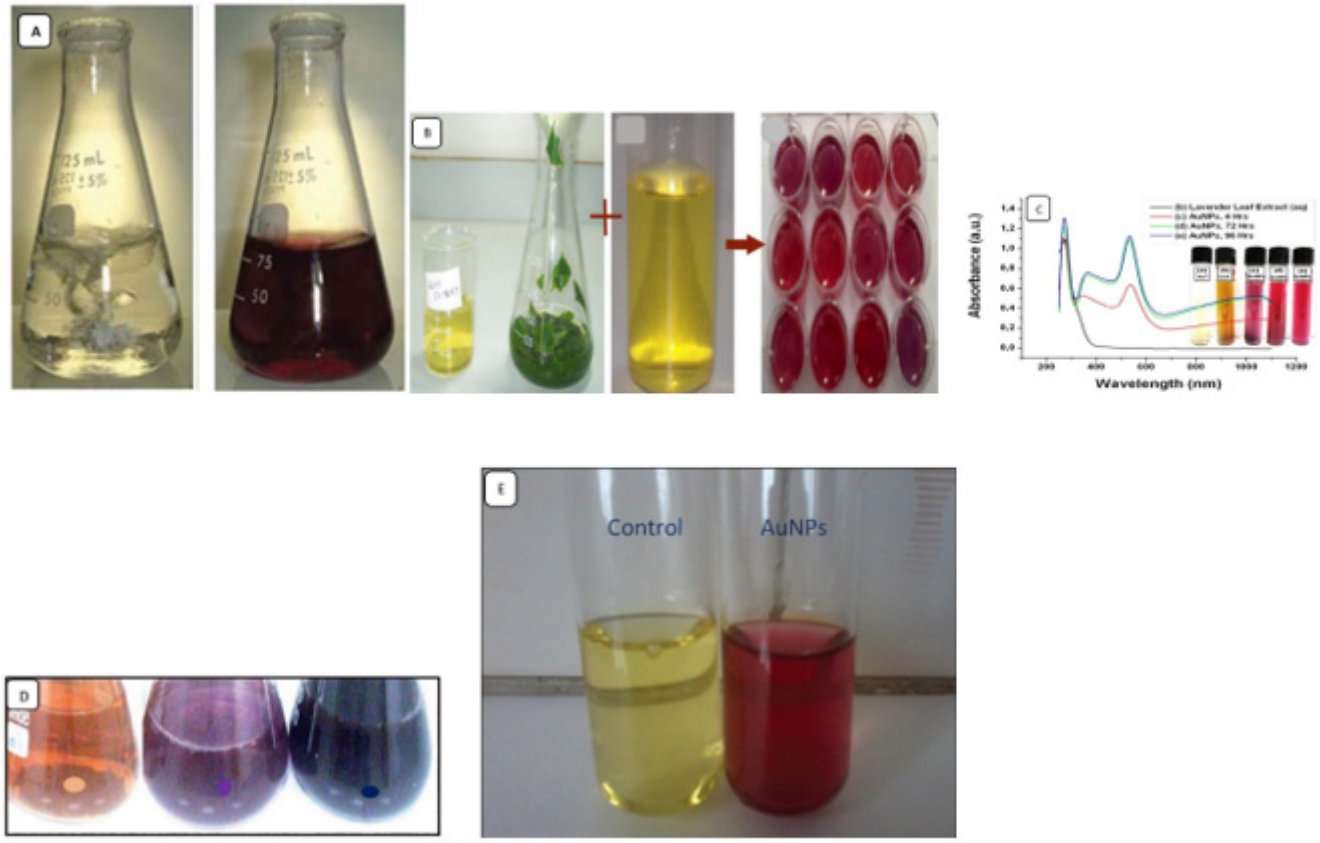

Figure 3: Visual observation of gold chloride solution and colourful green synthesized gold nanoparticles utilising an extract of (a) *Allium cepa* stem (b) *Hibiscus rosa-sinensis* leaf (c) *Lavandula angustifolia* leaf (d) Rhuscoriaria lanceolata fruit (e) *Alhagi maurorum* flower.

Table 2: Bioinspired Synthesis of Violet/Purple Gold Nanoparticles Utilising Extracts of Plant Parts.

Name	Part	Colour	Chemical Constituent	References
Rhuscoriaria lanceolata	Fruit	Purple	Tannins, flavonoids, tannins, terpenoids, xanthones, steroids, coumarins, essential oils and organic acids.	17
Salvia officinalis	Leaves fresh	Deep Purple/red	Alkaloids, carbohydrate, fatty acids, glycoside, coumarins, flavonoids, tannins, steroids and waxes.	19
Dillenia indica	Fruit	Purple	Steroids, glycoside, protein, alkaloid, fixed oils, flavonoids, Saponins, tanins, malic acid, arbinoglactan, and reducing sugar.	20
Withania somnifera	Leaves	Violet, dark pink	Alkaloids, steroidal lactones, saponins, glucosides, flavonoids, phenolics, glycosides.	21
Stevia rebaudiana	Leaves	Purple	Glycosides, flavonoid, chlorogenic acid, amino acids, fatty acids.	22
Eleutherococcus senticosus	Stem	Deep purple	Flavanoid, glycoside, lignan, glycan, saponin, hydroxycoumarins, Flavone.	23
Garcinia mangostana	Fruit peel	Purple	Anthocyanin glycoside, xanthone, tannins, flavonoids.	24

Pulit and his co-workers investigated the synthesis of GNP using a knotweed extract. Extract of *P. aviculare* also known as knotweed is a natural source of compounds with reducing properties such as elagic acid, gallic acid, ascorbic acid and anthocyanins.[32] SEM images of nanogold obtained with the participation of knotweed extract is characterized by a longitudinal, spherical and rectangular shape having a particle size from 20 and 200nm as shown in Figure 7a. Mishra *et al.* synthesized GNPs at ambient condition using *A. calamus* rhizome extract and two different concentrations of auric chloride solution, which showed improved antibacterial activity when it was coated on cotton fabric.[33] The SEM micrograph shows uniform size spherical ball

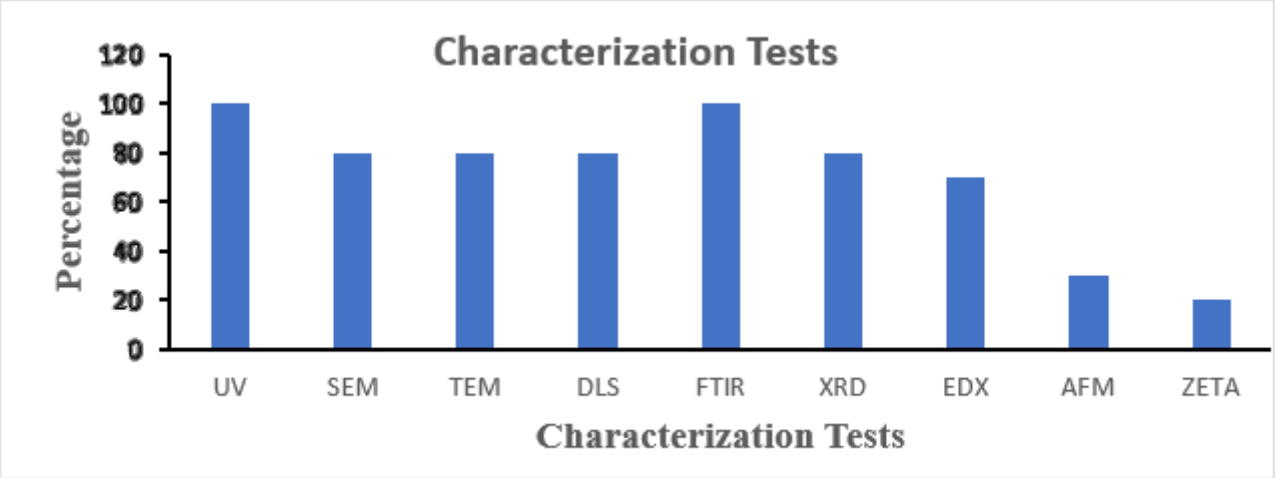

Figure 4: Characterization test performed (number of times as percentage) on herbal gold nanoparticles *n*=50.

morphology of 100nm using 0.001M chloroauric acid solution while 0.01M chloroauric acid solution yielded bigger spherical balls of particle size in the range between 100-500nm as shown in Figure 7b. Synthesis of GNPs using leaf extract of *N. khasiana* with GCS carried out by Bhau *et al.* demonstrated that as the incubation time increases, the absorbance increases, but then becomes steady, with a maximum absorbance at 599.78 nm after three hours.[34] The SEM images revealed a clustering of GNPs with diameters ranging from 50 to 80 nm in size as shown in Figure 7c. An eco-friendly technique for the fabrication of GNPs from GCS was also reported using *A. tetracantha* Lam. leaves extract.[35] The SEM image showed the formation of spherical structure of about 80 nm in diameter of GNPs using *A. tetracantha* leaves extract and confirmed the development of gold nanostructures as shown in Figure 7d. Looking at the above SEM images of the green synthesized GNPs it can be concluded that all herbal GNPs have a spherical morphology whatever be the conditions of synthesis.

TEM analysis of GNP synthesized using different plant extracts

The inner surface and shape, as well as the dimensional range and size distribution of GNPs, are extensively assessed using TEM analysis for imaging and analytical characterisation. The gold core of the GNP is photographed using high-resolution transmission electron microscopy (HRTEM), whereas TEM is a powerful and simple approach for determining the size and morphology of nanoparticles. The selected area electron diffraction (SAED) pattern is also employed in TEM equipment to distinguish crystalline structures from amorphous structures.

Elia *et al.* showed that GNP of different shape and size were produced by *P. granatum* fruit extract and *L. citriodora* leaf extract when 0.75mL of the 20% extract was mixed with 10 mL GCS. The fruit extract changed colour to deep purple in a matter of seconds, however the leaf extract required a brief heating to 40°C for several minutes.[17] For the fruit extract, the TEM images revealed diverse geometrical shapes of 150nm, such as triangles, pentagons, and hexagons, however for the leaf extract, the number of large particles was insignificant compared to the amount of irregular little particles of 20nm size as shown in Figure 8a and b. Singh *et al.* collected fresh *C. arietinum* leaf also called chickpea for the synthesis of GNP.[36] From what TEM images reveal, it was proved that nanoparticles of spherical, pentagonal and triangle shapes having sizes ranging from 30-80nm were produced as shown in Figure 8c. Abbasi *et al.* carried out green synthesis of GNP using aqueous bark extract of *M. tenuiflora* (Mt) using two different concentrations of the extract. 1.6 mL of GCS was mixed with two different concentrations namely 5.3mM for GNPMt1 and 2.6mM for GNPMt2.[37] The TEM micrograph shows an average size dispersion of 40nm for GNPMt1 with biggest diversity in shape and 150nm for GNPMt2 with uniform hexagonal shape as shown in Figure 8d. This diversity in shape was attributed to lack of stabilizing biomolecules in lower concentration of extract. In another recent study, sesquiterpenoids from *T. farfara* flower bud extract were successfully used as a reducing agent in the environmentally friendly synthesis of silver and gold nanoparticles.[38] TEM revealed that the most common size of silver nanoparticles is 10 nm to 15 nm (62% frequency), with an average size of 13.57±3.26 nm, whereas GNPs showed recurring particles of size 15 nm to 20 nm (43% frequency) and an average size of 18.20±4.11 nm. As shown in Figure 8e. The above TEM images of the green synthesized GNPs confirm the spherical shape of GNP along with hexagonal, pentagonal and a few prominent triangular shaped GNPs.

AFM analysis of GNP synthesized using different plant extracts

The surface morphology of GNP can be studied using AFM, which is a useful biophysical tool. It is used to characterise individual nanoparticles size information (length, breadth, and height) as well as other physical attributes (morphology

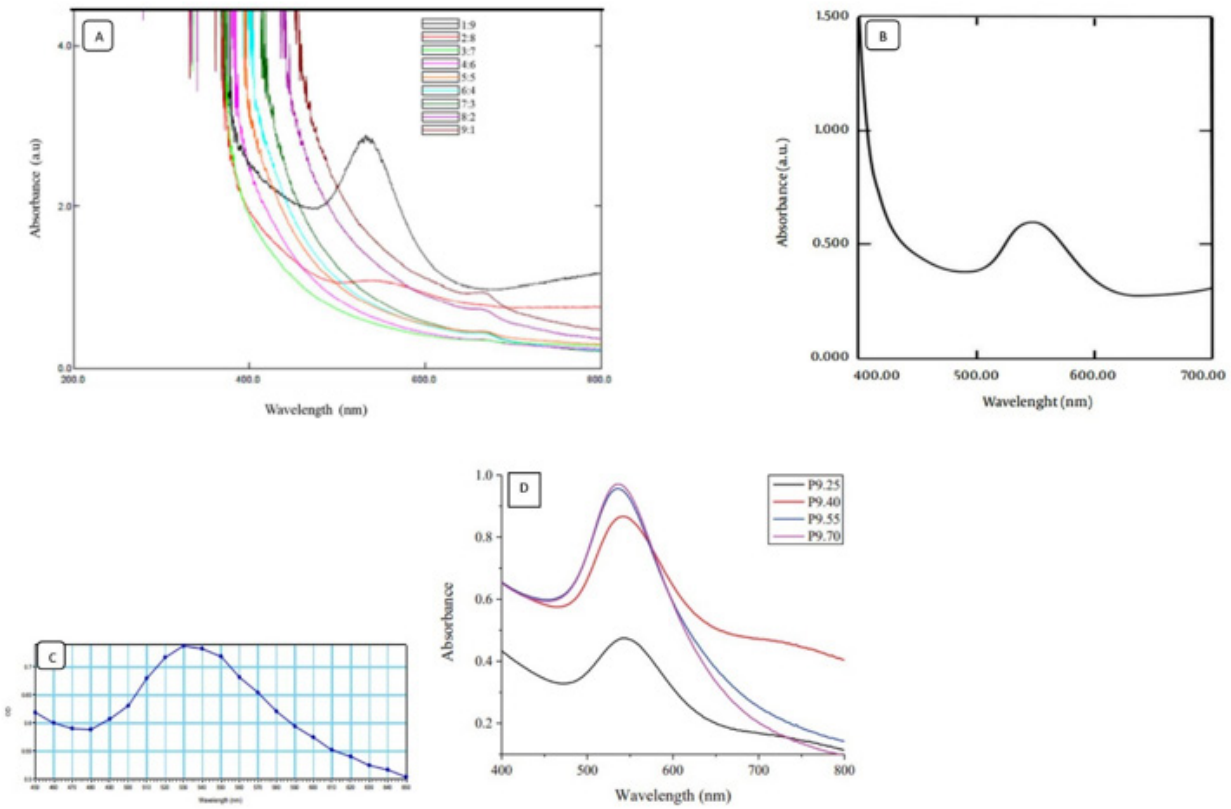

Figure 5: UV–vis absorption spectra of colloidal gold nanoparticles generated utilizing extracts of (a) *Wedelia trilobata* leaf (b) *Berberis vulgaris* and *Crocus sativus* stigma extract (c) *Terminalia. Arjuna* leaf (d) *Centella asiatica* whole plant.

and surface texture). Images from an AFM represent data in three dimensions, allowing for quantitative measurement of nanoparticle height.

Lee *et al.* elaborated synthesis of silver and GNP utilizing sesquiterpenoids obtained by counter-current chromatography obtained from *T. farfara* flower bud extract.[38] The bright coloured and spherically shaped particle of size 56.24±6.76 nm was obtained from the AFM images for silver nanoparticles while the particle size of GNP was found to be 41.96 ±10.28 nm as shown in Figure 9a. The cold-welding behaviour of nanoparticles on the mica substrate employed in AFM was implicated for the size difference between TEM and AFM images, but both TEM images and AFM images show similar results, mostly spherical shaped nanoparticles. Enhanced seed germination activity of *Gloriosa superba* using 1000 uM concentration of GNP synthesized from *T. arjuna* fruit extract was carried out by Gopinath *et al.*[39] AFM micrograph of the synthesized GNP clearly showed predominantly spherical shaped GNP having particles in the range 20-50nm which was confirmed using DLS and TEM as shown in Figure 9b. AFM images of plant extract mediated GNP confirms to a spherical morphology. As indicated in Figure 4 AFM is not a predominant choice amongst other characterization tests.

XRD analysis of GNP synthesized using different plant extracts

The XRD technique is a versatile, non-destructive analytical tool for determining the structural information of crystalline metallic nanoparticles and ensuring zero-valent nanoparticle production. When employing the XRD technique, the Debye–Scherrer equation is employed to calculate crystallite sizes. The SAED process, a supplementary analysis tool within the TEM instrument, can also be used to verify the crystalline nature and crystallite size of the formed GNPs.

The bioreduction property of gold ions into GNPs by ethnopharmacologically important *C. guianensis* flower extract is described in this report.[40] The angular orientations of the Bragg peaks at (111), (200), (220), and (311), respectively, suggest that the GNPs have face-centered cubic crystal structures as shown in Figure 10a. A facile synthesis of GNPs using fresh juice of *C. limonene* fruit was demonstrated by Pandey and his co-workers.[41] The Bragg reflections of colloidal GNPs on a glass cube showed intense peaks at (111), (200), (220), and (311) in the 2 range 30°-80°, which agrees with prior data on gold nanocrystals as shown in Figure 10b. GNPs were produced utilising *E. prostrata* leaf as a reducing and stabilising agent in the work of Rajakumar *et al.* and the absorption peak of the generated GNP was found to be located at 534nm.[42] The majority of the gold nanostructured

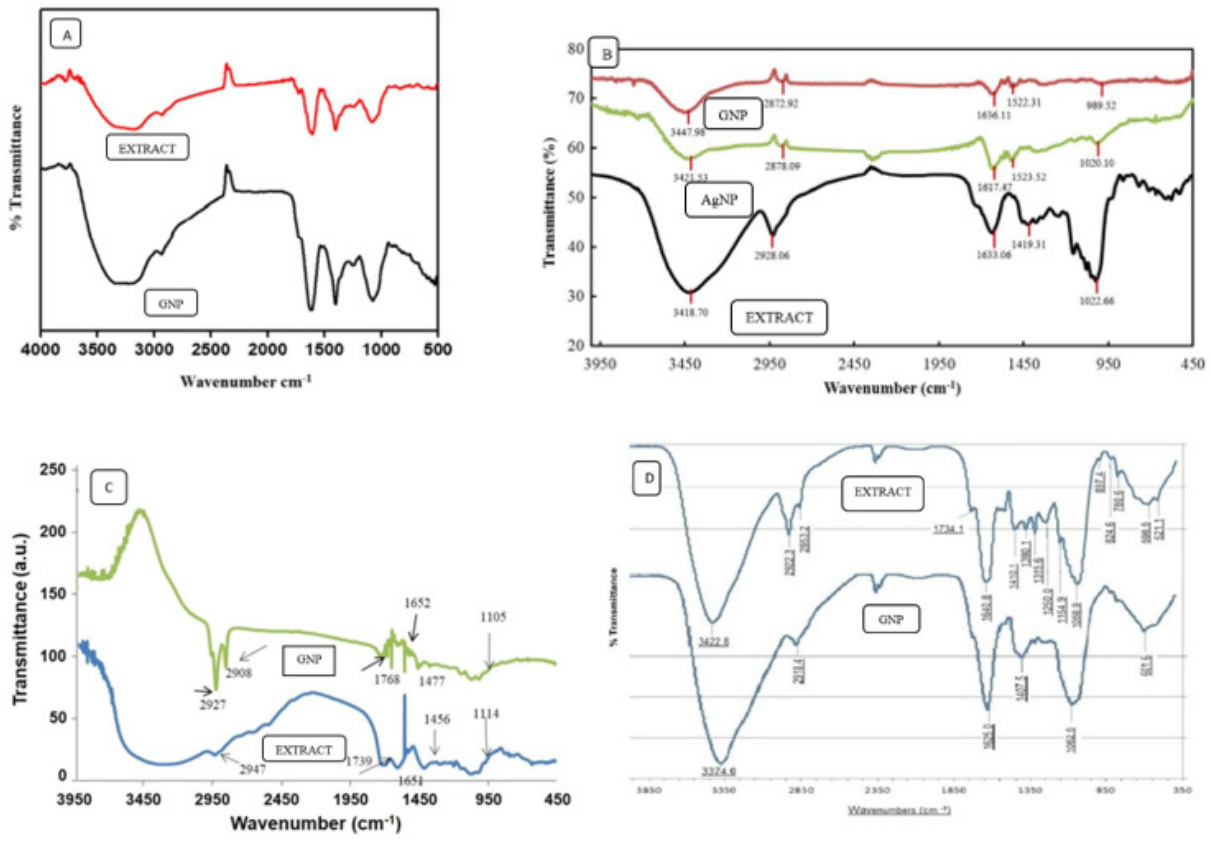

Figure 6: FTIR Spectrum of gold nanoparticle made using (a) *Gnidia glauca* flower (b) *Angelica pubescens* root(c) *Dillenia indica* fruit (d) *Laura nobilis* leaf.

samples were preferentially orientated along the (111) plane, according to the XRD pattern as shown in Figure 10c.

XRD data of the above mentioned green synthesized GPNs reveals prominent peaks at 111, 200, 220 and 311, in the 2 θ range 30°-80° which demonstrates that the GNPs generated are crystalline in nature. Amongst all the above peaks, the peak at 111 was found to have the maximum intensity.

Zeta potential characterization of GNP synthesized using different plant extracts

The zeta potential of nanoparticles is a physical property that provides information about their surface charge and stability. Values of the zeta potential can be positive or negative, but values of ± 30mV favour superior quality and such nanoparticles can be preserved much longer.

Anbarasu *et al.* produced spherical GNPs using *E. alsinoides* leaf at room temperature under dark condition.[43] With a zeta potential value of -28.7mV, these GNPs with an 80nm diameter showed good biocompatibility and stability for over 4 weeks as shown in Figure 11a. Gopinath *et al.* manufactured GNPs utilizing *T. arjuna* fruit extract.[39] The zeta potential was observed to be 21.9mV, which was well within the ± 30mV range required for a suspension to be physically stable as shown in Figure 11b. According to Xin Lee and co-workers Phenols, flavonoids, benzophenones, and anthocyanins from peel extract of *G. mangostana* fruit mediated the bioreduction of GCS directing the formation of mostly spherical, some hexagonal and triangular shaped GNP.[24] Pure *G. mangostana* peel extract had a zeta potential of -14.68mV, whereas GNPs produced with the extract had a zeta potential of -20.82mV as shown in Figure 11c. Plant peels are a cost-effective, efficient, and safe way to utilise waste material for synthesis of GNP.

Tripathi *et al.* investigated bioinspired GNP production from fresh *A. aspera* leaf extract and reported a 540 nm SPR absorption maxima.[44] At 25°C, the zeta potential of green produced GNPs was evaluated at several pH levels, particularly pH 6, pH 7, and pH 10. At pH 10, the zeta potential was observed to be -35.9 mV, with a zeta deviation of 7.41 mV and conductivity of 3.19 mS/cm, indicating good quality and relatively better stability of GNPs compared to pH 6 and pH 7. GNPs with a zeta potential of -35.9 mV were observed to be able to be stored for up to two months without affecting their quality or stability as shown in Figure 11d.

It can be observed that zeta potential of the synthesized GNP is more than the original extract and the zeta potential changes with the change in pH, but the value falls between -40 mV or +30 mV which confirms the stability of the nanosuspension.

Table 3: Peaks Having a Significant Shift in Position in the Extract when Compared to the GNP; Shift Values are Stated by Subtracting the Peak Position in the GNP Solution from the Peak Position in the Extract.

Plant	Original Peak	New Peaks/Shift Because of Bonding	Phytoconstituents	References
Gnidia glauca flower	3300cm^{-1}: hydroxyl group in alcoholic and phenolic compounds. 1607 cm^{-1} and 1450 cm^{-1}: C=C group of phenols 1220-1240 cm^{-1}: aliphatic amines. 1100 cm^{-1}: Alcohol group	2930 cm^{-1}: C–H stretches of alkanes.	Alkaloids, saponin, steroids, tannin, Coumarin, Flavonoids, diterpenes, cardiac glycosides, phenols and phytosterol.	12
Angelica pubescens root	3421.53 cm^{-1}: Stretching of O-H bond of alcohol groups 2878.09 cm^{-1}: C-H bond of alkanes. 1617.47–1523.52 cm^{-1}: aromatic C=C bond stretching due to the phenolic compounds 1020.10 cm^{-1}: Ether groups (C–O bond stretch).	3447 cm^{-1}: o-H bond of alcohol 2872 cm^{-1}: C-H bond of alkanes 1636 cm^{-1}: C=C bond 1522 cm^{-1}: phenolic compounds 989 cm^{-1}: C–O bond stretch	Flavonoids, sesquiterpenes, and phenols.	30
Dillenia indica fruit	2927 cm^{-1}:C-H alkanes, 2908 cm^{-1}: C=O carbonyl, 1768 cm^{-1}: N-H primary amines, 1652 cm^{-1}: N-O nitro group, 1558 cm^{-1}:C-C aromatic and 1477 cm^{-1}:C-O stretch for alcohols, 1105 cm^{-1}: Carboxylic acids, esters and ethers.	2947 cm^{-1}: C-H of alkane 1739 cm^{-1}: C=O carbonyl. 1651 cm^{-1}: for N-H primary amines, 1456 cm^{-1}: for C-C aromatic 1114 cm^{-1}: for alcohol, carboxylic acid, esters and ethers.	Polyphenols, tannins, alkaloids and flavonoids, betulin (pentacyclic triterpenoid) and betulinic acid.	19
Laura nobilis leaf	3422.8 cm^{-1}: Hydroxyl, 2922 cm^{-1}: aliphatic–CH– 1734 cm^{-1}: C=O stretch 1640 cm^{-1}: C=O(amide I).	3374.0 cm^{-1}: hydroxyl. 1734: carbonyl group interaction. 1625: stretching of C=O of the amide groups of protein.	Polysaccharides, flavones, flavonols, and glycosylated flavonoids.	31

EDX analysis of GNP synthesized using different plant extracts

The elemental composition of the GNP is established using an analytical technique known as EDX. For qualitative and quantitative element determinations, a spectrum of the energy versus relative counts of the observed X-rays is obtained and analyzed.

Reddy *et al.* have obtained brown coloured GNP which was synthesized using aqueous flower extract of *T. argentiea* and GCS.[45] The chemical nature of the synthesised GNP was established using an EDX scan. Gold gave a peak at 3 keV, and several faint peaks for C, O, and Cl were also detected as shown in Figure 12a. The reduction of gold ions to elemental gold is represented by the emission energy of 3 keV. The quantitative testing utilising EDX revealed a 52.27% gold content. A biomimetic

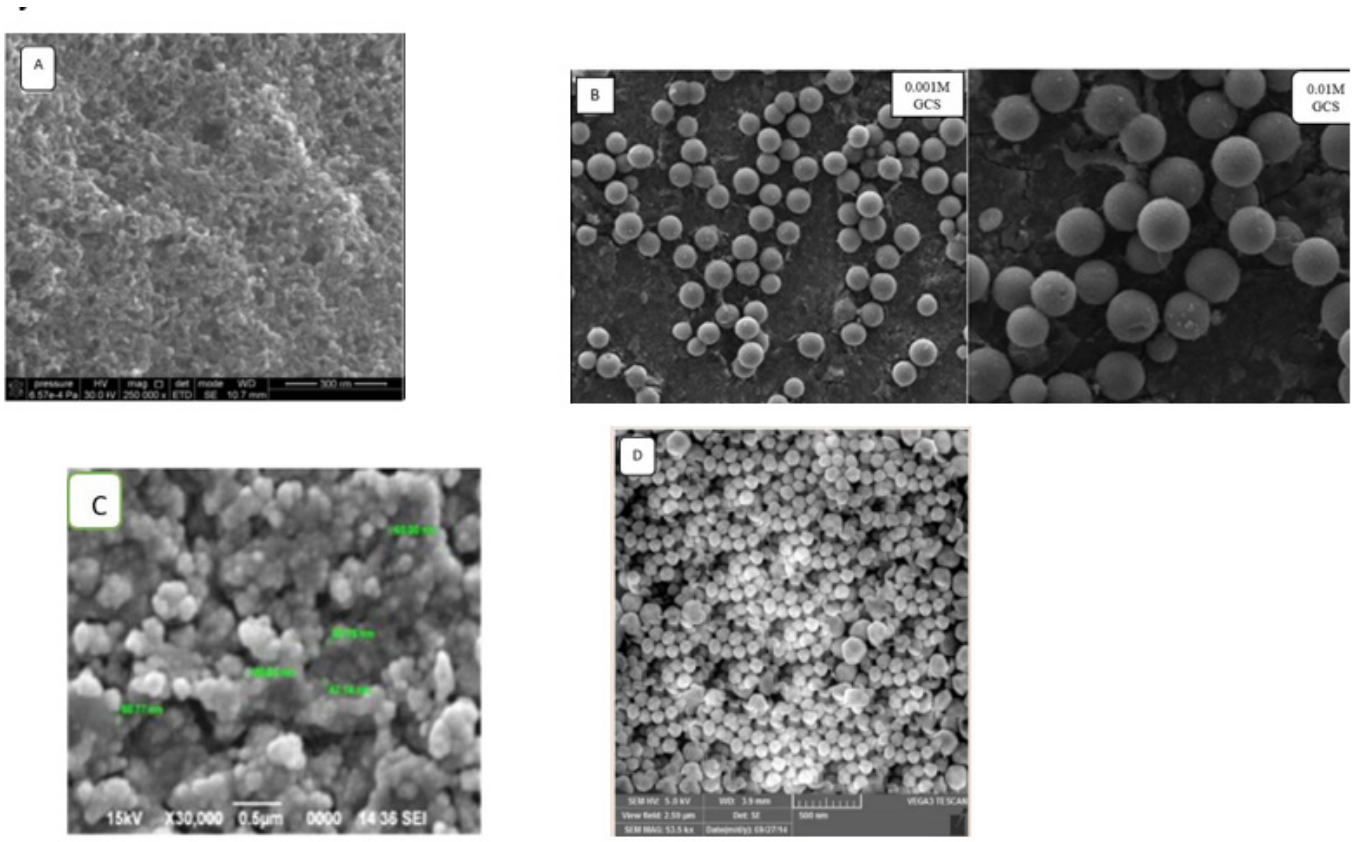

Figure 7: SEM images of gold nanoparticles after bioreduction with extracts of (a) *Polygonum aviculare* knotweed (b) *Acorus. calamus* rhizome (c) *Nepenthes Khasiana* leaf (d) *Azima tetracantha* leaf.

method of synthesis of GNP utilizing a terrestrial weed guduchi (*T. cordifolia)* by Abbasi *et al.*, revealed the presence of strong signal for gold atoms at approximately 2keV as shown in Figure 12b in the EDX spectra.[46] Weak signals from carbon, nitrogen, and oxygen atoms were also detected, which are most likely attributable to X-ray emission from proteins/ enzymes remaining in the residual plant extracts that were present alongside the nanoparticles. Using *E. prostrata* leaf extract as the reducing and stabilising agent, functionally stable and crystalline GNP were generated in 90 min.[42] The EDX study of the produced GNP demonstrated a conspicuous gold peak at around 3.6 keV, which is typical of metallic gold nanocrystallites absorption owing to SPR as shown in Figure 12c. Leaves of ornamental ground cover plant, *W. trilobata* have been collected by Dey *et al*. for the synthesis of GNP.[26] Strong optical absorption peaks were seen in the EDX image of the generated GNP at 1.66 keV, 2.08 keV, and 9.58 keV, which is the characteristic peak for crystalline gold nanoparticles. Due to the carbon-coated copper grid used to image the sample, additional signals from carbon and copper emerge as shown in Figure 12d. From the above EDX spectra obtained for the synthesized GNPs, the presence of strong signal at approximately 2 keV, 3 ke V and 9 keV confirms the presence of metallic gold.

DLS analysis of GNP synthesized using different plant extracts

DLS is one of the most widely used techniques for determining particle size, size distribution profile, and polydispersity index in a colloidal suspension.

DLS particle size analyser gave an average particle size of113.6±56.9 nm and PDI of 0.1256 for the GNP synthesized with *L. angustifolia* leaf commonly known as lavender at room temperature by Kumar *et al.* as shown in Figure 13a. The size distribution of the synthesized GNP ranged from 34-400nm as supported by the TEM study which clearly indicates formation of quasi-spherical and triangle particles.[18] Using *E. alsinoides* leaf extract, Anbarasu *et al.* observed the generation of spherical GNP with a diameter of 80 nm using SEM.[43] DSL study revealed that size distribution of the synthesized GNPs were in the range of 50-100nm with the highest fraction of 80.29nm as shown in Figure 13b, which matched the results of the SEM analysis. Markus *et al.* studied the synthesis of GNP utilising the Root of Chinese Herb *A. pubescens* Maxim.[30] The synthesis was carried out with different concentrations of root extract and metal ions, pH, reaction temperatures, and time. A wide variety of GNPs with a Z-average value of 109 nm and a PDI of 0.25 were observed utilizing DLS analysis in terms of intensity, number, and volume as shown in Figure 13c. According to Mapala *et al*., fresh flower

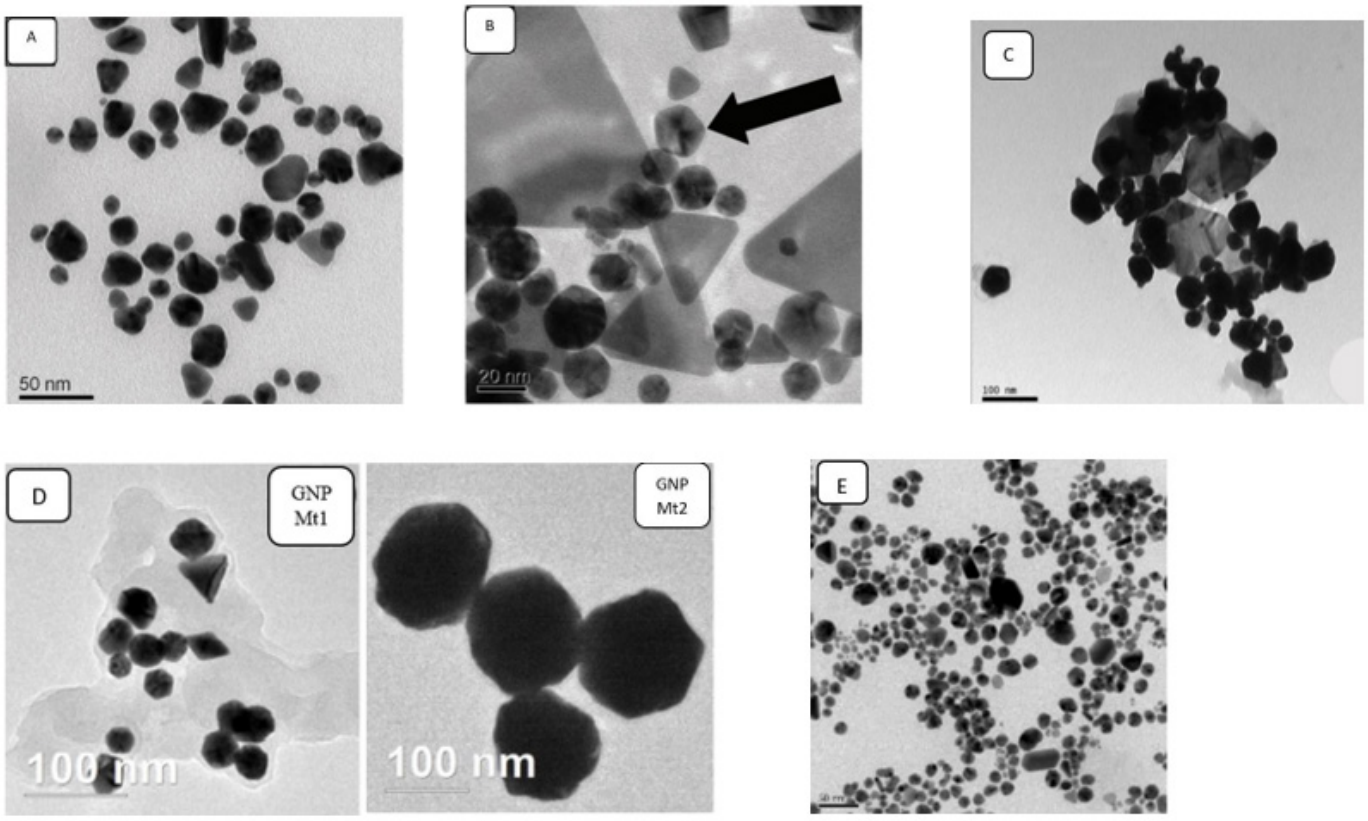

Figure 8: TEM images of gold nanoparticles synthesised utilizing extracts of (a) *Punica granatum* fruit (b) *Lippia citriodora* leaf (c) *Cicer arietinum* leaf (d) *Mimosa tenuiflora* bark i) GNPMt1 ii) GNPMt2 (e) *Tussilago farfara* flower bud.

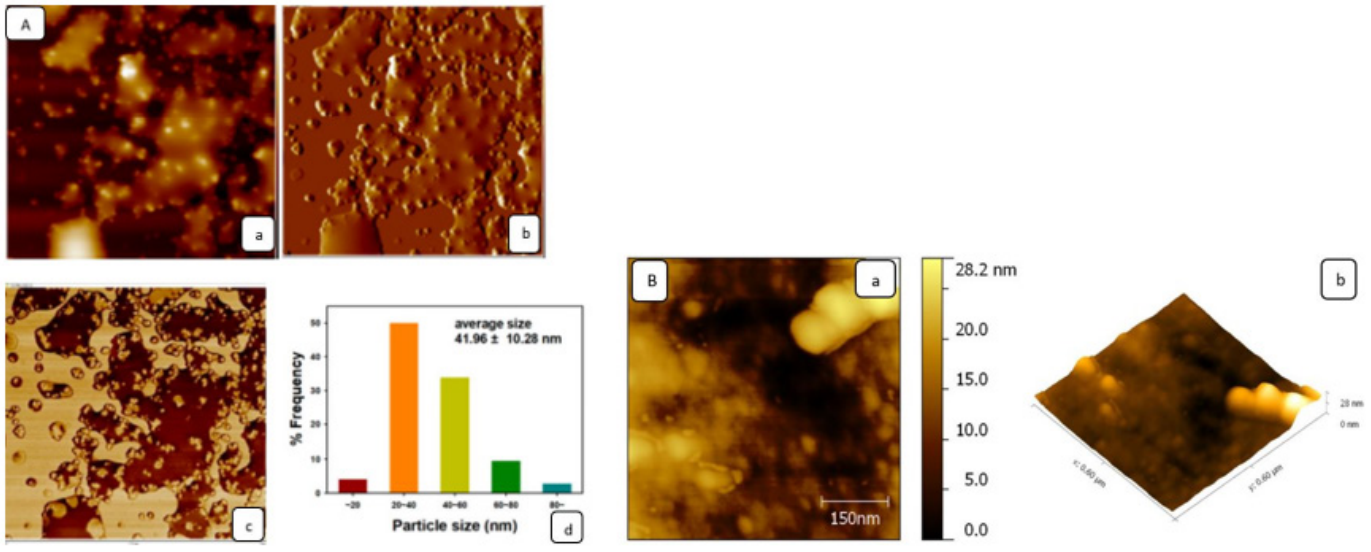

Figure 9: AFM images of gold nanoparticles synthesised from extracts of (a) *Tussilago farfara* flower bud *(b) Terminalia arjuna* fruit.

extract of *M. pudinca* when treated with GCS at room temperature and 100°C produced ruby red coloured GNP showing SPR peak at 538nm.[47] DLS study of the synthesized GNP gave a size range from 15nm to 62 nm with maximum particle having an average particle size of 24nm which was seconded using SEM and TEM studies as shown in Figure 13d. It was also observed that GNP obtained at 100°C showed presence of irregular shaped (triangular and disc shaped) nanoparticles compared to the well-defined spherical GNP obtained at room temperature. DLS analysis of plant extract mediated GNP reveals that the average particle size was not more than 120nm. TEM equipment possessing added

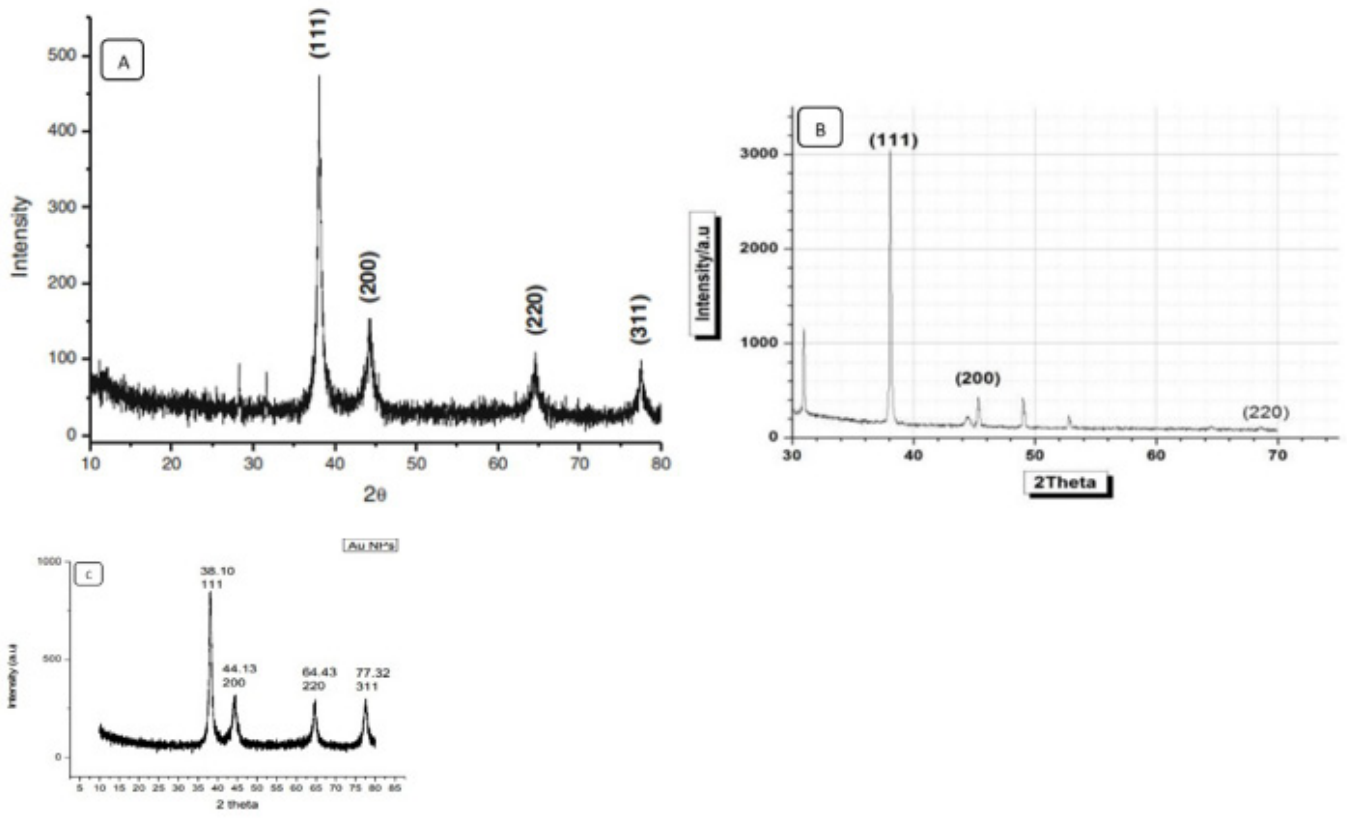

Figure 10: XRD spectrum of gold nanoparticles synthesized using extracts of (a) *Couroupita guianensis* flower (b) *Citrus limone* fruit (c) *Eclipta prostrata* leaf.

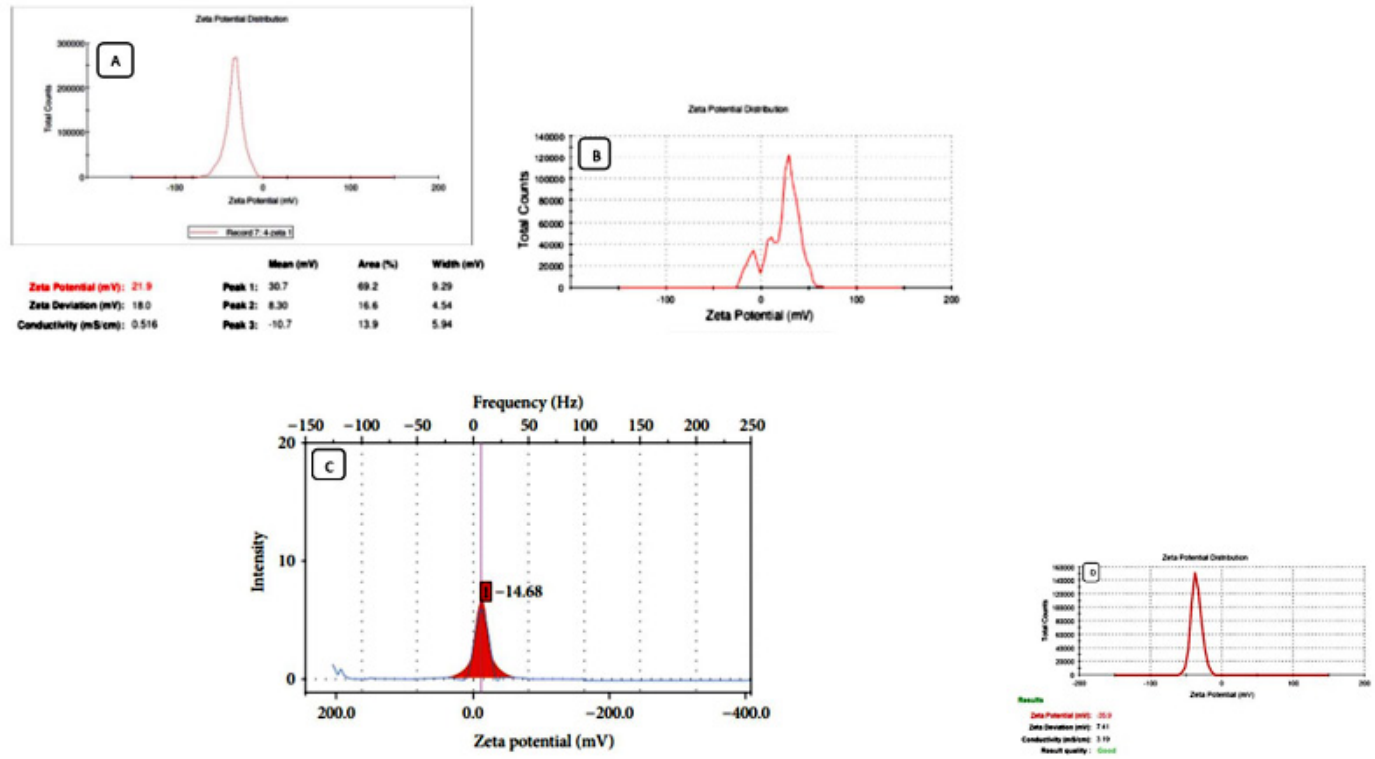

Figure 11: Zeta potential characterization of gold nanoparticles made utilizing extracts of (a) *Evolvulus alsinoides* leaf (b) *Terminalia arjuna* fruit (c) *Garcinia mangostana* fruit peel (d) *Achyranthes aspera* leaf.

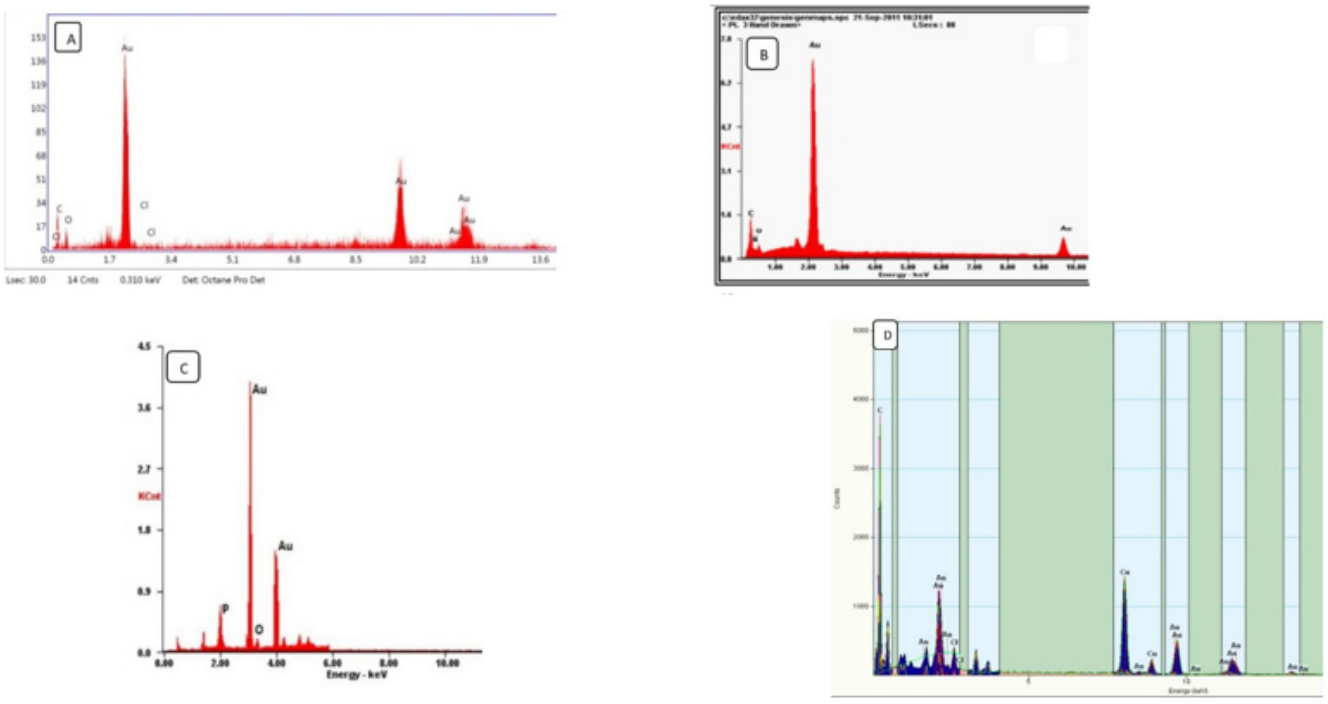

Figure 12: EDX Analysis of gold nanoparticles formed using extracts of (a) *Tabebuia argentiea* flower (b) *Tinospora cordifolia* stem *(c) Eclipta prostrata* leaf (d) *Wedelia trilobata* leaf.

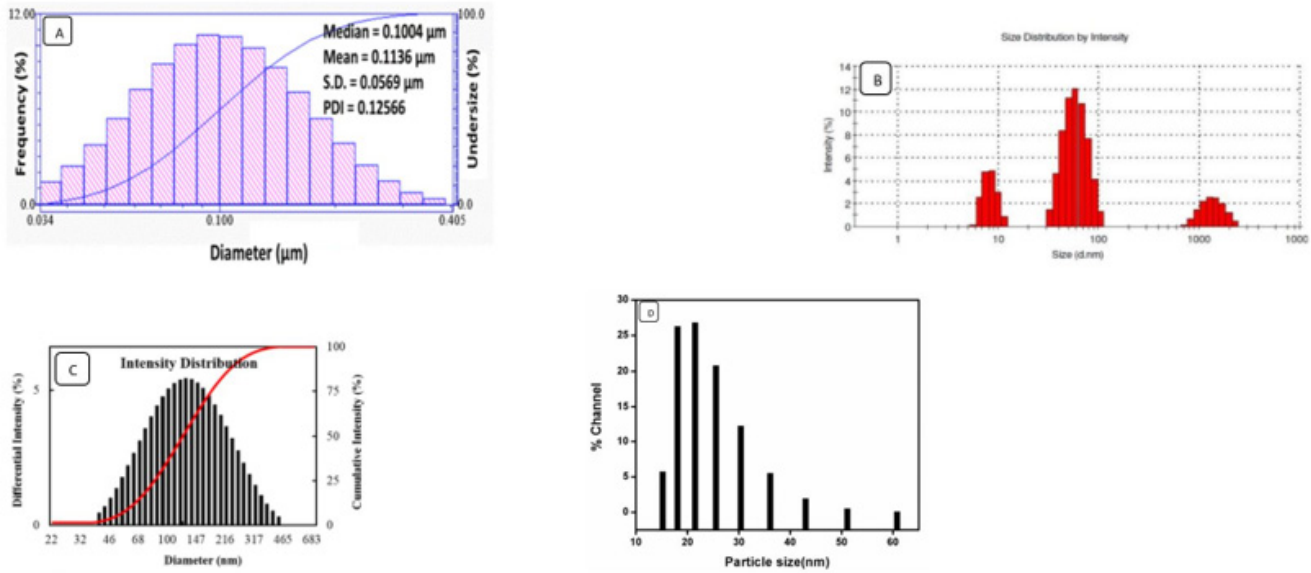

Figure 13: DLS Analysis of GNPs formed using extracts of (a) *Lavandula angustifolia* leaf (b) *Evolvulus alsinoides* leaf *(c) Angelica pubescens Maxim* root (d) *Mimosa pudinca* flower.

feature of particle size analysis makes DLS analysis not necessary, as a separate characterization test as evident in Figure 4.

Different applications of GNP produced from various plant extracts

Mishra *et al.* elaborated *A. calamus* rhizome extract mediated GNP synthesis.[32] When tested for antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacterial strains using the AATCC 100 test method, gold nanoparticles coated cotton fabric showed higher antibacterial activity compared to uncoated cotton and neat extract coated cotton against *E. coli*. The UV-blocking properties of uncoated cotton, neat extract coated cotton and GNPs coated cotton demonstrated that gold nanoparticle coated cotton fabric

Table 4: Summary of Plant Derived Metallic Nanoparticles and its Biomedical Applications.

Name	Part	Model	*In vivo/ In vitro*	Activity	References
Acorus calamus	Rhizome	GNP coated Cotton fabric.	*In vitro*	UV Blocking and antimicrobial activity.	32
Bauhinia tomentosa	Leaf	A-549, Vero, HEp-2 and MCF-7 cancer cells.	*In vitro*	Anticancer activity.	48
Cassia fistula	Stem bark	Rats with streptozotocin-induced diabetes.	*In vivo*	Treatment of hyperglycemia.	23
Hygrophila spinose	Leaf	Swiss male albino mice.	*In vivo*	Anemia and antioxidant.	49
Pueraria lobata	Root	SH-SY5Y cell lines.	*In vitro*	Anti-alcoholism.	50
Acalypha indica	Weed	BALB/c mice model with infected diabetic wounds.	*In vivo*	Wound healing.	51

provided excellent UV protection. These findings could be very beneficial to the medical textiles business.

Mukundan *et al.* obtained ruby red coloured silver and GNPs utilising aqueous leaf extract of *B. tomentosa*.[48] The anticancer capabilities of generated silver, gold nanoparticles, and aqueous leaf extract on Vero (Cercopithecus aethiops kidney epithelium normal), lung A-549 adenocarcinoma epithelial cells, laryngeal HEp-2 carcinoma epithelial cells, and breast MCF-7 adenocarcinoma cells *in vitro* were determined by MTT assay at concentration ranging from 7.8 to 1000 ug/mL. When investigated with Vero cells, silver nanoparticles, GNPs, and aqueous extract of leaves demonstrated cytotoxicity only at very high concentrations and were shown to be biocompatible. Compared to aqueous extract of leaves and AgNPs, the GNP had increased cytotoxicity only against laryngeal HEp-2 carcinoma epithelial cells at extremely low concentrations, but not against A-549 or MCF-7 cells.

Daisy *et al.* used an aqueous stem bark extract of *C. fistula* to make GNPs, which exhibited a significant drop in serum biochemistry markers as well as an increase in body weight, total protein levels and high-density lipoprotein levels and reversal of renal dysfunction in rats with streptozotocin-induced diabetes at a dose of 60mg/kg body weight than the ones treated with the aqueous extract, thus proving its potential as a hypoglycaemic agent for the treatment of diabetes mellitus and the complications that come with it.[49] Ghosh *et al.* reported synthesis of GNPs utilizing leaf extract of medicinally important herb *H. spinosa*.[50] In experimental anaemic swiss male albino mice model, the synthesised GNP showed enhanced haemoglobin percentage, total RBC count, haematocrit value, serum iron concentration and decreased total iron binding capacity proving its potential use in iron deficiency anaemia. According to Singh *et al.* GNPs when synthesized using *Pueraria lobate* (kudzu root) extract, guar gum extract and in combination of both the extracts at various temperatures yielded GNPs of different sizes and biological properties.[51] Evaluation of ethanol toxicity in SH-SY5Y neuronal cell line revealed that GNP synthesized using combination of both extracts were least harmful to SH-SY5Y cells at 50°C, but they were the most effective in mitigating the negative effects of ethanol on SH-SY5Y cells, thus proving the application of kudzu and guar gum extract GNPs in mitigating ethanol toxicity.

Using a BALB/c mouse model with infected diabetic wounds and stained microscopic pictures at different time intervals (day 2, day 7 and day 15), Bhoomi *et al.* reported phyto-engineered GNPs from the aqueous extract of *A. indica* weed for *in vivo* wound-healing activity. The wound region was totally re-epithelialized in 15 days, according to the study.[52] When compared to the control group, the re-epithelialization layer was completely covered by nanoparticles on the wound area, and collagen filled in the scar tissue. Thus, it can be noted from Table 4 that plant extract mediated GNPs have shown to possess anticancer, antimicrobial, anti-diabetic, diabetic wound healing, iron deficiency antioxidant activities by performing *in vitro* and *in vivo* studies.

CONCLUSION

During the current scenario nanotechnology encourages advancement in all spheres of life, hence biosynthetic route of nanoparticles synthesis will emerge as safer and better alternative to conventional methods. Since plants are widely distributed, readily available and at the same time safe to handle, there will be a wide scope for the development of green method of GNP synthesis. Green gold nanoparticle production has a significant advantage over chemical and physical methods: large scale synthesis without any need for high pressures, energy requirement, temperature and toxic chemicals. Different modern analytical techniques have been applied to characterize the GNP morphology. Plant-derived GNPs are mostly employed in biomedical, agricultural, biosensor and engineering applications, as well as drug delivery, wound healing, anticancer, antibacterial, tumour destruction and antifungal activity, foods, and herbal research among several others. The current review summarises the literature in order to gain a better understanding of the

production of gold nanoparticles using extracts from various plant parts, ranging from the flower to the root.

GNP has been synthesized by many researchers. Since every plant is a storehouse of secondary metabolites, determining which component worked as the reducing agent, what the capping material is and which nano-biomolecule is responsible for therapeutic efficacy becomes extremely challenging. In order to establish a logical methodology, more investigations employing isolated pure chemicals are needed to elucidate the specific mechanism of bio reduction. A systematic investigation is warranted to technologically engineer nanoparticles to gain better control over size, shape, and absolute monodispersity, which would promote nanoparticle applications in related disciplines. Aggregation of nanoparticles is also a critical element that must be carefully monitored because aggregated particles can produce a wide range of effects. As a result, novel stabilizing agents can be used in the synthesis to stabilise these nanoparticles.

Despite the diverse applications of biosynthesized metallic nanoparticles, toxicity profiles have yet to be thoroughly investigated. As a result, toxicity studies and environment issues of these nanoparticles impact on public health must be addressed as soon as possible. There is paucity of data on animal toxicity studies of synthesized herbal GNPs. Although many researchers have proved that gold nanoparticles are non-toxic in *in vitro* models, an overall systemic toxicity evaluation in *in vivo* models should be given priority to determine the safety of GNPs when it comes to their application and use. For *in vitro* studies, one requires small quantity of synthesized GNPs but in order to conduct animal studies, more quantities would be needed, which makes the process time-consuming, laborious and expensive, which could be the reason why not much research has been done on animal models using herbal GNPs. To conclude, with a new technology comes new challenges which has both pros and cons, resolving these challenges can make this technology challenging in research, development and applications. With the huge plant diversity and much more plant species yet to be exploited the scope for rapid and single step green GNP synthesis unfolds.

ACKNOWLEDGEMENT

We are truly indebted to the Principal KM Kundnani College of Pharmacy, Mumbai for their support to carry out the review work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Koliyote S, Shaji J. A Recent Review on Synthesis, Characterization and Activities of Gold Nanoparticles using Plant Extracts. Indian J of Pharmaceutical Education and Research. 2023;57(2s):s198-s212.