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Green Synthesis of Gold Nanoparticles: An Eco-friendly Approach

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Abstract—*In recent years, nanotechnology has become an immensely developing field due to their wide range of applications in various fields of science and technology. Among the various noble metallic nanoparticles gold nanoparticles remain one of the eminent nanoscale metallic materials due to their strong scattering and absorption, easy surface functionalization, catalytic properties, nontoxicity towards human, facile synthesis methods and low toxicity methods. Researchers have been influenced by the deleterious effects of current synthesis approach to focus on the creation of environmentally sustainable and green synthesis using nontoxic chemicals from natural sources, as plant extract and microbes like bacteria fungi, yeast etc. Eco-friendly synthesis of nanoparticles also known as "Green synthesis" using plant sources offers several advantages as compared to traditional methods like biocompatibility and effective application in biology and pharmacology, simplicity of methods used, utilization of natural bioresources, non-toxic method, no need of external capping and stabilizing agent, cost-effectiveness, reproducibility, simplicity in scaling upto large scale synthesis, well-explained morphology of biosynthesized nanoparticles, the elimination of high pressure, energy, high temperature and toxic chemicals which are necessary in the conventional synthesis methods. The biosynthesized AuNPs have better catalytic activity than chemically synthesized AuNPs without adding any hazardous substances during their synthesis and can also be used in drug delivery, cancer treatment, antioxidant and antimicrobial agents in biomedicine because of their functionally active properties*

Keywords: *Green synthesis, cost effective, gold nanoparticles, non-toxic.*

1. INTRODUCTION

Nanotechnology is a interdisciplinary field which includes biological constituents having physicochemical processes to synthesize nanomaterial with multifunctional properties and nanomaterials are those having at least one dimension at nanometer scale (0.1-100 nm) out of 3D space [1-2]. Nanotechnology and nanoparticles are widely used term and both play an important role in various fields such as medicine, biology, physics, chemistry and sensing, due to their unique properties [3]. In compared to other noble metal (Cu, Hg, Ag, Pt) nanoparticles, gold nanoparticles (AuNPs) are known to be the most stable having unique surface and optical properties, having various shapes like nanospheres, nanorods, nanocubes, nanobranches, nanoflowers, nanoshells, nanowires, and nanocages, by various techniques [4-5]. Due to biocompatible nature of AuNPs, these can directly interact with different kind of molecules having proteins, drugs, enzymes, nucleic acid, fluorescent dyes etc. on their surface for various biological and medical applications [6].

Nanomaterials can prove favorable to many technological and environmental challenges such as solar energy conversion, medicine, targeted delivery, therapy and wastewater treatment. In the process of global efforts to reduce hazardous waste, there is always requirement of a new synthesis route which is economical, cost effective, non-toxic and productive. Therefore, green approach is a technique that is nowadays used for the controllable synthesis of nanoparticles of well-defined morphology.

Therefore, in this review, we have summarized the approaches that are available for synthesizing AuNPs, as well as various characterization techniques that are used to characterize them, based on their unique and variable properties. We have also paid particular attention to the discussion of applications of AuNPs.

2. SYNTHESIS ROUTE OF MULTIFUNCTIONAL AUNPS

Nanoparticles production strategies can be categorized as top-down and bottom-up approaches. In top-down approach, nanoparticles are prepared from a suitable starting material by size reduction which is achieved by suitable physical and chemical treatments of starting material. In bottom up approach, nanoparticles are synthesized by joining smaller entities like atoms and molecules and in this approach, nanostructured building blocks of the nanoparticles are formed first and then assembled to produce the final particle [7]. Bottom up approach is usually carried out for the biosynthesis of NPs which includes various oxidation and reduction reactions. The first ever report on synthesis of AuNPs was published in 1857 by Faraday with light scattering potential of AuNPs confirmed by the change of red color and colloidal nature of nanomaterials [8]. The bottom up approach has been an emerging trend in recent years. There are three types of bottom–up synthesis approaches: chemical, physical and biological methods (table 1). The chemical approach includes chemical reduction, photochemical reductions, electrochemical techniques, and pyrolysis; physical methods like arc discharge and physical vapor condensation [9]; and biological methods include synthesis by bacteria, fungi, yeasts, plants, etc. are most commonly used (table 2). During the chemical synthesis of gold nanoparticles (AuNPs), three main components, *i.e.*, gold salt ($HAuCl_4.3H_2O$), a reducing agent (*e.g.*, sodium citrate), and a protecting or capping agent (*e.g.*, polyethylene glycol) are required for regulating the dimension of nanoparticles and avoiding their agglomeration. Though, these methods (physical and chemical) are accompanied with the use of overwhelming hardware, an enormous expenditure of energy, highly poisonous and harmful chemical components that generate natural hazards and are mostly not eco-friendly [10], so to compensate these effects, the twelve principles of green chemistry now have become an archetypal guideline for chemical technologists and chemists for developing less hazardous chemical synthesis [11].

Table 1: Methods of nanoparticles synthesis

Physical	Chemical	Biological
• Ultra Sonication • Irridation • Microwave • Electrochemica • Ion Beam method	• Chemical Reduction • Sol gel method • Insert condensation method	• Plant extract • Microbial culture • Agriculture mediated • Enzymes

The potentiality of a plant system in biological assisted synthesis of metal nanoparticles called “green synthesis” has now achieved a great role in the field of nanotechnology. The biosynthesis of NPs using plant material is cost effective, efficient and eco-friendly as the phytochemicals present in the plant extracts act as catalyst as well as stabilizers for the biosynthesized nanoparticles and no additional stabilizers or capping agents are needed [12-14].

Table 2: Synthesis of gold nanoparticles from different biosources

Biological source	Nanoparticles produced	Nano particles size(nm)	Reference
Plants:			
Medicago sativa	Au	4-10	[15]
Triticum aestivum	Au	10-30	[16]
Bacteria:			
Pseudomonas aeruginosa	Au	15-30	[17]
E. coli	Au	20-25	[18]
Yeast:			
Yarrowia lypolytica	Au	Upto 15	[19]
Magnusiomyce-s ingens	Au	Upto 90	[20]
Fungi:			
C. albicans	Au	20-40	[21]
Fusarium oxysporum	Au	8-40	[22]

3. CHARACTERIZATION TECHNIQUES OF AUNPS

After the biosynthesis of nanoparticles, characterization of nanoparticles is done to confirm the synthesis, size, shape, distribution, surface charge, surface area etc. according to their some unique properties like optical, electrical and chemical [23-24]. Many analytical techniques are used for the characterization of NPs such as ultraviolet (UV-VIS) spectroscopy, fourier transform infrared spectroscopy (FT-IR), X-ray diffractometry (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), etc. The characterization of AuNPs has been shown in figure 1.

Visual color change can be observed with the naked eye during the synthesis of AuNPs based on their principle of tunable surface plasmon resonance (SPR) band, which is monitored by using UV-Vis spectroscopy [25]. This technique can be used for the conformation of AuNPs synthesis by measuring their absorption spectra which depends upon various factors like size, morphology, shape and physico-chemical environment of biosynthesized AuNPs. Band width and spectral position of SPR is mainly dependent on size, shape, dielectric constant of the medium and the various functional groups adsorbed on the surface of NPs [26]. Band of the absorption spectra of biosynthesized AuNPs lies in the range of 500- 550 nm [27].

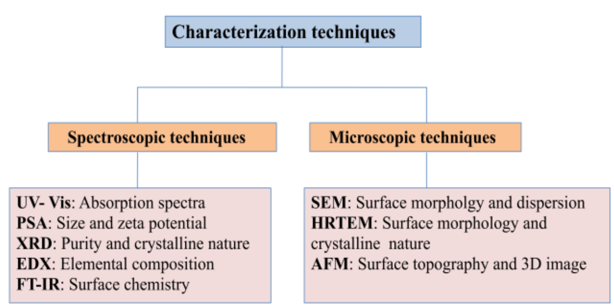


Fig 1: Characterization techniques of gold nanoparticles

Particle size analyzer (PSA) or Dynamic light scattering (DLS) is used to measure three characteristics of nanoparticles; size, polydispersity index and zeta potential [28]. Average hydrodynamic diameter and PDI of biosynthesized AuNPs of Lavender leaf aqueous extract were found to be ranged from 34 to 400 nm and 0.125, respectively, [29].

The crystalline structure, size and crystalllinity of the biosynthesized AuNPs are determined by X-ray diffraction (XRD) and it provides a rough idea of the particle size which is determined by applying the Debye-Scherer equation [30]. Crystallinity of biosynthesized AuNPs by using *Couroupita guianensis* extract was confirmed from XRD diffraction pattern which revealed the presence of FCC lattice of AuNPs [31]. The chemical composition of AuNPs can be confirmed by energy-dispersive X-ray spectroscopy (EDX).

FT-IR is a useful technique for identifying different types of chemical bonds in a molecule by producing an IR absorption spectrum which is like a molecular "fingerprint". It is used to evaluate different kind of functional groups of extract which are responsible for capping and stability of biosynthesized AuNPs [32]. Comparative FT-IR spectral analysis of dalspinin (DLP) and DLP-AuNPs confirmed the presence of coordination of dalspinin functional group like –OH and -C=O on AuNPs surface [33].

Due to the development of new advanced microscopic techniques such as SEM, TEM, HRTEM, and AFM, morphology of synthesized AuNPs can be well characterized [34]. TEM analysis is used for imaging morphology and distribution of nanomaterials with high resolution. It provides information about structure and crystallography nature of sample. HRTEM with EDX analysis and SAED pattern of AuNPs by using *Punica granatum* juice extract revealed the presence of face centerd cubic (FCC) lattice of metallic gold with triangular, spherical, pentagonal and hexagonal shaped NPs and size of NPs decreased with increase in extract concentration with diameter ranging from 23.1 to 35.8 nm [35].

The surface morphology, purity and chemical composition of biosynthesized AuNPs are determined by FESEM. Analysis of FESEM images of biosynthesized AuNPs by using Mentha and Pelargonium were found to have spherical, triangular and polygonal shape with average diameter of 34 and 33.80 nm, respectively [36].

AFM technique is more similar to the scanning probe microscopy and provides information about surface topography of AuNPs. 3D image of liquid environment can be obtained by using AFM [37].

4. APPLICATIONS OF AUNPS

In the above parts, different synthesis approach of AuNPs and their characterization based on optical and physicochemical properties have been discussed. Based on their characteristics, various applications of AuNPs have been explored, particularly in medical field, including deliver carriers (drug, gene and protein deliver), antioxidant, antimicrobial, diagnostics, imaging, and catalytic activities. In the following section, some of these applications will be discussed in detail.

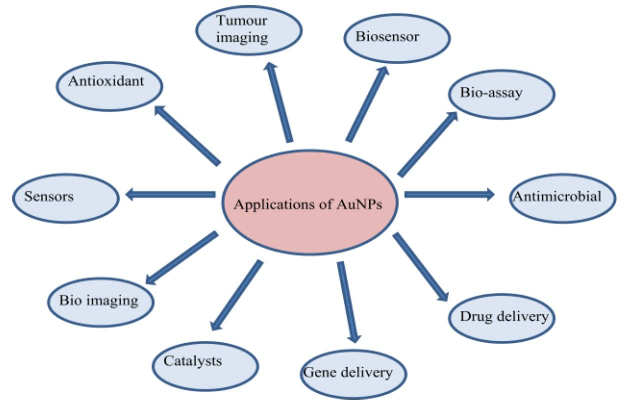


Fig 2: Various applications of gold nanoparticles

4.1 Catalytic activity

Biosynthesized AuNPs possess highly improved catalytic activity for the degradation of various dyes like methylene blue to colorless leucomethylen blue and p-nitrophenol (p-NP) to p-aminophenol (p-AP), rhodamine B, and gentian violet organic dyes [38], which is indicative of its great potential in chemical industry. These also act as effective catalyst in oxidation of alcohol [39-41] and alkanes [42-43] electrolysis NPs have high surface energy due to the presence of large number of surface active sites, so these can increase the rate of reaction by decreasing activation energy barriers. Possible reduction of p-nitrophenol to p-aminophenol in presence of AuNPs as catalyst is shown in figure:

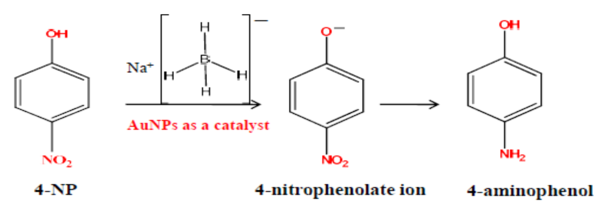
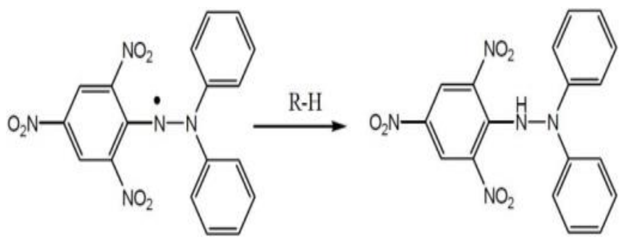


Fig 2: Reduction of 4-NP to 4-aminophenol by using AuNPs as a catalyst

The catalytic activity of NPs has promoted the synthesis of highly functionalized metal nanoparticles with characteristics shape, size, composition, either on their surface or adsorbed on other material [44]. Catalytic reduction of 4-NP was carried out by using biosynthesized AuNPs and AgNPs from *Breynia rhamnoides* [45] and rate constant was found to be in range of 0.002 to 0.009 s^{-1} by using different amount of AuNPs.

4.2 Antioxidant activity

Extracting solvents used for the phytochemicals like phenolics compounds had important effects on antioxidant activity of the extracts. Antioxidant activity of the extracts can be evaluated by DPPH radical scavenging method, total antioxidant activity, ferric reducing (FRAP) assay, Trolox equivalent antioxidant capacity (ABTS) assay, and reducing power (RP) assay [46]. By comparing the three methods (DPPH, ABTS, and RP), in which quercetin was used as a reference, it was observed that the quantified antioxidant activity was not the same. The values obtained by DPPH and RP assay were similar, whereas those obtained by ABTS were compartively lower. These differences may be due to the species analysed for the different assays [47]. Antioxidant activity by free radical scavenging method showed the DPPH solutions get reduced in the presence of antioxidants as a proton donor [48] and Rice-Evans *et al*., 1997 [49] stated that phenolic compounds or bioactive compounds present in plant extracts are known to be potent antioxidant due to their exclusive structural chemistry. Reaction of a stable DPPH free radical with an antioxidant compound is shown as:



Adewale *et al*., (2020) [50] evaluted antioxidant activity of biosynthesized AuNPs by using leaf extract of *Crassocephalum Rubens* and the result revealed that AuNPs (66.87%) showed lower antioxidant activity than crude extract (78.29%) with respect to Ascorbic acid (85.30%). Chahardoli *et al*., 2018 [51] biosynthesized AuNPs by using *Nigella arvensis* leaf extract and evaluated their antioxidant activity in concentration range of 100 – 500 ppm. Result revealed that antioxidant activity of plant extract (32 %) was greater than that of NA-AuNPs (12 %) at highest (500 ppm) concentration.

4.3 Antimicrobial activity

Metallic nanoparticles are widely used in biomedical sciences and engineering. Due to effective antimicrobial activity of NPs against various pathogens like bacteria, fungus, yeast etc., these NPs can be used as an alternative of traditional antibiotic to overcome their bacterial resistance [52]. Metal ions of NPs are slowly released and penetrates through the cell membrane, and then interact with the various functional groups of proteins, phospholipid, enzymes, lipids and nucleic acids, like –SH, –NH, and –COOH groups, thus induce morphological changes and inhibit enzymatic activity, changing or formation of non uniform pits on the cell wall leads to irreversible change in membrane impermeability, impairs the respiratory system and finally leads to cell death [53]. Some alterations which occur in nucleic acids are like mutations, additions, deletions, single and double-strand breaks and cross-linking with proteins [54]. Kasabwala *et al*., (2021) [55] synthesized and evaluated antimicrobial activity of AuNPs by using Neem and ginger plant extract and they found that maximum activity was shown against E. *faecalis* followed by *S. mutans*, *S. aureus* and *Candida Albicans.* Antibacterial activity of biosynthesized AuNPs by using *Platycodon grandiflorum* was found to be in range of 8 -13 mm against *E. coli* and *B. subtitlis* strains [56]. Antifungal activity of biosynthesized AuNPs by using seed aqueous extract of *Abelmoschus esculentus* was tested against *Puccinia graminis tritci*, *A. flavus*, *A. niger* and *Candida albicans* by standard well diffusion method and zone of inhibition was found to be in range of 8 -18 mm [57].

5. CONCLUSION

In summary, we can conclude that plant extracts contain several bioactive compounds that act as reducing agent and are involved in the reduction of metal ion to metallic nanoparticles of diverse morphology, but the exact chemical structure of these compounds are still unknown. This review mainly focus on phytosynthesis of gold nanoparticles, their characterization and their application, which may be relevant for future research on green synthesis of gold nanoparticles. The safety of using AuNPs remains a very controversial issue, as more important issues are raised, which needs to be properly addressed. In recent studies, researchers have reduced the toxicity of AuNPs by surface functionalization, improved their existing mode of synthesis, and have developed new and better methods. In conclusion, the unique properties of AuNPs like their optical properties having SPR band should be identified, to vast their applications in various fields.

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