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Review Article

# Biogenic Gold Nanoparticles (AuNPs) and its Biomedical application - Current & Future Prospects

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## Abstract

Green nanotechnology offers immense opportunities as it applies principles of green chemistry for synthesis of nanomaterial for various applications. Green gold nanoparticles (AuNPs) provide eco-friendly materials at low cost and toxicity, high chemical and thermal stability, enhanced degradation activity for environmental remediation and used in numerous biomedical fields. For biomedical application, the toxic chemical agents used for synthesis *via* conventional methods are a major deterrent. To address this, green synthesized of gold nanoparticles (AuNPs) were extensively studied. Continuous efforts have been focused on facile, low cost, pure, non-toxic and environment friendly approach for their synthesis. Their biocompatibility, photonic properties and their possible solubility in aqueous phases enabled the assimilation of AuNPs in diverse biomedical field. Different biological resources normally existing in the environment have been used for biosynthesis of biogenic nanoparticles that include bacteria, fungi, algae, yeast, cyanobacteria, actinomycetes, viruses and plants This review provides a comprehensive overview of synthesis and characterization of biogenic AuNPs with their broad applications in biomedical fields have also been elucidated along with their future prospects.

**Keywords**: Biogenic synthesis; Gold nanoparticles; Anti-bacterial activity; Biomedical applications

**Abbreviations:** NPs: Nanoparticles; nm: Nanometer; sp.: Species; EPS: Exopolysaccharides; DNA & RNA: Deoxy-Ribonucleic Acid/ Ribonucleic Acid; UV-vis: UV-Visible Spectroscopy; XRD: X-ray diffraction; FTIR: Fourier Transform Infrared Spectroscopy; GCMS: Gas Chromatography-Mass Spectrometry; HPLC: High Performance Liquid Chromatography; EDS: Energy Dispersive Spectroscopy; DLS: Dynamic Light Scattering; SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; AFM: Atomic Force Microscopy; SPR: Surface Plasmon Resonance; ZP: Zeta Potentials; mV: Milli Volts; SAED: Selected Area Electron Diffraction; pH: Potential of Hydrogen; ROS: Reactive Oxygen Species; MIC: Minimum Inhibitory Conc.; CFU: Colony Forming Unit; LPS: Lipopolysaccharides; JAK/STAT: Janus Kinase/Signal Transducers And Activators of Transcription; NF-kB: *Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells;* GFP: Green Fluorescent Protein; RME: Receptor-Mediated Endocytosis; MRI: Magnetic Resonance Imaging; CT scan: Computer Tomography Scan; LOD: Limit of Detection; LOQ: Limit of Quantification

## Introduction

Nano-science and technology is the new-fangled approach that becomes an inexorable element of the modern Era and are still garnering considerable interest in witnessing the ease of technology at the scientific and commercial level. The small-dimensions nanoparticles (1–100 nm) govern the entire research globally, due to its remarkable applications in physical, chemical, environmental and biological sciences [1]. Among all the synthetically classified nanoparticles, metal-based nanoparticles have enraptured, due to their unique physicochemical characteristics, highly active, reproducibility and antibacterial




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properties attributed to their enormous surface area to volume ratio [2,3]. Metallic nanoparticles that have achieved immense attention recently due to their imperative significance are aluminium, silver, gold, iron, zinc, copper and palladium [4]. Specifically, gold nanoparticles have an extensive history for medical purpose such as management of various aliments due to their biocompatible nature, high thermal and electrical conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and antimicrobial activity [5]. The characteristics of metal nanoparticles contribute to several biomedical purposes assisted by optical device, sensing components, photothermal therapy, catalysts and targeted drug delivery [2]. There are various studies that described the different mode of gold NPs formulation- include chemical, radiation, electrochemical, Langmuir-Blodgett, photochemical methods and biological techniques. Generally, conventional mode of synthesis has serious limitations like as upfront use of toxic reagents that are extremely harmful for the living system and environment, high cost, exposure to radiation, requires high-energy input, high temperature and less productivity. The chemical diluters used during fabrication later on leads to troubles in nanoparticles extraction and also exhibit considerable obstacles to biomedical applications. Furthermore, during the application of gold nanoparticles as drug delivery carriers the extra of precursor constituents may causes cytotoxicity of healthy cells. Some reports states that function of heart and its vasculature can be affected by AuNPs that have to be cautiously evaluated [6]. Therefore, there is an emergent need to develop an environmentally benign method for the gold nanoparticles synthesis. This draws attention to the researchers and industrial sector in the field of nanoparticle synthesis and assembly to utilize some biocompatible natural compounds for the reduction of Au-containing salts for the synthesis of Au nanoparticles is very important, that further generate numerous imperative pharmaceutical molecules relevant for various biomedical applications as toxic substances are abolished [6,7]. The biogenic synthesis method has several advantages with respect to clean, cost effective, effortless procedure and eco-friendly approach. The use of various fungi, bacteria and plant tissues have been stated for the biosynthesis of gold nanoparticles. The biological mode of AuNPs synthesis is categorically classified into two approaches: first category involves the use of microorganisms such as- algae, bacteria, and fungi while the other is based on the plant-based extracts as reducing and stabilising agents [8,9]. The advantage of plant-based extract to serve as excellent reducing and stablishing agents are responsible in reducing particle size and enhance their reactivity in a one-pot synthesis of AuNPs. Here, we discuss the formulation of biogenic gold nanoparticles through green synthesis. The synthesis of green AuNPs is explained and various environmental parameters affecting their synthesis are introduced.

Collectively, this review highlights the recent trends in the fundamental processes and mechanisms of biogenic synthesis AuNPs by using various biological systems (plants, algae, bacteria, fungus and etc). Consequently, till date, no detailed analyses and comprehend of the rationale factors affecting the green synthesis of AuNPs and their characterization has been studied. Further, we exclusively update the context of various factors affecting the biogenic synthesis of AuNPs and different characterization techniques used to determine the physiochemical properties of synthesized NPs and offer a deep understanding of NPs and their potential biomedical application in modern technology and in green environmental technology. The prominent applications of green AuNPs in the biomedical field involves anti-microbial agents, drug delivery agents, therapeutic agents, bio-sensing agents, and removal of environmental pollutants are also elaborated meticulously.

**Mechanism Involved in the Biosynthesis of Gold Nanoparticles**

In recent growing interest of industrial microbiology for green synthesis approach of nanoparticles with diverse range of microorganisms from bacteria, fungi, algae to actinomycetes, etc., has triggered the effective eco-friendly synthesis mechanism owing to vivid benefits such as effortless processing and management, reduced synthesis cost of medium for their growth, reduction and stabilisation ability of the biogenic compounds etc. known as main striking reasons for choosing biosynthesis as an alternative approach for nanoparticles synthesis**.** Here, Macro-scale cultivation in huge fermenters or on substrate (cellulosic wastes, agricultural wastes) will facilitate the surplus extraction of enzymes along with list of secondary metabolites in sustained way with least economical investment. For example, utilisation of such an inexpensive raw material will initiate cyclic waste management and drop down the increasing risk of environmental pollutants (heavy metals, dyes, and toxic chemicals etc.) too.

**Fungal Based Green Synthesis Approach**

Majority of fungal microorganism are known for their bioactive compounds that are obtained from them [10]. They are also known for their extreme tolerance, bioaccumulation and easy internalization of heavy metal ions from their surrounding nature. This allows us exploring edges where they can work as fungal nanofactories synthesizing desired nanoparticles with controlled size and morphology [10,11]. The underlying mechanism for synthesis of biogenic metallic nanoparticles using fungal species involves intracellular synthesis approach and extracellular synthesis approach. In intracellular synthesis approach the metal precursors, commonly known as primary metals are added in to the fungal culture where metal ions undergo sequential process. This sequential process has various events well described in *Verticillium* sp., initiating at electrostatic interaction of metal ion with fungal cell wall components [12], this enables easy entrapment of metal ion at interacting interface. Following this is enzyme linked synthesis of biogenic nanoparticles through bio reduction process of metal ions and its diffusion across fungal cell wall. The small sizes of nanoparticles so formed are difficult to isolate from fungal culture as they are synthesized within fungal species. This intracellular synthesis approach hereby has demerits as extraction cost added to the entire synthesis process, it involves chemical extraction, sequential centrifugation, filtration etc. in order to liberate the NPs in the required form, leaving behind the fungal debris [13,14]. Similarly extracellular synthesis approach involves many other methods such as presence of extracellular enzymatic reactions that are responsible for conversion of metal ions to their nanoparticles, presence of electron shuttle system doing the same in the presence of electrons *i.e*. acting as strong bioreducing agents [15]. Michaelis–Menten enzyme kinetics models, where the biological reduction of various metallic NP was initiated by the presence of protein having amino acid with -SH bonds with dehydrogenation process occurring when present amino acid reacts with metal precursor, further presence of such free amino acids are also responsible for capping and stabilization of nanoparticles thus adding advantage to the synthesis approach [16]. Soltani Nejad et al aimed to synthesis AuNPs using the extracellular approach in an endophytic fungus (*Phoma* sp.) that was isolated from peach trees (*Prunus persica*). Synthesized AuNPs has the nanosize range of 10–100 nm and absorbance peak were recorded at 526 nm. Further its application against other fungal species (*R. solani*) was also determined which confirms reduced formation of sclerotia by this species on increased concentration of nanoparticles [17]. Islam and coworkers have worked employing mycosynthesis method used for synthesis of Gold-selenide nanoparticles (AuSeNPs), using endophytic fungus *Fusarium oxysporum at ambient condition.* Hereby, AuSe NPs was reported to have a mean hydrodynamic nanoparticles size of 52 nm with crystalline nature. *Further* its anti-sporulant potency was also observed against the black fungal species (*Aspergillus niger)*. Thus minimize risk associated with re-infection, providing its wide utilization in developing newer fungicide or drugs with low cost and abundant supply [18] (Table 1).

### Bacterial Based Green Synthesis Approach

Bacteria as biological agents that can act as functional nanofactories, uses both intracellular and extracellular approach for NPs synthesis. In intracellular approach the synthesis occurs within bacterial cells, this route utilizes specialized group of enzymes like oxidoreductase, proteins, redox mediator shuttle system etc. for nanoparticles synthesis. In extracellular approach (exterior to bacterial cell) presence of bacterial biomass, bacterial specific supernatant, and bacterial derived extracts such as exopolysaccharides is obligatory for the synthesis of nanoparticles. Presence of functional moiety in various groups of enzymes, specialized proteins, derived extracts etc. is crucial for synthesis of biogenic NPs through bio-reduction reaction where they themselves act as reducing and capping agent. Further

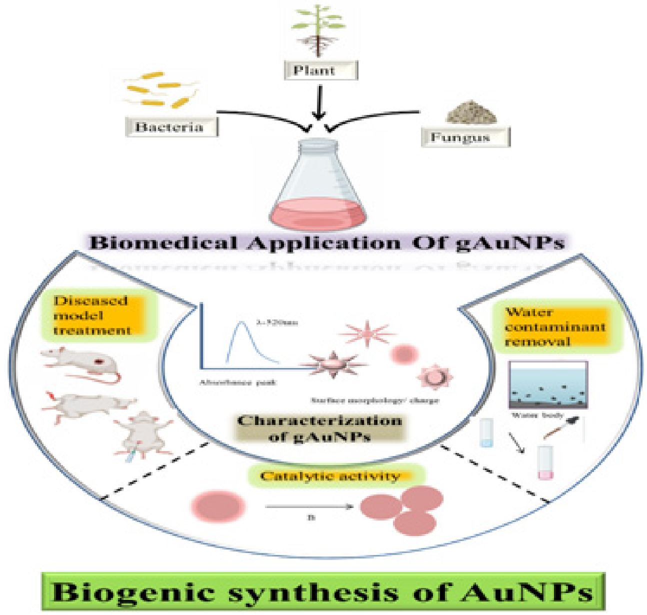


**Figure 1:** A short representation of biogenic synthesis of AuNPs and its application.

**Table 1:** List of various microbes and plant species involved in green synthesis approach.

| Green synthesis methods | Species | Mode of synthesis | Nanoparticles characterization | Inference | Ref |
|---|---|---|---|---|---|
| Bacterial based approach | *Micrococcus yunnanensis* | Extracellular | Surface morphology (Spherical shape) Size range (15–55nm) Zeta potential (−17.6±1.8 mV) | Change in color to ruby red, Indicates that the nanoparticles are synthesized. | [29] |
| | *Shewanella loihica* | Intracellular/ Extracellular | Surface morphology (Spherical shape) Size range (2–15nm) | Presence of extracellular electron transfer (EET) capability specifically Direct Electron Transfer (DET) and Mediated Electron Transfer (MET) mechanisms, here electrons act as strong reducing agent responsible for nanoparticles synthesis. | [30] |
| | *Caldicellulosiruptor changbaiensis* | Extracellular | Surface morphology (Spherical shape) Size range (20–60nm) | Their role as biosynthesizer can synthesize size-controllable AuNPs with enhanced bioactivity. | [31] |
| Fungal based approach | *Alternaria alternate* | Extracellular | Surface morphology (Spherical, triangular, hexagonal shape) Size range (12–29nm) | Fungi generate AuNPs by a process involving NADH-reductase, further it also stabilize it. | [32] |
| | *A. oryzae* var. *viridis* | Mycelial surface | Surface morphology (Spherical shape) Size range (10–60nm) | This confirms adsorption of gold ions on the fungal mycelium by electrostatic attraction/ion-exchange mechanism followed by enzymetic bioreduction of gold ions forming gold nanoparticles. | [33] |
| | *T. koningii* | Cell-free filtrate | Surface morphology (Spherical shape) Size range (10–14nm) | Confirms that the formation of nanoparticles are of narrow size distribution range, synthesised in presence of cell free filterate. | [34] |
| Plant based approach | *Caulerpa racemosa* | Extracellular | Surface morphology (Spherical-oval shape) Size range (13.7 to 85.4 nm) Zeta potential (-27.4 mV) | Color change from colorless to ruby-red. Presence of phytochemical in extract of C. racemosa is considered to be involved in synthesis process. | [35] |
| | *Eclipta alba* | Extracellular | Surface morphology (Spherical) Size (26 nm) Zeta potential (−12mV) | Evaluation of prepared plant extract with synthesized AuNPs depicts minor shifts at wave numbers in absorption bands. This indicate bioreduction of gold precursor salt and further capping of gold ions. | [36] |
| | *Nepenthes khasiana* leaf | Extracellular | Surface morphology ( triangular and spherical shape) Size (50 nm to 80 nm) | Plant extract mediated bioreduction | [37] |

the entire synthesis process led by bacterial nanofactories ensures its protection against metal toxicity through dissimilatory oxidation reactions, metal ion reduction, precipitation, complex formation mechanism etc. Further presence of redox mediator shuttle system offers independent and complex strategic regulation of electrons across the cellular membrane for synthesis of extracellular metal NPs. Bacterial secretions in form of EPS have multiple roles, in nature it shields them from noxious agents (chemicals, predators etc.) provide firm anchoring with the substratum, etc [19]. Recently, a strategy can be employed where presence of reducing sugars in EPS can chelated metal ions providing them with better stability, with enhanced features making them more suitable for biological purposes [20]. Study led by Kumari et al the reveal synthesis of AuNPs of various sizes ranging from 2–500 nm with varying surface morphology ranging from spheres, pentagons, hexagons, nanosheets simply by introducing alteration the physical parameters using bacterial species filtrate from *Trichoderma viride*, this confirms that slight variation in pH, temperature, time, concentration (bacterial filtrate) play significant role in determining morphology of NPs [21].

## Plants Based Green Synthesis Approach

Similarly, plants can also be utilized for nanoparticles synthesis using bio reduction reactions. Numerous parts of plant such as stem, barks, leaves, roots, seeds, latex, secondary metabolites, twigs, peels, fruits, seedlings, essential oils, tissues. etc. are rich source of plant phytochemical (polyphenols, flavonoids, sugars, enzymes, and proteins) readily involved in synthesis process acting as reducing and stabilizing agents [22]. Such biogenic synthesis can reduce the high cost of chemicals, environmental risk of being exposed to hazardrous chemicals, and can be used in developing "environmentally-friendly" nanofertilizers, nanopesticides, and nanoherbicides etc. However, the detailed description of mechanistic events is not yet well understood reflecting involvement of diverse phyto-constituents from plant extract working synergistically in the synthesis process mainly being polyphenols, organic acids, and proteins that are acting as main reducing agents [22,23]. Plant dependent synthesis of biogenic nanoparticles begins with plant-based extraction of phytochemicals and plant extract preparation. Here, the process of extraction is very critical as it is involved in parting the desired plant metabolites from the raw plant source in presence of specific solvents with no chemical modification and easy extraction method. It depends on extraction period, type of solvent, specific temperature, pH, solvent/ sample ratio, and particulate size of plant raw materials etc. Further effectiveness of the extraction methods that are being employed in biogenic nanoparticles synthesis relies on easy laboratory practices, timely and cost-effective production process. There are different extraction

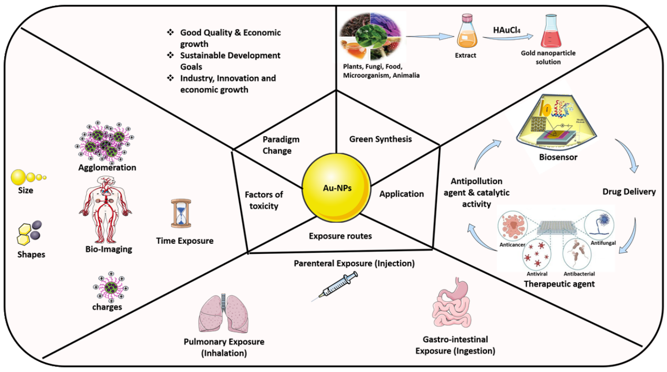


**Figure 2:** Schematic illustration of Green Synthesis, application, routes of exposure, factors responsible for toxicity, and paradigm changes of biogenic AuNPs production and their uses.

**Table 2:** Types of techniques for characterization of NPs.

| Type | Technique | Purpose | Ref |
|---|---|---|---|
| Nanoparticle preparation | Ultraviolet-visible spectrophotometry | Provide information regarding the size, stabilization, and aggregation of nanoparticles | [49] |
| Morphology and particle size | Dynamic light scattering | Determine the particle size distribution and PDI | [50] |
| | Transmission electron microscopy | Determine the shape, size and morphology of the nanoparticles | [51] |
| | Scanning electron microscopy | Determine the surface morphology by direct visualization | [52] |
| | Atomic force microscopy | Determine the size information in 3D along with other physical properties | [53] |
| Surface charge | Zeta potential | Determine the surface charge of the nanoparticles, and the nature of the materials encapsulated or coated on the nanoparticles | [54] |
| | Fourier transform infrared spectroscopy | Determine the functional groups and determine the emission, absorption, or Raman scattering of materials | [55] |
| | X-ray photoelectron spectroscopy | Characterizing the magnetic nanoparticles by providing imformation about mechanism occurs on its surface, involved with bonding of different elements in the magnetic nanoparticles | [56] |
| | Thermal gravimetric analysis | Determining the coating of surfactants or polymers to estimate the binding efficiency on the surface of magnetic nanoparticles | [56] |
| Crystallinity | X-ray diffraction | Identify and quantify crystalline forms or elemental compositions of nanoparticles | [57] |
| Magnetic properties | Vibrating sample magnetometry | Determine the magnetization of magnetic nanoparticles | [56] |
| | Superconducting quantum interference device magnetometry | Provide information regarding the magnetic properties of the nanoparticles | [56] |
| Other techniques used in nanotechnology | Chromatography and related techniques | Used for nanoparticles separation on the basis of their affinity towards the mobile phase | [58] |
| | Energy dispersive X-ray spectra | Identify the elemental composition of the nanoparticles | [59] |
| | Hyperspectral imaging | Determine the type of nanoparticles, the fate of these particles in water samples, their unique surface chemistry and functional groups added | [60] |
| | Laser-induced breakdown detection | Identify the concentration and size of colloids | [61] |
| | Mass spectrometry | Analyze fluorescent labeled nanoparticles | [62] |
| | Small angle X-ray scattering | Investigate the structure of solid and fluid materials in the nanometer range | [63] |
| | X-ray fluorescence spectroscopy | Determine the concentrations of elements present in solid, powdered, or liquid samples | [63] |

methods that are known for this purpose such as (a) solvent extraction (b) microwave dependent extraction (c) maceration extraction (d) ultrasound-assisted extraction [24,25]. The utilization of eco-friendly reagents and solvents, reducing high energy expenditure methods, employing non-toxic biomolecules, such as nucleic acids (DNA & RNA), enzymes, carbohydrates and proteins, as well as plant extracts, permit synthesizing biocompatible metallic NPs by depleting metal ions in aqueous solutions [26,27]. Effective role of different part of plants in extract form for biosynthesis of gold nanoparticles is in form of sequential steps; initial is activation phase, formation of reduced form of precursor metal salt to metal ion occur, this is followed by nucleation reaction involving its further reduction to metal atoms. In growing phase smaller nanoparticles impulsively coalesce into large nanoparticles size (a process commonly known as Ostwald ripening), amplifying the thermodynamic stability of synthesised gold nanoparticles. The duration of this growth phase is vital factor to be considered as aggregation of nanoparticles might lead to nanoparticles surface irregularities forming nanotubes, nanohexahedrons etc. with this it is entering into termination phase where energetically stable conformation is now finally confirmed. Such as case of nanotriangles which are known to have high surface energy and less stable configuration, but after stabilisation is provided in form of plant extract it might acquire a more stable truncated morphology hence, minimize the Gibbs free energy [28]. The advantages of plant-based approach on other green approach are contaminant free large-scale production of biogenic nanoparticles with definite shape, size and morphology along with desired fabrication [29]. Kamaraj, et al has worked on AuNPs that were synthesised from *Gracilaria crassa* (seaweed) using pre-prepared aqueous extract. During this ecological, rapid, and one step synthetic approach the nanoparticles has acquired spherical morphology, with nanodiameter of 32.0 nm ± 4.0 nm, NPs were highly stable and had a polycrystalline nature. However, its biological application against Anopheles *stephensi* larvae was also studies and was found to be very ecotoxic on exposure to different concentrations of prepared nanoparticles. Therefore, shedding radiance on its ecotoxicological potential of the prepared nanoparticles with enhance potential overcoming risk to aquatic biota [30]. The limitation associated with this method is raw materials nature that is responsible for restraining its production to the favourable conditions thus impact NPs formation Table 1.

**Characterization of Green Synthesis AuNPs**

The characterization of NPs attributes very important role to correlates the physicochemical properties with the biological effects and their toxicity [40,41]. Two approaches i.e., top-down or bottom-up approach are used for preparation of nanoparticles. Top-down approach helps in size reduction via chemical and physical process such as shape, physiochemical properties, size and surface structures [42] whereas Bottom-up method consent with atomic, subatomic and molecular level [43]. Different techniques come under consideration for their characterisation like UV-vis (UV-Visible Spectroscopy), XRD (powder X-Ray Diffraction), FTIR (Fourier transform Infrared Spectroscopy) [44], GCMS (Gas Chromatography-Mass Spectrometry), HPLC (High Performance Liquid Chromatography), EDS (Energy Dispersive Spectroscopy), Zeta potential, DLS (Dynamic Light Scattering), SEM (Scanning Electron Microscopy), TEM (Transmission Electron Microscopy), AFM (Atomic Force Microscopy) [45]. UV-vis, FTIR, XRD, EDS, DLS and Raman provides the information about composition of NPs, size and structure and also about crystal phase present in the NPs. UV spectra between the wavelengths from 300 to 800 nm describes the presence of several metallic nanoparticles such as AuNP ranging from 2 nm to100 nm. Maximum wavelength of AuNP is showing the peak from 500 to 580 nm [46]. Absorbance value sometimes increased with increase of time with redshift of the Surface Plasmon Resonance (SPR) band [47,48] (SPR band is visible on nanoscale range on the surface of some metals [49]) which indicate the formation of stable AuNPs occurs after 2-3 hours. This stability of AuNPs is also visualised with naked eyes as samples changed their colour from yellow to wine red to dark purple. These three different colours also depict the size, surface charge and other characteristic properties of AuNPs [50].

DLS evaluates the size distribution and zeta potential present on the surface of nanoparticles by analysis of the brownian motion [51]. Bottean and group revealed that AuNP synthesis using plant extract produces nanoparticles with different size and morphology [52]. This is due to the presence of various functional groups in the plant products. Hexane moiety is rich in benzophenones; however, ethyl acetate part is responsible for flavones, flavonoids and their derivatives like, vestitol, formononetin liquiritigenin & neovestitol. The average size of all kind of AuNPs fall in the narrow range of 8–15 nm depicting a narrow particle size distribution which can be validated by TEM. TEM showing smaller particles are generally in spherical shapes, but particles with larger size revealing different geometrical shapes, such as rods, pentagons or hexagons and sometimes triangles [53,54]. The reason behind geometrical shapes is biomolecules which are responsible for stabilizing and capping are very inefficient, therefore leading to the production of larger irregular-shaped nanoparticles [55].

Zeta Potentials (ZP) which is important for knowing the surface charge of gold nanoparticle which is critical for many applications including biosensing, drug delivery and cell imaging [56]. During the green synthesis, plant extract most of the time used as both a reducing and a capping agent. The capping of anionic biological based compounds on the surface of AuNPs having zeta potential in the range from -30 to -36 mV and this higher value to potential provides the strong electrostatic stability [57].

Elemental composition with atomic number Z>3 is complete by EDAX analysis [58]. Absorption band peak of all AuNPs were seen ~2.2keV, predominantly characteristics peak for gold absorption [59]. XRD characterise the crystallite or amorphous nature of NPs and crystalline nature of green synthesised AuNPs were also confirmed by Selected Area Electron Diffraction (SAED) patterns showing circular rings with coordinated of 111, 200, 220 and 311 Bragg's angle reflection planes [60].

FTIR analyse surface residues present on NPs and functional groups like phenol, flavonoid and hydroxyls which may attribute to the nanoparticles during the nano formulation, required for efficient stabilization and reduction. These groups sometime involved in the metal ion reduction and/or the capping for the colloidal stability [61-62]. The peak around 1700 $cm^{-1}$ and 1600 $cm^{-1}$ are very distinctive and showed hexane group and stretch vibration of $-COO^-$ represent carboxylate anion group to be also present in the nanoparticles. AuNPs also showed small peak around 3400 $cm^{-1}$ providing the information of oxidation of hydroxyl groups to carbonyl groups which involved in the reduction of Au ions [63]. This reduction occurs after the production of AuNPs may be due to the protein present in the samples had been utilised for the capping of nanoparticles leading to stability [64].

**Environmental Parameters Affecting Synthesis of Green AuNPs**

Numerous Literatures reported that dynamic character of the prepared nanoparticles changes with change of pH, pressure, time and other environment [79]. Some of the dominant factors are discussed below.

**pH:** pH influences the synthesis of nanoparticles as it influences the size and their texture [80]. Therefore, nanoparticle can be possibly altered and can be controlled by changing the pH of the solution. This is happened because of production of nucleation centre which elevated with increase of pH followed by increase in the reduction of metal ion into metal nanoparticles. Therefore, in basic pH ranges, synthesis of green gold nanoparticles is efficient process with high stability [81]. But under acidic condition, Low pH could lead to poor stabilization and agglomeration by over nucleation. These NPs are employed in multiple fields in order to having stable NPs under environmental conditions. This alteration of pH is also facilitated to activate the phytochemical compounds, which involves in electrons donation to the metal, reducing $Au^{3+}$ to Au [50]. pH also affects the functional group present in the extract and their rate of reduction [51]. Similarly, Armendariz and group also demonstrated that AuNP synthesised by *Avena sativa* produced large sized nanoparticles about 25-85 nm when pH 2 was used but size get smaller in case of pH 3 and 4 [81]. They speculated AuNPs aggregated to form large NP at low pH instead of nucleated and in case of higher pH (3 or 4) Au have high affinity for functional groups like hydroxyl and carbonyl, which facilitate the greater number of Au (III) complexes and available for bound with plant extract at the same time. Effect of pH also studied in green synthesis of AuNPs by *Cacumen platycladi* leaf extract [43]. It was observed that pH influences the intensity of absorbance peak by increase pH range but with this size of the AuNPs decreased too. The author also noticed that high pH is responsible for speedy rate of reduction of chloroaurate ions, with enhanced nucleation and decreased in heterogenicity of nanoparticles.

**Temperature:** Temperature is also another parameter. High temp is required during the synthesis *via* physical method of about >350°C, while chemical approach maintains temperature below 350°C. Green technology is more convenient as it needs ambient temperatures or <100°C. Temperature also play essential role in the nature of the NP formed [83]. Sneha and team observed that at temperature 20°C form triangular shaped AuNPs prepared by *Piper betle* leaf extract and in contrast, high temperature 30-40°C formed nanoparticles of size 5-500 nm [84]. Although with further increasing temperature up to 60°C, it has been noted the spherical shaped of AuNPs is dominantly more than the triangular and octahedral shaped nanoparticles. Sheny et al., provide information about stability, to prepared stable AuNPs, reaction in low temperature is more preferable than high temperature [85]. Pamies and colleague revealed that at temperature 33°C or below, small part of copolymer chains aggregated to form the particles and remaining major part are free in the solution [86]. As the temperature rises up to 42°C, copolymer chains started to interact with each other and form micelle-like-structure with size of about 100 nm. The synthesis of AuNPs at this temp is very optimum in term of particles stability. Size elevated with value of 300 nm if temperatures further increase up to 55- 70°C but interestingly does not affect the stability of the particles [86].

**Pressure:** Shape and size of the NPs can change or alter if the optimum pressure applied to the reaction medium. At ambient pressure, metal ions easily get reduced and faster using biological agents [87]. Therefore, pressure can be concluded as one of the factors required for green gold synthesis. Atmospheric Pressure Plasma (APP) when interact with Liquid phase during synthesis in non-equilibrium state, synthesis of nanoparticles showing exceptionally versatility. Interaction of water with high density gas leading to exchange of electron and eventually create a cascade of intermediate nonlinear chemical reactions and production of reactive radicles like H• and OH•. These reactive species and solvated electrons are very beneficial for preparation of successful nanoparticles like AgNPs, AuNPs, $Cu_2O$ NPs, $Fe_3O_4$ NPs and other metal nanoparticles [88].

**Time:** In green technology, the quality of nanoparticles to be formed are also influencing by incubation time of the reaction required for the production of AuNPs [89]. In the same way, we can alter the characteristics of nanoparticles with alteration of time duration of light exposure and storage conditions, etc. [90] and these variations in the time may resulting aggregation of particles for longer storage; it may decrease or increase their size during storage, their shelf life, and other factors which affects the efficacy potential [91]. Noruzi and colleague investigated that biosynthesis of AuNPs by Rosa hybrid petal was rapid and completed their reaction within 5 min [92] on the other hand, Dwivedi and Gopal [Dwivedi and Gopal, 2010] reported that AuNPs and AgNPs synthesis by Chenopodium album leaf started the reactions in 15 min. They also observed that with increasing contact time is strongly responsible for the sharpening of the intensity peaks in both AuNPs and AgNPs. In additions, some of the reports also suggested that there are changes in the size and shape of the NPs with the increase of their reaction time [93]. Apart from this incubation and reaction time, time required for centrifugation is also an important factor. To stop the reaction and also for eliminating the unwanted components, duration of centrifugations plays a major role. Balasubramanian et al., investigated the size of prepared nanoparticles which shows distinct variation with a duration of 5min- 3 hrs of centrifugation process [94].

**Proximity:** NPs when come closer to each other or nearer to the other nanoparticles surface, there is maximum probability of alteration in their properties. This phenomenon is very useful for the nanoparticles to make it more tuned and efficient. These proximity effect could be implied on the particle charge, the interactions of the substrate, and also magnetic nature of the NPs [95].

**Other environment factor:** The surrounding environment also affects the nanoparticles properties. Alone nanoparticles can form core-shell nanoparticles by reacting/ absorbing with different substance from environment *via* oxidation or corrosion [96]. Therefore, it affects the physical properties, their structure and chemistry of the prepared nanoparticles.

**Gold Nanoparticles for Biomedical Application**

Biogenic synthesis of AuNPs has been gained considerable attention among researchers and still it is facilitating the progressive development in various biomedical fields due to its unique biocompatible, optical, physicochemical properties. Some of the important application of biomedical field comprises of bioactive, chemical, physical and environmental sciences. In this section, we briefly discuss the role of green synthesized gold nanoparticles in the fields of therapy, anti-microbial activity, drug/gene delivery, catalytic activity and biosensing as enlisted in Table 3 and Figure 3.

**Table 3:** Tabular representation of application of Biogenic Gold nanoparticles in various fields.

| Nanoparticles source | Characteristics | Activity/ Effect | Outcome | Ref |
|---|---|---|---|---|
| *Ziziphus spina-christi (Leaf extracts)* | 14.64-32.95 nm | **Biological Activity (Anti-bacterial)** *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Enterococcus faecalis* | *Z. spina-christi* extracts were used as a reducing and stabilizing agent for the biosynthesis of Au, Ag and Au- Ag alloy NPs in this study. AgNPs combined with *Z. spina-christi* leaf extract had a synergistic bactericidal effect on the biosynthesized Au-Ag alloy NPs. As an alternative to conventional antibacterial materials, this hybrid nanomaterial has been developed. | [139] |
| *Agaricusbisporus* (Edible mushroom extracts) | 25nm | **Biological Activity (Anti-fungal)** *A. flavus* and *A. terreus*. | *Agaricusbisporus* extract act as capping and reducing agents used to synthesized gold nanoparticles via hydrothermal method displayed excellent anti-fungal activity against *Aspergillus flavus* in comparison to *Aspergillus terreus* | [140] |
| *Leptadeniahastata (Leaf extracts)* | 5 to- 30 nm | **Biological Activity (Anti-fungal)** *Aspergillus fumigatus* | LH-AuNPs exhibited remarkable antifungal properties against *A. fumigatus*, involving the inhibition of radial growth of fungal hyphae and green pigmentation and caused cell walls damage. The *.i.v.* administration of LH-AuNPs in the IPA murine model demonstrated evident therapeutic ability for lung repair with negligible *in vivo* cytotoxicity. | [141] |
| *Curcuma wenyujin* (Rhizome) | 286.5nm | **Biological Activity (Anti-cancer effect)** Human renal cell carcinoma (A498 cells) | Herbal-based gold nanoparticle with the traditional medicinal herb effectively induced apoptosis in the renal cancer cell line A498 as collectively confirmed by analysed flow cytometry, RT-PCR, and immunoblotting data. | [142] |
| *Dendrobium officinale* | 32.95 nm | **Biological Activity (Anti-cancer effect)** Liver cancer (HepG2 and L02 cells) | This study demonstrated that Do-AuNP had better anti-tumor efficiency compared with DO extraction alone without increasing toxicity *in vivo* and *in vitro*. | [143] |
| Curcuma wenyujin | 127.7nm | **Biological Activity (Anti-cancer effect)** MDA-MB231/HER2 cell line | CW-AuNPs showed the effective anticancer property by elevated the level of ROS in breast cancer cells which in turn suppressed the HER2/neu, key oncogene expression and prevented the cancer cell proliferation | [144] |
| *Artemisia capillaris* | 16.88±5.47~29.93±9.80 nm | **Catalytic activity (4-Nitrophenol reduction)** | The extract of AC-AuNPs was collected by centrifugation to determine their proficiency in the reduction reaction. This finding showed that the extracts excellently the catalytic activity by up to 50.4 %. | [145] |
| *Sargassum* spp | 15–30 nm | **Catalytic activity (Degrading organic dyes in water)** | This green synthesized AuNPs were excellent in efficiency and degradation of environmental pollutant dye such as methylene blue, methyl orange, and methyl red. | [146] |
| *Oryza sativa* (seeds) | 180-150nm (Size) 12-25nm (pore size) | **Sensors (non-enzymatic sensing)** | The biogenic synthesized nano-porous gold nanoparticles were observed to be extremely proficient for sensing glucose as compared to their metallic counterpart. The 3D porous meshwork like structure with a higher surface area, could be scrutinize for wider application such as biosensors, sensors, and other industrial applications. | [147] |
| *Lignin*(matrix) | 15.67±1 nm | **Sensors (Colorimetric sensing)** | L-Auf (1-5) NPs can also be employed as an effective sensor for the rapid and specific detection of Pb2+ ions in real environmental samples based on colorimetric analysis. | [137] |

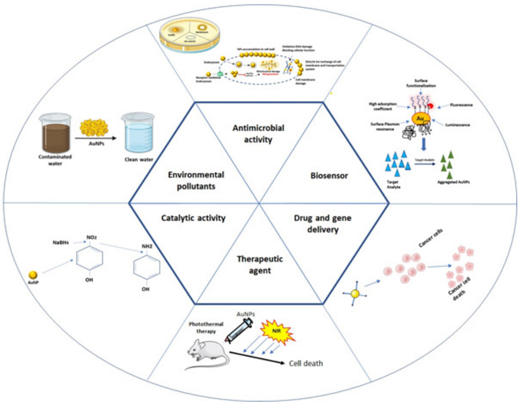


**Figure 3:** Schematic representation of green synthesis AuNPs as a therapeutic agent in mediating anti-microbial and anti-cancer effect and excellent delivery system for the facilitation of drug and gene to the targeted site. This also plays significant role in diagnostic purposes such as Biosensors and responsible for the removal of environmental pollutants through catalysis process.

### Anti-Microbial Activity

AuNPs are known to treat wide variety of pathogen such as bacteria, fungi, parasites, and others. It exhibited potent anti-bacterial activity when encounters bacterial cell wall due to their surface charge effect. Usually, nanoparticles disrupt the microbial surface via electrostatic flux, and results into the release Reactive Oxygen Species (ROS). This leads to the degradation of protein, DNA, and mitochondrial dysfunction, finally cause fungal and bacterial death. Hence, NPs induce apoptosis via mediating mitochondrial oxidative stress and impede ATP synthesis [97-99].

Many researchers have shown that AuNPs could combat microbes successfully as shown in Table 3. Gold nanoparticles synthesised from extracellular metabolites of different bacterial strain (*Rastrelligerkanagurta, Selachimorpha sp.,* and *Panna microdon*) isolated from Indian marine fishes (Sample 1-5) that exhibited remarkable anti-bacterial and anti-mycobacterial activity against various panel of microorganisms like *Mycobacterium tuberculosis* and *Serratia marcescens, Staphylococcus epidermidis, Proteus sp., Candida albicans, Salmonella typhi, Pseudomonas fluorescens, , Escherichia coli Micrococcus luteus, and Staphylococcus aureus*. This study revealed that sample 1, 3 and 5 delivered enhanced anti-bacterial activity against gram positive and gram-negative bacteria. Further, AuNPs synthesized from sample 1 demonstrated 61.47%, 82.52%, and 75.5% RLU inhibition towards *M. tuberculosis* H37Rv, HR-sensitive *M. tuberculosis*, and HR-resistant *M. tuberculosis*, respectively. Whereas sample 5 enhanced RLU reduction against *M. tuberculosis* H37Rv (91.13%), HR-sensitive *M. tuberculosis* (86.72%), 276 and HR-resistant *M. tuberculosis* (77.10%). While the sample 3 did not showed any anti-mycobacterial properties. Therefore, based upon the sample types exhibited anti-bacterial and anti-mycobacterial activity against human pathogens [100]. Recently, AuNPs was also produced from flower extract of *Jatropha integerrima*. Successively, the formulation exhibited maximal and minimal anti-bacterial effect against *E. coli* and *B. subtilis.* This occurred because of gram-positive bacteria encloses with a rigid cell wall and that prevent the easy penetration of NPs across cell wall. Therefore, the gram-negative bacteria are destroyed more rapidly at lower concentration because $Au^{2+}$ triggers the ROS (Reactive Oxygen Species) generation, such as hydroxyl radicals (-OH), which contributes to increasing the membrane permeability. They reported the MIC of AuNPs against *B. subtilis, S. aureus, E. coli*, and *K. pneumoniae* were observed to be 5.0, 10, 2.5, and 2.5 µg/mL, respectively [101]. The antimicrobial agents AuNPs are accountable for the disruption of membrane, ROS generation and protein/DNA damage of target microorganisms. The mechanisms of action of anti-bacterial action of nanoparticles are significantly dependent upon the size of nanoparticles. Due to the small size and large surface area, AuNPs easily attach to the cell membrane and penetration of bacteria causes the cell membrane disruption as suggested by Arief et al, explored the anti-bacterial activity of synthesized green gold nanoparticles (AuNPs) using *Uncaria gambir* Roxb. leaf extract based upon different size and shape range (29nm, 11nm, 23nm and 31nm) as confirmed by disc diffusion assay or Kirby Bauer method. This study demonstrated that smaller size of AuNPs (11nm) exhibited the higher zone of inhibition against *S. aureus* and *E. coli* as responsible for the degradation, distortion of bacterial cell wall and ultimately cell death [102,148].

Lomelí-Rosales et al has worked on reducing gold precursor salt using aqueous extracts from different plant part such as root, stem, and leaf of *Capsicum chinense* with conceptual approach reliving agro-industrial burden by channelizing the generated waste to biogenic synthesis of nanoparticles assisted by microwave radiation and UV light radiation, antioxidant and antimicrobial properties were also tested. The antioxidant activity showed a decrease of 44.7% after the synthesis of the AuNPs-leaf, the concentration of reducing sugars decreased by 67.7% in the extract samples after completion of nanoparticle synthesis. At last, the growth of *S. aureus*, *E. coli*, *S. marcescens* and *E. faecalis* was also compromised by subsequent application of green gold nanoparticles in clinics for effective regime against fast growing antibiotic resistance [103].

Donga et al., used seed extract of *Mangifera indica* which act as a strong reducing agent by aiding the reduction of gold to AuNPs and studied in 14 strains of pathogenic microorganisms consisting of gram negative, gram positive, fungal strain and its clinical isolates demonstrated the significant toxicity against gram positive bacterial and fungal strain. This study also showed the remarkable antioxidant activity against cancer cell line in dose dependent manner (HeLa cancer cell line, MCF-7 cell line and Normal fibroblast cell). Therefore, green AuNPs provide the anti-microbial, anti-cancer and antioxidant properties [104]. Mandhata et al in their study has shown extracts from *Anabaena spiroides* (N2-fxing cyanobacterium) from brackish-water at Puri, Odisha is well utilized for synthesis of gold nanoparticles with nanosize range with absorbance peak recorded at 538 nm. It was also reported to have definite antimicrobial activity against other bacterial species *K. oxytoca, MRSA* and *S. pyogenes* [105].

Due to the emergence of fungal infections, there is a constant need to develop novel therapeutic approaches to overcome the outcome of harmful diseases. Gold nanoparticles possessed eminent anti-fungal activities against human fungal pathogens. In such study, biogenic AuNPs isolated from root and leaf extracts of *Vetiveria zizanioides* and *Cannabis sativa* tested against various panel of fungal pathogens (*Penicillium* sp., Aspergillus sp., *Aspergillus flavus, Aspergillus fumigates*, and *Fusarium* sp. and *Mucor* species) using standard disk diffusion assay. It has been found that green AuNPs exhibited a remarkable zone of inhibition as compared to chemically synthesized AuNPs. Further, chemical composition, shape, size and photoactivation factors that responsible for the anti-microbial properties. Therefore, the small sized NPs possessed higher toxicity against fungal pathogen due to the large surface area to volume ratio and surface connectivity contributed to retarding the normal functioning of DNA like synthesis, repair and replication caused the cell death [106]. Moreover, the eco-friendly synthesis of gold nanoparticles using the leaves extract of *Viola betonicifolia,* the secondary metabolite of plant extract act as both reducing and capping agents. The characterization of synthesized NPs confirmed by DLS, FTIR, TEM, XRD, EDX and UV-Vis spectroscopy. They examined the anti-bacterial, anti-fungal, antioxidant and cytotoxic activity against all the tested microbial organisms associated with biogenic synthesized AuNPs and commercially available chemical synthesized AuNPs. Successively, the finding showed that biogenic AuNPs displayed 4.14, 4.32, 4.50, and 4.65 $\log_{10}$ depletion in CFU rate in *S. aureus, B. subtilis, E. coli, and P. aeruginosa*, respectively, with more than 82% killing proficiency through the action of complete disruption of cell membrane, leakage of intracellular biomolecular functionality and enhanced ROS generation in compared to chemically available AuNPs. Further, the antifungal activity of AuNPs was determined by Sabouraud-Gentamicin-Chloramphenicol (SGC) fungus agar plate against *C. albicans, A. fumigatus, A. flavus, and A. niger* with reduced CFU by 3.32, 4.0, and 3.55 $\log_{10}$ respectively. The study also concluded that biogenic AuNPs were inhibited the biofilm of both bacterial and mycological strains and accountable for the anti-cancer activity against breast cancer cell line (MCF-7) [107]. Therefore, the study stands a embark for the application of green gold nanoparticles to employed in the clinical sector. There are some studies which defines the success of green synthesized AuNPs against various parasitical diseases. The green AuNPs synthesized from Nigrospora oryzae a phytopathogenic fungus exhibited anti-Helmintic efficacy as tested on tapeworm (cestodes). This study illustrated the alterations in the ultrastructure and biochemical characteristics of the treated parasites as compared to non-treated [108]. Further clinical studies are required on the anti-microbial efficacy of green gold nanoparticles, to substantiate and explored the results of *in vitro* study and pave the way for novel transition to commercialize the viable therapeutics.

**Therapeutic Agents**

The most eminent application of AuNPs in biomedical field is to treat cancer, arthritis, and other disorder. The physicochemical properties of NPs (for example as small size, charge, surface area, functional groups and targeting agents) considered to be promising approach to overcome the various biological obstacles by easily penetrated to tumour site and thus defining the EPR (enhanced permeation effect) [109]. Recently, Wang et al., synthesized gold nanoparticles using *Scutellaria barbata via* green method. This formulation displayed effective anti-cancer property against pancreatic cancer cells (PANC-1) by enhanced level of ROS that leads to the apoptotic mode of cell death [110]. Various concentration of *Arthospira platensis* Exopolysaccharides (EPS) were employed to synthesized biogenic gold NPs (AuNPs1, AuNPs2, AuNPs3). AuNPs3 was formulated using EPS along with L-ascorbic acids exhibited higher stability in compared to other AuNPs.  AuNPs3 and AuNPs1 showed the anti-bacterial effect with zone of inhibition percentages of 88.92% and 83.13%, respectively. Moreover, this also concluded that AuNPs3 was highly biocompatible to the non-cancerous cells while its highly sensitive to the breast cancer cells (MCF-7 cells) with 70.2% of inhibition rate. This AuNPs responsible for the down regulation of Bcl2, Ikapα, and Survivin genes in breast cancer (MCF-7) treated-cells and showed the abilities to arrest cancer cells in the S phase (77.34%) and enhanced the cellular population in the sub G0 phase. However, Transmission Electron Microscopy (TEM) data demonstrated that AuNPs3 and AuNPs2 were successfully confined in cytoplasm, perinuclear region, and cell vacuoles [111].

Similarly, Hosny et al. in their present study has synthesized phyto-fabricated AuNPs that was prepared using aqueous extract of *T. capensis* leaves thus, encourage doctrine of green chemistry being less perilous as that of chemical syntheses, much more safer solvents and auxillaries choices, designing as per renewable feedstocks. Here, findings reveal that phytoformation of AuNPs has spherical shape with a nanosize range (10–35 nm), absorbance peak (515 nm), and zeta potential (−24.5 mV). Antioxidant efficiency was evaluated to be 70.73% and anticancer activity was also studied against breast cancer cells (MCF7 cell line). Therefore, suggest a new and sustainable route for the green synthesis of AuNPs [112].

Moreover, gold nanoparticles were fabricated using *Curcuma Manga* (CM) extract under the influence of photothermal therapy demonstrated the killing of breast cancer cells (MCF-7). It also showed that green synthesized gold nanoparticles exhibited high photothermal heating efficiency as compared to citrate-AuNPs (chemically synthesized). Therefore, this concluded that green synthesized AuNPs combined with electromagnetic radiations, mediated apoptosis in malignant cells [113]. Currently, Liu et al used *Euphrasia officinalis* extract to prepared green synthesised AuNPs, these NPs were investigated in inflammatory diseases. This showed the significant reduction in LPS-mediated inflammation by inhibiting the JAK/STAT pathway via suppressing the phosphorylation and degradation of inhibitor kappa-B-alpha, that prevent the nuclear translocation of NF-kB p65 [114]. Various studies mentioned that green AuNPs also displayed anti-diabetic effect. One such study revealed that AuNPs orally injected to the diabetic induced animal model maintains the metabolic activity and restores the normal functioning of cholesterol and triglycerides almost the control levels. Moreover, Rats diabetic model exposed with green synthesized AuNPs from leaf extract of *D. viscosa* were able to enhance the hyperglycaemia and recovers the normal body weight through reducing hepatic gluconeogenesis by suppressing the expression level of hepatic phosphoenolpyruvate carboxykinase (PEPCK) gene. This finding showed that after treatment the favourable changes occurred in the streptozotocin-induced diabetic rats (HFD/STZ) that exhibited the normal level of glucose concentration in blood serum, improved the lipids profile and transaminase activity [115]. Some *in vitro* study concluded that propanoic acid functionalized AuNPs exhibited not only the anti-diabetic effect but also inhibited the protein-tyrosine phosphatase 1B, α-glucosidases, and α-amylase activity. Successively, prevent the cells from oxidative damage and normalize the unregulated insulin signalling pathways [116]. Recently, various studies suggested that green synthesized AuNPs displayed the virucidal activity against various viral infection such as HIV, Measles virus (MeV), Swine Flu, SARS virus, chikungunya virus. The green synthesized AuNPs using garlic extract *(Allium sativa)* as a reducing agent (AuNPs-As) inhibited the MeV replication in Vero cells with 50% effective concentration ($EC_{50}$) of 8.829 μg/mL and their selectivity index (SI) observed was 16.05. Therefore, this study concluded that AuNPs-As inhibited the viral spreading by inhibiting the viral particles directly in MeV and other related enveloped viruses [117]. Therefore, Versatility nature of green AuNPs used as an excellent therapeutic agent for wide variety of diseases.

**Drug and Gene Delivery**

The efficacy and competency of drugs is precisely based on the targeted delivery and its solubility. Due to the imperceptible effects and well-being issues its usage is limited in clinical translation. Therefore, green synthesized gold nanoparticles emerged as a novel delivery system to incorporate wide variety of cargoes (drugs, DNA, RNA, peptides, and proteins) to facilitate the controlled and sustained release to their targeted site for the treatment of various ailments. AuNPs are also susceptible to surface modification by conjugation with molecules either by covalent or non-covalent bonding [109,118].

Biosynthesized gold nanoparticles from extract of *Camellia sinensis* (Black tea) had been reported for the enhancement of chemosensitivity of doxorubicin against colon cancer cell line HCT116. This study suggested that BTE-GNP improved the cell cytotoxicity at lower doses of doxorubicin *via* a ROS-dependent mitochondrial pathway of apoptosis where elevated pro-apoptotic protein expression thus enables it to act as an excellent chemosensitizer [119].

Moreover, formulation of gold nanoparticles using *A.hirsutus* extract, bio-capped with activated folic acid and loaded chlorambucil (AuNPs-FA-CHL) studied against normal epithelial cells and human cancer cells (HeLa, RKO and A549) showed remarkable cytotoxicity against human cancer cells in targeted manner comparison to AuNPs and drug alone [120].  Protein coated AuNPs extracted from *Tricholomacrassum* were found to be most suitable applicant for gene delivery, based on plasmid DNA-AuNPs complex successfully delivered Green Fluorescent Protein (GFP) into mouse sarcoma cancer cells was possible. The low haemolytic activity of AuNPs, showed its biocompatible nature. Overall, this study concluded that AuNPs as potential delivery of apoptotic agents for gene delivery in cancer therapeutics [121]. Targeted delivery of anti-steroidal drugs (Diclofenac) to inflamed cells using green synthesized gold and iron nanoparticles as delivery vehicle isolated from *Phyllanthus emblica* and *Syzygium cumini* extract. Both the nanoparticles reported to be most effective anti-bacterial agents against gram positive and gram-negative bacteria. However, gold nanoparticles exhibited robust anti-inflammatory potentials and protected DNA from damage when compared to iron nanoparticles hence, it also aided as DPPH free radical scavengers (antioxidant properties) [122].

### Catalytic Activity

The AuNPs surface can be employed as selective oxidation site for the synthesis of nano-catalyst. The various application of nano-catalyst attracted researchers especially in non-toxic and fuel cells [123].

Ramakrishna et al., reported the green formulation of gold nanoparticles derived from marine brown algae from the aqueous extract of *T.conoides* and *S. tenerrimum*. This AuNPs act as systematic catalyst for the reduction of aromatic nitro compounds and organic dye molecules. It also demonstrated that *T.conoides* have strong catalytic activity than *S. Tenerrimum* [124]. Recently, in a study biosynthesis of gold nanoparticles from *Alpinianigra* leaves extract exhibited stronger catalytic properties in catalysing the reduction of anthropogenic pollutant dyes in the presence of sunlight.

The percentage of degradation facilitated by AuNPs in Methyl orange and Rhodamine B were 83.25% and 87.64% respectively. This photodegradation process obeys pseudo first order kinetic reaction [125]. Similarly, the reduction of methylene blue using biogenic AuNPs extracted from *Polyscias scutellaria* leaf catalysed in same pseudo-first order reaction with a reduction rate constant ($k_{obs}$) of 0.0233 $min^{-1}$ under the effect of UV irradiation [126]. Recently, Nguyen and coworkers depicted the green-synthesis protocol of gold NPs using mushroom (Ganoderma lucidum), the pre-prepared extract is employed both as reducing and capping agents. The NPs thus prepared are considered superb for catalytic, colorimetric detection of Fe3+ ions in water bodies and display antimicrobial potential hence is excellent contender for biotechnological applications [127]. Work led by Alikhani et al investigated novel biogenic approach stenciled with used of *Rosa canina* fruit extract. Presence of phytochemicals (polyphenols, flavonoids, tannins etc.) assisted the green reduction of Au ions to stable Au NPs with wide application as a proficient catalytic system causing reduction toxic water pollutant dyes like Methylene Blue (MB), Rhodamine B (RhB) and 4-nitrophenol (4-NP) etc. [128].

### Removal of Environmental Pollutants

The biogenic fabrication of AuNPs as a bequeathed rapid explorer for mitigating the environmental pollutants in a sustainable manner. The green AuNPs synthesized from yeast (*Saccharomyces cerevisiae)* used to degrade halogenated herbicide (quinclorac), which demonstrated the dichlorination by converting quinclorac to 8-quinoline-carboxylic acid [129]. Moreover, Das et al., prepared Nanogold-Bioconjugate (NGBC) used to purify contaminated waters. Gold nanoparticles fabricated on the surface of *Rhizopus oryzae,* fungal strain exhibited antimicrobial activity and strong adsorption efficacy against different organophosphorus pesticides.

Therefore, the functionality of NGBC in single operation obtained potable water free from pesticides and pathogens [130]. Microwave assisted green gold nanoparticles synthesized from *Myristica fragrans* act both as stabilising and capping agents. The catalytic efficiency of this NPs enabled the degradation of 4-nitrophenol and methyl orange dye with $NaBH_4$ [131]. Nadaf and coworker briefly described facile bacteriogenic approach for synthesis of AuNPs using Bacillus marisflavi, this was further employed as catalytic agent to address environment issues in form of polluted dyes such as congo red and methylene blue etc. in the presence of sodium borohydride [132].

### As a Sensor

Due to the advancement in nanotechnology, AuNPs has been utilized as potent sensitive biosensor for the analysis of biological and environmental factors based on their peculiar properties such as radiative and electromagnetic nature. The surface of AuNPs enables the direct conjugation with recognised agents, such as antibodies for their early detection of respective surface biomolecules. AuNPs offers the remarkable sensitivity in detecting the cancerous cells, bacteria, pollutants, pathogens, viruses, biological toxins and molecules. Although, AuNPs exhibit various exclusive properties such as SPR (surface plasmon resonance) leads to the strong electromagnetic fields that enhances the radiative properties- Absorption and Scattering. Therefore, AuNPs can be readily employed in optical imaging. Innumerable forms of sensors can be engineered based on their distinct features such as- colorimetric, surface plasmon resonance, fluorescent, electrical, electrochemical, and Bio-barcode assay sensors [133].

Fluorescent-plant-based markers utilised for AuNPs extracted from *Medicago sativa*, *H. ambavilla, Olax Scandens*, and *H. lanceolatum,* have been employed for sensing cancers as reviewed by Sargazi et al [134]. AuNPs offer long-term monitoring and imaging the target cells due to the longer circulation time, permit easy decorated with moieties that are identified by cancer cell surface receptor and enhanced uptake through Receptor-Mediated Endocytosis (RME) in comparison to other mode currently used in the clinical field.

Therefore, these green AuNPs are currently being applied in imaging diagnoses such as, fluorescence imaging, NIR imaging, X-ray imaging and photoacoustic imaging at cellular level [134]. AuNPs used to detect amino acids, proteins, nucleic acids, and enzymes based on colorimetric and fluorometric method. Oleyl chitosan functionalized AuNPs hybrid is also used in an imaging technique such as MRI and CT-scan for detecting biomolecules [135]. Recently, Nazarpour et al. designed a electrochemical sensors using eco-friendly AuNPs/rGO nanocomposite extracted from *Eucalyptus tereticornis* solution as a both reducing and stabilising agents for the determination of tryptophan amino acid based on response surface methodology approach. The tryptophan is the most predictive biomarker for lungs cancer. The Limit of Detection (LOD) and Limit of Quantification (LOQ) at a minimum concentration in a calibration was found to be 0.39 and 1.32 μmol/L, respectively. This electrochemical sensor exhibited excellent reproducibility, high selectively, and sensitivity for detecting the tryptophan and can be employ for the analysis of Try in human plasma, serum, and saliva [136].

Lignin- mediated green formulation of functionalized AuNPs (L-$Au_f$NPs) under the effect of laser irradiation and sono-chemical process is employed as colorimetric sensor for the recognition of lead ($Pb^{2+}$) ions in aqueous media. L-$Au_f$NPs demonstrated the change in colour from red wine to purple when encounters $Pb^{2+}$ ions [137]. Similarly, biogenic synthesis of AuNPs from *Camellia sinensis is* applied as a colorimetric and plasmon resonance sensor for detection of presence of specific divalent metal ions ($Ca^{2+}$, $Sr^{2+}$, $Cu^{2+}$, and $Zn^{2+}$) in aqueous solution [138].

## Conclusion

The green production of AuNPs is an evolving technique, in present day the development of reliable, unconventional, remarkable, and sustainable solutions is promising for the researchers and scientists with broad range of application in various biomedical fields. This review summarises the current understanding of synthesis and characterization of biogenic AuNPs in the field of biomedical application that holds eminent potential to address and explore the diverse areas such as chemistry, medicine, technology and industry.

Special interest is focused on the prospective effect of AuNPs in mediating the anti-microbial, anti-oxidant, anti-cancer and anti-diabetic effect. Along with this it also offers suitable platform for efficient delivery (drugs and gene) system and potent sensitive biosensor for detecting various ailments. The synthesis based on green reducing agents is executed in a modest way without pre-requisite need for higher temperature and pressure. Innumerable evidenced demonstrated the interdisciplinary ways for the production of biogenic AuNPs from several plant and microorganism extracts including the use of harmless components that mediates the reduction, capping of AuNPs and removing the risk associated to environmental pollutants. Usually, plant based reducing agents provide fastest reaction in comparison with microbial based green synthesis. Therefore, the biological synthesis of nanoparticles is extremely quicker, cost- effective process that can be developed as an economic and valuable substitution for the commercialization and large-scale production of biogenic gold NPs suitably applied in biomedical and clinical fields. Conclusively, emergence of green and sustainable nanotechnology provides a promising eco-friendly strategy and its utilization in multiple fields, but still its implementation as an in-vivo delivery vehicle and its efficacy along with clinical studies are critically required.

The emerging of green production in biomedical fields enables in reducing the drug toxicity and improve the efficacy of drug targeting without off-effects.

### Future Perspectives

However, scaling up the green NPs for commercialization has still become an exhaustive challenge because there are various factors that still need to be addressed and optimized to achieve accuracy in shape, size, and homogeneity of nanoparticles such as pH, temperature, type and growth stage of microorganism, concentration, reaction time and growth medium. Hence, comprehensive consideration of biochemical components and molecular mechanism of reducing agents involved in green synthesis should be focused meticulously. Thus, Green gold Nanoparticles (GNPs) offer a remarkable size and unique surface properties that allows its use as an ideal vehicle for targeted and specific drug delivery. Thereby, various research should be carried to garner deep knowledge of their distribution and mechanisms of action of AuNPs to treat various ailments.

The fluorescence properties of AuNPs can be explored as marker in diagnosis of various diseases. More and more studies should be focused to evaluate the toxic façade of green nanoparticles and their bioaccumulation before using it in different practical applications to validate its precise duration and residual power and side effects in an in-vivo system.

## Author Statements

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### Author Contribution

KN, PY, LB, MY, KD and NKP conceived and wrote the manuscript. AKV edited the manuscript. All authors contributed to manuscript writing, revision, read and approved the submitted version.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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