

## Review Article

# Green Synthesis of Metallic Nanoparticles and Their Multifaceted Applications: A Review Study



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### Article info:

**Received:** 25 May 2025

**Accepted:** 18 Sep 2025

### Keywords:

Bacteria, Fungi, Green synthesis, Metallic Nanoparticles (NPs), Metal ions, Plant

## ABSTRACT

**Background:** Nanotechnology, specially, metallic nanoparticles (NPs) has successively revolutionized many aspects of biomedical and industrial practices due to their distinct physiochemical and functional characteristics. Nonetheless, traditional methods of synthesis typically create toxic wastes and threaten the environmental safety. Green synthesis has since been developed as a sustainable solution, utilizing biological entities, including plants, bacteria, and fungi, in an environmentally-friendly way to produce NPs.

**Objectives:** This work critically examine green synthesis methodologies to determine the biological processes, and evaluate their effects on the quality of NPs, their bioactivity, and use in medical and industrial practice.

**Methods:** Google Scholar and PubMed were used for the literature review till April 30, 2025. The used keywords were “metallic nanoparticles,” “metal ions,” “green synthesis,” “bacteria,” “fungi,” and “plant.” The development of the manuscript was based on full-length research and review articles, which describe the rationale, technologies, methods and basic principles of “green chemistry.”

**Results:** The analysis of green methods of synthesizing NPs showed that it is possible to produce metal NPs (e.g. Ag, Au, Cu, Fe, Pd, Se) of controlled size and shape consistently. The biogenic sources were found to be reliably able to generate sufficiently pure and monodisperse particles and protocols. These NPs have potential antimicrobial, antioxidant, and anticancer efficacies. Also, the improved techniques of biodistribution evaluation suggest that they could be used in the medical field.

**Conclusion:** The review confirmed various strategies researchers have performed to manufacture metallic NPs using green synthesis method.

**Citation** Banalia A, Puri D. Green Synthesis of Metallic Nanoparticles and Their Multifaceted Applications: A Review Study. *Pharmaceutical and Biomedical Research*. 2025; 11(4):277-298. <http://dx.doi.org/10.32598/PBR.11.4.1418.1>

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## Introduction

Over the past 20 years, nanotechnology has greatly ranked among the most important technologies studied and rapidly expanding sciences because of its applicability in several human welfare domains [1].

The term “Nano” was obtained from Greek word “nanos,” which means diminutive. The units of measurement for nanoparticles (NPs) are nanometers. They are popular because of their sub-microscopic particle-size and large surface area [2].

There are four main categories of NPs depending on their chemical makeup: Carbon-based (carbon nanotubes and nanofibers, etc.), bioorganic-based (liposomes, micelles, etc.), metal- and metal oxide-based (Ag, Cu, etc.), and composite based [3]. NPs have extraordinary physical, thermal, optical, magnetic electrical, and chemical, properties compared to their non-nano material [4]. They’ve wide scope in Pharmaceuticals, as well as catalysis, householding products, environmental sensors, automotive industries, and nanobiotechnology. Additionally, nanotechnology helps the early diagnosis of serious illnesses like cancer. Examples of NPs include dendrimers, metal NPs, liposomes, fullerenes, and nanodroplets [5].

Metallic NPs are an important and most-studied class of materials with various applications. Numerous studies are being conducted on the production of metal ion NPs from microorganisms and botanical extracts [6]. Silver NPs, AgNPs, are the most often used of all the produced NPs and found in over 25% of consumer goods. Research shows that gold NPs (AuNPs) has biological uses as muscle relaxant, antibacterial, and enzyme control. AgNPs prevent gram-positive and gram-negative bacteria from growing and acting. Copper NPs (CuNPs) possess great promise as drug delivery vehicles, anti-cancer drugs, and enhancers of photodynamic treatment. Palladium NPs (PdNPs) are used as catalysts, dye degradation, and anti-microbial. Finally, iron NPs (FeNPs) inhibit bacterial growth [7]. NPs can be synthesized by three different techniques: physical, chemical, biological (Figure 1) [5].

This review explores how biology and physical sciences collaborate to produce metal NPs in a “green” manner for different applications. According to the research, bacteria and plant extracts are novel sources of producing NPs. To achieve this goal, it is essential to use natural resources (such organic means) and the best solvent systems.

## Advantages of green synthesis over synthetic method [8–10]

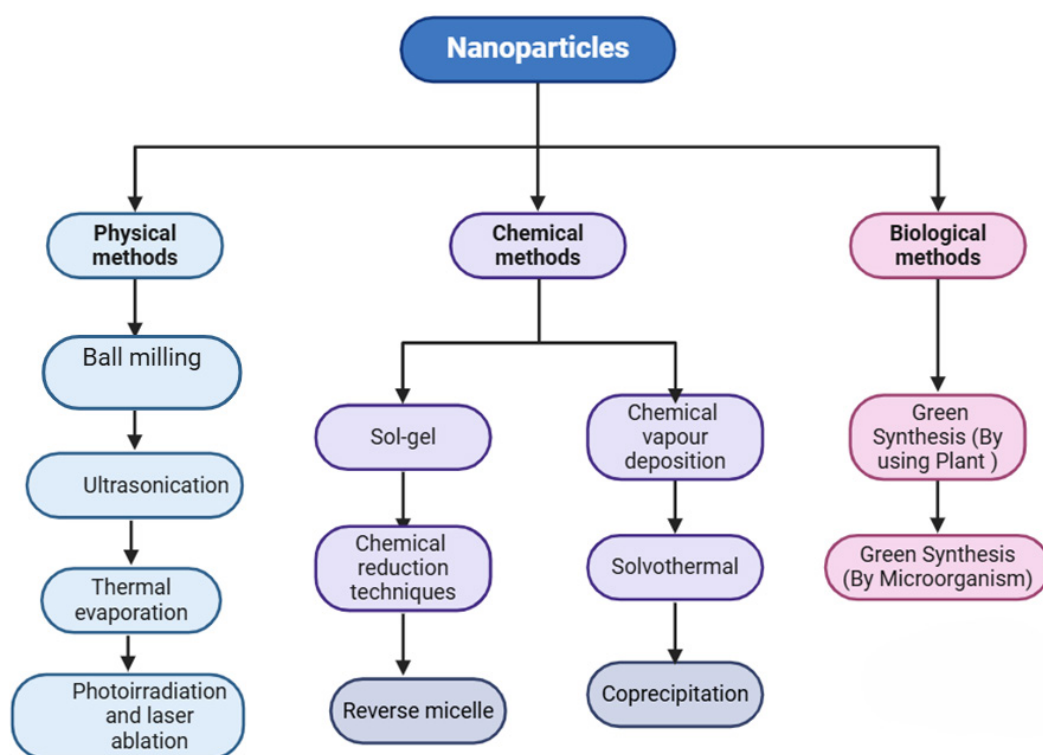
Green synthesis has the following advantages

- 1) By using fewer dangerous components and toxic organic solvents, green chemistry provides substantial economic and environmental benefits over conventional synthetic processes.
- 2) They improve the sustainability in drug synthesis by using renewable resources while preserving the efficacy and quality of medications.
- 3) Green synthesis reduces the use of resources and improves the atom economy.
- 4) This method lowers toxicity and eliminates using dangerous procedures.
- 5) It uses cutting-edge techniques, including water as a solvent and microwave synthesis.
- 6) Real time monitoring can take place.
- 7) During green synthesis, catalysts are utilized sparingly and have a high rate of small-scale reaction.

## Green synthesis

Although the traditional methods have long been used for decades, research has shown that green methods are more outstanding in producing NPs because they are easier to outline, less expensive, and less likely to fail [10]. Numerous resources, including plants and their extracts, algae, fungi, yeast, bacteria, and viruses, can be used to carry out the green synthesis of NPs [11]. Proteins (amino acids), phytochemicals (alkaloids, flavonoids, reducing sugars, polyphenols), and other substances are present in the biomaterials and serve as capping and reducing agents throughout the synthesis process for generating metal NPs from their precursor metal salts [12]. The reduction of the metal precursor to its subsequent NPs may be initially confirmed by observing the color shift of the colloidal solution [9, 10]. Many biotechnological uses, such as bioremediation and bioleaching, have been created since bacteria can interact with, extract, and collect metals from their surroundings [3].

Because of lipid based amphipathic membrane, they can interact with their environment, promote variety of oxidation-reduction reactions, and allow biochemical transformations. Utilizing plants as opposed to other environmentally beneficial biological systems, such as bacteria and fungus, such doing away with costly and time-consuming isolation and processing techniques [13]. Use of plants and their extracts is more safer and efficacious for the production of NPs than other biological systems of producing NPs [14]. Summary of concepts and principles behind the green chemistry are shown in Figures 2 and 3. Different metallic precursors used for the synthesis are given in Figure 4. Finally, Table 1 pres-



**Figure 1.** Techniques used to synthesize NPs

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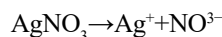
ents the differences between synthesis of NPs by green chemistry and classical chemistry. The major advantage of green chemistry over classical chemistry is that green chemistry is more eco-friendly

### Chemical transformations during green synthesis

The primary ingredients of AgNPs green synthesis are silver metal ion solution (0.1-10 mM) and a reducing biological agent. This eco-friendly method avoids the use of toxic chemicals [15]. Reaction should be completed at room temperature to mild heating and pH 7–10 is optimal for many systems. The green synthesis of AgNPs included the reduction of silver ions ( $Ag^+$ ) to elemental silver ( $Ag^0$ ) using plant extracts, microbes, or other biological agents, which is followed by agglomeration into clusters. These clusters eventually form metallic colloidal silver particles [16]. In most cases, the reducing agents or other constituents present in the cells act as stabilizing and capping agents. This process includes the following steps [17].

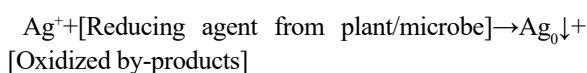
a. Silver salt dissociation:

In the first step, silver nitrate is dissociated into silver ion.

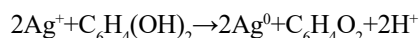


b. Reduction of  $Ag^+$  to  $Ag^0$  by phytochemicals:

In the presence of reducing agent like plant extract, microbes, or biomolecules,  $Ag^+$  is reduced to  $Ag^0$ .



Example is using a polyphenol like catechol.

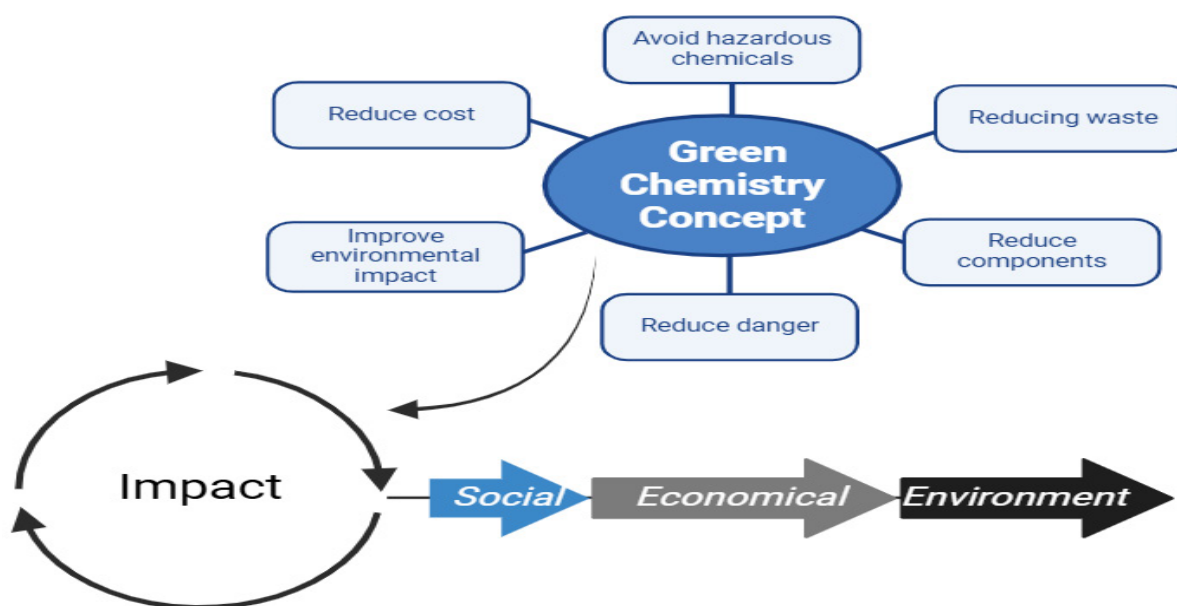


c. Nucleation and growth:

In this step, reduced  $Ag^0$  atoms are nucleated to form small clusters and then these clusters grow into NPs.

d. Stabilization (capping):

At the end, biomolecules (like proteins, terpenoids, etc.) cap the NPs to prevent aggregation.



**Figure 2.** Concept of green chemistry

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### Synthesis of metallic NPs using bacteria

Numerous bacteria have demonstrated the capacity to synthesize metallic NPs; each has specific pros and cons. Critical metals need to enter the cytoplasm through the cell wall (extracellular and intracellular) [18]. Because of their capacity to reduce metal ions, bacteria are excellent options for creating NPs. Prokaryotic and actinomycetes bacteria have been used extensively in the synthesis of metal/metal oxide NPs [19].

Bacteria can participate actively in creating NPs, serve as a bioscaffold for mineralization, or function as a biocatalyst for the synthesis of inorganic materials. During the incubation period, bacteria in broth medium can produce extracellular or intracellular nanomaterials [10]. Bacterial species with different morphologies and the internal and exterior environment of a cell frequently affect the crystalline and non-crystalline phases of particle creation [20, 21].

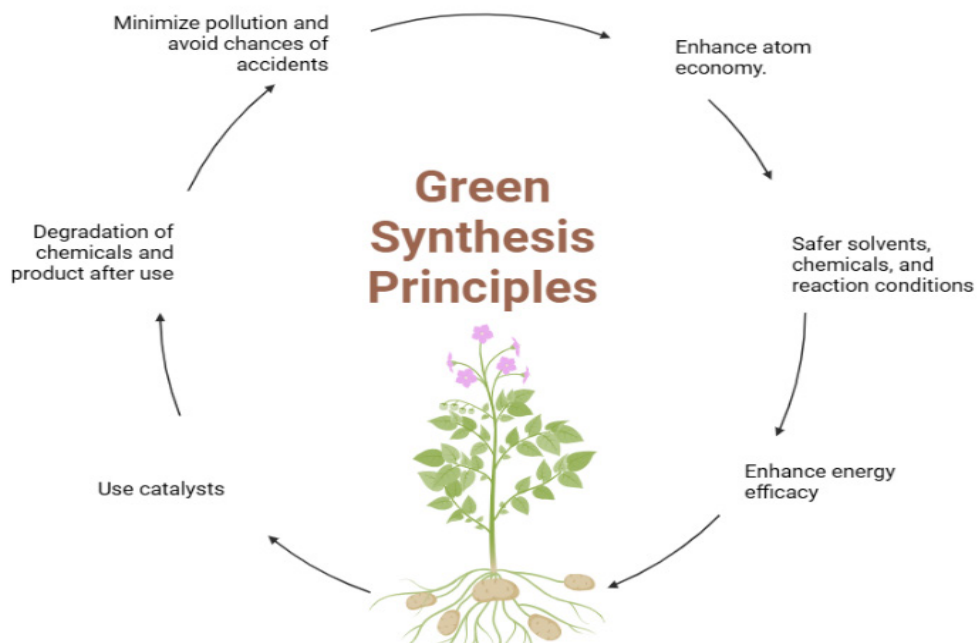
Shivaji et al. synthesized AgNPs using bacterial culture that remain stable for 8 months in dark. Bacteria used in this experiment were *Bacillus indicus*, *B. cecembensis*, *Arthrobacter kerguelensis*, *A. gangotriensis*, *P. antarctica*, *P. proteolytica*, and *P. meridiana*. Created NPs were bactericidal [22]. Sharma et al. synthesized gold NPs using *Marinobacter pelagius* [23]. Tiwari et al. manufactured copper NPs using copper-resistant *B. cereus*. Synthesized NPs shows antimicrobial effects [24]. Hasan et al. synthesized iron nanoparticle using *B. proteolyticus* UPMC1508. Created NPs were bactericidal and anti-

cancer [25]. Liu et al. synthesized selenium NPs using *B. paramycoides*. Created NPs shows anti-bacterial and anti-oxidant properties [26] (Table 2). Figure 5 shows the process of NPs synthesis using bacteria.

### Synthesis of NPs using fungi

Fungi are considered good candidates because they can produce monodisperse NPs with highly defined dimension, various chemical compositions and sizes. Also, fungi release greater quantity of proteins that lead to a higher level of nanoparticle production [66]. Fungi can produce various compounds with different applications. Over 6400 bioactive chemicals are produced by ascomycetes, imperfect fungi, and other microscopic filamentous fungi [67]. Fungi are at the forefront of research for the production of biological metal NPs because of their tolerance and capacity for metal biomagnification [68-73]. The ability to synthesize enormous amounts of proteins and enzymes, some of which may be utilized for the quick and sustainable production of NPs, gives fungi an edge over other microbes [67].

Raut et al. synthesized silver NPs using fungi, such as *Cladosporium cladosporioides*, *Penicillium chrysogenum*, and *Purpureocillium lilacinum*. Created NPs shows anti-microbial NPs [72]. Iranmanesh et al. synthesized gold NPs using 12 fungi; out of 12 fungi, 8 could successfully synthesize gold NPs. Further out of 8 fungi, 3 are investigated: *Fusarium oxysporum*, *Aspergillus flavus*, *Rhizoctonia solani*, and *Verticillium dahliae* [72]. Kamal et al. synthesized iron NPs using fungi,



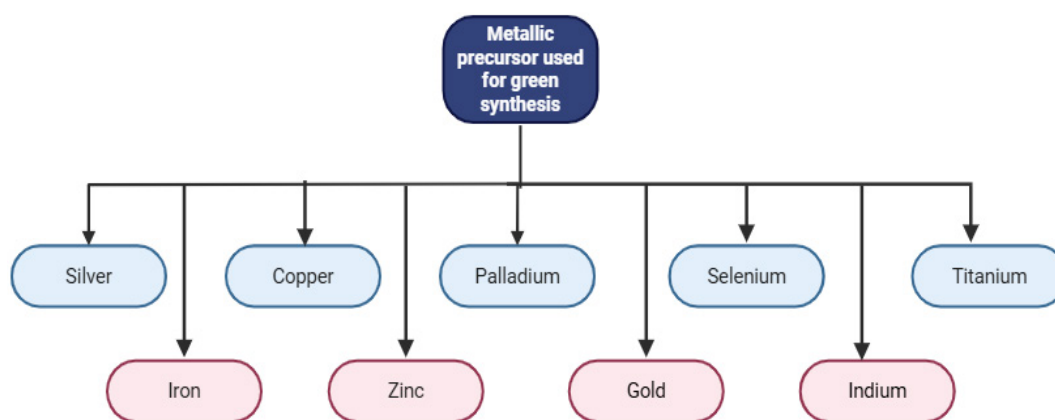
**Figure 3.** Principles of green chemistry

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ie, Daedalea mushroom. It is first time that iron NPs are synthesized by using mushroom. Created NPs were analyzed against the pathogenic fungus *Aspergillus niger* [74]. Fatima and Wahid synthesized copper NPs using fungi *Schizophyllum commune*. Created NPs were analyzed against multidrug-resistant organisms (MDROs) like *Escherichia coli*, *Salmonella abony*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* [75]. Hussein et al. synthesized selenium NPs by using 4 different fungi: *Aspergillus quadrilineatus*, *Aspergillus ochraceus*, *A. terreus*, *Fusarium equiseti*. Synthesized NPs were antibacterial and anti-fungal [76] (Table 3). Figure 6 shows the process of synthesized of NPs using fungi.

### Synthesis of NPs using plant and their extracts

Although the capacity of plant extracts to reduce metals has been known since the early 1900s, little is known about the specifics of the reducing chemicals at play. Compared to whole plant tissue, using plant extracts to make NPs is simpler. Plant extract-mediated synthesis is a growing area of interest [25]. These days, plant extracts serve as capping and reducing agents throughout the nanoparticle manufacturing process, which is way better than microbial, chemical synthesis [109-114].



**Figure 4.** Metallic procedure used for the synthesis of metallic NPs

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**Table 1.** Differences between synthesis of nanoparticles by green chemistry and classical chemistry

Classical Chemistry	Green Synthesis
Utilizing organic solvents and reagents	Utilizing more secure solvents and reagents
Developing potentially harmful NPs	Developing NPs that are likely to be harmless
Synthesis that may pose risk	Safer synthesis methods
Inefficient atomic economy	Higher atomic efficiency
Significant waste build-up	Mitigating the build-up of waste
Utilizing non-renewable raw materials	Utilizing more secure solvents and reagents

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Zahir et al. synthesized silver NPs using aqueous plant extract of *Euphorbia prostrata*. Created NPs were analyzed with pesticidal activity against *Sitophilus oryzae* L [115]. Hazarika et al. synthesized palladium NPs using leaf extract of *Garcinia pedunculata* Roxb. Created NPs were analyzed with anti-microbial activity *Cronobacter sakazakii* strain AMD04 [116]. Majumdar et al. synthesized gold NPs by using leaf extract of *Acacia nilotica* (Babool). Created NPs were analyzed with catalyst property which is used to reduce 4-nitrophenol to 4-aminophenol [117]. Vasantharaj et al. synthesized iron NPs using *Ruellia tuberosa* (RT) leaf extract. Created NPs were analyzed with potent anti-bacterial activity against *K. pneumoniae*, *E. coli* and average anti-bacterial activity against *S. aureus* [118]. Alao et al. synthesized copper NPs using ethanolic extract of *Kigelia africana* fruit. Created NPs were analyzed with anti-microbial activity against *E. coli*, *Shigella* sp., *S. aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* [119] (Table 4). Figure 7 shows the synthesis of nanoparticles using plant and their extract.

### Characterization of metallic NPs [164-178]

Characterization of NPs can be done using ultraviolet (UV) (UV-Vis spectroscopy), Fourier transforms infrared spectroscopy (FT-IR), scanning electronic microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), and X-ray diffraction

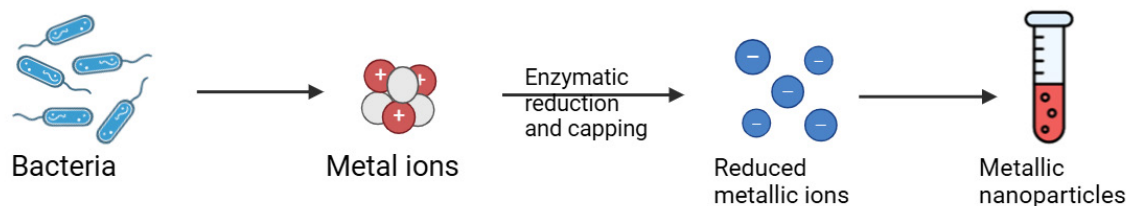
(XRD). Figure 8 shows a summary of techniques used for the characterization of metallic NPs.

UV-Visible: Examining the optical properties of NPs requires the use of UV-visible absorption spectroscopy. This method makes it easier to analyze the size of NPs and enables the quantitative evaluation of their creation. In essence, it entails examining a sample's reaction to electromagnetic waves having wavelengths between 190 and 700 nm.

FT-IR: FT-IR spectroscopy is used to get information about various functional groups based on the peak positions observed in the spectrum. Additionally, this analysis may provide information about the stability and capping of NPs.

SEM: It is used to investigate NPs. The synthesised NPs' size, shape, morphology, and distribution are all ascertained using this analytical method. The morphological structural alterations before and after the therapy are assessed using the SEM analysis.

TEM: When compared to SEM, TEM has two important advantages: It delivers higher resolution and permits more thorough analytical examinations. The necessity of a high vacuum environment, the need for a very tiny sample size, and the time-consuming nature of sample preparation—all of which are critical for TEM—are disadvantages, though.

**Figure 5.** Synthesis of NPs using bacteria (Biorender.com)**PBR**

**Table 2.** Synthesis of NPs by using bacterial and their applications

Species	NPs	Size (nm)	Applications	Ref.
<i>Streptomyces griseoplanus</i>		19-20	Anti-fungal	[25]
<i>Bacillus brevis</i>		41-68	Anti-bacterial	[26]
<i>Pseudomonas sp.</i>		20-70	Anti-bacterial	[27]
<i>Ochrobactrum anthropi</i>		38–85	Anti-bacterial	[26]
<i>Pantoea ananatis</i>	Silver	8.06-91.31	Anti-bacterial	[28]
<i>Bacillus endophyticus</i>		5	Anti-microbial	[29]
<i>Massilia sp. MAHUQ-52</i>		15-55	Anti-microbial	[30]
<i>Paenarthrobacter nicotivanovans</i>		13-27	Anti-microbial	[30]
<i>Exiguobacterium mexicanum PR 10.6.</i>		5-40	Anti-bacterial	[31]
<i>P. haeundaensis</i>		20.93±3.46	Anti-proliferative and Antioxidant	[32]
<i>Streptomyces sp.</i>	Gold	5-50	Anti-malaria	[33]
<i>Streptomyces hygrosopicus</i>		10-20	Anti-bacterial	[11]
<i>Streptomyces viridogens</i>		18-20	Anti-bacterial	[11, 34, 35]
<i>Xanthomonas campestris</i>		20-80	-	[36]
<i>Actinomycetes</i>		30-60	-	[37]
<i>E. coli</i>	Iron	18±2	Anticoagulant	[38,39]
<i>B. cereus</i>		29	-	[37]
<i>Ralstonia sp. SK03</i>		1.2-2	Treatment of hepatocarcinoma	[40]
<i>Streptomyces sp.</i>		80-180	Anti-bacterial	[41]
<i>Pseudomonas fluorescens</i>	Copper	-	Anti-microbial	[42]
<i>B. cereus</i>		-	Anti-microbial	[43]
<i>Serratia</i>		-	Anti-microbial	[44]
<i>Streptomyces sp.</i>		-	Anti-microbial	[45]
<i>Actinomycetes</i>		61.7	Anti-bacterial	[46]
<i>Priestia megaterium</i>		57.73	Anti-tumor	[47]
<i>Pantoea agglomerans</i>			Anti-oxidant	[48]
<i>P. aeruginosa ATCC 27853</i>		96	Anti-oxidant and dietary supplement	[49]
<i>Bacillus subtilis BSN313</i>	Selenium		Anti-bacterial and anti-oxidant	[50]
<i>B. cereus</i>		120	Prevent malignancies	[51]
<i>Enterococcus faecalis</i>		29-195	Antibacterial	[52]
<i>E. coli ATCC 35218</i>		100-183	Dietary supplement	[20]
<i>Desulfovibrio desulfuricans</i>	Palladium		Biomanufacturer of novel targeted catalysts	[53]

Species	NPs	Size (nm)	Applications	Ref.
<i>Pseudomonas putida</i> (DSM6125)		3-30	-	[54]
<i>Paracoccus denitrificans</i> (DSM413T)		3-30	Catalytic activity	[51]
<i>Clostridium butyricum</i> (LMG1217)		-	-	[21]
<i>Citrobacter braakii</i> (ATCC6750)		-	-	[21]
<i>Enterococcus faecium</i> (PhIP-M1-a)	Palladium	-	-	[55]
<i>E. coli</i> (ATCC25922)		-	Chemotherapeutic agents	[56]
<i>Shewanella oneidensis</i> MR-1		6.2	-	[57]
<i>Shewanella oneidensis</i> ATCC700550		5-10	-	[58]
<i>Aeromonas hydrophila</i>		57.72	-	[59]
<i>Bacillus licheniformis</i>	Zinc	200	-	[60]
<i>Lactobacillus sporogenes</i>		5-15	-	[61]
<i>Rhodococcus pyridinivorans</i>		100-120	-	[62]
<i>Aeromonas hydrophila</i>		40.50	Anti-bacterial	[63]
<i>Bacillus mycoides</i>	Titanium	40-60	-	[64]
<i>Lactobacillus</i>		24.63	-	[65]

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XRD: Materials' atomic structures can be examined using XRD. This method is useful for figuring out a substance's qualitative and quantitative properties. The size and structure of crystalline NPs are identified and confirmed using XRD analysis. To determine the particle size of nanomaterials, XRD data are subjected to the Debye–Scherrer formula, which links the width of the Bragg reflection to the subsequent equation:  $K\lambda/\beta \cos \theta = d$ . The Scherrer constant,  $K$ , the X-ray wavelength,  $\beta$ , the full width at half maximum, diffraction angle (half of the Bragg angle) connected to the lattice plane, and particle size,  $d$ , which is measured in nanometers (nm), are all represented in this equation.

DLS: Small particle size and distribution may be examined with DLS on a scale ranging from submicron levels to 1 nm. This method depends on how light and NPs interact. Narrow size distributions, particularly those between 2 and 500 nm, may be measured using it.

### Biodistribution of NPs

Biodistribution is a technique to determine whether the compound of interest is distributed throughout the body of animal or human and how long it stays in tissue or the body.

Determining the biodistribution of the NPs after in vivo treatment in humans and animals is a crucial step in the translational evaluation of nanomedicines. Several meth-

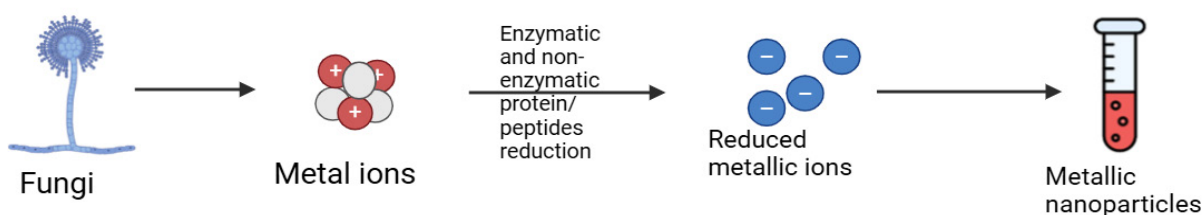


Figure 6. Synthesis of NPs using fungi (Biorender.com)

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**Table 3.** Synthesis of NPs by using fungi and their applications

Species	NPs	Size [nm]	Applications	Ref.
<i>Macrophomina phaseolina</i>		-	Anti-bacterial	[8]
<i>C. cladosporioides</i>		-	Anti-bacterial	[73]
<i>Fusarium verticillioides</i>		-	Anti-bacterial	[74]
<i>Alternaria</i> sp.		-	Anti-fungal	[75]
<i>Pleurotus cornucopiae</i> var.		-	Anti-fungal	[76]
<i>Pleurotus djamor</i> var. roseus		-	Anti proliferative	[72]
<i>Sclerotinia sclerotiorum</i> MTCC8785	Silver	-	Anti-bacterial	[77]
<i>Colletotrichum gloeosporioides</i>		-	Anti-proliferative	[78]
<i>P. chrysogenum</i>		-	Anti-microbial	[79]
<i>Penicillium italicum</i>		-	Anti-bacterial and anti-fungal	[80]
<i>Arthroderma fulvum</i>		-	Anti-fungal	[81]
<i>Aspergillus versicolor</i>		-	Anti-fungal	[82]
<i>Fusarium oxysporum</i>		30-35	Anti-bacterial	[83]
<i>A. flavus</i>		-	Anti-cancer	[84]
<i>R.solani</i>	Gold	10-100	Anti-bacterial and anti-fungal	[84, 85]
<i>Fusarium Solani</i>		40-45	Anti-cancer	[86]
<i>A. terreus</i>		10-16	Anti-bacterial, Anti-fungal, Anti-oxidant	[87]
<i>Alternaria alternata</i>		9±3	Anti-bacterial	[88]
<i>A. flavus</i>		-	Anti-microbial	[89]
<i>A.niger</i>	Iron	10-30	Anti-fungal	[70]
<i>Cladosporium herbarum</i>		10-30	Anti-fungal	[70]
<i>Daedalea</i> mushroom		16.8	Anti-Fungal	[90]
<i>P. chrysogenum</i>		10-30	Anti-fungal	[91]
<i>Aspergillus fumigatus</i>		6	Anti-bacterial	[91]
<i>A. niger</i> (Tiegh)		-	Anti-cancer, anti-diabetic, anti-microbial	[92]
<i>Aspergillus oryzae</i>	Copper	55	Anti-microbial	[93]
<i>Botrytis cinerea</i>		-	Anti-fungal	[94]
<i>Fusarium oxysporum</i>		84-130	Anti-bacterial	[69]
<i>Hypocrea lixii</i>		24.5	Anti-microbial	[95]
<i>Myrothecium gramineum</i>		-	Anti-fungal	[96]
<i>Yarrowia lipolytica</i>	Selenium	110	Anti-microbial and anti-oxidant	[10]

Species	NPs	Size [nm]	Applications	Ref.
<i>A. flavus</i>			Antiviral, anticancer, anti-biofilm, and antibacterial fields	[97]
<i>Penicillium citrinum</i>			Antioxidant, anti-microbial, anticancer	[98]
<i>Candida albicans</i>	Selenium		Anti-fungal	[99]
<i>Aspergillus oryzae</i>		55	Anti-microbial	[100]
<i>Penicillium verhagenii</i>		25-75	Anti-microbial	[101]
<i>Agaricus bisporus</i>	Palladium	13-18	Anti-bacterial, anti-inflammatory, anti-oxidant	[102]
<i>A. terreus</i>	Zinc	54.8-82.6	Anti-bacterial and anti-cancer	[103]
<i>Candida albicans</i>		25	-	[104]
<i>Aspergillus eucalypticola</i> SLF1		33	Anti-microbe, anti-oxidant	[105]
<i>A. niger</i> DS22	Platinum	10.4-45.8	Wound healing activity	[106]
<i>A. flavus</i>		62-74	Anti-bacterial	[107]
<i>Alternaria solani</i>		15	Anti-coagulant, anti-platelet	[108]

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ods are available to assess the biodistribution of NPs. Existing methods of evaluating biodistribution of NPs with their pros and cons are discussed below [149–152].

## Histology

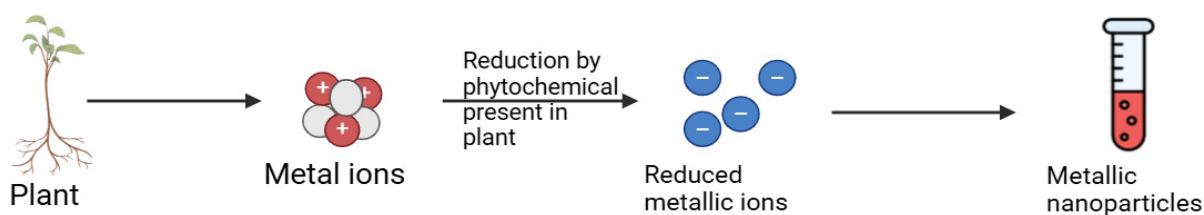
### Pros

1) A comparatively economical approach; 2) typically regarded as a qualitative method for assessing biodistribution; 3) facilitates the examination of extensive tissue samples; 4) enables the investigation of the precise cellular interactions of NPs within tissues, and 5) does not necessitate the use of contrast materials.

### Cons

1) Low-resolution imaging of NPs inside tissue slices is possible with “light and fluorescence microscopy; 2) A small number of tissue slices are typically analyzed

to determine the biodistribution of NPs over an entire organ, 3) this method is time-consuming and labor-intensive; 4) tissue structure and resolution may be harmed by the freezing procedure used for cryostat sectioning, especially when light microscopy is being employed; 5) human mistake is a possible while preparing and analyzing slides; 6) It might be difficult to distinguish between particular cell types and NPs in tissue slices; 7) labeling NPs with fluorescent dyes for fluorescence imaging of histological sections may change the NPs' ‘physicochemical’ characteristics and impact how they behave in vivo, and 8) when fluorescently tagged, NPs are exposed to light during the in vivo injection procedure, photobleaching may happen, which might cause problems with tissue harvesting and processing.



**Figure 7.** Shows the synthesis of NPs using plant and their extract ([Biorender.com](https://www.biorender.com))

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**Table 4.** Synthesis of NPs by using plant and their extract and their applications

Species	NPs	Size (nm)	Applications	Ref.
<i>Artemisia vulgaris</i> (leaves extract)		25	Anti-bacterial	[115]
Aloe vera		20	Anti-bacterial	[116]
<i>Pseuderanthemum reticulatum</i> (aqueous leave extract)		17.83	Anti-oxidant and Anti-aging	[117]
<i>Moringa</i> leaves		<22	Anti-bacterial	[118]
<i>Juniper</i> leaves	Silver	<22	Anti-fungal	[118]
<i>Artocarpus heterophyllus</i> Lam (seed)		10.78	Anti-bacterial	[119]
<i>Ocimum sanctum</i> (Tulsi)			Anti-microbial	[120]
<i>Artemisia nilagirica</i>		70-90	Anti-bacterial	[121]
<i>Boerhavia diffusa</i>		25	Anti-bacterial	[122]
<i>Mentha piperita</i>		90	Anti-bacterial	[123]
Banana peel extract		300	Anti-microbial	[124]
<i>Hibiscus cannabinus</i>		13	Anti-bacterial	[125]
<i>Curcuma pseudomontana</i>	Gold	20	Anti-bacterial and anti-inflammatory	[126]
<i>Solanum nigrum</i>		50	Anti-bacterial	[127]
<i>Nerium oleander</i>		2-10	Anti-oxidant	[128, 129]
<i>Eucalyptus</i>		80	Anti-bacterial, anti-oxidant	[130, 131]
<i>Tridax procumbens</i>		80-100	Anti-bacterial	[132]
<i>Dodonaea viscosa</i>		50-60	Anti-bacterial	[132]
<i>Lawsonia inermis</i> and <i>Gardenia jasminoides</i>	Iron	-	Anti-bacterial	[133]
<i>Spinacia oleracea</i>		-	Anti-bacterial	[134]
Cabbage		12.99-22.72	Anti-bacterial	[132]
<i>Lawsonia inermis</i> and <i>Gardenia jasminoides</i>		-	Anti-bacterial	[135]
<i>Ocimum sanctum</i>		-	Anti-bacterial	[136]
<i>Eucalyptus</i>		-	Anti-microbial	[41, 137]
<i>Ananas comosus</i>		-	Anti-microbial	[138]
<i>Vitis vinifera</i>		3-6	Anti-microbial	[139]
<i>Punica granatum</i>	Copper	40	Green peach aphid repellent	[140]
<i>Azadirachta indica</i> , <i>Hibiscus rosa-sinensis</i> , <i>Murraya koenigii</i>		-	Anti-oxidant and anti-cancer	[141]
<i>Brassica oleracea</i> var <i>acephala</i>		-	Anti-microbial, anti-cancer and anti-oxidant	[142]
<i>Beta vulgaris</i> L		-	Anti-bacterial and anti-cancer	[45]
Green tea		-	Anti-biotic and anti-bacterial	[143]

Species	NPs	Size (nm)	Applications	Ref.
Hawthorn		113	Anti-tumor	[144]
Ginger (extract)		-	Anti-bacterial	[115]
<i>Withania somnifera</i>		-	Anti-bacterial, anti-oxidant, and antiproliferative	[145]
Aloe vera		7-48	Chemopreventive agent in cancer diagnosis	[146]
<i>Allium sativum</i>	Selenium	8-52	Anti-bacterial	[147]
<i>Pelargonium zonale</i>		50	Anti-bacterial	[148]
<i>Emblica officinalis</i>		-	Anti-microbial	[149]
<i>Clausena dentata</i>		46.32-78.88	Mosquito repellent	[150]
<i>Diospyros montana</i>		4-16	Anti-bacterial and anti-fungal	[150]
<i>Moringa oleifera</i>		-	Anti-bacterial	[111]
<i>G. pedunculata Roxb</i>		-	Anti-bacterial	[152]
<i>Phoenix dactylifera</i>	Palladium	13-21	Anti-bacterial	[153]
<i>Filicium decipiens</i>		2-22	Anti-bacterial	[154]
<i>Gardenia jasminoides</i> Ellis		3-5	Anti-oxidant	[150]
<i>Moringa oleifera</i>		27±2	Anti-bacterial	[155]
<i>Aloe vera</i> (Liliaceae)		18	-	[156]
<i>Azadirachta indica</i> (Meliaceae)		9.6-25.5	-	[157]
<i>Coptidis rhizoma</i>	Zinc	2.9-25.2	-	[158]
<i>E. crassipes</i> (Pontederiaceae)		32-36	-	[159]
<i>Ocimum basilicum</i> L. var.		50	-	[160]
<i>Aloe vera</i> (L.) <i>Burm. f.</i>		60	-	[161]
<i>Azadirachta indica</i> A. Juss.	Platinum	124	Photocatalytic activity	[158]
<i>Citrus sinensis</i> (L.) Osbeck		19	-	[162]
<i>Dandelion</i>		-	-	[163]

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### Electron microscopy

#### Pros

1) Able to provide thorough information at very high magnification about the biodistribution of NPs; 2) facilitates the observation of nanoparticle accumulation within cells and their specific localization in cellular organelles; 3) typically regarded as a semi-quantitative approach, and 4) this approach is mostly used to evaluate the cellular interaction of NPs in vitro; just a few

research studies use it to examine the biodistribution of NPs following in vivo delivery.

#### Cons

1) A more costly method compared to conventional histology; 2) inability to assess large tissue samples; 3) this technique is labor-intensive; 4) typically, a small number of extremely thin tissue slices are analyzed to determine the distribution of NPs throughout an entire organ; 5) a relatively large quantity of NPs must be consumed; 6) to accurately detect the nanomaterial within tissues and

cells, another identification technique are needed; 7) the application of high-voltage electron beams may have an impact on the characterisation of soft materials; 8) images may have burn-in regions, which might result in artifacts, and 9) not all nanoparticle kinds may be suitable for the sample preparation method.

### Liquid scintillation counting (LSC)

#### Pros

1) A technique that is sensitive, specific, and quantitative, and 2) LSC can assess the biodistribution of NPs at the level of organs or tissues.

#### Cons

1) This approach can be quite demanding, particularly due to the requirement to process and dissolve the collected tissues before conducting LSC analysis; 2) it may not accurately represent the biodistribution of the entire organ if only a small segment is sampled for LSC; 3) LSC provides little information about the precise cellular interactions or the buildup of NPs in the tissues, and 4) the cocktail used, together with variables like sample composition, volume, temperature, and the counting device employed, all affect the data's quality and consistency.

### Measurement of drug concentration in tissues

#### Pros

1) A numerical evaluation of biodistribution that may be used to analyze whole or partial tissue samples; 2) it can serve as a supplementary quantitative tool to reinforce the biodistribution findings obtained through qualitative methods, and 3) using contrast agents to enhance imaging outcomes, integrating imaging molecules into NPs, or being exposed to ionizing radiation are not necessary for this method.

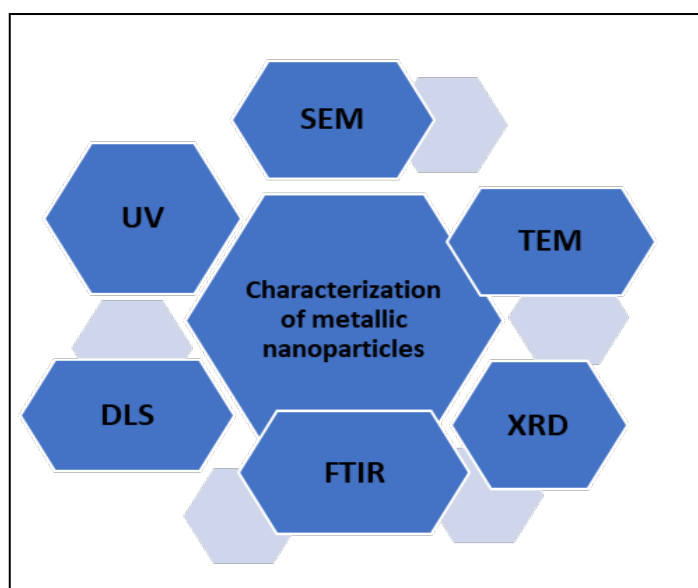
#### Cons

1) Assessing the payload's biodistribution is the main objective of this indirect method; 2) if the drug separates from the NPs too quickly after in vivo delivery, it might, however, provide conflicting findings; 3) the quality of tissue preparation and the extraction procedure, which may be both time-consuming and tedious, have a major impact on the accuracy of drug concentration readings, and 4) additionally, this method does not offer insights into the real-time biodistribution at various time intervals in animal subjects.

### In vivo optical imaging

#### Pros

1) A straightforward and non-invasive method that is easy to implement; 2) quick image capture durations; 3) eliminates the need for exposure to ionizing radiation;



**Figure 8.** Techniques used for characterization of metallic NPs

4) allows for real-time imaging and can be conducted at various time intervals; 5) it is possible to assess the biodistribution of NPs at the tissue or organ level; 6) the resulting pictures typically exhibit high sensitivity along with improved spatial and temporal resolution, and 7) This approach is typically thought of as a qualitative evaluation of biodistribution.

#### Cons

1) Less than 1 cm of tissue can be penetrated, and as tissue depth grows, it may become less effective; 2) Comparatively lacks the spatial resolution of CT and MRI imaging methods; 3) fluorophores can be used to mark NPs, changing their physicochemical properties and in vivo behavior; 4) numerous fluorophores are prone to photobleaching during imaging procedures, which can diminish their sensitivity; 5) because tissue autofluorescence is a significant obstacle that might impede the interpretation of results, fluorophores should have greater signal-to-background ratios; 6) does not yield information about the specific cellular association or accumulation of NPs within tissues, and 7) is unable to visualize individual NPs, focusing instead on measuring overall fluorescence intensity.

#### Computed tomography (CT)

##### Pros

1) Produces reliable, high-quality images that may be used to assess the NPs' biodiversity; 2) it has no restrictions on the invasion of tissue and provides comparatively quick picture capture times; 3) generally considered a qualitative evaluation of biodistribution; 4) identifying nanoparticle's biodistribution at both the tissue and organ levels, and 5) it is possible to track the distribution of NPs throughout the body both in real time and at different intervals of time.

##### Cons

1) Includes being exposed to ionized radiation; 2) Lacks details on the certain cells interactions of NPs; 3) to increase clarity and distinguish between different tissue types, contrast-enhancing imaging substances are frequently required; 4) there may be potential complications when NPs tagged with contrast agents are utilized alongside other contrast imaging agents to enhance anatomical and tissue imaging; 5) the sensitivity of nanoparticle contrast agents is lower than that of other imaging modalities, such as nuclear imaging, and 6) when contrast agents are added to NPs, their physicochemical

properties and behavior within a biological organism can be altered.

#### Magnetic resonance imaging (MRI)

##### Pros

1) Non-invasive and straightforward method; 2) eliminates the risk of exposure to ionizing radiation; 3) generates images with superior spatial resolution in comparison to techniques like optical; 4) improves the ability to distinguish between fat, muscle, water, and soft tissue by providing more contrast for soft tissues compared to CT; 5) unrestricted by the depth of the tissue, penetration is unbounded; 6) capable of assessing NPs distribution at the tissue level, and 7) enables real-time evaluation of NP biodistribution across various time intervals.

##### Cons

1) A comparatively expensive method; 2) exhibits slow image capture and extended post-processing durations; 3) typically necessitates a significantly larger volume of contrast agents due to its potential low sensitivity; 4) not suitable for individuals with metallic implants or devices, and 5) the integration of contrast agents into NPs may modify their physicochemical characteristics and in-vivo performance.

#### Nuclear medicine imaging (PET and SPECT)

##### Pros

1) Capable of quantitative evaluation of biodistribution; 2) it is possible to track the biodistribution of NPs in real time; 3) this method allows for the imaging of biochemical processes; 4) it is not limited by the constraints of tissue penetration; 5) because this extremely sensitive method uses less radiolabels, it has less of an effect on adjacent tissues and cellular activity; 6) PET offers greater sensitivity compared to SPECT and delivers more precise localization of radiation events; 7) by substituting positron-emitting isotopes with naturally existing atoms, PET can better see molecular processes; 8) SPECT is capable of simultaneously imaging many radionuclide probes and is easier to get, and 9) SPECT examination are considerably more cost-effective than PET scans, largely due to the simpler preparation of its radionuclides, easier availability, and generally longer half-lives compared to those used in PET.

## Cons

1) A comparatively expensive method; 2) consists of ionizing radiation exposure; 3) shows sluggish rates of picture capture; 4) because radiolabels deteriorate with time, they are not appropriate for longitudinal investigations; 5) provides a poor spatial resolution and insufficient anatomical details, which often necessitates its combination with other imaging techniques like MRI or CT; 6) because certain NPs may exhibit differing compatibility and imaging performance across various approaches, the choice of radionuclide and radiolabelling methodology needs to be carefully considered; 7) compared to PET, SPECT has a reduced photon detection efficiency and resolution, and 8) PET generally necessitates the use of a cyclotron or generator.

## Conclusion

Since the traditional techniques for creating NPs are expensive and provide highly hazardous products, it is urgent to lower the risk of environmental toxicity from the various substances that are employed in both chemical and physical processes. Green synthesis is one of the alternate techniques that has been found to discover to create NPs. In this review, green synthesis of Au, Ag, Fe, Cu, and Pd NPs with their applications has been discussed. Synthesis of metallic NPs by using green method, i.e, plant and their extract, bacteria, fungi has shown enormous promise in a number of fields, including industry and medicine.

## Ethical Considerations

### Compliance with ethical guidelines

This article is a review paper with no human or animal sample.

### Funding

This review received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Authors' contributions

Conceptualization, study design, data collection, and draft manuscript: Ankush Banalia; Data analysis and data interpretation: Dinesh Puri.

### Conflict of interest

The authors declared no conflict of interest.

## Acknowledgments

The authors are thankful to the School of Pharmacy, **Graphic Era Hill University** for offering guidance and technical.

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