



Green synthesis of metal nanoparticles using plant extracts and their biomedical applications: an overview

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Received: 3 March 2026 / Accepted: 4 June 2026
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Abstract

The green synthesis of metal nanoparticles (NPs), particularly silver (Ag), gold (Au), copper (Cu), and iron (Fe), has attracted considerable attention owing to their inherent biocompatibility, low toxicity, and tunable physicochemical properties. These features make them highly promising for diverse biomedical and environmental applications. In this study, we investigate the synthesis of these four metallic NPs using environmentally sustainable reduction techniques and comprehensively evaluate their biological activities, with particular focus on anticancer and antibacterial properties. Their potential roles in therapeutic interventions, drug delivery systems, and antimicrobial applications are highlighted. In addition, we discuss the growing demand for eco-friendly nanotechnology in food packaging, where such green-synthesized NPs can be effectively employed. Conventional nanoparticle synthesis methods, which rely on synthetic chemicals and physical processes, often pose environmental and health risks. In contrast, the use of plant extracts or metabolites for the synthesis of Ag, Au, Cu, and Fe NPs offers a safer, more economical, and readily accessible alternative, consistent with the principles of sustainable chemistry, which aim to minimise harmful by-products. The notable antimicrobial efficacy and non-toxic nature of these green-synthesized NPs position them as valuable components in sustainable food packaging solutions. Overall, this research emphasizes the importance of environmentally benign synthesis routes for producing nanoparticles with enhanced biological functionalities, thereby contributing to the development of sustainable and efficient strategies in both biomedicine and food technology.

Keywords Green synthesis · Nanoparticles · Silver nanoparticles · Gold nanoparticles · Copper nanoparticles · Iron nanoparticles · Antibacterial activity · Biocompatibility

Introduction

Metal nanoparticles (NPs) have emerged as a significant area of study due to their unique physicochemical properties, which are notably different from those of their bulk counterparts. The characteristics, including improved catalytic performance, optical features, and antimicrobial properties, have spurred significant investigation into their uses across diverse domains, such as healthcare, electronics, and catalysis. Traditional methods for synthesizing NPs often involve harsh chemicals and high energy inputs, raising environmental concerns. In recent years, there has been a growing interest in developing eco-friendly and sustainable approaches for NP synthesis. One such promising approach involves utilizing plant extracts as reducing and stabilizing agents. This review will discuss the various aspects of plant extract-mediated synthesis of metal NPs, including

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synthesis approaches, the role of phytochemicals, characterization techniques, and their biomedical applications. The nanoparticles are classified into organic, inorganic, and carbon-based.

Nanometallic materials, comprising metals and alloys with nanocrystalline grains (5–100 nm), exhibit unique physical and chemical properties stemming from nanoscale dimensions. These properties, such as increased surface area and quantum effects, differentiate them significantly from their bulk counterparts. Consequently, nanometals find widespread applications across diverse fields, including biology, medicine, and engineering. For instance, gold nanoparticles (Au NPs) demonstrate promising applications in enzyme regulation, antimicrobial activity, and muscle relaxation. Silver nanoparticles (Ag NPs) exhibit potent antibacterial properties against gram-positive and gram-negative bacteria. Similarly, iron nanoparticles (Fe NPs) effectively inhibit bacterial growth and offer efficient remediation for matrices contaminated with organic matter, metals, and dyes. Palladium nanoparticles (Pd NPs) also showcase versatility in applications such as dye degradation, antimicrobial activity, and catalysis.

Green synthesis leverages the capabilities of living organisms, such as fungi, algae, bacteria, and plants, to generate nanoparticles. Rich in phytochemicals, plant extracts have emerged as promising sources for nanoparticle synthesis, acting as both reducing and stabilizing agents. This approach aligns with the growing emphasis on sustainable and green technologies. Nanoparticle synthesis can be broadly categorized into two distinct approaches: bottom-up and top-down. The bottom-up approach involves the assembly of nanoparticles from individual atoms or molecules, while the top-down approach involves reducing more extensive materials into nanoscale dimensions.

The synthesis of nano-sized materials constitutes an essential chemical procedure. At present, the preparation of nano-sized materials involves the utilisation of chemical and physical methods. Nevertheless, these options may not represent the most favourable selection owing to their elevated expenses and the possibility of environmental contamination. As a result, there is a pressing need to develop alternatives to the current methods that prioritise environmental sustainability throughout the entire production process. This is particularly relevant for the synthesis of nano-sized materials, a topic that has garnered significant attention from the global research community. The conventional synthesis methods, whether physical or chemical, are typically conducted under very severe conditions. Conversely, biological processes are typically carried out at ambient temperature and pressure, highlighting their simplicity, energy efficiency, and lower toxicity or harm to both humans and the environment [1].

The thermodynamic stability and standard reduction potentials (E^0) of some metals, particularly silver (Ag), gold (Au), copper (Cu), and iron (Fe), are the primary factors that determine which metals are chosen for green synthesis. Phytochemicals, including terpenoids, flavonoids, and polyphenols, serve as the main reducing agents in plant-mediated synthesis. These biomolecules may effectively donate electrons to metal ions with positive or somewhat negative reduction potentials because they have particular redox potentials. Because their reduction is thermodynamically spontaneous ($\Delta G < 0$) when combined with the oxidation of plant phenolics, noble metals such as gold ($E^0 \text{Au}^{3+}/\text{Au}^0 = +1.50 \text{ V}$) and silver ($E^0 \text{Ag}^+/\text{Ag}^0 = +0.80 \text{ V}$) are readily reduced to their zero-valent metallic states at room temperature. In a similar vein, iron and copper have reduction potentials ($E^0 \text{Cu}^{2+}/\text{Cu}^0 = +0.34 \text{ V}$; $E^0 \text{Fe}^{2+}/\text{Fe}^0 = -0.44 \text{ V}$) within the reducing capability of typical plant extracts, yet more reactive than noble metals [2]. On the other hand, highly electropositive metals such as magnesium ($E^0 \text{Mg}^{2+}/\text{Mg}^0 = -2.37 \text{ V}$) and aluminium ($E^0 \text{Al}^{3+}/\text{Al}^0 = -1.66 \text{ V}$) pose serious challenges for green synthesis [3–5]. Their highly negative reduction potentials indicate a strong resistance to reduction in aqueous plant media, often requiring extreme conditions or powerful chemical reductants that deviate from “green” protocols [2]. The green synthesis of metal oxides, such as zinc oxide (ZnO) and titanium dioxide (TiO_2), has become the focus of contemporary research due to constraints in generating zero-valent forms of highly active metals. Instead of pure metallic clusters, the plant extracts in these situations promote the production of stable oxide lattices [6]. This field’s expansion into metal oxide nanostructures offers sustainable pathways to produce functional materials for photocatalysis, antimicrobial coatings, and advanced biomedical sensors.

Nanoparticle synthesis approaches can be broadly categorized into top-down and bottom-up methods. Physical methods, including evaporation–condensation, electrolysis, diffusion, laser ablation, sputter deposition, pyrolysis, plasma arcing, and high-energy ball milling, are classified as top-down approaches, which entail reducing larger materials to nanoscale dimensions. While effective, these methods often have limitations, including low production rates, high energy consumption, and expensive operational costs.

Chemical synthesis methods, encompassing chemical reduction, micro-emulsion/colloidal, electrochemical, and thermal decomposition, represent a widely employed bottom-up approach. Chemical reduction, involving the reduction of metal salt precursors using reducing agents like sodium borohydride, potassium bitartrate, formaldehyde, methoxy polyethylene glycol, and hydrazine, is a widespread technique due to its relatively simple operational requirements. Stabilizing agents such as dodecyl benzyl

sulfate and polyvinyl pyrrolidone are often employed to control particle size and prevent agglomeration. Despite their economic viability for large-scale production, chemical methods usually involve toxic chemicals and generate harmful by-products, raising concerns regarding environmental impact and limiting their applicability in clinical and biomedical settings.

Synthesis approaches for metal NPs

Nanoparticle synthesis primarily employs "bottom-up" and "top-down." Bottom-up approaches, where the catalysts act as reducing agents, build nanoparticles from individual atoms or molecules in a controlled manner. Conversely, top-down approaches, which involve thermal decomposition, mechanical grinding, and sputtering, reduce bulk materials to nanoscale dimensions. While top-down methods can lead to surface defects, bottom-up approaches often offer better control over nanoparticle size and homogeneity. Nanoparticles can be synthesized through various methods, including chemical, physical, and biological approaches. Chemical methods involve reducing agents like ascorbic acid and borohydride to reduce Metal ions in solution. Physical processes, such as arc discharge and sputtering, generally require high energy but offer advantages like narrow size distribution. However, these methods often involve hazardous chemicals and can be energy-intensive. Whereas Biological synthesis uses organisms such as plants, bacteria, or fungi to prepare nanoparticles. Metal nanoparticle synthesis methods are broadly classified into two main categories: top-down and bottom-up approaches, as illustrated in Fig. 1.

Top-down approaches

Top-down approaches to nanoparticle synthesis involve breaking down bulk materials into smaller particles. These methods include mechanical milling, where materials are ground or milled to reduce particle size. Laser ablation utilizes high-energy laser beams to vaporize the target material, forming nanoparticles in the vapor phase. Additionally,

lithographic techniques, including photolithography and electron-beam lithography, can be employed to create patterned structures on a substrate, effectively defining the size and shape of the resulting nanoparticles.

Bottom-up approaches

Bottom-up approaches to nanoparticle synthesis involve building nanoparticles from individual atoms or molecules. These methods include chemical vapor deposition (CVD), where a thin material film is deposited onto a substrate in a gaseous phase. Physical vapor deposition (PVD) also involves depositing thin films in a vacuum. The sol-gel method, another prominent bottom-up approach, consists of hydrolysis and condensation reactions to form a gel, which is subsequently processed to obtain nanoparticles.

General methods for the synthesis of metal nanoparticles

Plant-mediated nanoparticle synthesis commences with the dissolution of a desired metal salt in a suitable solvent, typically water or ethanol, to create a solution. Subsequently, this metal salt solution is introduced to a plant extract. Within this reaction mixture, the plant extract assumes a dual role: as a reducing agent, it facilitates the conversion of metal ions into their elemental form, and as a stabilizing agent, it prevents the aggregation of the newly formed nanoparticles.

The driving force behind this transformation lies within the phytochemicals present within the plant extract. These bioactive compounds, such as flavonoids, terpenoids, and alkaloids, possess inherent reducing properties. Upon interaction with the metal ions, these phytochemicals donate electrons, causing the reduction of the metal ions to their elemental state. This reduction process initiates the formation of minute nuclei, serving as the foundation for subsequent nanoparticle growth. These nascent nuclei then undergo a process of continuous growth and aggregation. Additional metal atoms, generated through further reduction reactions, are incorporated into the existing nuclei, increasing

Fig. 1 Top-down and bottom-up synthesis approaches for the synthesis of metal nanoparticles

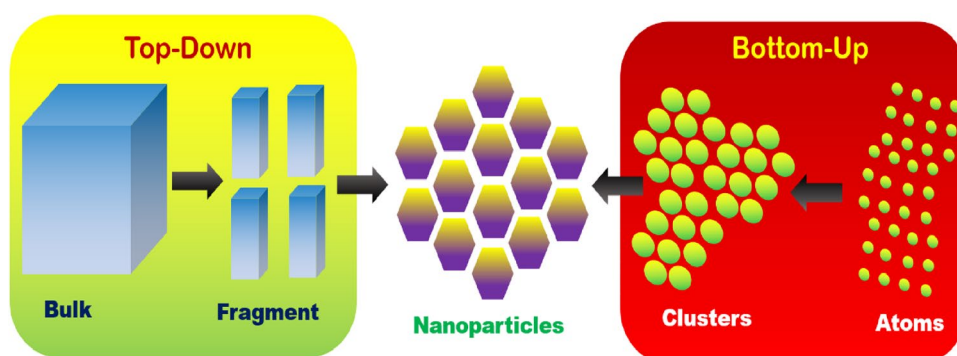


Table 1 Comparison of various synthesis methods for metal nanoparticles

Synthesis Method	Advantages	Disadvantages
Biological	Eco-friendly, cost-effective, diverse sources	Scalability issues, Variability in yield
Chemical	High control over size and shape	Toxic chemicals, Environmental concerns
Physical	Precise control over particle characteristics	High energy consumption, Expensive equipment

their size. Simultaneously, the stabilizing agents within the plant extract interact with the nanoparticle surface, preventing their uncontrolled agglomeration and ensuring the formation of stable, well-defined nanoparticles. This intricate interplay between reduction, nucleation, growth, and stabilization ultimately culminates in the successful synthesis of nanoparticles utilizing the inherent capabilities of plant extracts Table 1.

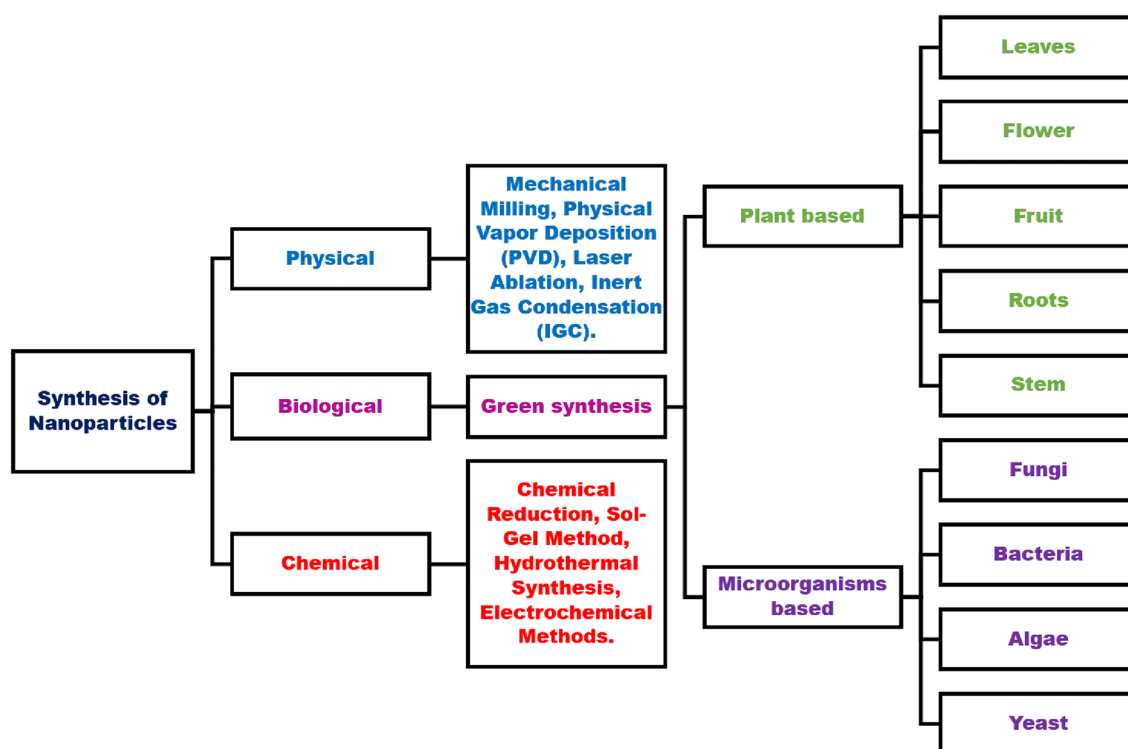
The biosynthesis of nanoparticles has emerged as a dynamic and promising field, encompassing a range of biological methods for synthesizing these materials using living organisms. This approach offers several advantages, including eco-friendliness, cost-effectiveness, and the potential for large-scale production. This review will delve into key subtopics within nanoparticle biosynthesis, providing

an in-depth exploration of relevant details and illustrative examples, as shown in Fig. 2.

Green synthesis of metal nanoparticles

Silver nanoparticles (Ag NPs)

Silver (Ag) is a ductile and malleable element with atomic number 47. It is a little more durable than gold. This element is fundamental to our planet's structure. It exists naturally as a native [7, 8]. Elements can exist as alloys with other metals (e.g., gold) or as minerals (e.g., chlorargyrite and argentite). Silver has four different oxidation states: Ag^0 , Ag^{1+} , Ag^{2+} , and Ag^{3+} . Generally, chemically impermeable elements can react with nitric acid or highly concentrated sulfuric acid to generate soluble silver salts. Although it has good heat and electricity conductivity, its limited use in the electrical sector is owing to its high cost. Silver nanoparticles (Ag NPs) can be synthesized using plant extracts from various sources, such as *Aloe vera*, *Azadirachta indica* (Neem), and *Citrus sinensis* (Orange) [8–10]. Ag NPs, produced with extracts from *Morus alba* L. (80–150 nm), *Panax ginseng* (5–15 nm), *Dolichos lablab* (4–16 nm), *Alternanthera bettzickiana* (5–15 nm), *Thymus vulgaris* (30 nm), *Nauclea latifolia* (12 nm), *Malus domestica* (16 nm), *Cestrum nocturnum* (20 nm), *Reishi Mushroom* (15–22 nm), *Elaeagnus*

**Fig. 2** General methods for the synthesis of nanoparticles

umbellata (40 nm), *Dionaea muscipula* (5–10 nm), *Tagetes erecta* (10–90 nm), and *Garcinia mangostana* (15–20 nm) [9, 11–22] demonstrate diverse applications, including antibacterial, anticancer, antiviral, antimicrobial, antioxidant, and antifungal properties. Figure 3 shows the Synthesis of metal nanoparticles using plant extracts such as *Phyllanthus urinaria*, *Pouzolzia zeylanica* and *Scoparia dulcis*.

Gold nanoparticles (Au NPs)

Gold nanoparticles (AuNPs) are minuscule particles of gold with an atomic number of 79, typically ranging from 1 to 100 nm in diameter. Their unique properties, such as strong optical absorption, surface plasmon resonance, large surface-to-volume ratio, and biocompatibility, make them valuable in various fields [23, 24]. It shows many properties, such as size, surface, and shape. Due to their high reactivity, AuNPs find applications in medicine for drug delivery and cancer therapy, electronics as conductive materials, and

sensing and catalysis. Gold nanoparticles (Au NPs) can be synthesized using plant extracts from sources like *Camellia sinensis* (Tea), *Eucalyptus globulus*, and *Punica granatum* (Pomegranate) [25, 26]. AuNPs synthesized using *Camellia sinensis* (10 nm) for antibacterial purposes [25], *Nigella arvensis* (3–37 nm) exhibiting antibacterial, antioxidant, cytotoxic, and catalytic properties [27], *Viola betonicifolia* leaves for antimicrobial, antioxidant and cytobiocompatible activities [28], *Gelidium pusillum* (12 ± 4.2 nm) for anticancer activity [29], *Hibiscus sabdariffa* (15–45 nm) demonstrating antiacute myeloid leukemia effects [30], *Pimenta dioica* (13 ± 4 nm) for anticancer applications [31], *Croton sparsiflorus* (16.6–17 nm) serving as UV-protection, antibacterial, and anticancer agents [32], *Litsea cubeba* (8–18 nm) for the catalytic reduction of 4-nitrophenol [33], and *Desmodium gangeticum* (16 ± 4 nm) displaying antioxidant properties [26]. Figure 4 shows the *Viola betonicifolia* leaves for synthesis illustration.

Fig. 3 Synthesis of metal nanoparticles using plant extracts. Reproduced/adapted from Ref. [23] under the terms of the Creative Commons Attribution License (CC BY)

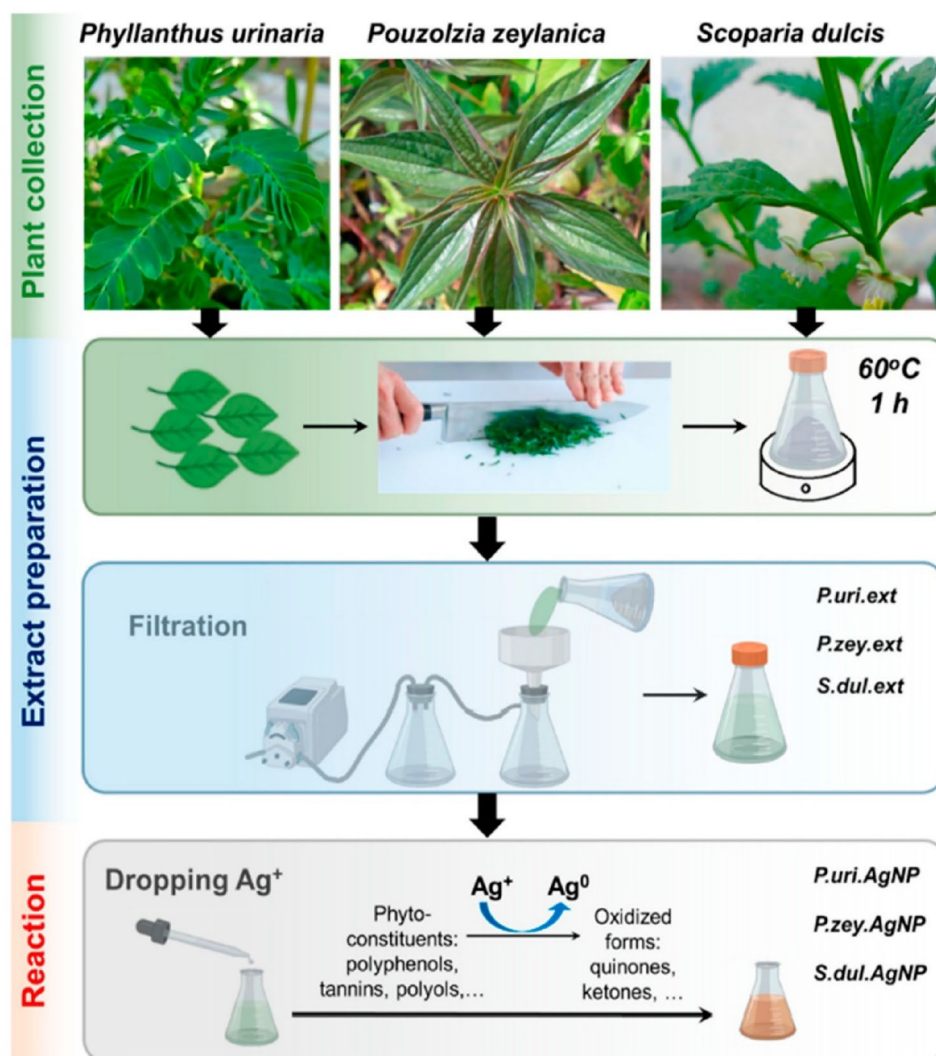
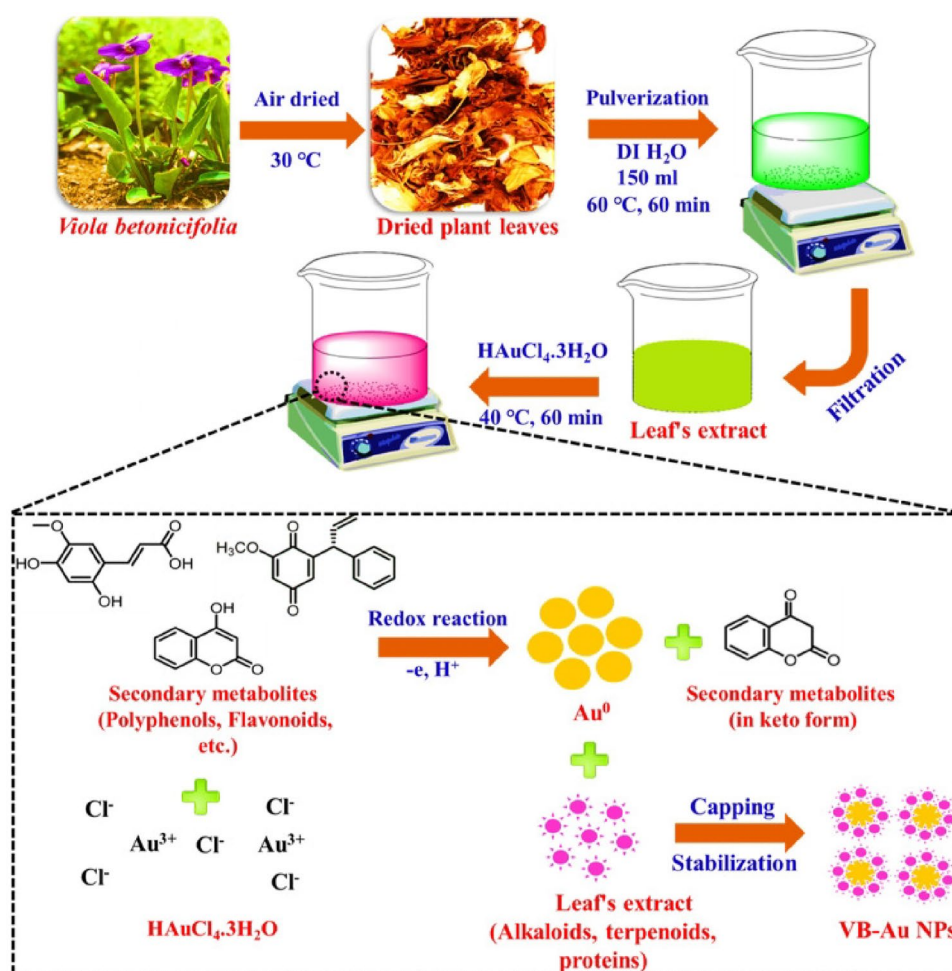


Fig. 4 Schematic illustration of the possible synthesis mechanism for green-synthesized VB-Au NPs using *Viola betonicifolia* leaf extract. Reproduced/adapted from Ref. [28] under the terms of the Creative Commons CC BY-NC 3.0



Copper nanoparticles (Cu NPs)

Copper nanoparticles (Cu NPs) are generated from copper, a light transition metal. This means that they cannot be made directly from basic copper salts. Capping agents, such as surfactants, are critical for controlling particle size [34]. Moses divided the biological creation of copper nanoparticles into two categories: microorganisms and plant extracts [35]. Copper nanoparticles were created using non-toxic chemicals such as L-ascorbic acid [36]. The Cu nanoparticles produced with L-ascorbic acid were less than 2 nm in size. Cu nanoparticles have been created by researchers using plant extracts from the Eucalyptus sp. leaves, Thymus vulgaris L., and G. biloba Linn, as reported in publications by [37–39]. Extracellular proteins found in microbe extracts, such as those from the white-rot fungus (*Stereum hirsutum*), may play an important role in the stabilization and formation of copper nanoparticles [40]. Copper nanoparticles derived from Eucalyptus sp. shown outstanding homogeneity, although their prospective applications were not investigated. Thymus vulgaris L. leaves have a high concentration of phenolic compounds and flavonoids, which help

to reduce copper salts and ensure excellent stability against agglomeration. Flavonoids can release hydrogen atoms or electrons, increasing their antioxidant action. The copper nanoparticles derived from Thymus vulgaris L. leaves also contained bentonite, which contributed to their high level of dispersion. Bentonite/Cu NPs acted as a catalyst, reducing Congo red (CR) and methylene blue (MB) with remarkable efficiency. The bentonite/Cu nanoparticles demonstrated the ability to be recycled five times without losing efficacy. Copper nanoparticles (Cu NPs) was synthesised using aloe Vera flowers, with proteins contained in the aloe flower functioning as a covering on the particles' surfaces, aiding Cu NP creation [41]. Citron juice extraction is simple and cost-effective, producing Cu NPs that inhibit the growth of bacteria like *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. acne*, and *S. typhi*, and fungi such as *F. graminearum*, *F. oxysporum*, and *F. ulmorum* [34]. Psidium guajava L. fruits, rich in vitamin C, are used to synthesize Cu NPs, which are stabilized with Macrogol 6000 to prevent oxidation and exhibit antimicrobial activity against *Staphylococcus aureus* and *E. coli* [42]. Black soya bean extract, containing various biological compounds, is used to synthesize CuO

NPs that inhibit the growth of Hela cells by disrupting the mitochondrial membrane [43]. The sol–gel method using coffee powder and extracts from *Azadirachta indica* leaves and *Callistemon viminalis* flowers is also employed to synthesize CuO NPs [44]. CuNPs have been produced utilizing various plant extracts for multiple uses. Copper nanoparticles (NPs) developed from *Thymus vulgaris* (23–94 nm) are utilized for the breakdown of organic dyes CR MB [39], whilst NPs derived from *Glycine max* (26.6 nm) exhibit activity against HeLa cells [43]. Additional plant sources encompass *Coffea Arabica* (20–60 nm) [45], *Aloe Vera* (40 nm) [41], *Ginkgo biloba* (15–20 nm) for Huisgen (3+2) Cycloaddition [38], *Citrus medica* (10–60 nm) [34], *Malva sylvestris* (5–30 nm) [44], *Psidium guajava* (15–30 nm) [42] demonstrating antibacterial properties, and *Pseudomonas stutzeri* (50–150 nm). *Tribolium castaneum* (10–70 nm) for insecticidal applications, *Stereum hirsutum* (5–20 nm), *Solanum nigrum* (25–35 nm) exhibiting antioxidant properties, *Spinacia oleracea* for the reduction of BOD and COD [46], *Cupressus sempervirens* (9–31 nm) for dye removal, *Vaccinium* spp. (61.1–100.6 nm) for arsenate removal, *Quercus palustris* Münchh. (20–80 nm), *Urtica dioica* (21–71 nm), *Eichhornia crassipes* (20–80 nm), *Camellia sinensis* (20–80 nm) for dye elimination, and *Urtica dioica* for the removal of Cr (VI) [47, 48].

Ferrous nanoparticles (Fe NPs)

Green synthesized iron nanoparticles (Fe NPs) are increasingly being recognized for their potential as agent in environmental remediation. Iron nanoparticles can be synthesized employing conventional methods. Conventional synthesis techniques involved the use of toxic, corrosive, and flammable substances, necessitating specialized apparatus and contributing to increased costs. The biosynthesis of nanoparticles utilizing natural product extracts presents a cost-effective, straightforward, and eco-friendly alternative to conventional synthesis techniques, effectively addressing current challenges in the field. This process employs non-hazardous substances as reductants or capping agents, and it does not produce hazardous waste [49, 50]. Fe NPs serve as an oxidant in the creation of multiwalled carbon nanotubes (MWCNTs) that are combined with a thiophene polymer sheath to form composite nanocables, all while using the cationic surfactant decyltrimethyl ammonium bromide (DTAB) [51]. These nanoparticles were also used to target bacteria that lead to stomach problems, thanks to their impressive antibacterial properties [52]. Nanoparticles, regardless of their simplicity or complexity, possess distinctive physical and chemical characteristics. They are becoming increasingly significant in various fields, including food and health, manufacturing and materials, environmental

applications, electronics, and mechanical industries [53, 54]. It's important to recognize that when it comes to the chemical synthesis of nanoparticles, many of the chemicals, solvents, and substrates involved can be quite toxic and pose risks to both the environment and human health. Additionally, this approach to synthesizing nanoparticles could lead to the creation of harmful byproducts [55]. In this situation, to tackle this challenge, the synthesis of green nanoparticles can be seen as a different method to overcome the limitations associated with using chemicals [56].

Fe NPs have been synthesized using *Lawsonia inermis* and *Gardenia jasminoides* (21 & 32 nm) exhibiting antibacterial activity [57], *Citrus Limon* (3–300 nm) for Cr (VI) removal [58], *Mentha spicata* L (20–45 nm) for the removal of As (III) & As (V) [59], *Passiflora tripartita* var. (19.3–25.3 nm) showing catalytic effect for the synthesis of 2-arylbenzimidazole [60], *Murraya koenigii* (59 nm) for fermentative hydrogen production [61], *Terminalia chebula* (<80 nm) [62], *Carica papaya* (33 nm) for eutrophic wastewater treatment [52], *Psoralea corylifolia* (39 nm) for targeting cancer cells [59], *Ocimum sanctum* (35–60 nm) [63], *Padina pavonicathivy* (10–19.5 nm) [64], *Psidium guajava* (1–5 nm) exhibiting antibacterial activity, *Citrus* (15–80 nm) for antibacterial applications [65], *Malus pumila* (50–100 nm) for decolorization of dye [66], *Camellia sinensis* L (28.5 nm) showing antioxidant properties [67], *Syzygium cumini* (40–52 nm) exhibiting antibacterial, antifungal, and aflatoxin B1 adsorption properties [68], *Juglansregia* (12.6 nm) for cytotoxic assay [69], *Pyrus sinkiangensis* (10–90 nm) for Cr (IV) removal [70], *Cymbopogon citratus* (9±4 nm) for nanotoxicological studies [71], and *Laurusnobilis* (8.03±8.99 nm) exhibiting antimicrobial activity [72] as tabulated in Table 2.

Role of plant extracts in the synthesis of metal NPs

The green synthesis of nanoparticles utilizes plant and animal-derived secondary metabolites, such as alkaloids and flavonoids, as reducing, capping, and stabilizing agents. This eco-friendly approach offers several advantages over conventional methods, including enhanced affordability, reduced environmental impact, and improved biocompatibility. By harnessing the inherent bioactivity of these natural compounds, researchers can create nanoparticles with tailored properties for applications in various fields, such as drug delivery, diagnostics, and environmental remediation [1]. Plant-mediated biosynthesis offers a compelling alternative for nanoparticle production, overcoming the challenges associated with microbial culture maintenance and preservation. This eco-friendly approach leverages the

Table 2 Ag, Au, Cu and Fe nanoparticles and their applications

Metal NPs	Shape /size (nm)	Precursor Material	Application	Ref
Ag	80–150	<i>Morus alba L</i>	Antibacterial	[13]
	5–15	<i>Panax ginseng</i>	Anticancer and antiviral	[14]
	4–16	<i>Dolichos lablab</i>	Antimicrobial and anticancer	[15]
	5–15	<i>Alternanthera bettzickiana</i>	Antimicrobial and anticancer	[16]
	30	<i>Thymus vulgaris</i>	Anticancer and antioxidant	[17]
	12	<i>Nauclea latifolia</i>	Antimicrobial and antioxidant	[18]
	16	<i>Malus domestica</i>	Antimicrobial	[19]
	20	<i>Cestrum nocturnum</i>	Antioxidant and antibacterial	[20]
	15–22	<i>Reishi Mushroom</i>	Antifungal	[21]
	40	<i>Elaeagnus umbellata</i>	Antimicrobial	[9]
	5–10	<i>Dionaea muscipula</i>	Antioxidant	[22]
	10–90	<i>Tagetes erecta</i>	Antimicrobial	[11]
	15–20	<i>Garcinia mangostana</i>	Antimicrobial	[12]
Au	10	<i>Camellia sinensis</i>	Antibacterial	[25]
	3–37	<i>Nigella arvensis</i>	Antibacterial, antioxidant, cytotoxicity, and catalytic	[27]
	12±4.2	<i>Gelidium pusillumappli</i>	Anticancer activity	[29]
	15–45	<i>Hibiscus sabdariffa</i>	Antiacute myeloid leukemia	[30]
	13±4	<i>Pimenta dioica</i>	Anticancer activity	[31]
	16.6–17	<i>Croton sparsiflorus</i>	UV-protection, antibacterial, and anticancer agents	[32]
Cu	8–18	<i>Litsea cubeba</i>	Catalytic reduction of 4-nitrophenol	[33]
	16±4	<i>Desmodium gangeticum</i>	Antioxidant	[26]
	23–94	<i>Thymus vulgaris</i>	Organic dyes CR MB	[39]
	26.6	<i>Glycine max</i>	HeLa cells	[43]
	20–60	<i>Coffea Arabica</i>	—	[45]
	40	<i>Aloe Vera</i>	—	[41]
	15–20	<i>Ginkgo biloba</i>	Huisgen (3+2) Cycloaddition	[38]
	10–60	<i>Citrus medica</i>	Antibacterial activity	[34]
	5–30	<i>Malva sylvestris</i>	Antibacterial activity	[44]
	15–30	<i>Psidium guajava</i>	Antibacterial activity	[42]
	50–150	<i>Pseudomonas stutzeri</i>	—	[35]
	10–70	<i>Tribolium castaneum</i>	Insecticidal	[46]
	5–20	<i>Stereum hirsutum</i>	—	[40]
	25–35	<i>Solanum nigrum</i>	Antioxidant	[48]
	—	<i>Spinacia oleracea</i>	Reduction of BOD & COD	[73]
	9–31	<i>Cupressus sempervirens</i>	Dye removal	[74]
	61.1– 100.6	<i>Vaccinium Spp</i>	Removal of Arsenate	[75]
	20–80	<i>Quercus palustris Münchh</i>	—	[76]
	21–71	<i>Urtica dioica</i>	—	[77]
	20–80	<i>Eichhornia crassipes</i>	—	[47]
	20–80	<i>Camellia sinensis</i>	Elimination of dyes	[78]
	—	<i>Urtica dioica</i>	Removal of Cr (VI)	[57]
	21 & 32	<i>Lawsonia inermis and Gardenia jasminoides</i>	Antibacterial activity	[58]
	3–300	<i>Citrus Limon</i>	Removal of Cr (VI)	[59]

Table 2 (continued)

Metal NPs	Shape /size (nm)	Precursor Material	Application	Ref
Fe	20–45	<i>Mentha spicata L</i>	Removal of As (III)& As (V)	[60]
	19.3– 25.3	<i>Passiflora tripartita var</i>	Catalytic effect For synthesis of 2-arylbenzimidazole	[62]
	59	<i>Murraya koenigii</i>	Fermentative hydrogen production	[61]
	<80	<i>Terminalia chebula</i>	—	[62]
	33	<i>Carica papaya</i>	Eutrophic wastewater	[36]
	39	<i>Psoralea corylifolia</i>	Cancer cells	[43]
	35–60	<i>Ocimum sanctum</i>	—	[63]
	10–19.5	<i>Padina pavonicathivy</i>	—	[64]
	1–5	<i>Psidium guajava</i>	Antibacterial	[65]
	15–80	<i>Citrus</i>	Antibacterial	[79]
	50–100	<i>Malus pumila</i>	Decolorization of dye	[66]
	28.5	<i>Camellia sinensis L</i>	Antioxidant	[67]
	40–52	<i>Syzygium cumini</i>	Antibacterial, antifungal, aflatoxin B ₁ adsorption	[68]
	12.6	<i>Juglans regia</i>	Cytotoxic assay	[69]
	10–90	<i>Pyrus sinkiangensis</i>	Cr (IV) removal	[70]
	9±4	<i>Cymbopogon citratus</i>	Nanotoxicological	[71]
	8.03±8.99	<i>Laurusnobilis</i>	Antimicrobial	[72]

inherent capabilities of plants, simplifying the synthesis process and enabling large-scale production without the risk of contamination.

Incorporating plant materials such as fruits, seeds, flowers, leaves, stems, gums, and roots, along with essential oils, in the creation of nanomaterials opens up the possibility of utilizing a diverse array of secondary metabolites that plants produce. These compounds, including polyphenols, alkaloids, terpenoids, quinones, and tannins, can play a significant role in the process of ion bioremediation [80]. Moreover, specific plant species utilize phytochelatin, glutathione, and oligomers to mitigate the toxicity of heavy metals like cadmium and arsenic through hyperaccumulation mechanisms. The mechanisms discussed encompass the bioactivation of metals within the rhizosphere, enhanced metal uptake facilitated by cell membrane transporters, phytochelatin-mediated chelation of metals, the creation of metal complexes with proteins such as metallothionein metal-binding proteins, and the retention of metals within the cell's vacuole organelle [81]. In this context, metallic nanomaterials and nanocomposites have been created from various plants, including monocotyledonous and dicotyledonous ones. Microorganisms need reducing and stabilizing agents like hydroquinone ($C_6H_6O_2$), naphthoquinone ($C_{10}H_6O_2$), and anthraquinone ($C_{14}H_8O_2$) metabolites, along with extracellular enzymes such as NADH-dependent enzymes, for the production of nanomaterials [82].

Conversely, these molecules function as reducing agents (such as flavonoids, tannins, terpenoids, and alkaloids) or stabilizing agents (including carbonyl, carboxyl, and amine groups) within bimetallic nanocomposites [83]. These metabolites have the ability to reduce. In the process of creating nanomaterials, these chemicals serve as stabilizers and

reducing agents which facilitate the reduction and formation of nanomaterials by providing electrons to metal ions present in the precursor solution [84]. Plant extracts comprise a variety of biomolecules, such as proteins, polysaccharides, and organic acids, that can function as agents for capping and stabilizing nanoparticles. The biomolecules attach to the surface of the nanocomposites, providing stability and preventing aggregation. Additionally, the capping agents influence the dimensions, morphology, and surface characteristics of the resulting nanocomposites [1]. The green synthesis of plant extracts is one of several strategies that considerably improve nanomaterial stability and activity [84]. Leaves, often referred to as the “food factories” of plants, have emerged as a promising source for nanoparticle synthesis. Their readily available nature and straightforward extraction procedures make them ideal for researchers. Numerous studies have successfully utilized leaf extracts from various plants, including *Azadirachta indica* [10] (neem), *Mentha piperita* (peppermint) [85] and *Ocimum sanctum* (holy basil) [86] to synthesize silver nanoparticles. Furthermore, investigations have extended beyond leaves to explore other plant parts. For instance, ethanolic extracts of marigold flowers [11] and kinnow fruit [87] effectively synthesized silver nanoparticles. Similarly, fruit peels from *Garcinia mangostana* (mangosteen) have been utilized to synthesize silver nanoparticles with an average size of 15–20 nm [12]. The versatility of plant-mediated synthesis is further demonstrated by the successful production of various nanoparticles, including gold-iron, copper, and silver-iron core-shell nanoparticles, using extracts from *Punica granatum* [88].

Plant extracts are veritable treasure troves of bioactive compounds, encompassing a diverse spectrum of

phytochemicals such as flavonoids, terpenoids, alkaloids, and phenols. These compounds are pivotal in nanoparticle synthesis, acting as multifaceted agents. A key function of these phytochemicals is their ability to act as reducing agents. A significant number of these compounds contain electron-rich functional groups, including hydroxyl (-OH) and carbonyl (C=O). These electron-rich groups readily donate electrons to the metal ions present in the solution. This electron transfer process effectively reduces the metal ions to their elemental form, a crucial step in the nucleation and growth of nanoparticles [89, 90]. Beyond their reducing capabilities, phytochemicals also exhibit remarkable capping and stabilizing properties. As the nanoparticles form, these compounds can interact with the nanoparticle surface through various mechanisms, such as hydrogen bonding, electrostatic interactions, or van der Waals forces. This surface interaction effectively prevents the uncontrolled aggregation of nanoparticles, which can significantly alter their size and shape. By acting as capping agents, these phytochemicals effectively control the growth and morphology of the nanoparticles, forming stable and well-defined nanostructures [90–92]. In essence, the diverse array of phytochemicals present within plant extracts drives the reduction of metal ions. It plays a crucial role in controlling the synthesized nanoparticles' size, shape, and stability. This multifaceted role of phytochemicals underscores the unique advantages of plant-mediated nanoparticle synthesis, offering a sustainable and eco-friendly approach to nanotechnology.

Factors influencing the green synthesis of metal NPs

The green synthesis of metal nanoparticles is a complex process influenced by several critical factors. The concentrations of metal ions and plant extract significantly impact the reaction kinetics and the final properties of the nanoparticles. The reaction's medium's pH plays a crucial role in influencing the stability and reactivity of metal ions and phytochemicals. Temperature significantly influences the reaction kinetics, while the intensity and wavelength of light directly affect the rate of photochemical reactions and the final properties of the nanoparticles. These factors interact intricately, and their optimal values must be carefully considered to achieve controlled synthesis of nanoparticles with desired properties such as size, shape, and stability. Plant-mediated nanomaterials occur in plenty of sizes and shapes and are more stable as illustrated in Fig. 5. Plant extracts contain flavonoids, terpenoids, and phenolic acids, which act as reducing agents for nanomaterial production [92].

The creation of nanoparticles (NPs) is intricately influenced by a range of physicochemical characteristics, encompassing temperature, time, pH, optical properties, substrate concentration, and enzyme sources. When it comes to the specific synthesis of silver nanoparticles (AgNPs), temperature emerges as a key physical determinant. Some methodologies leverage high temperatures, such as autoclaving silver nitrate and starch solutions at 121 °C and 15 psi to facilitate AgNP formation [93, 94]. In contrast, other eco-friendly approaches harness the reducing capabilities of plant extracts at ambient room temperature to generate both

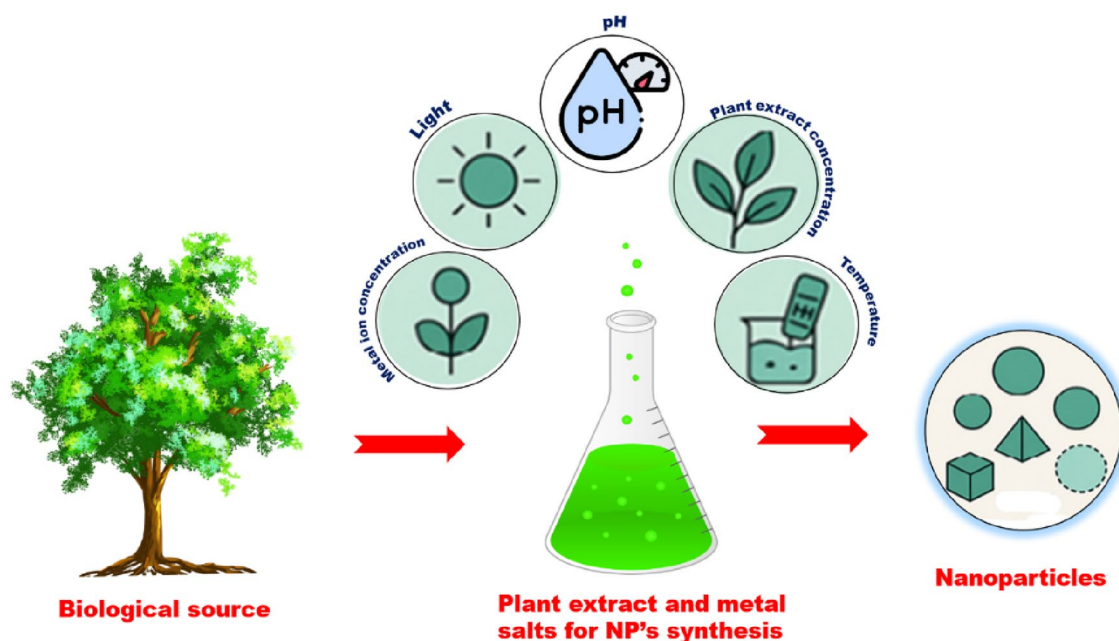


Fig. 5 Factors influencing the green synthesis of metal nanoparticles

silver and iron NPs [94]. Beyond synthesis, temperature also plays a role in the longevity of the resulting AgNPs, with storage typically recommended between 18 and 25 °C for sustained stability [95]. The concentrations of the silver precursor (often silver nitrate) and the reducing agents, frequently the diverse bioactive compounds present in plant extracts [96, 97], are also pivotal in AgNP development. Higher silver salt concentrations generally promote the formation of larger NPs due to an increased pool of silver ions available for reduction [98], while lower concentrations can lead to smaller particles. Likewise, the quantity of plant extract employed can modulate NP size and the kinetics of silver ion reduction [97]; however, an overabundance might induce aggregation and compromise NP quality. Chemical reduction methods, such as those employing Tollens' reagent, underscore the significance of reagent concentration, where the amount of ammonia and the nature of the reductant significantly impact AgNP formation, with ammonia's strong complexation with silver ions being a crucial factor [99]. Finally, pH stands as another influential physical parameter that governs the morphology, particularly the size and shape, of the synthesized NPs [100]. Notably, in certain reactions, a pH of 11.5 has been observed to favor the production of smaller particles from disaccharides compared to monosaccharides, and a lower reaction pH has been correlated with reduced polydispersity in the resulting nanoparticle population.

Characterization of metal NPs

Various characterization techniques are employed to analyze nanoparticles synthesized through green approaches. These techniques include spectroscopic methods such as UV–visible spectroscopy, FTIR, Raman spectroscopy, PL, and UV-DRS; microscopic techniques such as TEM, SEM, FE-SEM, and AFM; analytical techniques such as EDAX, XRD, and XPS; and other techniques such as TG–DTA, ATR, and DLS. These techniques provide valuable information about the synthesized nanoparticles' size, shape, composition, optical properties, and other crucial characteristics. These analyses have revealed that the synthesized metal nanoparticles exhibit predominantly spherical morphologies with average diameters ranging from 5 nm to several tens of nanometers [15, 16].

UV–visible spectroscopy

This technique is fundamental for detecting the presence of nanoparticles by observing a characteristic surface plasmon resonance (SPR) band, which arises from the collective oscillation of free electrons within the nanoparticle.

This technique can also be used to monitor the kinetics of nanoparticle formation. This technique measures the diffuse reflectance of light from a sample, providing information about the optical absorption properties of the nanoparticles. It is beneficial for characterizing nanoparticles in solid form or embedded in a matrix. Ultraviolet–visible (UV–Vis) spectroscopy is a crucial technology for numerous purposes, including evaluating optical properties, production, and stability of nanoparticles. This method is beneficial because of its simplicity, user-friendliness, speedy analysis, sensitivity, and selectivity [101]. UV–Vis spectroscopy measures the absorption of visible or ultraviolet light by different substances in solution [102]. A sample solution analysis, including solely a crude plant extract, revealed no distinctive peak. The sample of botanically produced nanoparticles exhibited distinct peaks at various wavelengths. The absorption band for silver nanoparticles (AgNPs) generally falls within the range of 417 to 448 nm [103, 104]. Silver nanoparticles show notable surface plasmon resonance (SPR) properties due to the collective oscillation of free electrons on their metallic surfaces. The size of the particles within the visible spectrum determines the range of wavelengths they can absorb, which then influences their oscillations. As the nanoparticles develop, the absorption maximum shifts to longer wavelengths, experiencing a redshift [105]. Surface plasmon resonance (SPR) is a critical technique commonly used to study silver nanoparticles (AgNPs), thanks to its remarkable sensitivity to their unique optical characteristics. Upon exposure to incoming light, the electrons located on the surface of the nanoparticle exhibit coordinated movement, resulting in surface plasmon resonance (SPR). This interaction results in a unique absorption peak in the UV–visible spectrum [106]. The location and intensity of this peak are shaped by factors like the size and shape of the particles, as well as the medium around them. Similarly, another research showed the efficiency of UV–Vis absorption spectroscopy in identifying a specific absorption peak at 396 nm for produced AgNPs. This finding illustrates surface plasmon resonance's accuracy in assessing nanoparticles' properties [107]. Moreover, sensors based on surface plasmon resonance (SPR) have been created to identify silver nanoparticles (AgNPs) in various settings, such as food and environmental samples. This showcases the adaptability and importance of this method in analyzing nanomaterials [108].

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis provides valuable information about the functional groups on the surface of the nanoparticles and the biomolecules involved in their synthesis. By comparing the FTIR spectra of the plant extract before and

after nanoparticle synthesis, one can identify the biomolecules responsible for the reduction and stabilization of the nanoparticles. ATR is a technique used to obtain FTIR spectra of solid or liquid samples in direct contact with the ATR crystal. It is beneficial for characterizing the surface chemistry of the nanoparticles. This approach analyzes biomolecules to identify the functional groups involved in nanoparticle formation by generating a molecular sample's fingerprint via its absorption and transmission spectra [109]. Several functional groups were found on the surface of silver nanoparticles (AgNPs) produced from the floral extract of *H. trichophylla* [110]. The identified peaks were N–O (1385 cm^{-1}), C–H (2921 and 2847 cm^{-1}), O–H (3397 – 3410 cm^{-1}), and C–N (1626 cm^{-1}), signifying the presence of essential biomolecules in the nanoparticle creation process [111] as illustrated in Fig. 6.

Zeta potential analysis

The zeta potential analysis is essential in understanding silver nanoparticles (AgNPs), providing insights into their surface charge and stability in colloidal solutions. This approach determines the electrostatic potential at the sliding plane of suspended particles. Generally, readings over $+20\text{ mV}$ or below -20 mV indicate sufficient electrostatic repulsion to sustain stability [114]. Various investigations show that AgNPs produced using different techniques have zeta potential values ranging from -14.2 mV to -32.2 mV . This range suggests a notable level of electrostatic repulsion and shows that these particles maintain good colloidal stability. These measurements are crucial because nanoparticles with zeta potential values close to zero tend to clump together. This aggregation can negatively impact their stability and effectiveness [115].

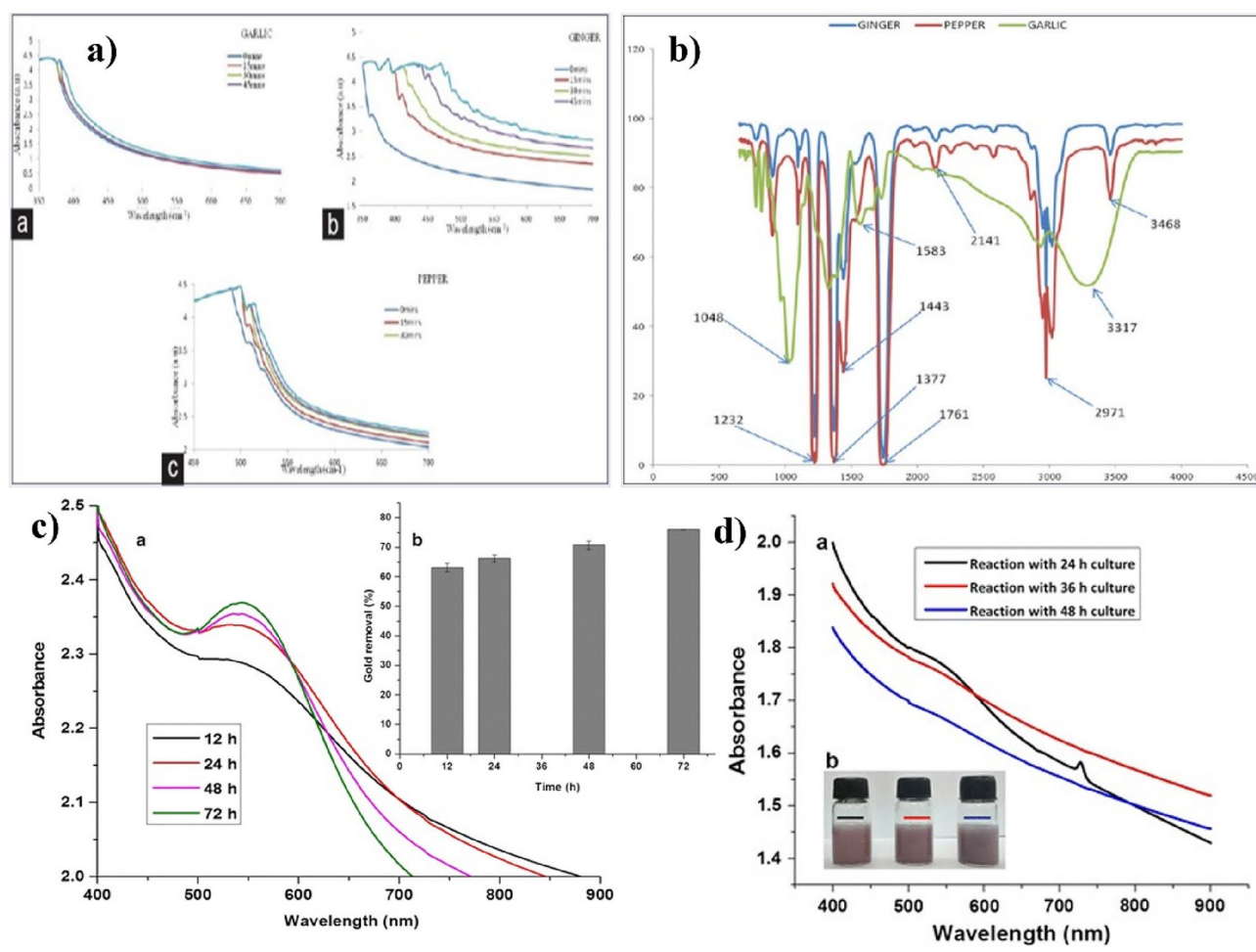


Fig. 6 **A** UV- Vis of AgNPs derived from ginger, cayenne pepper and garlic **B** FTIR spectra of AgNPs derived from ginger, cayenne pepper and garlic Ref. [112] under the terms of the Creative Commons CC BY-NC-SA 4.0. **C** Kinetics of intracellular formation of gold nanoparticles using resting cells of *Candida parapsilosis* ATCC 7330 **a** UV-

vis spectrum analysis and its Gold accumulation with respect to time **D** UV/vis spectrum analysis of the gold nanoparticles biosynthesized using whole cells (24, 36 and 48 h culture cells) of *Candida parapsilosis* ATCC 7330, visual representation of the reaction Ref. [113] under the terms of the Creative Commons CC BY 4.0

Scanning electron microscopy (SEM)

SEM provides three-dimensional images of the nanoparticle surface, revealing details about their morphology, surface roughness, and agglomeration state. FE-SEM technique offers higher resolution images than conventional SEM, enabling more detailed characterization of the nanoparticle surface features.

Scanning electron microscopy (SEM) is a crucial technique for analyzing the two-dimensional surface morphology of nanoparticles. Atomic force microscopy (AFM) generates accurate three-dimensional images [116], whereas scanning electron microscopy (SEM) provides a robust method for examining the size, shape, and surface characteristics of biosynthesized nanoparticles. Researchers can gain a clearer understanding of the structural diversity of nanoparticles using SEM analysis. Silver nanoparticles (Ag NPs) were generated using *Achillea millefolium* extract, which displayed several morphologies, including rectangular, cubic, and spherical shapes [117] as shown in Fig. 7.

Transmission electron microscopy (TEM)

TEM provides high-resolution images of the nanoparticles, used for the determination of their size, shape, and

morphology. It can also be used to study the internal structure of the nanoparticles and identify any defects or impurities. Transmission electron microscopy (TEM) is a complex analytical method using an electron beam to create high-resolution images of nanoparticles. This approach allows a comprehensive study of nanoparticles, revealing their shape and intrinsic framework. Apart from investigating nanoparticles' aggregation or cluster behavior, it is applied to investigate their particle size distribution, a fundamental factor controlling their physical and chemical properties [118] as shown in Fig. 8. The size of nanoparticles mainly defines their possible uses and capabilities. Many research have precisely calculated the average particle size using TEM. Using *Tussilago farfara* flower extract, Lee et al. (2019), successfully synthesized silver (Ag) and gold (Au) nanoparticles. The synthesized nanoparticles exhibited particle sizes ranging from 13.57 and 18.20 nm [23].

Atomic force microscopy (AFM)

Atomic force microscopy (AFM) is often used to investigate the shape, size, sorption capabilities, aggregation, dispersion, and structural characteristics of nanomaterials. There are three different ways to perform scans: contact mode, non-contact mode, and intermittent sample contact

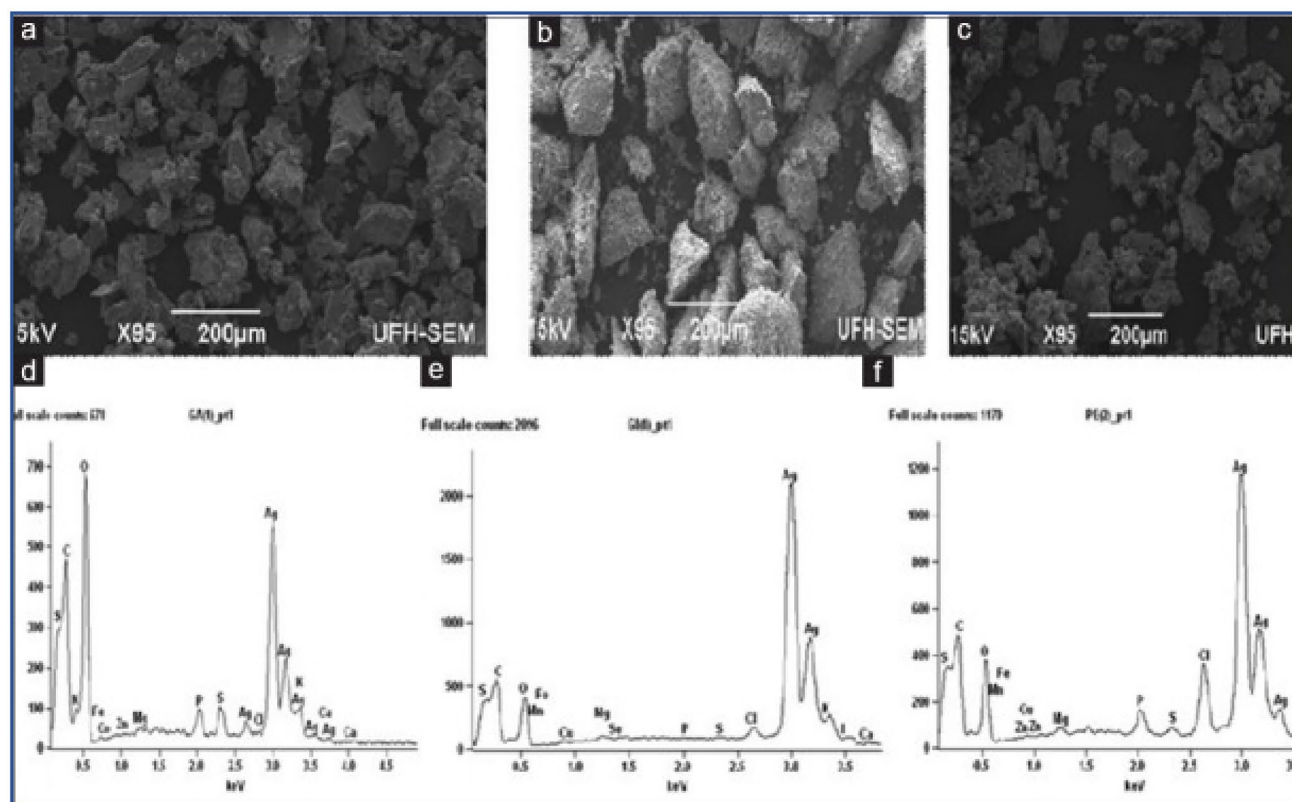


Fig. 7 SEM of Ag NPs derived from **A** garlic, **B** ginger, and **C** cayenne pepper; (d-f) EDAX of Ag NPs derived from **D** garlic, **E** ginger, and **F** cayenne pepper. [112] Reproduced/adapted under the terms of the Creative Commons CC BY-NC-SA 4.0

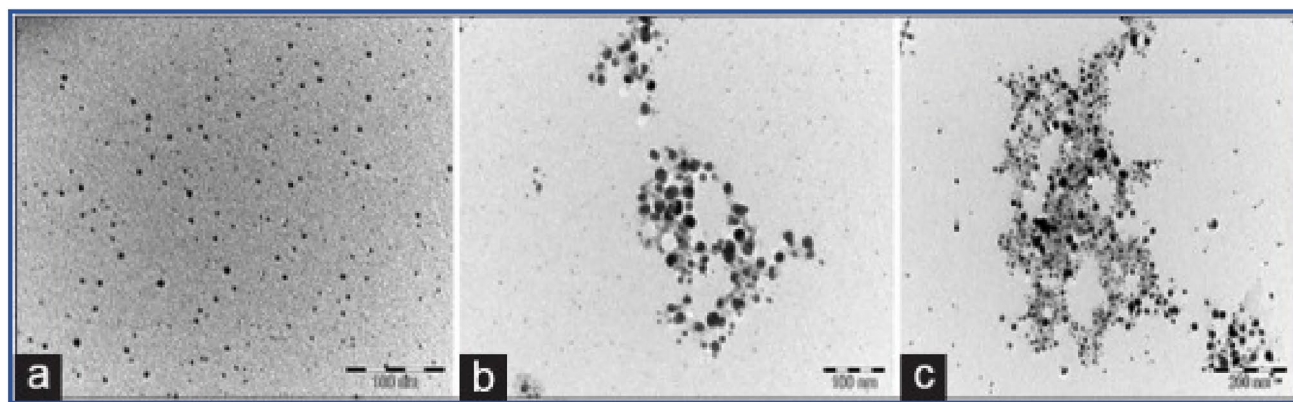


Fig. 8 TEM images of Ag NPs from **A)** garlic, **B)** ginger, and **C)** cayenne pepper. Reproduced/adapted under the terms of the Creative Commons CC BY-NC-SA 4.0

mode [7]. AFM is a valuable tool for exploring how nanomaterials interact with supported lipid bilayers in real time, something traditional electron microscopy methods can't achieve [119]. Unlike electron microscopy, AFM reduces damage to diverse native surfaces, enables for imaging at subnanometer resolution even in wet conditions, and does not require surfaces to be oxide-free or electrically conductive for measurements [120].

X-ray diffraction (XRD)

XRD provides information about the phase and crystal structure of the nanoparticles. It can determine the nanoparticles' size and crystallinity and identify any impurities or defects in the crystal lattice. X-ray diffraction (XRD) is a vital method applied to research on crystal structure, crystal planes, and nanomaterial crystallite size. This method relies on X-rays' ability to expose complete information about internal crystal structure and penetrate objects deeply [24]. Studies will disclose diffraction peaks at specific angles for crystalline material matching the atoms' arrangement inside the crystal lattice [121]. Many studies observed characteristic diffraction peaks in the XRD analysis at 38.5° , 50.7° , 65.2° , 78.4° , and 81.2° , which match the crystal planes (111), (200), (220), (311), and (222, respectively). The results of this work reveal that the created silver (Ag) nanoparticles from *A. haussknechtii* extract have a face-centered cubic (FCC) structure [122].

X-ray photoelectron spectroscopy (XPS)

XPS provides information about the chemical composition and electronic state of the elements present in the nanoparticles. It can be used to study the oxidation states of the metal ions and the nature of the chemical bonds between the nanoparticles and the surrounding environment. X-ray photoelectron spectroscopy (XPS), often called electron

spectroscopy for chemical analysis (ESCA), is a valuable method for analyzing surfaces and quantitatively determining empirical formulas. XPS is beneficial for offering quantitative and qualitative insights and details about the different forms of chemical composition found on sensor surfaces [123]. In a high vacuum environment, X-rays are directed at the surface of the nanomaterial, leading to the release of electrons. The XPS spectrum is created by measuring the number of released electrons and examining their kinetic energy. By analyzing these measurements, we can uncover the electron binding energy, which helps us understand different chemical states. XPS can identify and characterize specific functional groups like P=S, aromatic rings, C–O, and C=O. This technique offers valuable insights into the chemical structure of starburst macromolecules and other nanomaterials [124].

Dynamic light scattering (DLS)

DLS can be used to determine the size distribution and hydrodynamic diameter of the nanoparticles in solution. It can also be used to study the stability of the nanoparticle dispersion. Analyzing the biological activity of synthetic nanomaterials depends much on their physicochemical characterizing; hence, radiation scattering methods are often used for this aim [124]. Dynamic light scattering (DLS) with a measurement scale ranging from submicrons to nanoscales can help one find the size distribution of the small particles in a suspension or solution. Evaluation of limited particle size distributions between 2 and 500 nm benefits significantly from DLS, a technique based on how light interacts with particles [125]. Among several techniques for characterizing nanoparticles, DLS is still the most often used based on Rayleigh scattering from the suspended nanoparticles [126]. DLS analyzes light distributed by a laser passing through a colloidal solution. One well-known method for measuring particle and molecule size and distribution is DLS. It is

widely used to describe nanoparticles, particularly their size control. Mainly, DLS has been used to examine, as reported in several research, the size of magnetic nanoparticles distributed in liquid medium Brownian motion, causes the measured nanoparticle size using DLS to be typically more significant than that obtained using transmission electron microscopy (TEM) [127]. Estimating the average size of nanoparticles in liquid solutions is significantly benefited by this method [128].

Phytochemicals in plant extracts

Plant extracts serve as veritable treasure troves of bioactive compounds, encompassing a diverse spectrum of phytochemicals and some phytochemicals, as illustrated in Fig. 9. This rich tapestry includes flavonoids, renowned for their potent antioxidant and anti-inflammatory properties; terpenoids, a structurally diverse class exhibiting antimicrobial and antitumor activities; alkaloids, nitrogen-containing compounds often associated with significant pharmacological effects; phenols, known for their antioxidant and antimicrobial properties; tannins, polyphenols characterized by their astringent taste; and glycosides, compounds comprising a sugar moiety linked to a non-sugar component, often exhibiting diverse biological activities. This intricate assemblage of phytochemicals contributes significantly to the multifaceted biological activities observed in plant extracts, making them invaluable resources with applications spanning various fields, including medicine, food science, and cosmetics [129].

Phenols

Phenolics have one or more aromatic rings and hydroxyl groups. These chemicals are widespread across the plant kingdom and are the most common secondary metabolites in plants, with over 8,000 known phenolic structures ranging from basic molecules like phenolic acids to complex, highly polymerized substances like tannins. Plant phenolics defend against UV radiation as well as risks from infections, parasites, and predators, and they also contribute to plant colors. They are found in all plant organs, making them an essential component of the human diet. Phenolics are commonly found in a variety of plant foods, including fruits, vegetables, cereals, olives, legumes, and chocolate, as well as drinks like tea, coffee, beer, and wine. They contribute significantly to the overall sensory features of plant-based diets. Phenolics contribute significantly to the bitterness and astringency of fruit and fruit juices due to the interaction between phenolics, notably procyanidin, with the glycoprotein in saliva. Anthocyanins, one of six subgroups of plant polyphenol components known as flavonoids, contribute to the brilliant orange, red, blue, and purple hues observed in a variety of fruits and vegetables, including apples, berries, beets, and onions. Phenolics are known as essential molecules that influence flavor and color differences in white, pink, and red wines. They interact with oxygen and play an important role in wine preservation, maturation, and aging processes. Plant phenolics encompass phenolic acids, flavonoids, tannins, and the less prevalent stilbenes and lignans. The majority of polyphenols in our diets are flavonoids. Flavonoids' core structure is defined by the flavan nucleus, which is made up of 15 carbon atoms grouped into three rings (C6-C3-C6) labeled A, B, and C. The oxidation state of the middle C ring divides flavonoids into six separate

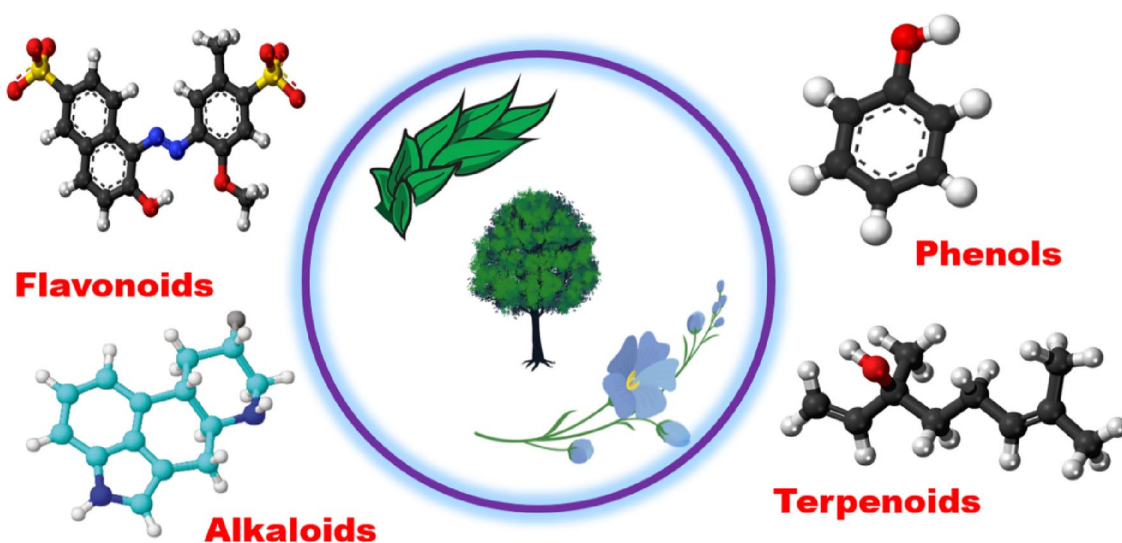


Fig. 9 Phytochemicals in Plant Extracts

subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins. Each subgroup's structural diversity can be traced to the amount and order of hydroxylation, methoxylation, prenylation, or glycosylation. Among the prevalent flavonoids are quercetin, a flavonol found in onion, broccoli, and apple; catechin, a flavanol present in tea and various fruits; naringenin, the primary flavanone in grapefruit; cyanidin-glycoside, an anthocyanin-rich in berry fruits such as black currant, raspberry, and blackberry; and daidzein, genistein, and glycitein, which are the main isoflavones in soybean [130].

Flavonoids

Flavonoids are a prominent family of natural compounds, specifically classed as plant secondary metabolites distinguished by their polyphenolic composition. They are typically present in fruits, vegetables, and some drinks. They have different positive biochemical and antioxidant properties connected to multiple disorders, including cancer, Alzheimer's disease (AD), and atherosclerosis [131]. Various polyphenolic compounds classified as flavonoids effectively chelate and reduce metal ions to their NP form. Metal ions can be reduced because they possess significant functional groups, such as carbonyl and hydroxyl moieties. As a result, it was shown that an aqueous extract of *Rumex dentatus*, rich in phenolic and flavonoid compounds, facilitated the straightforward reduction of Ag^+ ions to Ag^0 . A study indicates that releasing reactive hydrogen atoms from flavonoids during their transformation into keto-enol tautomers facilitates the reduction of metallic ions to their nanoparticle form. For example, flavonoids and rosmarinic acid produced from *Ocimum basilicum* extract have been claimed to accelerate the ketoenol process, which is critical in the formation of NPs. Moreover, the internal conversion of ketones to carboxylic acids in flavonoids likely accounts for reducing Au^{3+} metal ions. In contrast, flavonoids easily attach to the surfaces of developing nanoparticles due to their ability to chelate metal ions like Cu^{2+} , Zn^{2+} , Fe^{2+} , Fe^{3+} , Pb^{2+} , Cr^{3+} , and Co^{2+} . This indicates that they influence early nucleation, restrict aggregation, and promote the bioreduction of metal ions. The hydrogen interacts with the Ag^+ ion, leading to the keto-enol tautomerism mechanism that reduces Ag^+ to its Ag^0 form. This mechanism has been demonstrated as the method for forming AgNPs, utilizing phytochemicals as both reducing and stabilizing agents [132].

Terpenoids

Terpenoids, which are found in practically all classes of living beings, are the biggest category of natural chemicals

with amazing structural variety. They are produced from the carbon backbones of 2-methylbuta-1,3-diene (isoprene units, C5) that have been rearranged into linear and cyclic structures. They are divided into monoterpenes (2 C-5 units), sesquiterpenes (3 C-5 units), diterpenes (4 C-5 units), triterpenes (6 C-5 units), and so on. They are classified into six basic classes: tocopherols, taxanes, ingenanes, artemisinins, sterols, and cannabinoids [133]. Terpenoids, a class of small secondary metabolites found in plants, are among the most abundant natural substances. Terpenoids have various pharmacological actions, including anti-cancer, anti-inflammatory, anti-viral, anti-bacterial, and anti-malarial properties [134]. Recent insights reveal that two key processes, the mevalonate pathway (MVA) and the methylerythritol phosphate (MEP) pathway, work alongside glycolysis to transform glucose into two five-carbon molecules. These molecules, dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), are essential building blocks for terpenoids. Each pathway consists of a cascade involving 18 enzymes [135]. A study revealed that terpenoids are often associated with forming metallic nanoparticles, as FTIR results indicate. A survey by Shankar and colleagues 2003 indicated that terpenoids found in leaves played a crucial role in forming AuNPs during the reaction between geranium leaves and chloroaurate ions. The group reached a comparable conclusion: the significant presence of eugenol in *Cinnamomum zeylanicum* (cinnamon) extracts played a substantial role in the bioreduction of HAuCl_4 and AgNO_3 by these extracts. Previous studies indicate that the deprotonation of the hydroxyl group in eugenol leads to the formation of an anion, which is subsequently oxidized by metal ions, resulting in the reduction of these ions to metallic nanoparticles. Additionally, it was demonstrated that the steroidal saponin diosgenin acted as both a capping and reducing agent in the synthesis of Ag NPs [132].

Alkaloids

The indolizidine alkaloids, ergoline alkaloids, benzenoids, and phenolic compounds found in the extracts of *I. pes-caprae* roots exhibited the stability and reduction potential of Ag ions to AgNPs in research carried out by Subha and colleagues (2015). The team reported that when they performed FTIR analysis on this extract combined with silver salt, they observed peaks at 1660, 1043, and 635 cm^{-1} . The peaks observed were associated with the stretching vibrations of $\text{C}=\text{O}$ in protein amides, the $\text{C}-\text{OH}$ stretch vibrations found in cyclic alcohols, and the aromatic $\text{C}-\text{H}$ vibrations related to free quinones that come from polyphenolic compounds present in the extract. The group highlighted that there is a limited number of academic publications addressing the role of alkaloids in the transformation of metal ions

into nanoparticles. Further inquiries are essential in this domain moving forward. While this study does not encompass significant findings on synthesizing metal nanoparticles through alkaloids, various plant extracts have shown alkaloids' capability in producing metal NPs. A survey conducted by Almadiy and Nenaah (2018) has demonstrated the synthesis of Ag NPs utilizing potato steroidal alkaloids and their effectiveness against phytopathogenic fungi. This highlights the necessity of exploring the alkaloids of the genus *Phyllanthus* to synthesize metal NPs. Additionally, Almadiy and colleagues (2018) demonstrated the synthesis of Ag NPs utilizing harmala alkaloids. The study also examined NPs for their insecticidal efficacy against khapra beetles. This indicates alkaloids can reduce metal ions to their nanoparticle forms [132].

Phytochemical analysis

Phytochemical analysis involves identifying and quantifying the different classes of compounds present in plant extracts. Common tests involve chromatographic techniques that help in detecting and identifying metabolites from plant materials. These methods have evolved considerably in recent years, thanks to advancements in analysis time, detection limits, and separation characteristics. Depending on the biological question at hand, one can differentiate between targeted strategies and non-targeted approaches [136]. The phytochemical is commonly analysed by Thin Layer Chromatography, Gas Chromatography-Mass Spectroscopy, and Liquid Chromatography-Mass Spectroscopy.

Gas chromatography-mass spectroscopy

GC-MS is a method used to analyze and break down mixtures of volatile organic compounds, helping us identify each individual component within those mixtures. This technique is favored in archaeological organic chemistry because it is sensitive, fast, versatile, affordable, and capable of identifying a wide range of compounds in a mixture. This method can be used to examine any material that includes volatile organic compounds [137]. Gas chromatography-mass spectrometry (GC-MS)-based metabolomics is a powerful technique for identifying and measuring small molecular metabolites, which are typically less than 650 daltons. This includes a variety of substances such as small acids, alcohols, hydroxyl acids, amino acids, sugars, fatty acids, sterols, catecholamines, drugs, and toxins. Often, chemical derivatization is employed to enhance the volatility of these compounds, making them suitable for analysis through gas chromatography. This unit demonstrates how GC-MS-based metabolomics seamlessly combines targeted

assays for precise quantification of specific metabolites with untargeted metabolomics, allowing for the discovery of new compounds. With the support of extensive spectral libraries and carefully validated standard operating procedures, GC-MS is capable of identifying and semi-quantifying more than 200 compounds in human body fluids such as plasma, urine, or stool samples during each study. Deconvolution software allows us to identify over 300 additional signals that were previously unknown. These signals can be annotated using precise mass instruments and suitable data processing methods, much like what we see in liquid chromatography-MS untargeted profiling (LC-MS). GC-MS is a well-established technology that employs traditional detectors, known as 'quadrupole,' alongside advanced mass spectrometers referred to as 'triple quadrupole' and precise mass instruments called 'quadrupole-time of flight.' This unit explores various facets of metabolomics using GC-MS technology: First, we prepare samples from mammalian sources. Next, we gather the necessary data. After that, we conduct quality control to ensure accuracy. Finally, we process the data for analysis [138].

Liquid chromatography-mass spectrometry (LC-MS)

LC-MS has become a standard technique, thanks to the development of electrospray ionization (ESI), which offers an easy and reliable way to connect different components. This approach can be used for various biological molecules, and by employing tandem MS along with stable isotope internal standards, we can create assays that are both highly sensitive and accurate. To reduce the impact of ion suppression, it is essential to implement certain optimization methods. Quick scanning speeds enable a remarkable level of multiplexing, allowing for the measurement of numerous compounds in just one analytical run. As instruments become more affordable and reliable, LC-MS is beginning to take on a vital role in various fields of clinical biochemistry, standing alongside traditional liquid chromatography and other methods like immunoassay [139, 140]. LC-MS has emerged as a go-to technique, largely due to the advancement of electrospray ionization (ESI), which provides a straightforward and dependable method for linking various components. This method is applicable to a range of biological molecules. By using tandem mass spectrometry in conjunction with stable isotope internal standards, we can develop assays that achieve impressive sensitivity and accuracy. To lessen the effects of ion suppression, it's important to adopt specific optimization techniques. The ability to scan quickly opens up exciting possibilities for measuring many different compounds all in a single analytical run. With instruments becoming more accessible and dependable, LC-MS is starting to play an important role in

different areas of clinical biochemistry, complementing traditional liquid chromatography and other techniques such as immunoassay [140].

Test for alkaloids

Alkaloids are groups of nitrogenous organic compounds of natural origin that are proven to have a pharmacological effect on various living organisms. Many studies on the antibacterial activity of alkaloids indicate their significant application against many infectious diseases [141]. Piperine, an alkaloid isolated from *Piper nigrum* and *Piper longum*, when co-administered with ciprofloxacin, was proven effective against *Staphylococcus aureus*, *E. coli*, and yeast *Candida albicans* [142]. The aqueous extract of *Berberis heterophylla* did not show any antimicrobial activity, but the alkaloid berberine derived from the same plant showed potential antifungal activity against different *Candida* species [143]. Berberine is also effective against Methicillin-resistant *Staphylococcus aureus* bacteria [91]. The alkaloid Tomatidine shows antibacterial activity against *Staphylococcus aureus* grown in the presence of *Pseudomonas aeruginosa* [144]. Sanguinarine, an alkaloid derived from rhizomes of *Sanguinaria canadensis* L. (blood root), was effective against various gram-positive and gram-negative bacteria [145].

Test for polyphenols

Polyphenols are the simplest bioactive compounds that constitute the primary class of phytochemicals in fruits, seeds, flowers, and vegetables. More than 8000 phenolic phytochemicals have been identified and classified into subgroups depending on biological activity, structure, and origin [146]. Flavonoids, phenolic acids, coumarins, and tannins are polyphenols active against many human pathogens.

Test for flavonoids

Flavonoids are secondary metabolites that have been reported to possess different pharmacological properties. Seven flavonoids, including luteolin, chrysin, and quercetin, have been isolated from *Halostachys caspica*, and antimicrobial activity was tested against gram-positive gram-negative bacteria and fungi [146]. Baicaline was effective against Methicillin-resistant *Staphylococcus aureus* bacteria [147]. Kaempferol and Myricetin showed good antimicrobial activity against *K. pneumonia* and *S. aureus* [148]. Flavonols like catechin, epicatechin, and epigallocatechin gallate are the main constituents of tea leaves that are active against *S. aureus* [149], *Serratia marcescens* [150], *P. aeruginosa* [149], *H. pylori* [151]. However, these flavonols show

moderate to minimum resistance against gram-negative bacteria like *E. coli* [144], *S. thypi* [152], *Porphyromonas gingivalis* [153], and *P. aeruginosa* [154]. This is because of the presence of a lipopolysaccharide layer in gram-negative strains.

Test for phenolic acids

Phenolic acids are a non-flavonoid phenolic compound found abundantly in cereals, berries, legumes, and herbs. Many kinds of research show that phenolic acids can exhibit excellent anti-inflammatory, anti-oxidant, anti-cancer, and anti-microbial activity. D. Shao studied the mechanism of antibacterial activity of gallic acid against *Escherichia coli* [Gram-negative] and *Streptococcus mutans* [Gram-positive] by varying concentration, incubation time and temperature. Overall, gallic acid was most effective when incubated at 37 °C for 24 h and at 25 °C for 48 h [155]. In another study, gallic acid, chebulinic acid, and other alkaloids were isolated and separated from *Sapium baccatum*, and their antimicrobial efficiency was tested against plant pathogenic bacteria [156]. Several phenolic acids, including tannic acid, Vanillic acid, Ferulic acid, and Caffeic acid, have been identified in the chloroform extract of *Ardrographis paniculata* and tested against 4 g-positive and 5 g-negative bacteria. The results showed better antibacterial activity against gram-negative bacteria [157].

Test for tannins

Tannins are a large group of complex polyphenols found in almost every part of the plants (leaves, bark, root, fruits, and wood). They can be hydrolyzable and condensed tannins, both known to be used in traditional medicines. Gallotanin was separated from *Terminalia chebula* fruit and tested against various uropathogenic bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*) by Bag et al. [158]. Tannins derived from *Rhizophora apiculata* barks have been known to possess excellent antiviral and antibacterial activity [159]. The main phenolic compounds in red raspberries are ellagitannins (57%) and anthocyanins (42%), displayed bactericidal activity against *Corynebacterium diphtheriae*, *S. epidermidis*, *Streptococcus* group A and *S. pneumoniae* and inhibitory activity against *C. sporogenes*, *S. aureus*, *B. subtilis* and *Moraxella catarrhalis* [160]. Proanthocyanins are condensed tannins that are proven to be effective against pathogens like *E. coli* [161], *S. epidermidis* [30], and *P. aeruginosa* [162].

Test for terpenoids

Terpenoids are a chemically diverse group of natural products with many applications. A.C. Guimaraes et al. analyzed 33 individual terpenes and terpenoids from essential oils and screened for antimicrobial efficiency against *B. cereus*, *S. Typhimurium*, *E. coli*, and *S. aureus*. Of 16 compounds, including terpineol, eugenol, carveol, citronellol, and geraniol, exhibited bacteriostatic effects against all four bacteria, particularly gram-negative ones [163]. In another study, Liu et al. evaluated and analyzed the antimicrobial activities of essential oils (thymol, berberine, eugenol, and cinnamaldehyde) against *S. typhimurium* and *L. monocytogenes* [164]. Several other common terpenoids like Citral, Limonene, Farnesol, Ursolic acid, Oleanolic acid, and vanillin were found effective against various pathogens, including *S. aureus*, *L. monocytogenes*, *E. coli*, *E. faecium*, *S. enterica*, *P. aeruginosa*, *S. epidermidis*, *B. pseudomallei* and *M. tuberculosis* [165, 166].

Test for organosulphur

Organosulphur compounds are present abundantly in nature and have proven to have anti-cancer, anti-aging, anti-inflammatory, and antimicrobial activity. Cauliflower, garlic, onion, meat, broccoli, eggs, cabbage, and fish are rich sources of sulfur compounds for humans. Park et al. identified the antimicrobial activity of isothiocyanate against *Lactobacillus casei*, *C. albicans*, *S. mutans*, *S. sobrinus*, *Lactobacillus casei*, and *S. aureus* [167]. Other isothiocyanate compounds such as berteroin, hirsutin, sulforaphane, alyssin, and erucin were effective bactericidal against *Helicobacter pylori* [168, 169]. Phenyl-isothiocyanate was combined with erythromycin and ciprofloxacin against *S. aureus* [170]. 2-phenyl ethyl-isothiocyanate, which was derived from watercress tissue, was tested against various isolates of *E. coli*. The results showed increased antibacterial activity of the antibiotic when combined with plant extracts and pure compounds [171]. Also, the compound 2-phenyl ethyl-isothiocyanate demonstrated that it is effective against gram-positive and gram-negative bacteria [172]. Allicin, one of the essential sulfur compounds, allicin, has excellent antibacterial activity against *S. epidermidis*, *P. aeruginosa*, *S. epidermidis*, and *S. aureus* [173, 174].

Storage of metal NPs

The appropriate storage of synthesized nanoparticles is crucial for maintaining their stability and preventing degradation. The stability of nanoparticles can be influenced by various factors, including temperature, light exposure, and

the presence of contaminants. Utilizing inert containers and maintaining low temperatures, such as 4 °C, can effectively minimize degradation. The appropriate storage of synthesized plant-derived nanoparticles (NPs) is crucial for maintaining their stability and preventing degradation. Factors such as agglomeration, oxidation, biological contamination, light sensitivity, and temperature fluctuations can compromise their functionality over time, thereby impacting their effective application across various fields. To address these challenges, appropriate storage techniques are essential. This includes maintaining nanoparticles in suspension with stabilizing agents under dark and refrigerated conditions to minimize degradation and agglomeration, or converting them into a dry powder through lyophilization, which significantly enhances long-term stability by eliminating moisture. The choice of storage method should be tailored to the specific type of nanoparticle and its intended application. Furthermore, regular characterization using techniques such as TEM, DLS, and UV–Vis spectroscopy is critical for evaluating their stability over time, thereby ensuring sustained efficacy.

Biomedical applications of metal NPs

Using plant extracts to create metal nanoparticles (NPs) has become a more affordable and eco-friendly option compared to traditional chemical and physical techniques. Diverse plant sources, abundant in bioactive chemicals, function as reducing and stabilizing agents in synthesizing these nanomaterials. The radial diagram emphasizes the diverse applications of nanoparticles (NPs), highlighting their roles as antimicrobial agents, in drug delivery, biosensors, tissue engineering, dye degradation, cancer therapy, bioimaging, wastewater management, and multicolour optical coding as illustrated in Fig. 10. This illustrates the extensive utilization of nanotechnology across medicine, environmental science, and biotechnology. Metal NPs synthesized using plant extracts have shown promising biomedical applications, including:

Anti-bacterial activity

Ag NPs and Au NPs exhibit vigorous antibacterial activity against various bacterial pathogens. Incorporating silver nanoparticles into *Ipomea carnea* along with a cellulose acetate membrane resulted in a structured antimycobacterial membrane, which demonstrated a 4 mm zone of inhibition against *Mycobacterium smegmatis* [175]. Boerhaaviadifusa-mediated Ag NPs demonstrated greater sensitivity towards *Flavobacterium branchiophilum* compared to other fish bacterial pathogens, such as *Aeromonas hydrophila* and

Fig. 10 Applications of Metal Nanoparticles



Pseudomonas fluorescens [176]. Lingo-berry and cranberry juice-assisted silver nanoparticles show greater effectiveness against *S. aureus*, *B. subtilis*, and *B. cereus*, while demonstrating reduced activity against *C. albicans* and food-borne *B. cereus* [177]. The Ag NPs made from the inflorescence of *Cocos nucifera* significantly hindered the growth of *V. alginolyticus*, *K. pneumoniae*, *P. aeruginosa*, *B. subtilis*, and *P. shigelloides*. Microscopic evidence shows how Ag NPs attach to bacterial cell walls [178]. AgNPs made with lemon peel extract shown antidermatophytic action against *T. mentagrophytes* and *Candida albicans* with a maximal zone of inhibition of $12 \pm 0.3SD$ and $11 \pm 0.5SD$, respectively, but no activity against *T. rubrum* [179]. Triangular, hexagonal, and spherical Ag NPs of size between 78 and 98 nm formed by the leaf extracts of *Caesalpinia coriaria* showed better antibacterial activity against *Escherichia coli* (12 mm) and *Pseudomonas aeruginosa* (18 mm) [180]. The apoptosis of *C. albicans* and *S. cerevisiae* induced by *P. oleracea*-mediated silver nanoparticles may result from the generation of reactive oxygen species and a reduction in hydroxyl radical production, which is initiated by the phytoconstituents that coat the synthesized silver nanoparticles [181]. In-vivo analysis of biochemical and histological

parameters provides evidence for the antibacterial effect of AgNPs synthesized using *Leucas aspera* on fish models (*Aeromonashydrophila* and *Catla catla*) [182]. The extract of *Sphaeranthus amaranthoides*, when used to create silver nanoparticles, has been shown to boost antimicrobial activity. This enhancement occurs because it disrupts the outer membrane of bacteria, interferes with their respiration, and reduces the levels of ATP inside the cells, ultimately causing the bacterial cell wall to break down. The differences in how growth is inhibited in Gram-positive and Gram-negative bacteria could be linked to the permeability of their cell membranes [183, 184]. The silver nanoparticles created with *Vinca rosea* leaf extract demonstrated encouraging effectiveness in inhibiting *Staphylococcus aureus*, *Lactobacillus*, *Escherichia coli*, and *Pseudomonas fluorescens* at a concentration of 10 μL [184].

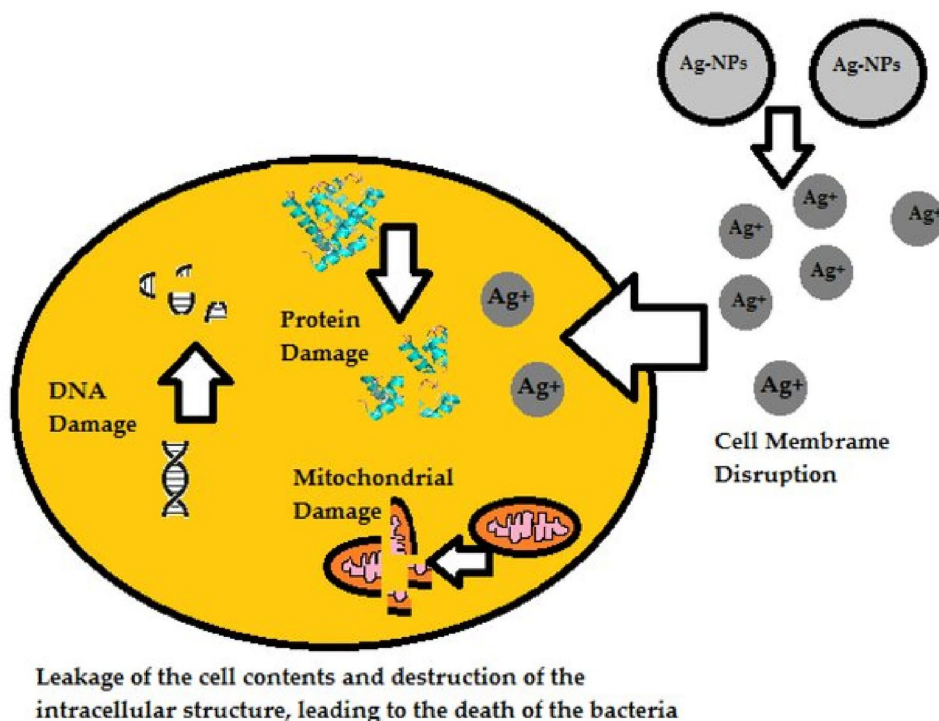
Bacteria pose a significant threat to humans and animals, causing diseases like food poisoning, pneumonia, nosocomial infections, and sepsis, resulting in 5 million deaths annually [185]. Antibiotic-resistant bacterial infections are projected to exacerbate this problem, with a forecasted \$3.4 trillion annual GDP gap by 2030 [186]. This necessitates a deeper understanding of antibacterial mechanisms and their

effectiveness in combating bacterial infections [187]. Current antimicrobial drugs have limitations due to antagonistic interactions, lack of diversity, and the development of drug resistance [188]. Emerging resistant microorganisms pose a serious threat to public health [189]. Researchers are developing new antibiotics from natural and synthetic sources to combat these resistant organisms [190], which can also overcome microbial resistance to traditional antibiotics [191]. Phyto-mediated synthetic nanomaterials have shown promise in drug delivery and combating resistant diseases due to their robust antibacterial properties. Green synthesized nanocomposites often demonstrate superior antibacterial activity to their monometallic counterparts due to synergistic interactions between the different nanoparticles. Due to their synergistic antimicrobial efficacy, bimetallic nanocomposites have shown more effective antibacterial treatments than traditional agents against various Gram-negative, Gram-positive, and viruses [192]. Formulating new antibiotics from synthetic and natural sources aids in combating resistant microorganisms [193]. The pictorial representation of the mechanism is shown in Fig. 11.

Nanocomposites can induce the formation of superoxide (O_2^-) and hydroxyl ($OH^\bullet$) free radicals, which cause significant DNA and protein damage in bacteria [194]. These free radicals damage biological macromolecules like proteins, carbohydrates, fats, and nucleic acids [195]. Combining silver and copper oxide nanoparticles can enhance their antibacterial activity [196]. A magnetic copper oxide doped silver / zeolitic-imidazolate/ polyacrylonitrile/ zeolitic

nanocomposite, synthesized using mulberry leaf and Aloe vera aqueous extracts, has demonstrated strong antimicrobial potential against Gram-positive *B. subtilis* and *S. aureus*, as well as Gram-negative *E. coli* [197]. Silver and graphene oxide nanocomposites, due to their large surface area, bind effectively, inducing oxidative stress and membrane damage [198]. The proposed mechanism suggests that silver's toxicity kills bacteria while graphene oxide encapsulates them. Ag-Au bimetallic nanoparticles synthesized from *Artocarpus heterophyllus* fruit extract exhibited more potent antibacterial activity against Gram-negative bacteria and weaker activity against Gram-positive bacteria [105]. We utilized plant extracts to effectively reduce and stabilize copper-nickel hybrid nanocomposites, which demonstrated impressive antibacterial properties [199]. Nanocomposites show impressive antibacterial properties against a range of bacteria, such as *Neisseria gonorrhoeae*, *Escherichia coli*, *Yersinia pestis*, *Chlamydia trachomatis*, *Pseudomonas aeruginosa*, and *Vibrio cholera* [200, 201]. Many studies have shown that they are effective at stopping the growth of both Gram-negative and Gram-positive bacteria [202]. Silver-iron bimetallic nanocomposites synthesized using *Gardenia jasminoides* leaf extract were effective against *S. aureus* and *P. aeruginosa*, showing greater efficacy than monometallic nanoparticles due to the synergistic effect [203]. The Pd-Ag nanocomposites created with the help of *Terminalia chebula* fruit extract showed promising antibacterial effects against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. Gold-silver bimetallic nanoparticles created

Fig. 11 Antibacterial activity of silver nanoparticles Reproduced/ adapted from Ref. [208] under the terms of the Creative Commons CC BY 4.0



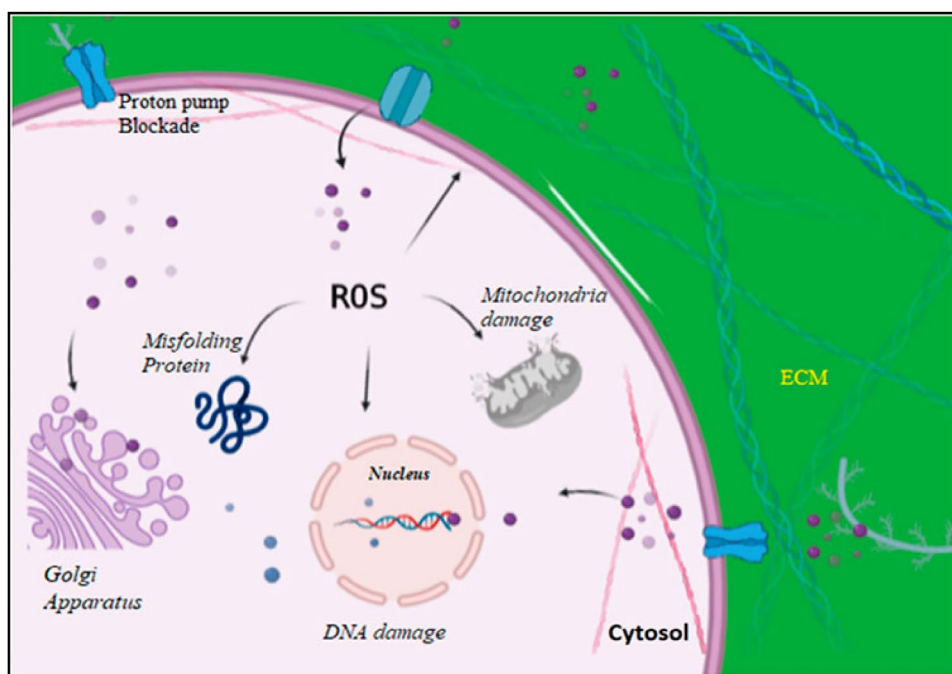
using *Ocimum basilicum* leaf extract demonstrated antibacterial properties against a range of bacteria, such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Researchers have suggested that Au–Ag nanocomposites can directly interact with bacterial cell membranes, as their size closely resembles that of microbial systems. Nanomaterials tend to be less likely to cause bacterial resistance compared to antibiotics, as they showcase a variety of antibacterial mechanisms [204]. However, challenges like toxicity, complex and expensive synthesis, and agglomeration must be addressed for the widespread application of nanomaterials as bactericides [205]. Nanomaterials smaller than 10 nm can enter bacteria, affecting DNA and enzymes and ultimately causing cell death, while larger nanomaterials aggregate on cell surfaces and impair cellular uptake [206]. Although Gram-negative and Gram-positive bacteria present significant health challenges, the promising antibacterial properties of nanocomposites provide a safe and affordable alternative. The photocatalytic properties of nanomaterials contribute to bacterial disinfection by generating reactive oxygen species (ROS) [204]. Nanocomposites employ a range of antibacterial strategies, such as destabilizing cell walls, photocatalysis, binding to DNA, causing physical and mechanical damage, inhibiting bacterial metabolism, and inducing oxidative stress through reactive oxygen species. These diverse mechanisms create challenges for bacteria in developing resistance [204]. The synergistic effect of nanocomposites, focusing on specific bacterial cells, enhances antibacterial activity compared to nanoparticles. For example, silver/copper nanocomposites showed greater inhibition zone diameters (9–30 mm) against *E. coli* and *S. aureus* compared to copper nanoparticles alone (12–18 mm) [207]. Therefore, nanocomposites have proven to be more efficient than mono-metallic nanoparticles.

Anti-fungal activity

Antifungal agents, irrespective of their mechanisms of action, possess limitations concerning their spectrum of activity, medication interactions, resistance emergence, pharmacokinetics, pharmacodynamics, and intrinsic toxicity. Moreover, their physicochemical characteristics, such as hydrophobicity resulting in diminished water solubility and selectivity complications arising from resemblances between human and fungal cells, present obstacles to therapeutic efficacy and efficiency [209, 210]. Nanotechnology is an evolving discipline with extraordinary characteristics that have transformed the biomedical sector [211]. NPs can inhibit the growth of various fungal species. Fungal infections vary from superficial dermal infections to systemic infections impacting significant organs. Worldwide, more

than 300 million individuals are afflicted by severe fungal diseases, leading to almost 1.4 million fatalities each year. Disseminated infections are predominantly attributed to *Cryptococcus*, *Pneumocystis*, *Aspergillus*, and *Candida* species, which cause cryptococcosis, pneumocystis pneumonia, aspergillosis, and candidiasis, respectively [212]. Numerous fungi may biosynthesize nanoparticles, and several metallic nanomaterials possess intrinsic antifungal properties against pathogenic fungi in humans and plants [213]. Nanomaterials can enhance the therapeutic efficacy, safety, and adherence of conventional antifungal medications. Metal oxide or nanoscale metal nanocomposites have garnered significant interest for antifungal applications due to their distinctive characteristics, including photocatalytic, antifungal, and antioxidant activities [8]. Metals such as copper, silver, platinum, and palladium have been utilized to fabricate various metal-chitosan nanocomposites for antifungal applications. This hybridization can augment the characteristics of each constituent in the composites. Progress in nanotechnology has facilitated the development of nanoscale metal oxides and related composites, including TiO_2 , ZnO/TiO_2 , ZnO , and $\text{Fe}_3\text{O}_4/\text{TiO}_2$ [214]. Nanomaterials provide benefits owing to their economical synthesis and environmental sustainability. Nanoparticles counteract fungus via many methods of action [215]. Chitosan-Au nanocomposites were environmentally manufactured as a biodegradable and somewhat non-toxic antifungal agent against *Candida albicans* [216]. Monodisperse and highly crystalline gold and silver nanoparticles, synthesized by an eco-friendly biosynthetic method, have shown considerable efficacy against *Candida albicans* [217]. The green synthesis of ZnO-chitosan nanocomposites utilizing *Prosopis farcta* extract exhibited significant anti-candida efficacy [218]. Magnesium-Zinc Oxide nanoparticles produced from *Ficus religiosa* leaves showed enhanced antifungal efficacy against *Aspergillus niger* [219]. The mechanism is pictorially represented in Fig. 12. Silver-zinc oxide nanocomposites, produced from fenugreek leaf extract, were evaluated for antifungal efficacy against *Candida albicans*. The disk diffusion method demonstrated an inhibitory zone diameter of 10.5 ± 0.707 mm at a dosage of 20 mg/ml [220]. The synthesized Ch-AgNPs demonstrated robust antifungal capabilities when evaluated against *Fusarium oxysporum*, *Curvularia lunata*, and *Aspergillus niger* in solid media formats. These findings align closely with existing literature regarding the deployment of chitosan-based nanocomposites to manage fungal pathogens. Specifically, previous research highlights their effectiveness against postharvest threats like *A. niger* and *Aspergillus flavus*, as well as preharvest agricultural pathogens including *F. oxysporum*, *Rhizopus stolonifer*, and *Botrytis cinerea* [221–223] as shown in Fig. 13.

Fig. 12 Mechanism of action of Ag NPs against fungi. Ref [224] under the terms of the Creative Commons CC BY



The suggested antifungal mechanism of nanocomposites entails the rupture of the fungal cell wall, resulting in cellular debris, swelling, and substantial cellular alterations [225]. Alterations in membrane potential signify cell membrane injury. Intact cell membranes inhibit the passage of phosphatidyl inositol, permitting its entry solely through compromised or necrotic cells. Cells undergo a specific degree of stress [226]. Oxidative stress can induce cell membrane degradation, compromising vital membrane functions by enhancing permeability and discharging cellular constituents, indicating that reactive oxygen species (ROS) can facilitate damage to DNA, proteins, and cell membranes, ultimately resulting in cell death. Membrane permeability and rupture of the cell membrane lead to the loss of soluble proteins. Anionic proteins may be unable to infiltrate the soluble protein fraction. Polyelectrolytes in nanocomposites can engage with proteins to create insoluble complexes [227]. Chitosan-based nanocomposites have antifungal activities owing to their polycationic characteristics, which can disrupt fungal cells, infiltrate their nucleus, and impede protein, energy expression, and production [228]. A study evaluating the antifungal efficacy of nanocomposites vs individual nanoparticles against filamentous fungus revealed that copper/silver nanocomposites exhibited inhibition zone widths of 10–12 mm against *C. albicans*. Monometallic copper nanoparticles exhibited a 10 mm inhibition against the identical strain [229]. Consequently, nanocomposites demonstrate synergistic effects, rendering them more efficient than monometallic nanoparticles due to the cooperative interactions of many metals.

Anti-viral activity

Some NPs have shown potential in inhibiting viral replication. Global public health faces substantial problems as a result of both established and newly emerging viral diseases that cause widespread illness and death. In contrast, many viruses still lack effective antiviral medicines. Current antiviral drugs frequently provide obstacles, including their inability to treat specific viral infections [146, 147] effectively. Remdesivir is the only medicine with full FDA approval for treating COVID-19 in hospitalized patients and those with mild-to-moderate infections at a higher risk of developing severe sickness [230, 231]. As the COVID-19 pandemic continues to impact our lives, researchers are looking into using silver nanoparticles to prevent virus spread. Furthermore, nanocomposites have demonstrated outstanding potential in tackling this problem. Patients with cirrhosis who have chronically normal alanine transaminase levels are nevertheless at risk of developing hepatocellular cancer. This highlights the critical necessity for the speedy development of innovative therapeutic techniques. While good treatment can help regulate disease development, this condition highlights the necessity of developing novel therapeutic methods. A visual depiction of the mechanism can be found in Fig. 14.

Recently, nanomaterials derived from plants have shown promise as a viable alternative for treating viral infections. Green tea contains a high concentration of EGCG and polyphenolic catechins, which are plant-derived substances. The green synthesis process is an excellent choice because it promotes safety and is gentle to the environment. Plants

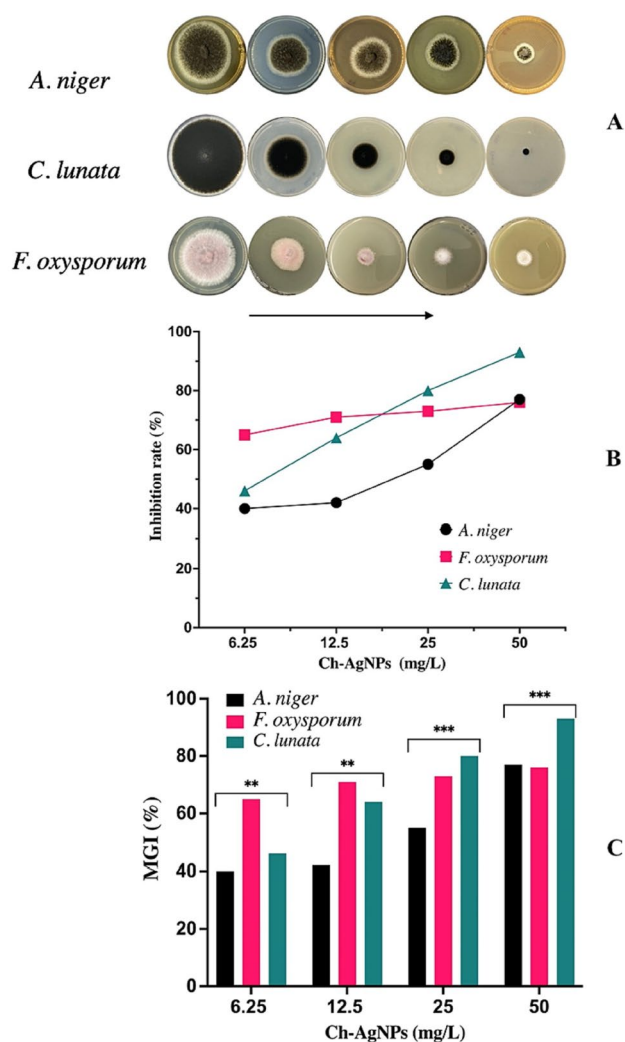


Fig. 13 The mycelium growth inhibition of *A. niger*, *C. lunata*, and *F. oxysporum* on PDA plates with different concentrations of Ch-AgNPs from left to right (0, 6.25, 12.5, 25, and 50 mg/L) (A), The correlation between Ch-AgNPs concentration and the MGI rate (B), MGI% of tested fungi with different concentration Ch-AgNPs (C). All values shown in the graph are means of triplicates ($\pm$ SD). (*) indicates significant differences ($P < 0.05$) in comparison to their controls respectively. Ref. [223] under the terms of the Creative Commons CC BY 4.0

are noteworthy for their capacity to fight cancers, slow cell growth, and reduce inflammation; they also have antibacterial, antioxidant, and antiviral capabilities. Furthermore, they are high in multifunctional bioactive chemicals, which add to their benefits. Furthermore, drug resistance development impacts synthetic antivirals' efficacy [233]. Researchers believe that nanocomposites could provide viable medical treatments for various viral diseases. Nanocomposites have demonstrated promising antiviral action against several viruses, including HIV and influenza. A proposed application uses a cotton fabric reinforced with zinc oxide nanoflowers as a possible membrane filter in facial masks. This idea attempts to improve respiration while simultaneously

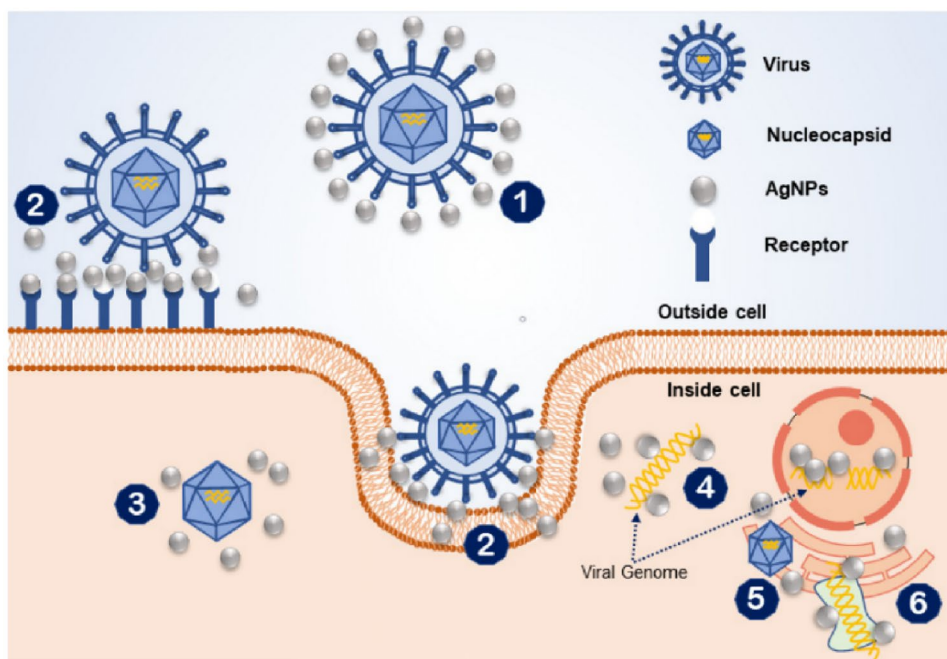
reducing the spread of COVID-19. Computational simulations indicate that these zinc oxide nanoflowers can capture the SARS-CoV-2 spike proteins [234]. A study found that silver nanoparticles coated with chitosan have antiviral properties against swine flu. Unlike pure chitosan, which has no antiviral properties, the Ag-chitosan nanocomposite dramatically reduced viral activity. Chitosan composites may also directly inhibit interactions with host cells [235]. Biologically generated silver nanoparticles and nanocomposites show significant promise as antiviral therapies, with the ability to battle a wide range of viral infections while limiting toxicity and adverse effects. The nanocomposite's active sites are essential in antiviral action by binding to and damaging viral surface proteins [236]. This approach effectively limits viral infection by preventing the virus from reaching host cells during the initial phases of the disease [237]. Researchers discovered that targeting mRNA breakdown can inhibit viral multiplication.

Nanoparticles' ability to cause harm is determined by several characteristics, including their concentration, size, and structure. Surface chemistry, particularly surface charge, significantly impacts the efficacy of antiviral therapies. These characteristics impact toxicity, but surface chemistry is also essential. When we compare single nanoparticles, nanocomposites stand out because they have a more significant surface area and active sites, making them a more enticing alternative to monometallic nanoparticles [238].

Anti-oxidant activity

Metal NPs can scavenge free radicals, protecting cells from oxidative damage. Antioxidants are unique chemical compounds crucial in slowing down or halting oxidation, even when present in very small quantities compared to an oxygen electrode [239]. They act as the body's natural first responders, quickly stepping in to shield biomolecules from the harmful effects of free radicals. As we come across more reactive oxygen species (ROS), the importance of antioxidants grows increasingly vital. Different factors like pollution, smoking, medications, stress, illness, and even exercise can raise our exposure to free radicals. Antioxidants frequently serve an important function in antibiotics, acting as helpers that fight inflammation, fungi, bacteria, and viruses. These substances have garnered significant attention due to their capacity to capture free radicals, which are associated with various diseases stemming from reactive oxygen species. Synthetic antioxidants are often preferred over natural ones due to their greater effectiveness and cost efficiency [240]. Free radicals are molecules that possess an unpaired electron in their outer shell, which gives them a tendency to react vigorously in chemical processes. Elevated levels of free radicals can negatively impact our health and hinder

Fig. 14 Illustration of the possible antiviral mechanisms of silver nanoparticles (AgNPs): (1) interaction of AgNPs with the viral surface; (2) interaction with the cell membrane, thereby blocking viral attachment; (3) inhibition of viral cellular pathways; (4) interaction with the viral genome; (5) inhibition of viral genome replication; and (6) inhibition of cellular factors (e.g., protein synthesis) required for viral replication. Reproduced/adapted from Ref. [232] under the terms of the Creative Commons CC BY 4.0



the healing process. The substances our metabolism creates can engage with DNA, proteins, and fatty acids, resulting in oxidative stress. This process is linked to several health issues that affect people. Oxygen has a fascinating and multifaceted role in our bodies. On the one hand, it plays a role in generating reactive oxygen and nitrogen species (ROS/RNS), but on the other hand, an excess of these can result in significant health problems. This encompasses issues within the central nervous system, such as neurodegeneration, which can play a role in conditions like Alzheimer's disease and the impacts of aging [241–243]. Reactive oxygen species (ROS) are naturally produced during vital metabolic processes, like the electron transport in mitochondria, and their levels are typically regulated by antioxidants that help maintain balance [244, 245]. Antioxidant activity occurs in two primary ways. The first involves single electron transfer, where the antioxidant reduces compounds by donating an electron. Next, we have hydrogen atom transfer, a process in which the antioxidant releases a hydrogen ion to neutralize reactive oxygen species (ROS). Plant-based antioxidants can effectively reduce or bind metal ions, crucial in stabilizing nanomaterials. This process is essential for the development of metal nanomaterials and metal oxides. Free radicals, such as the peroxy radical ($\text{ROO}\cdot$), hydroxyl radical ($\text{OH}\cdot$), and superoxide anion ($\text{O}_2^{\cdot-}$), are incredibly reactive and play a crucial role in influencing biological systems [246, 247]. Figure 15 illustrates the mechanism through a pictorial representation.

Using safe and eco-friendly plant-based chemicals, the green synthesis of nanomaterials offers a sustainable approach to material creation. Plant alkaloids are known for

their remarkable ability to serve as sustainable sources of bioactive compounds, which can be harnessed to develop metallic nanomaterials [249]. Reactive oxygen species are highly reactive molecules that can cause oxidative stress, potentially damaging our cells, tissues, and organs. Nanocomposites possess an impressive capability to capture these molecules due to their unique properties [250, 251]. The effectiveness of these nanostructures as antioxidants depends on several factors, such as their chemical composition, properties, surface charge, crystalline structure, particle size, and surface coating [252]. Nanocomposites are an innovative way to develop new nano-antioxidants that provide improved health benefits. Some nano-antioxidants demonstrate improved abilities to neutralize free radicals and lower oxidative stress compared to their natural versions. This indicates they can maintain stability and resilience even in tough microenvironments [253]. Nano-encapsulation, or nano-delivery, enhances how our bodies process natural antioxidants by slowing their breakdown during stressful conditions. Researchers have developed *Olea cuspidata* Ag@MgO nanocomposites that boast a large surface area, achieved by incorporating silver onto a magnesium oxide support [254]. The MgO matrix was crucial in creating a balanced and well-distributed environment, which helped boost the production of reactive oxygen species (ROS) and improved antibacterial and antioxidant effects. Green nanochemistry is about developing methods that minimize the biological risks of nanomaterial production while enhancing human health benefits. The magnesium–zinc oxide nanocomposites developed using *Ficus religiosa* demonstrated remarkable antioxidant activity,

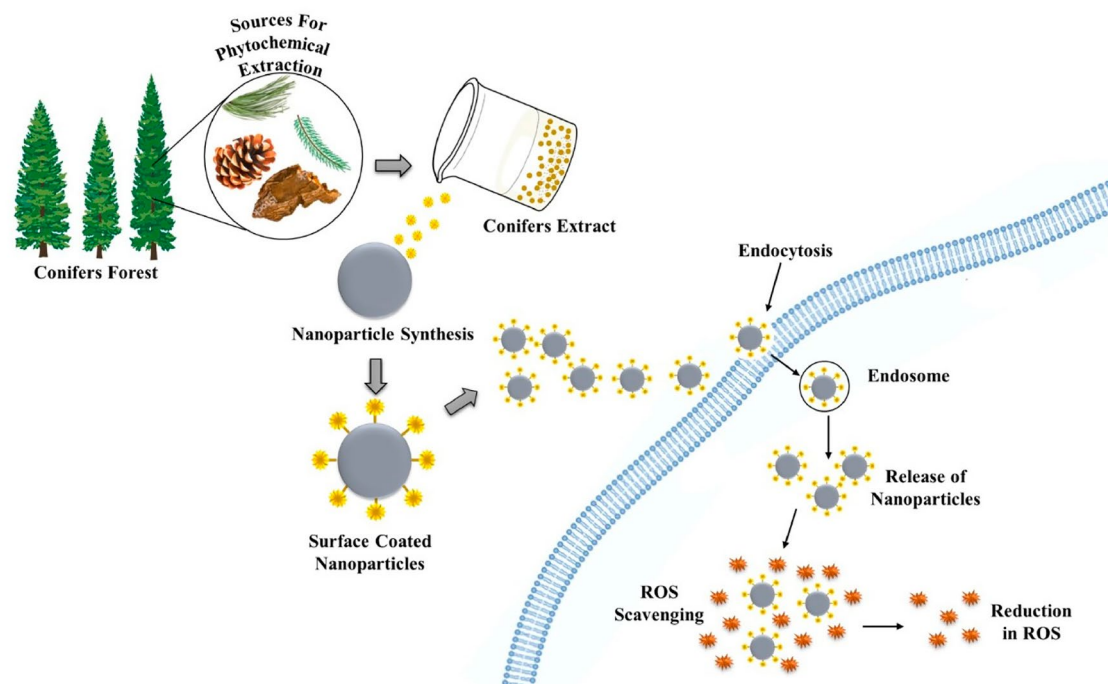


Fig. 15 Antioxidant mechanism of nanoparticles (NPs). Reproduced/adapted from Ref. [248] under the terms of the Creative Commons CC BY 4.0

reaching an impressive 87% inhibition at a 500 $\mu\text{g/ml}$ concentration. Their anti-inflammatory effects were terrific, achieving 89% at the same concentration, and they also showed notable anti-diabetic properties with an 82% inhibition. Interestingly, when the concentrations were increased, the levels of inhibition became even more pronounced. ZnO and Ag nanoparticles possess an inherent capability to capture free radicals. The antioxidant qualities of Ag nanoparticles arise from the functional groups present on their surface. Adding silver to zinc oxide nanoparticles creates a dynamic duo that enhances their ability to combat free radicals and elevate antioxidant capacity [255]. The Se-ZnO nanocomposites made with aloe vera showed a remarkable ability to capture hydroxyl radicals, and this ability grew stronger as the selenium content increased. The $\text{Pd}_3\text{In}_2@\text{GO}$ nanocomposites demonstrated encouraging antioxidant activity [256]. Nanoparticles from *Peganum harmala* demonstrated more potent antioxidant activity than their single-metal counterparts and showed promising anticancer effects against breast (MCF-5) and lung (A549) cancer cells. The Ag-Pt nanocomposites obtained from *Crocus sativus* demonstrated improved antioxidant properties when compared to Ag or Pt nanoparticles individually [257]. Nanocomposites made from *Salvadora persica* have demonstrated antioxidant properties as well. Zn monometallic nanoparticles showed an impressive antioxidant potential of 72% when evaluated through the DPPH method. On the other hand, copper/zinc bimetallic nanomaterials showed a scavenging potential of 89.5% when tested at a concentration

of 1500 $\mu\text{g/ml}$, and it was observed that increasing the concentration led to an even greater scavenging activity [258]. These examples suggest that nanocomposites could improve the bioavailability and absorption of antioxidants more effectively than individual nanoparticles [248].

Anti-cancer activity

Metal NPs can induce apoptosis in cancer cells and inhibit tumor growth. Cancer stands as one of the foremost causes of death around the world, frequently associated with changes in proto-oncogenes and tumor suppressor genes [259, 260]. Some of the most frequently encountered types of cancer are those that impact the prostate, lung, liver, colon, stomach, cervix, thyroid, and breast [260]. Colorectal cancer is a major cause of cancer-related deaths and affects a large number of both men and women. It also has a high mortality rate worldwide. Today's cancer treatments, including chemotherapy, radiation, and various medications, often come with significant side effects that can significantly impact patients' quality of life. This has led to an increasing interest in exploring complementary and alternative therapies. Although there are other options available, such as immunotherapy and hormone therapy, these treatments can also lead to unwanted side effects [261, 262].

Drug delivery systems that utilize nanotechnology have surfaced as an exciting option to tackle these challenges. Nanotechnology's role in medicine has grown remarkably, presenting opportunities for safer, more precise, and

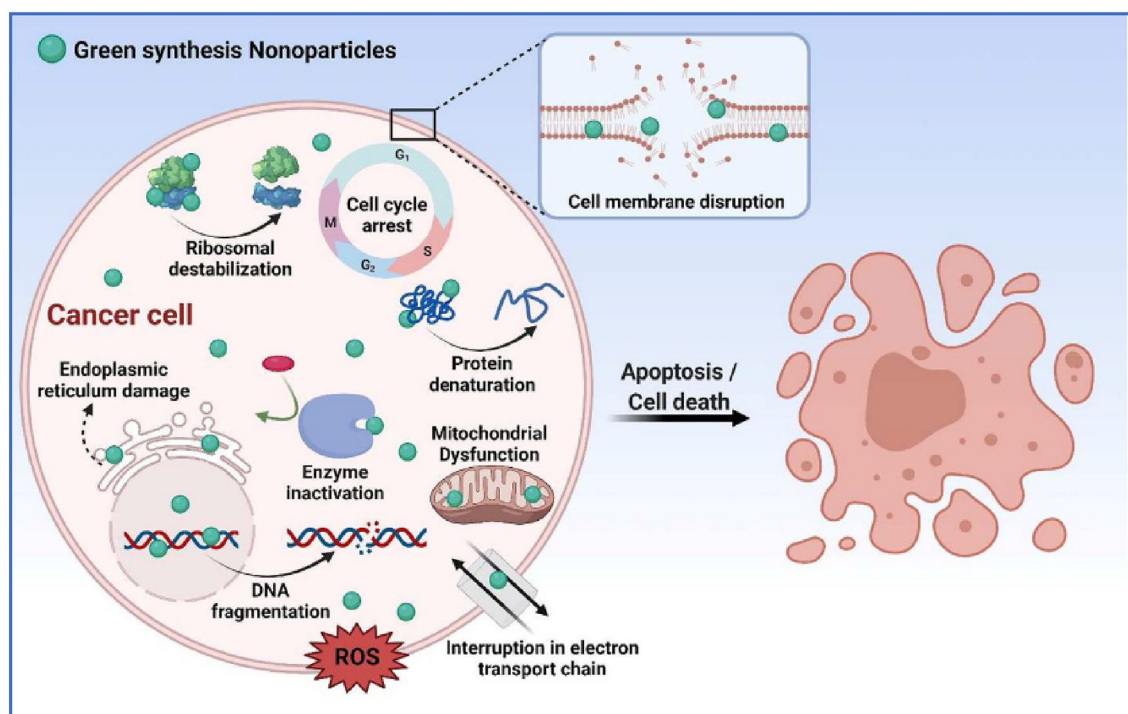


Fig. 16 Mechanisms underlying the anticancer activity of metallic nanoparticles (NPs). Reproduced/adapted from Ref. [265] under the terms of the Creative Commons Attribution License (CC BY)

effective cancer diagnosis and treatment approaches. Using nanomaterials for drug delivery brings several benefits, including better absorption in the body, targeted treatment of cancer cells, fewer side effects, and a lower chance of drug resistance [263, 264]. Figure 16 provides a visual representation of the mechanism.

Plant-based treatments and their derivatives have helped us gain deeper insights into how cancer develops. The green synthesis of nanocomposites shows great potential in cancer treatment by specifically targeting tumor cells and possibly enhancing the effects when used alongside chemotherapy drugs [266]. These nanocomposites may also show promise in combating drug-resistant cancer cells. Combining chemotherapy or radiotherapy with natural compounds from plants is seen as significant. Research indicates that nanocomposites made with alkaloids and sterols derived from medicinal plants may work together to enhance anticancer effects. Due to the precise configuration of their hydroxyl groups, Flavones play a significant role in inhibiting cell growth and kinase activity. They are involved in the cellular processes that contribute to the development and progression of cancer [267]. Imagine creating nanocomposites that blend superparamagnetic nanomaterials with 2D graphene derivatives to enhance drug delivery. These materials can hold many drugs and allow for carriers that magnets can control. Multifunctional moieties play a crucial role in effectively targeting and managing the release of encapsulated

anticancer drugs. Nanogels, a fascinating nanocomposite form, can be taken up by specific target cells. This process helps limit their build-up in healthy tissues, reducing side effects [268]. Nanomaterial drug delivery systems can potentially lessen the harmful effects on healthy cells during chemotherapy, ultimately leading to better patient survival rates. Using molecular interactions, nanocomposites can be crafted to engage with particular cells, tissues, or organs. This precise delivery happens when drugs loaded in nanocomposites interact with receptors on cancer cells. Many studies have shown that bimetallic nanocomposites can have anticancer effects. For instance, trimetallic nanocomposites made from Au-Pt-ZnO and synthesized using *Arctium lappa* extract demonstrated anticancer activity at a concentration of 10 mol [269]. In a similar vein, nanocomposites made from Au-Ag and derived from *Desmodium gangeticum* showed promising results in combating prostate (DU 145) and cervical (HeLa) cancer cells [270]. The nanocomposites of CuO and GO-CuO, created using the extract from *Acalypha indica* leaves, showed notable anticancer effects on the HCT-116 cell line [271]. The Mo-ZnO/reduced graphene oxide nanocomposites created with the help of *Phoenix dactylifera* fruit extract demonstrated improved anticancer impact compared to pure ZnO nanoparticles. This was observed in colon (HCT116) and breast (MCF7) cancer cells, with the effects linked to the p53, caspase-3 pathway, and reactive oxygen species. Trimetallic nanocomposites

made from Ru, Ag, and Pd, created with the help of garlic tunicate leaf extract, demonstrated the ability to inhibit the growth of several cancer cell lines [272]. These nanocomposites can home in on tumor cells, thanks to a phenomenon known as enhanced permeability and retention. This occurs because tumor cells often have new blood vessels and larger openings, making them different from normal cells. They can slow down the growth of cancer cells by stopping them from dividing and encouraging them to undergo programmed cell death. This happens when cyclins and cyclin-dependent kinases are reduced in activity [273]. These tiny materials can also trigger the formation of reactive oxygen species, which may result in oxidative harm and cell death by damaging membranes and causing the condensation of the nucleus. Apoptosis can be initiated through two main pathways: intrinsic and extrinsic. Mitochondria changes and DNA damage characterize the intrinsic pathway. Higher levels of reactive oxygen species, halting the cell cycle, and activating caspase-3 can contribute to programmed cell death in cancer cells. Further studies showed that the Au–Ag nanocomposites created from *Desmodium gangeticum* demonstrated remarkable effectiveness against prostate tumors (DU 145) and cervical tumors (HeLa) [274]. Biosynthesized bimetallic Ag@ZnO nanocomposites showed the ability to induce programmed cell death in cervical cancer cells. In the groups treated with biosynthesized bimetallic Ag@ZnO NCs at the IC₅₀ concentration of 5 µg/mL, the percentage of apoptotic cells was 79.68%. In contrast, ZnO-NPs needed more than 500 µg/mL to achieve similar effects, possibly indicating a significant difference in their efficacy [275]. Bimetallic and trimetallic nanoparticles are gaining attention in biomedical applications because they are biocompatible, versatile, and chemically inert, making them quite advantageous in this field. Their combined effects often result in greater antibacterial and anticancer activities than those seen with single-metal counterparts [266].

Plant tissue culture

Plant tissue culture techniques can be used to obtain consistent and reproducible sources of plant material for NP synthesis. This approach can produce plant extracts with specific phytochemical compositions. Plant tissue culture (PTC) overcomes the inherent unpredictability associated with wild-harvested plants by providing a sterile and highly controlled supply of biomass, thereby offering a transformative platform for the green synthesis of nanoparticles (NPs). PTC techniques, such as callus and cell suspension cultures, ensure genetic homogeneity and a consistent metabolic profile, unlike field-grown plants that are susceptible to seasonal and regional variations in phytochemical composition. This level of control can be achieved by modifying

the growing environment, including the composition of the nutrient medium and the use of elicitors to selectively enhance the production of secondary metabolites, such as terpenoids, phenolic acids, and flavonoids. For example, *Agrobacterium rhizogenes*-induced hairy root cultures provide an autotrophic, genetically stable system capable of producing large quantities of specific metabolites for effective metal ion reduction and nanoparticle stabilization [276]. By optimizing these in vitro systems, researchers can precisely control the size, shape, and stability of metallic nanoparticles, such as Ag, Au, and ZnO, thereby enabling the reliable and large-scale production of biocompatible nanomaterials for industrial and biomedical applications.

Summary and future outlook

In conclusion, the use of plant extracts nanoparticles for the synthesis of metallic nanoparticles (NPs) represents an environmentally friendly and sustainable alternative to conventional chemical approaches. The diverse phytochemicals present in plants act as both reducing and stabilizing agents, enabling the controlled fabrication of NPs with tunable size, shape, and functional properties features that are critical for their performance in various applications. Future research should focus on identifying novel plant resources, standardizing extraction and synthesis protocols, and improving reproducibility to achieve greater precision in tailoring NP characteristics. Moreover, expanding the scope of applications, particularly in biomedicine, food technology, agriculture, and environmental remediation, while ensuring rigorous safety and toxicity assessments will be essential for their broader adoption. Ultimately, plant extract-mediated synthesis offers a promising and rapidly evolving platform in green nanotechnology, with the potential to drive sustainable innovations and contribute to a healthier and more eco-friendly future.

Author contributions A.R.: Literature Search, Data Collection, Writing—Original Draft; A.K.S.: Literature Search, Writing—Review & Editing; R.D.N.: Validation, Reviewing & Editing; N.C.: Funding Acquisition, Reviewing & Editing; S.A.: Conceptualization, Resources, Supervision, Validation, Funding Acquisition, Reviewing & Editing.

Funding Not applicable.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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