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Recent Updates On Green Synthesized Silver Nanoparticles: Preparation Technologies, Properties, And Applications In Biomedical Sector

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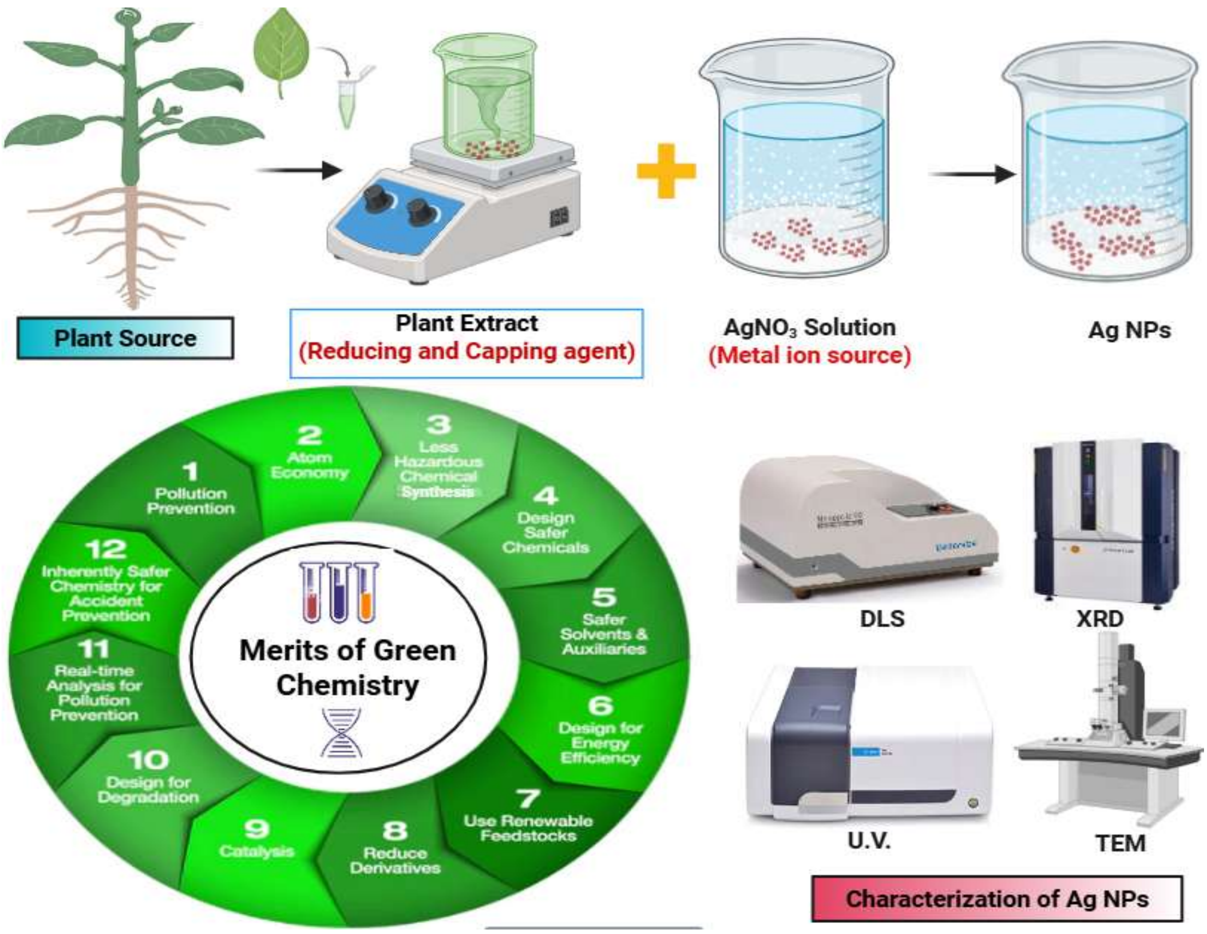
Abstract
Nanotechnology is attracting significant interest as a novel research domain focused on the production of nanomaterials for applications across several sectors. Green-synthesized AgNPs are the most environmentally benign and sustainable choice, and their nanoscale size and exceptional capacity to alter physical, chemical, and biological characteristics have made them very popular. Amongst the diverse forms of nanoparticles, green synthesized AgNPs have become prominent as the most prevalent and extensively employed owing to their remarkable qualities. The implementation of plant-derived materials reductants enhances the attractiveness of silver nanoparticle manufacturing. This review highlights the various therapeutic properties of AgNPs produced through plant-mediated methods. Furthermore, an effort is undertaken to present a clarified description of the operational processes that govern the pharmacological effects of green AgNPs. This manuscript comprehensively covers the preparation technology, significance of green synthesis with characterization techniques used for evaluation and broadly describes antibacterial qualities, wound healing, anticancer, antioxidant potential, and tissue engineering with regulatory challenges
Keywords: *AgNPs; Green synthesis; Regulatory framework; Wound healing; Antibacterial activity, Eco-friendly.*

Graphical abstract



INTRODUCTION

Nanotechnology is attracting significant interest as a novel research domain focused on the production of nanomaterials for applications across several sectors, including biomedical, cosmetics, pharmaceuticals, sensors and food technology. Nanoparticles (NPs) are solid particles at the nanometer scale (<100 nm) that exhibit superior physical characteristics relative to bulk molecules, influenced by their size and shape [1]. Various types of metal and metallic oxides nanoparticles have been comprehensively investigated as they have larger characteristics, including an efficient dispersion in solution and a high surface-to-volume ratio [2]. Amongst the different metal nanoparticles, green-synthesized AgNPs are gathering substantial attention among the researchers as a result of their extensive applications in pharmacology, microbiology, chemistry, cell biology and food technology [3]. Various approaches, including hydrothermal method, thermal decomposition, microwave-assisted combustion, sol–gel method, microemulsion, co-precipitation and chemical vapor deposition has been utilized for silver nanoparticle fabrication [4]. “Green synthesis” implies the utilization of ecologically benign components, including plants, fungus, and bacteria, in the nanoparticle synthesis. Green synthesis, involves bioactive agents for instance, flavonoids, terpenoids, alkaloids and polyphenols, which play a fundamental role in nanoparticle formation [5]. Nanoparticles synthesized using green technology are more advantageous than those produced using chemical and physical approaches. Green-synthesized nanoparticles requires less energy, is environmentally benign, and harmless. It possesses the maximum stability and a less difficult technique [6]. Diverse elements of plant like leaves, flowers, fruits, roots, and rhizomes are reported for fabrication of AgNPs. The efficacy of nanosilver in eliminating harmful bacteria has attracted significant interest, it can impede growth of both gram-ve and gram +ve bacteria [7]. The antimicrobial efficacy of several antibiotics was improved by incorporating AgNPs. It is as well an efficient fungicide and possesses antiviral effects. AgNPs (AgNPs) are often utilized in various products, including wound dressings, surgical instruments, bone cements, and implantable devices [8]. Numerous approaches for instance chemical, biological, and physical methods can be employed for nanoparticle synthesis. Stabilizing agents and concentrated reductants are employed for nanoparticle production through physical and chemical approaches, posing health risk in humans and affects the ecosystem. Green synthesis procedure supports sustainability as it utilizes renewable sources, works in ambient conditions [9, 10]. This paper aims to presents thorough synthesis of AgNPs using plants and to investigate their potential biomedical applications including antimicrobial, anticancer, and wound healing properties. The review paper thoroughly examines the current understanding of the possible application of plant materials in conjunction with AgNPs. Furthermore, it offers a significant database that might act as a basis for future researchers seeking to investigate the environmentally sustainable manufacture of AgNPs using plant-based techniques.

Techniques for nanoparticle production

In recent years, both plants and microorganisms have been subject of extensive research for the green-synthesised AgNPs (AgNPs), yielding diverse sizes, shapes, stabilities, and antibacterial properties [11]. Two primary strategies for nanoparticle production includes the top-down strategy that depends on physical approaches, and the bottom-up technique that employs biological and chemical activity. The top-down technique entails the diminution of large mass of materials into nanoscale size with the help of cutting, milling, and scratching. This method is incapable of accurately controlling nanoparticle at atomic level formation and instead produces nanomaterials by breaking down the bulk materials [12]. Various physical methods for nanoparticle fabrication encompass, pulsed wire discharge, vacuum vapor deposition, laser ablation, and mechanical milling [13]. The bottom-up method includes amassing the structure of nanoparticles by starting with individual atoms, molecules, or clusters. Chemical reduction, microwave-assisted processes, chemical free reduction, hydrothermal approaches, co-precipitation, microemulsion techniques and electrochemical methods are often utilized for nanoparticle creation by chemical means [14]. The biological method of synthesis of nanaoparticles include plants, fungi, bacteria, yeast etc. Plant extracts with diverse pH levels are added to solutions with changing concentrations of silver salts as metal precursors, thereafter subjected to heating at different temperatures, leading to the production of AgNPs [15]. The top-down strategy includes physical and as well as chemical processes, while the bottom-up method is only concerned with chemical procedures. The physical top-down approach encompasses the conventional methodology of metallurgy, which may be classified into two categories: direct and indirect processes [15]. The selection of a non- toxic stabilizer, an efficient reducing substance, and an environmentally benign solvent constitutes the three essential prerequisites for the nanoparticles synthesis. Various synthetic methods, encompassing physical, chemical, and biosynthetic processes, have been implemented to manufacture nanoparticles [16]. The conventional chemical approaches employed are prohibitively expensive and involve hazardous materials that pose significant environmental risks. Numerous nanoparticles have been derived from different plant parts like, leaves, roots, stems, seeds, and fruits as these components contain phytochemicals that performs dual function as both stabilizers and reducers in the extracts. Stabilizing and capping agents are vital in the green synthesis of AgNPs, as they help prevent the particles from clumping together and maintain their uniform size and structural stability [17]. AgNPs can now be synthesized in an eco-friendly and sustainable manner through green synthesis, that uses green technology which avoids toxic reducing agents or stabilizers by employing biological organisms such as fungus, algae, and bacteria to aid in the reduction of Ag ions into nanoparticles [18]. Plant extracts, recognized for their abundant secondary metabolites, are particularly advantageous as capping and reducing agents, such as, the application of *Azadirachta indica* and *Eucalyptus globulus* extracts for fabrication of AgNPs led to improved antibacterial action, as well as regulated size and morphology. Similarly, *Aspergillus niger* and various fungi have been utilized for synthesis of AgNPs elucidate the role of fungal proteins in the maintenance pathway [19]. Furthermore, algal-mediated production demonstrates potential; under mild conditions, *Chlorella vulgaris* can facilitate the synthesis of AgNPs [20]. These qualities depict green synthesis as an advantageous choice for environmental and medicinal applications. Research has shown that AgNPs generated using environmentally benign ways has powerful antibacterial, anticancer and antifungal action, rendering them appropriate for many medical applications [21].

Green nanoparticles synthesis vs conventional methods of silver nanoparticle synthesis

Nanoparticles' unique physicochemical properties enable tailored medicine delivery to certain regions, minimising negative effects. Because of their quick elimination, poor solubility, and systemic toxicity, traditional drug delivery techniques usually show little therapeutic value [22]. By enhancing medication release mechanisms, targeting, and bioavailability, nanoparticles have revolutionised modern medicine. These characteristics include regulated drug release mechanisms and variable surface characteristics [23].

Green nanoparticle synthesis

Green synthesis differs from former synthesis techniques because it is more sustainable, non-polluting, non-hazardous and safer for the environment. Employing biobased resources like as microorganisms, plants, and agricultural waste aids in the preparation of environmentally friendly NPs which promote a sustainable environment [24]. Various natural sources, for instance, bacteria, yeast, plants and fungi, may be utilised to create nanoparticles. Furthermore, both unicellular and multicultural organisms are capable of synthesising extracellular and intracellular inorganic nanoparticles [25]. The green techniques used for synthesis and nanoparticle size control utilizing different herbal extracts for the manufacturing of nano ferrites, comprising of photocatalysis, metal ions may be turned into nanoparticles with the help of biomolecules included in plant extracts. This biogenic reduction of metal ions to base metal may be accelerated quite quickly at suitable pressure and temperature [26]. In comparison of traditional ways, green synthesis is more sustainable and yields fewer hazardous byproducts [27].

Fig.1 Process of production of green AgNPs

Conventional methods of silver nanoparticle synthesis

Due to their unusual surface-to-volume ratio, nanosized metallic particles have been used for an array of purposes and may significantly affect biological, physical and chemical properties. Owing to their distinctive features, they have found employment in a variety of products, including as antimicrobials, medical device coatings, pharmaceuticals, industrial, home, and healthcare-related items [28].

Conventional methods to prepare AgNPs:

1. **Physical Method:** Utilising physical agents like heat, electrical discharge and electromagnetic irradiations are examples of physical technique [29].
2. **Chemical Method:** Chemical synthesis of AgNPs typically involves three key components: a silver salt (metal precursor), stabilizing or capping agent and reducing agent. The process includes two main stages—nucleation and growth—where shape and size of the nanoparticles are largely determined. To obtain monodispersed AgNPs with uniform size, simultaneous nucleation is essential, followed by uniform growth. Reaction conditions such as temperature, pH, and the choice of reagents critically influence these stages [30].
3. **Biological Method:** Nontoxic molecules (proteins, carbohydrates, antioxidants, etc.) made by living things, for instance bacteria, plants, yeast and fungi (Fig. 1) take the place of toxic reducing agents and stabilisers in the biological production of AgNPs [31].

Characterization techniques for plant-derived AgNPs

AgNPs (AgNPs) possess very distinctive properties, which mark their valuable applications in various industrial and consumer products. In materials science and engineering, characterization techniques help to inspect the properties of materials at both the macroscopic and microscopic levels. These techniques deliver info about chemical, electrical and physical properties of material. These, in turn, aid in understanding how the materials behave and help in developing new ones with special features. Characterization is employed to investigate the properties of raw material, check material quality at the time of processing and production, and test how well they perform in different applications [32].

UV–vis Spectroscopy- This is the versatile and popular method for governing extracellular green production of NPs (nanoparticles) in reaction mixture. It is an enormously useful tool that can be employed for the main evaluation of the produced nanomaterial [33].

Fourier transform infrared (FTIR) spectroscopy- This technique is mainly used for studying the surface chemical constitution or functionalization of NPs during characterization, especially in organic ones. It is also useful in detecting contaminants on the surface of the material when high purity is sought. For plant-based NP production, for instance, Ag NPs fabricated using *Camellia sinensis*, the FTIR analysis shows *Camellia sinensis* phytocompounds presence, for example catechin and caffeine, on Ag NPs surface, may be accountable for Ag ion reduction to Ag nanoparticles or may act as stabilizing agents [34].

X-ray Diffraction (XRD) - This technique analyses the crystal structure of the AgNPs, wherein the X-rays interact with the crystal lattice of the material when they are directed at it and scatter in a specific pattern. These pattern, according to BRAGG'S LAW, reveals details about internal material structure. Thus, the crystalline nature and the normal size of Ag nanoparticles are established. By applying the Scherrer equation in XRD, the broadening of diffraction peaks (which are produced during the interaction of X-rays with the crystalline structure) is analysed, and the crystallite size is determined. This property helps in the development and application of nanoparticles in numerous disciplines [35].

Energy dispersive x-ray spectroscopy (EDS or EDX)- This method analyses the elemental composition of the plant-derived AgNPs, providing semi-qualitative and qualitative information about the chemical elements in them. In a study on AgNPs synthesised by lactobacillus, an XRD pattern was observed at 32.32° corresponding to the FCC (face-centered cubic) structure of Ag, a characteristic of FCC arrangement. EDX further confirmed the existence of Ag in the nanoparticles [36].

Transmission electron microscopy (TEM) - TEM delivers thorough data regarding the surface morphology of NPs by studying their shape, size, and arrangement in the structure. This technique uses electrons to achieve very high resolution, which helps to visualise details at the atomic level [37].

Field emission scanning electron microscopy (FESEM)- A potent method for imaging, useful for studying the size, shape, distribution, and surface morphology of NPs. A focused electron beam scans the sample surface, obtaining high resolution that helps observation of finer details at the nanoscale. When coupled with EDX, NP's structural and chemical properties are evaluated and applied in sectors like medicine, environmental sciences and electronics [38].

Dynamic Light Scattering (DLS) - This technique involves analysis of NPs in the submicron range and works on the principle of Brownian Motion, wherein particles scatter light while moving randomly in a liquid. DLS measures the diffusion coefficient caused by the rapid fluctuations in the scattered light intensity by particles of different sizes. Measurements of the sizes are then done by the Stokes-Einstein equation [39].

Particle size and zeta potential analysis- Zeta potential is used to ascertain the surface charge of NPs in colloidal solutions, thus evaluating and optimizing the formulation of NP based products**.** The stability of NPs is measured by zeta potential. [40].

Thermogravimetric analysis (TGA)- TGA determines the change in mass when heated or cooled in a controlled environment. This technique assesses the thermal stability of materials and can detect other thermal events such as phase transition, decomposition, and oxidation. The resulting data is represented as a thermogram, unique to each material that helps in determining its composition [41].

Applications of green AgNPs in biomedical sector

Silver and its compounds have fascinated humans for thousands of years because of their exceptional antibacterial and therapeutic qualities. Ancient civilizations, including the Greeks, provide evidence of the early acknowledgment of silver's therapeutic properties. Hippocrates, a renowned physician regarded as the "Father of Medicine," used silver treatments to address ulcers and promote wound repair [42]. Silver therapies have persisted over the decades, notably utilized in burn management and the prevention of wound infections in 19th century. This ancient viewpoint highlights the lasting significance and many use of silver's medicinal capabilities through various periods. The advent of antibiotics in the 1940s signified a transformative change in medicine, offering effective means to combat bacterial infections [43]. Antimicrobial resistance (AMR) has emerged as a significant worldwide health problem since the 1980s, further intensified by the rise of multidrug-resistant bacteria. Scientists and clinicians are diligently investigating alternate methods to address bacterial infections in light of this escalating threat [44]. In current era, nanotechnology has emerged as a prolific domain for scientific inquiry and invention. Silver has garnered heightened attention in this emerging sector owing to its distinctive nanoscale characteristics. AgNPs have attracted considerable interest in several domains, especially medicine, due to their exceptional antibacterial properties and prospective therapeutic uses [45] (Table 1).

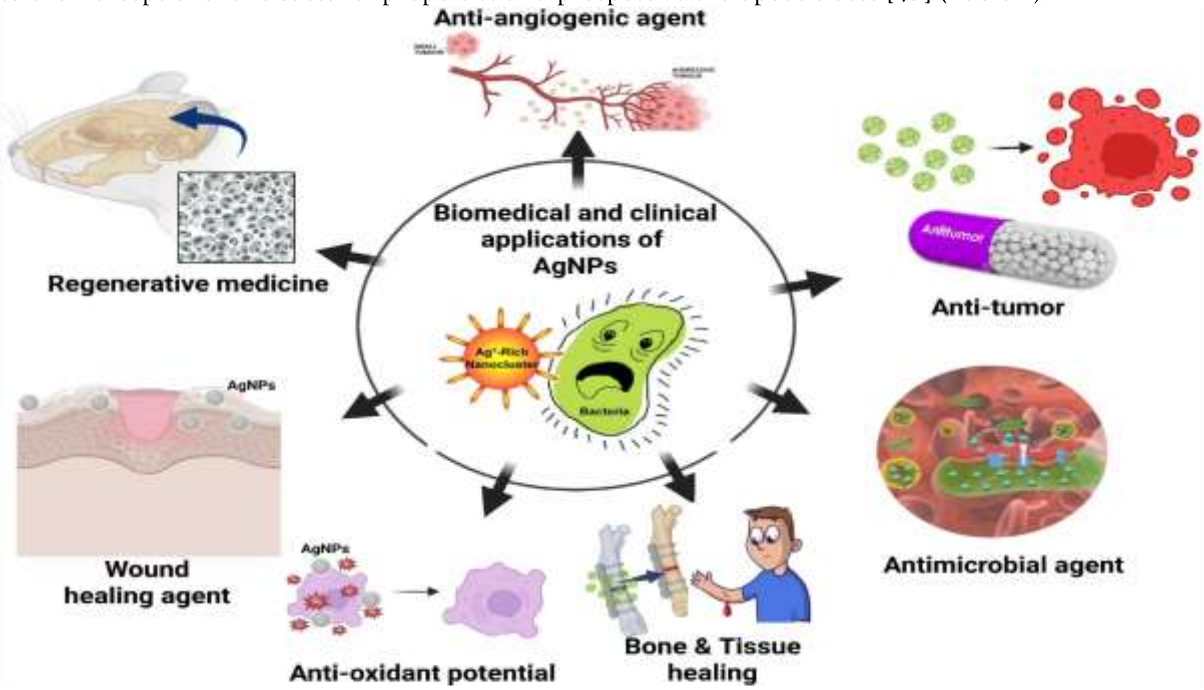


Fig 2: Various biomedical and clinical applications of AgNPs

Table 1 Various herbal components used in green AgNPs

Components for producing green AgNPs	Characterization Techniques	Antimicrobial study/Pre-clinical study	Outcome	Reference
Neem and turmeric extracts	TEM,SEM,XRD	Candida albicans, Staphylococcus aureus, and Lactobacillus,	Formed nanoparticles displayed substantial antimicrobial action for *Staphylococcus aureus* and other species, therefore this research investigation clearly stated the immense potential of AgNPs using plants for fabricating eco-friendly antimicrobial agents.	[46]
L. angustifolia	FTIR, EDX,SEM, and XRD	Rats	The findings indicated that La-AgNPs possessed an ideal size and, when administered orally, resulted in a substantial reduction in gastric ulcer index, alongside an elevation in pH. Furthermore, tissue levels of nitric oxide (NO), and glutathione were elevated, whereas serum levels of NO and reactive oxygen species, together with histological findings, corroborated these observations. This formulation may serve as a viable therapeutic option for gastric ulcer	[47]
Clerodendrum serratum	XRD,FTIR,TEM	Acinetobacter baumannii and Pseudomonas aeruginosa	The formulated nanoparticle-based gel preparations markedly improved in vivo wound repair and inhibiting multidrug-resistant pathogens while concurrently facilitating tissue regeneration. So, the formulation is good for infected wounds.	[48]
Glycyrrhiza glabra linn root extract	TEM,FTIR,UV	Rats	This nanoformulation exhibited considerable wound repair efficacy via facilitating contraction of wound and expediting restoring skin over a 21-day period	[49]
Rubus sanctus schreber	(FT-IR), the phytochemical (XRD), (SEM), (TEM),	*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*	It may be deduced that biogenic AgNPs were functionalized, exhibiting considerable promise as anti-cancer and antibacterial agents for industrial and medicinal use.	[50]
Banana and pineapple peel extracts	FTIR (XRD), (AFM) and Zeta potential.	*Pseudomonas aeruginosa* and *Escherichia coli*	AgCuNPs synthesized by traditional green synthesis techniques. Furthermore, the nanoparticles successfully sterilized tilapia skin while preserving its collagen structure, indicating their potential for use in biomaterial sterilization.	[51]

Rhamnus prinoides (Gesho)	FTIR, TGA XRD	*Klebsiella pneumoniae*, and *Streptococcus pyogenes*.	AgNPs produced from *R. prinoides* leaf extract exhibit excellent antibacterial, photocatalytic and antioxidant properties, positioning them as viable candidates for biomedical applications.	[52]
Ferula gummosa Boiss. gum extract	TEM, DLS, EDX, FESEM, and FTIR	*Staphylococcus aureus* and *Bacillus subtilis*.	The formulation exhibited notable antibacterial efficacy for various bacterial species. The antioxidant experiments demonstrated good antioxidant activity. These findings present a viable approach of CS-AgNPs for prospective medicinal applications.	[53]
Salvia sclarea L.	UV, SEM, FTIR Zeta potential, , XRD, and TEM	*Bacillus cereus* and *Candida krusei*, *Candida albicans*.	The conclusion emphasizes on the ability of S. sclarea extract as a cost-effective and accessible resource for the synthesis of biogenic AgNPs with diverse uses.	[54]
Lignin	UV, XRD and TEM	*S. aureus* and *E. coli*	The formed nanoparticles possessed 24 nm of size and displayed effective results after combining lignosulfonate and silver particles. It enhances antimicrobial and antioxidant action and it is less toxic compared to pure AgNPs.	[55]

Antibacterial action

AgNPs offers significant benefits in the fight against bacterial infections. Their diminutive size promote intensified interactions with bacteria, resulting in significant antibacterial efficacy. Furthermore, AgNPs has distinctive physical features that compromise bacterial cell membranes and obstruct vital cellular functions [56]. These drugs demonstrate broad-spectrum activity against many bacteria, encompassing both gram-positive and gram-negative species, therefore tackling the escalating issue of antibiotic resistance and the constraints of conventional therapies. The revival of silver at the nanoscale underscores its promise as an effective instrument in combating bacterial diseases [57]. This work demonstrates the eco-friendly manufacture of AgNPs utilizing *Nothapodytes foetida* leaf extract (Nf-AgNPs) and examines its antibacterial, and anticancer properties. The formulation displayed prominent anticancer efficiency with superior efficacy in inhibiting both Gram-negative and Gram-positive bacteria. The results validate that formed formulation can function as antibacterial as well as anticancer agent for biomedical purposes [58]. The biosynthesis of AgNPs from *Pyrus betulifolia* Bunge branch extracts was carried out, where plant bio actives such as phenols, proteins, and sugars served as reducing and capping agents for the AgNPs. Results of various analytical investigations depicted absorbance of UV–vis spectra at 425 nm, a distinctive feature of AgNPs, signifying the effective synthesis of the nanoparticles. Subsequent analysis using TEM revealed that the produced AgNPs were homogenous, 19.9 nm spherical particles and a zeta potential of −18 mV. FTIR verified that many functional groups were accountable for reduction and stabilization during the biosynthesis procedure. The antibacterial efficacy of the produced AgNPs indicated that the AgNPs were very efficient against both bacteria and fungi. The outcomes of the MTT and hemolysis assays demonstrated that the synthesized AgNPs exhibit great biocompatibility, rendering these green-synthesized AgNPs suitable for diverse applications [59].

Antioxidant action

AgNPs generated by green methods, for instance plant extracts, demonstrate distinctive features that augment their antioxidant capabilities. This facilitates the efficient neutralization of free radicals and the mitigation of oxidative stress in biological systems, as corroborated by several studies [60]. This research generated AgNPs(AgNPs) with Cuscuta epithymum extract, using maceration. The production of AgNPs was confirmed using UV-Vis at 425 nm. The nanoparticles displayed a spherical morphology with 15-60 nm diameter. The antioxidant action of AgNPs was determined using the DPPH test (IC50=45.55 mg/L), whereas formulation exhibited significant antimicrobial action. It may be inferred that *C. Epithymum* extract may convert Ag+ ions to form AgNPs, which exhibit remarkable antioxidant, antibacterial, and anti-tumor properties [61]. This work aims to illustrate the photo irradiation-facilitated green production of AgNPs, employing aqueous extract of *Xylocarpus granatum* (**XG**) bark. Additionally, 500 μg ml−1 and 100 μg ml−1 of the extract exhibited antioxidant activity. Similarly, 500 μg ml−1 of extract exhibited approximately 35% chelating potential for iron and copper against 0.1 mM FeSO4 and 0.4 mM CuSO4, respectively. The nanoparticles were mostly spherical, including roughly 17.5% silver atoms and 37% carbon atoms, suggesting the incorporation of silver atoms into the extract nanocomposite, which may facilitate prospective therapeutic applications of the nanocomposite. Consequently, the present study deduces that the extract's antioxidant and metal-chelating capabilities may enhance its nanotherapeutic uses [62].

Anticancer action

Silver nanoparticles designed employing environment viable methods display efficient anticancer action and considerably hinders the development of many cancer cell lines, including those of breast, lung, and prostate cancers. These nanoparticles obstruct signalling pathways in cancer cells and induce apoptosis, indicating their potential use in cancer treatment [21]. Current investigation focusses on production of AgNPs using extracts from the leaves, stems, and roots of *Pelargonium radens*. The produced AgNPs exhibited a pristine crystalline structure with dimensions ranging from 40 to 60 nm. The FTIR investigation identified the infrared bands of the functional groups ranging from 2360 cm^{-1} to 1047 cm^{-1}. This study examines the *in vitro* anticancer efficacy of AgNPs derived from the roots, stems, and leaves of the *Pelargonium radens* plant against breast, cervical, and lung cancer cell lines. The antitumor efficacy of the produced AgNPs was evaluated using a cell viability test. The produced AgNPs utilizing *Pelargonium raden* extracts exhibited enhanced anticancer efficacy against cancer cell types [63]. This investigation involves the production of green AgNPs (AgNPs) using O. *gratissimum* (gr-AgNPs), O. *tenuiflorum* (te-AgNPs), and O. americanum (am-AgNPs), along with an assessment of their antibacterial and anticancer activities. SEM examination revealed spherical particles with an average size of 69.0 ± 5 nm. Synthesized AgNPs promoted apoptotic cell death in MCF-7 in a concentration-dependent manner. At a higher dosage (20 μg), AgNPs demonstrated efficacy comparable to that of the market standard Doxorubicin (DOX). Am-AgNPs have superior in vitro anticancer and antibacterial efficacy compared to te-AgNPs and gr-AgNPs. The study documented *Ocimum americanum* for its anticancer effects, utilizing a chemical profile (GCMS) and comparing it with previously described species. The action against certain cancer cells clearly shown that various species exhibit different differences in efficacy. The studies have also highlighted the greater potential of biogenic AgNPs in healthcare [64].
This work examined the green production of AgNPs utilizing an extract from green tea to evaluate the cytotoxic effects of these nanoparticles on malignant murine melanoma cell lines (B16F10) and normal murine cell lines (L929). The spontaneous production of AgNPs (AgNPs) using extract of green tea was confirmed by UV–vis spectroscopy, DLS, TEM, and AFM methods. The results indicate the effective production of evenly dispersed AgNPs, producing nanoparticles with an average size of 91 nm. MTT assay

findings indicated that Green tea-AgNPs decreased B16F10 cell viability. Moreover, Green tea-AgNPs augmented the rate of apoptosis in B16F10 cells. The study findings indicate that Green tea-synthesized AgNPs (Green tea-AgNPs) have increased anti-cancer efficacy exclusively against melanoma cells. This indicates that the Green tea-AgNPs generated by this process has significant promise for in anti-cancer research [65].

Wound repair

Wound disrupts skin's protecting barrier that may arise from several sources, including chemical exposure, physical trauma, thermal harm, immunological reactions, or microbial invasion. This disturbance often results in alterations to the skin's structure and function [66]. Effective wound repair is crucial for reinstating the skin's usual functionality and basic integrity. Multiple findings highlight the need of effective wound repair methods for returning the skin to its ideal physiological condition. Wound repair is a complex and dynamic progression that includes four distinct steps: hemostasis, inflammation, proliferation, and wound remodeling, culminating in scar tissue development. These phases enable complex interactions among diverse cellular populations, soluble mediators, cytokines, and other components. The healing activity starts immediately after injury, illustrating the body's capacity to swiftly engage repair systems in reaction to tissue damage [67]. This study used the root of *Premna integrifolia* L for synthesis of AgNPs in wound healing. AgNPs were biosynthesized using a 5% hydroalcoholic root extract enriched in secondary metabolites, combined with 1 mM AgNO3 and exposed to daylight for 10 minutes. They exhibited an optimal surface plasmon resonance (SPR) at 447 nm. The aldehyde, primary amine and phenol functional groups in the extract facilitate the reduction of silver cations to nanoparticles. The nanoparticles exhibited a spherical morphology with stability. The nanoparticles exhibited an excellent roughness profile and contained 57.55% elemental silver.

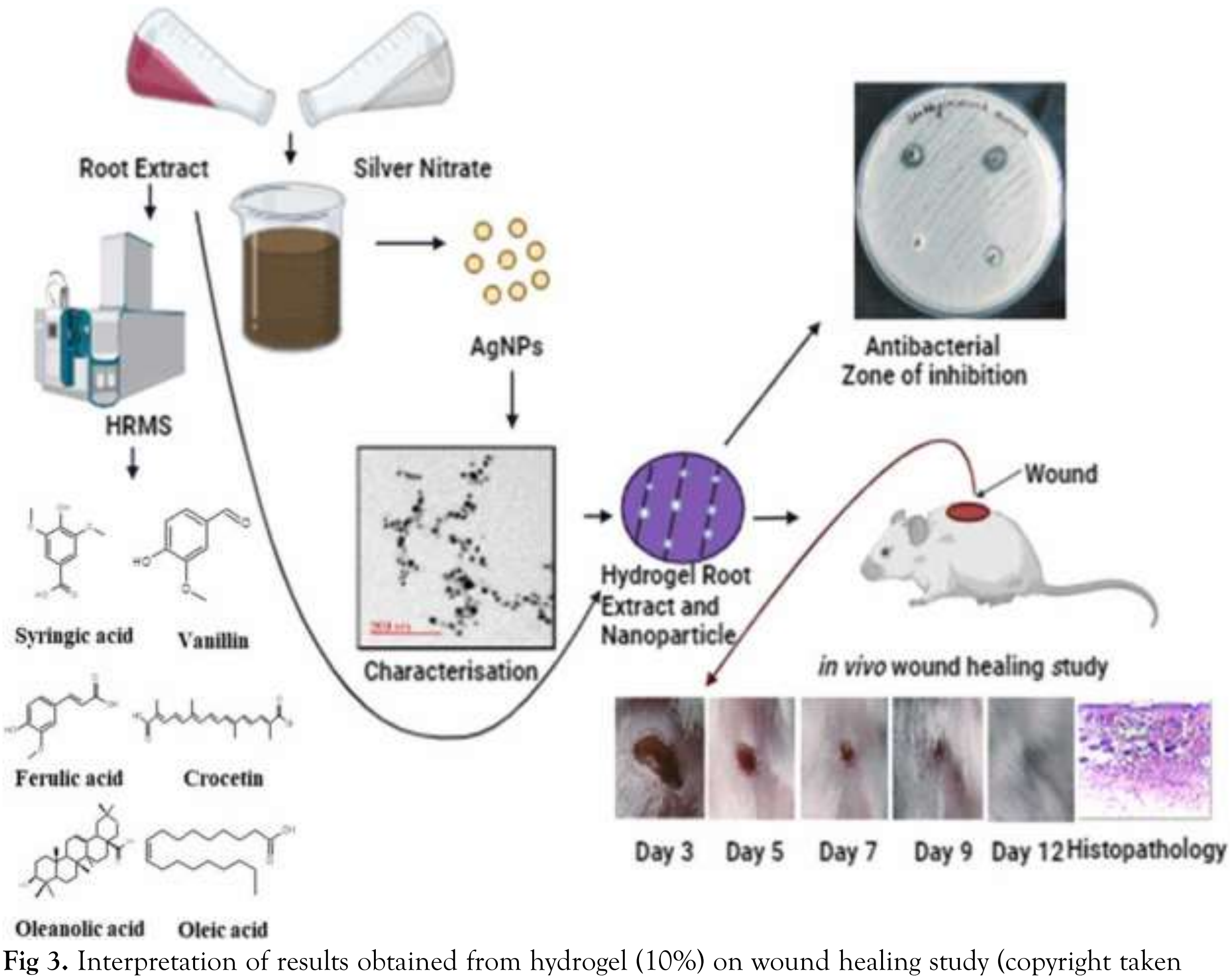


Fig 3. Interpretation of results obtained from hydrogel (10%) on wound healing study (copyright taken for this figure) [68]

The nanoparticles-hydrogel (10%) and the root extract-hydrogel (10%) achieved healing rates of 99.9% and 97.3% for the wound infected with *S. aureus*, respectively. The nanoparticle-hydrogel effectively stimulated re-epithelialization in the dermis of Wound infected with *S. aureus* in comparison to the extract-hydrogel. The nanoparticle-hydrogel accelerated the pace of wound closure [68]. This study illustrates biogenic production of AgNPs using *Ageratum conyzoides* extracts, termed AC-AgNPs, and effectively merges the hemostatic and anti-inflammatory attributes of *Ageratum conyzoides* with the intrinsic antibacterial efficacy of AgNPs. *In vitro* coagulation studies demonstrate that AC-AgNPs possess a significant hemostatic impact, attributable to their size, concentration, and negative charge, while exhibiting little cytotoxicity and hemolysis. Results demonstrated that AC-AgNPs encourage platelet aggregation and activation, stimulate the kallikrein-kinin system, reduce activated partial thromboplastin and prothrombin time, and elevate fibrinogen levels. These results demonstrate that AC-AgNPs influence several signaling pathways, encompassing both endogenous and external coagulation pathways, the complement system, and platelet activation and aggregation. Moreover, the hemostatic effectiveness of AC-AgNPs is evidenced in murine models of tail amputation and hepatic damage, whereby AC-AgNPs markedly diminish both blood loss and bleeding duration. This research demonstrated that AC-AgNPs exhibit potent anti-inflammatory, antibacterial and hemostatic properties, hence promoting wound repair. The biogenic production of AgNPs from medicinal plants may serve as a multifunctional hemostatic agent for practical use [69].

Table 2 Green synthesized AgNPs for wound healing

Biological entity with nanoparticles	**Wound repair mechanism**	**Species**	**References**
Fructus Aurantii	Hastening cellular migration in addition to neovascularization with collagen formation	Rats	[70]
Juglans regia L extract	Enhanced collagen formation	Wistar rats	[71]
Leaves of Cotoneaster nummularia	Antioxidant and antibacterial effect	Male rabbits	[72]
Potentilla fulgens	Re-epithelization and increased collagen levels	Rats	[73]
Azadirachta indica (AAgNPs) and *Catharanthus roseus* (CAgNPs) leaf extracts	Epithelium restoration with collagen deposition	BALB/c mice	[74]
Liquorice extract	Substantial antibacterial action for *E. coli* and MRSA.	Rabbits	[75]
Garlic bulb (*Allium sativum*)	Granulation and epithelialization processes.	BALB/c mice	[76]
Azadirachta indica extract of leaves	Antibacterial effect, Collagen deposition	Adult male albino mice	[77]
Leaves of *Tridax procumbens*	Fibroblasts differentiation hastens the contraction of wound	Mice	[78]

Tissue Engineering

Human tissue comprise intricately structured cells having designated functions and they are associated ECM, a protein-based milieu comprising of glycosaminoglycans that offers three-dimensional support for cell adhesion and proliferation, controls intercellular message, and moderates cell physiology. Tissue engineering (TE), a component of regenerative medicine, involves the creation of nonviable, complex biocompatible systems capable of restoring the structural integrity and functionality of injured tissues through restoration, replacement, or regeneration [79-81]. Green (AgNPs) are suitable option for TE applications due to their effective interaction with biological systems and their specific therapeutic functions. Additionally, they exhibit versatile and tunable properties that meet particular criteria, such as (i) biocompatibility, a multifaceted characteristic dependent on the reciprocal interactions between AgNPs and host cells or tissues. Considering their reduced toxicity to healthy cells, ease of surface functionalization, and superior antibacterial properties, the influence of AgNPs on tissue engineering is noteworthy [82]. This work involved the functionalization of bovine bone grafts (BG) for dental applications with AgNPs generated by the bioreduction approach utilizing extracted essential oil (EEO) and commercial orange essential oil (CEO). The main constituent found in EEO and CEO was D-limonene having concentration reaching 95%. The UV–Vis spectroscopy of nanoparticles revealed a maximum absorbance peak at around 400 nm, whereas transmission electron microscopy indicated typical diameters of 50 nm and 80 nm for NPs-EEO and NPs-CEO, respectively. The physicochemical stability of the nanoparticles was examined for a period of 60 days at a refrigerated temperature of 6 ± 2 °C, revealing that the nanoparticles with essential oil exhibited good antioxidant action evaluated using the suppression of DPPH and ABTS free radicals, as well as the Fe^{3+} reducing power (FRAP test). NPs exhibited superior antioxidant activity outcomes using DPPH and FRAP methodologies in comparison to individual essential oils. Nanoparticles and functionalized bone grafts exhibited superior antagonistic effects compared to individual essential oils against the seven bacteria examined. Consequently, one may assert the biological significance of the produced formulation as an antibacterial agent in dental biomaterials, representing a viable option to mitigate infection processes in bone transplants [83]. This research presented a novel approach for synthesizing genipin-crosslinked gelatin/nanosilver scaffolds using the environmentally friendly in situ production of AgNPs via heat treatment. All gelatin/nanosilver solutions exhibited antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli*. Nonetheless, only the most elevated dose exhibited antifungal properties against *Candida albicans* infections. Scaffolds were fabricated using a lyophilization procedure from this solution, and their antibacterial properties were assessed. This straightforward one-pot method for manufacturing scaffolds with antimicrobial and anti-biofilm characteristics may facilitate significant advancements in tissue engineering applications [84].

Challenges associated with silver nanoparticles

Nanotechnology has drastically changed a variety of scientific domains by making nanoscale materials manipulation possible. Of all synthesis strategies, the green synthesis of nanoparticles, in particular plant-based approaches are widely investigated due to the greenness of the process, economic viability, and sustainability [85]. Plants carry a diverse store of bioactive molecules for instance, alkaloids, terpenoids, and phenolics as reducing and stabilizing agents throughout the synthesis of nanoparticles. Despite increasing promise, numerous challenges restrain scalability, reproducibility, and extensive exploitation of plant-derived nanoparticle synthesis. One of the biggest challenges in plant-mediated synthesis of nanoparticles is the lack of uniformity in the phytochemical nature of plant extracts. The biomolecule concentration is different because of plant variety, geographical location, season, plant age, and used part (leaf, stem, root, etc.) [86]. All these directly influence the size, shape, and stability of the produced nanoparticles, hence uniformity is a major problem.

Although plant-mediated synthesis is extensive, the specific biochemical processes underlying metal ion reduction and nanoparticle stabilization are still unclear. This complicates optimization and control of the synthesis process. For instance, the influence of single phytochemicals on nanoparticle morphology is mostly speculative [87]. Scalability and low yield of AgNPs obtained is greatest challenge. Conditions like the time of the reaction, extract concentration, pH, and temperature require close monitoring while scaling-up, which gets challenging with respect to heterogeneous plant matrices. In addition to that, batch-to-batch difference introduces variance in the characteristics of the nanoparticles [88]. Post-synthesis purification is important to eliminate excess plant residues and metal salts. Plant-mediated synthesis, however, tends to form complex mixtures, and thus purification is cumbersome and time-consuming. Additionally, the long-term stability of plant-mediated synthesized nanoparticles is doubtful, as phytochemicals could degrade with time, resulting in aggregation or alteration of properties [89].

Although deemed green, biosynthesized nanoparticles can still be toxic based on size, shape, surface charge, and concentration. Few in vivo studies exist to completely know their biosafety. Additionally, coatings derived from plants can interact in an unpredictable manner with biological systems [90]. An overall toxicological profile is still unknown.

Regulation of particle size and form is important for their functionality in biomedical, environmental, or agricultural uses. But since plant extracts are complicated and undefined, uniform and specific morphologies are difficult to achieve. This lack of order restricts their use in precision areas such as targeted drug delivery [91].

Presently, there is no standard regulatory protocol for the use of plant-mediated nanoparticles, particularly in biomedical applications. This uncertainty discourages commercial investment and restricts the translation of laboratory results to clinical and industrial environments [92]. Future studies should include detailed phytochemical analysis of plant extracts with methods such as LC-MS, NMR, and FTIR. Knowing what particular metabolites can affect the properties of nanoparticles can aid in standardizing protocol for synthesis. Synthetic versions of these biomolecules can also be made to minimize variations. Biotechnological methods like metabolic engineering and synthetic biology may be used to genetically engineer plants to overproduce the desired phytochemicals. Genetically modified plants that possess stable and homogeneous metabolite profiles could potentially increase reproducibility in the synthesis of nanoparticles [93]. For obtaining uniform and scalable synthesis, microfluidic systems may be employed. They provide excellent control over reaction parameters for instance, flow rate, temperature, and mixing. Integration of plant extracts within such systems might assist in reaching reproducibility at the industrial scale [94]. Development of environmentally friendly purification techniques like membrane filtration, centrifugation using green solvents, or magnetic separation will help in effective recovery of clean nanoparticles without the loss of properties. Detailed toxicological analysis employing in silico models with *in vivo* testing must be done to ascertain the safety of plant-derived nanoparticles. Long-term exposure tests and studies on cell interactions will enable biosafety assurance, particularly in medical applications [95]. Artificial intelligence and machine learning can be embedded in nanoparticle synthesis processes to forecast outcomes on the basis of plant extract content and synthesis parameters. Data-driven predictive synthesis has the potential to significantly lower trial-and-error based experimentation and increase consistency. International regulatory agencies should frame guidelines for the synthesis, characterization, and use of green nanoparticles [95]. Interdisciplinary collaboration involving academia, industry, and government is needed to allow safe and ethical exploitation of nanotechnology [96, 97].

CONCLUSION AND FUTURE PROSPECTS

AgNPs have arisen in contemporary and future contexts, with diverse applications including cardiovascular implants, dentistry, medicine, therapies, biosensors, agriculture, and others. But due to toxicity issue of AgNPs more emphasis was given to green synthesis of nanoparticles due to its eco-friendliness, biocompatibility, and cost-effectiveness. However, its widespread commercial application is somewhat hindered by factors such as extract variability, lack of mechanistic understanding, challenges in scaling up, and little toxicity concerns. Addressing these problems using sophisticated analytical, genetic, and engineering techniques, with robust regulatory frameworks, would facilitate the development of sustainable and commercially viable green nanotechnology. Green synthesis surpasses chemical and physical processes due to its cost-effectiveness, environmental friendliness, and scalability for large-scale production. Nature possesses excellent and innovative techniques for creating highly efficient miniature useful materials. An increased awareness of green chemistry and the use of environmentally friendly technologies for the synthesis of metal nanoparticles, particularly AgNPs, has fostered a drive to create sustainable approaches. Organisms, from simple bacteria to sophisticated eukaryotes, can be employed for the manufacture of nanoparticles with specific size and form. Nonetheless, the proliferation of micro-organisms and extensive formulation residue presents challenges in comparison to others. The poor synthesis rate and restricted size and form distributions prompted the study to focus on the usage of plants. The use of plants for the synthesis of Ag-NPs offers benefits over other biological entities, as it may circumvent the protracted process associated with microorganisms and the challenges of maintaining their cultures, which may diminish their efficacy in nanoparticle creation. Additional benefits of synthesis from plant extracts include the supply of a sanitary working environment, protection of health and the environment, reduced waste, and the production of more stable goods. Ag-NPs generated by green methods possess significant implications in nanotechnology across several applications. Through continuous multidisciplinary study and development, plant-derived nanoparticles are poised to significantly impact fields such as medicine and environmental remediation in the next decades.

List of abbreviations

AgNPs: Silver nanoparticles
MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
FTIR: Fourier transform infra-red
ECM: Extracellular matrix

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