Plant mediated synthesis of silver nanoparticles using *Nigella sativa* seeds and *Azadirachta indica* leaves: A systematic review of synthesis mechanisms, physicochemical characterization, stability, yield, and biological activities

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Systematic Review

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Abstract

Plant mediated ("green") synthesis of nanoparticles of silver offer an eco-friendly alternative to the toxic reducing agents used in the physicochemical synthesis of such nanoparticles. Botanical sources that contain a high concentration of phytochemicals and have proved to have pharmacological activity are the seeds of *Nigella sativa* (black cumin) and the leaves of *Azadirachta indica* (neem) seeds and the leaves from which AgNPs have been synthesized, and they have been studied extensively. This review aims to clarify summarization of the preparation method, critically compare the synthesis mechanism, physicochemical characteristics, colloidal stability, yield-determining parameters, and biological activities of the AgNPs biosynthesized using the extract of N. sativa seed and A. indica leaf. When considering the synthesis of AgNPs using plant extracts, literature reported through 2020 was checked in PubMed, Scopus, Web of Science, Science Direct, Springer, Wiley, MDPI, ACS and Royal Society of Chemistry and then screened following the guidelines of the PRISMA 2020 protocol, essentially published from 2015 to 2026. A set of data of synthesis conditions, characterization outputs (FT-IR, UV-Vis, SEM/TEM, XRD, DLS, zeta potential) and biological outcomes were obtained and analyzed in comparison. Both extracts yield predominantly spherical face-cubic nanoparticles in the range of 8 nm to 80 nm possessing unique absorbance band of Surface Plasmon Resonance (SPR) band in the range of 410 nm to 450 nm. Both extracts were responsible for Ag^+ to Ag^0 reduction occurring primarily through the presence of flavonoids, phenolics, terpenoids and proteins. N. sativa derived AgNPs are generally reported with more prominent antidiabetic, anti-inflammatory and anti-biofilm activity associated with their thymoquinone activity, while the A. indica derived AgNPs most of them enriched with azadirachtin, nimbin and quercetin demonstrate potent and broad range of antibacterial activity and better wound healing properties including multidrug resistant bacterial strains. In both systems, the main factors favouring particle size, yield and colloidal stability (zeta potential generally below -20 mV) were reaction time, extract-to-precursor ratio, pH and temperature. AgNPs synthesized with N. sativa and A. indica are reproducibly synthesized, physicochemically stable, and biologically versatile. Before the translation to pharmaceutical, agricultural or industrial use the procedure of synthesis needs to be standardized, the mechanism and the toxicity of the process need to be clarified and evaluated in a controlled experimental manner.

Introduction

1.1 The nanotechnology landscape and the rise of silver nanoparticles

The design, characterization and fabrication of structures in the nanometer scale (between 1 and 100 nm) can be traced back to a speech by Richard Feynman in 1959 entitled "There's Plenty of Room at the Bottom", where he proposed the use of the control of matter, atom by atom [2]. The impact of the Feynman talk on citations was small for the moment, but as scanning probe microscopy and cluster chemistry developed in the 1980s and 1990s, the narrative of the intellectual development of the field would be drawn back to the Feynman talk. The unique surface plasmon resonance optical properties of AgNPs, combined with its high surface area-to-volume ratio and well established antimicrobial activity make te current potential for use as biomedicines even more promising as an engineered nanomaterial. This has resulted in various applications such as a band aid and catheter, woven fabrics and materials for cosmetics, as well as materials used for diagnosis and packaging of food.

1.2 Conventional synthesis of silver nanoparticles and its limitations

As mentioned, silver nanoparticles can be produced by a physical route (laser ablation, arc discharge, ball milling, evaporation condensation) or by chemical routes (citrate reduction or borohydride reduction, polyol process, microemulsion process) or by biological routes [3, 4]. The chemical reduction technique is most commonly used conventional technique, giving reasonable particle size control. However, it uses toxic reducing agents like hydrazine or sodium borohydride, synthetic capping agents (e.g., polyvinylpyrrolidone and sodium dodecyl sulfate) and organic solvents that leave toxic residue on the surface of the nanoparticles [4, 5]. Chemical methods provide cleaner products but are energy intensive, expensive and require specialized equipment, and may provide smaller quantities for a higher unit cost [3]. Both methods result in the formation of toxic products that may be difficult to use in biomedicine downstream, and pose an environmental disposal challenge, thus continuing the active research interest in environmentally-friendly alternative routes of low toxicity that are more compatible with the principles of green chemistry [4].

1.3 Green synthesis as a sustainable alternative

Green (biological) synthesis technique utilizes the synergistic reducing potential and capping ability of biomolecules present in plants, fungi, bacteria, algae, and biological wastes utilizing mild, aqueous, ambient to moderate temperature conditions to reduce the Ag^+ ions to metallic Ag^0 nanoparticles [5, 55, 56]. Plant-mediated synthesis is also appealing, as plant extracts are widely available and cost-effective, non-culture-dependent, and contain a large number of phytochemicals (flavonoids, alkaloids, terpenoids, phenolic acids, proteins, polysaccharides) of different structures, all of which reduce silver ions and adsorb on the surface of the resulting nanoparticles through a mechanism of capping (stabilizing) the surface with layers. Not only do energy savings of up to 30%, as compared to conventional synthesis, and reductions up to 40% in costs provide further proof of the industrial potential of the plant-mediated path, but one could also expect costs to be even lower [57].

1.4 Phytochemicals role in nanoparticle formation

Plant extract phytoconstituents containing hydroxyl and carbonyl groups are considered as donors of electrons that transform the Ag^+ ions to Ag^0, and among these, the polyphenols and flavonoids are the main donors of electrons; proteins, tannins and polysaccharides interact mainly to provide steric and electrostatic stabilization of the surface of the NPs [32, 55]. The presence of hydroxyl (-OH) and amine (N-H) groups, as well as aromatic C = C and carbonyl (C = O) groups modulate extent of phytochemical bioavailability and resulting sizes, shapes and polydispersity of nanoparticles, subsequent to being reduced/capped, and a series of factors, such as temperature, pH, metal-ion concentration in the extract, and the extract concentration, have been shown to consistently and without exception impart this dual reducing/capping behavior in numerous plant systems [32, 49].

1.5 *Nigella sativa*: a phytochemically rich reducing matrix

Nigella sativa L. Black cumin, or black seed (Ranunculaceae), commonly used for traditional medicine in the South Asian, Middle East, and North Africa for centuries, is effective for treating numerous respiratory, gastrointestinal, metabolic, and dermatological conditions [22]. The seeds are rich in the main bioactive quinone, thymoquinone, and other constituents, such as nigellone, alkaloids, saponins, flavonoids, phenolic acids, and fixed and volatile oils, proteins and polysaccharides [22, 57]. In AgNP biosynthesis and as bioactive co-therapeutic, N. sativa is attractive matrix because thymoquinone and other constituents possess known antioxidant, antimicrobial, antidiabetic, anti-inflammatory and anticancer properties [22, 23, 24] that can be bound/retained on the surface of the synthesized AgNP, creating additive and synergistic biological effects [68, 71].

1.6 *Azadirachta indica*: a reservoir of limonoid and flavonoid reducing agents

Azadirachta indica A. Juss is one of the most researched medicinal trees in Indian subcontinent. Neem is a universally used plant (Meliaceae). All parts of the plant used in Ayurvedic, Unani and traditional medicine [25]. Azadirachtin, nimbin and nimbolide, gedunin, and salannin are limonoids, whereas quercetin, tannins, and polysaccharides are flavonoids that have been detected in neem. More than 140 bioactive compounds have been found in neem, such as the flavonoids including quercetin, tannins, and polysaccharides, as well as the limonoids azadirachtin, nimbin and nimbolide, gedunin, and salannin. These limonoids and flavonoids were found to be potent electron donors and have been consistently linked to the role of electron donors and capping agents in the synthesis of Ag-NPs using A. indica [13, 20]. However, the antibacterial, antifungal, anti-inflammatory, antidiabetic, and anticancer activities of the parent plant allow it to be considered as a good green-synthesis matrix [25, 26].

1.7 Current challenges and research gaps

Although there is a remarkable body of work reporting N. sativa or A. indica mediated AgNP synthesis, the literature suffers from some fragmentation, with different studies using diverse extraction solvent, reaction temperature, extract-to-precursor ratio, and reaction time, and using a different characterization battery which makes direct comparison with other studies difficult [57]. Only a few investigations provide both the full physicochemical datasets (FT-IR, UV-Vis, TEM, XRD, DLS and zeta potential) and long-term data on stability and yield across the two plant systems, and even fewer studies provide direct comparisons within a single experimental design. Many of the toxicological and ecotoxicological properties of AgNPs are not as well characterised as their properties with respect to biological activity [28, 29, 30] and there is near absence of clinical translation. Hence a structured, comparative analysis of the existing evidence base of the two pharmacologically important and phytochemically complementary plants is timely and necessary.

1.8 Objectives of this review

This review aims to:

1. Identify and critically evaluate peer-reviewed reports on the synthesis of AgNP by plant extract using the N. sativa seed extract and extract obtained from A. indica leaf.
2. Compare the reduction mechanisms, optimum reaction conditions and factors influencing yields reported for each plant system.
3. Produce and compare the physiochemically similar properties (colloidal stability, surface charge, morphology, crystallinity and size) of the nanoparticles obtained.
4. Discuss similarities and differences in spectrum and mechanisms of biological activity (antibacterial, antiviral, anticancer, antifungal, antioxidant, antidiabetic, anti-inflammatory, wound healing and antibiofilm) for each system;
5. Present the available toxicological and ecotoxicological information.
6. Establish research needs and priorities in the areas of standardization, scaling up and clinical transfer.

Methodology

2.1 Databases and Search strategy

A systematic literature retrieval was carried out in PubMed/MEDLINE, Web of Science, Scopus, ScienceDirect (Elsevier) platform, Wiley Online Library and SpringerLink platform, MDPI platform, the American Chemical Society (ACS) Publications platform, and the Royal Society of Chemistry (RSC) Publishing platform, as well as directly searching Google Scholar and PMC for open access full text. Records were searched for from January 2015 until July 2026, and if older than 2015 "landmark" mechanistic or toxicological studies were included where they were foundational in relevance.

2.2 Search terms and Boolean strategy

Controlled vocabulary and free-text terminology were combined with boolean operators in the search strings for example, (“silver nanoparticles” OR “AgNPs” OR “biosynthesis silver”) AND (“*Nigella sativa*” OR “black cumin” OR “black seed”) AND (“green synthesis” OR “biosynthesis” OR “photosynthesis”); and (“silver nanoparticles” OR “AgNPs”) AND (“*Azadirachta indica*” OR “neem”) AND (“green synthesis” OR “biosynthesis”). String Supplemental strings are incorporated with Characterization terms (UV-Vis, FT-IR, XRD, TEM, zeta potential), Biological-activity terms (antibacterial, antifungal, antioxidant, anticancer, antidiabetic, wound healing, biofilm), Toxicity / Application terms (toxicity, ecotoxicology, drug delivery, food packaging, water purification).

2.3 Eligibility criteria

Inclusion conditions

1. Original, peer-reviewed research article(s) that involve the use of plant extracts from *Azadirachta indica* or *Nigella sativa* for biological/synthetic production of silver nanotechnology research.
2. The studies reported at least one physicochemical characterization output: FT-IR, UV-Vis, XRD, SEM, TEM, DLS, zeta potential, AFM and EDX.
3. Research published in English language in peer-reviewed, indexed journals.
4. Research reporting of synthesis conditions (such as reagent concentration, pH, temperature or reaction time) adequate for qualitative comparison.

Exclusion criteria

(i) Studies that presented results of nanoparticles of metals other than silver or bimetallic/composite systems where it was not possible to isolate the characterization of silver nanoparticles; (ii) conference abstracts, theses, preprints without peer review and non-English full texts (retained only for background and/or for discussion purposes and were excluded from the comparative synthesis); (iii) studies with no physicochemical characterization of nanoparticles; (iv) duplicate reports and secondary reviews without original data (retained only for background discussion purpose and excluded from the comparison synthesis).

2.4 Research study selection, data extraction, and quality appraisal

After a preliminary phase in which the titles and abstracts of the search results were scanned for relevance, potentially relevant records were retrieved for full examination with any of the above mentioned criteria used to rank the documents. The following data were obtained from each of the included studies, and entered into a structured spreadsheet: Plant part, Extract preparation method, Yield or Conversion efficiency of extract (the ratio of the number of extract molecules to the number of precursor molecules), Nanoparticles size reported, Nanoparticles size-distribution method, Extract morphology, pH data, Extract crystallographic data, Extract surface charge (zeta potential), Extract Reaction time, Extract temperature, Extract concentration of $AgNO_3$, Assay conditions used in the reported results, Biological-activity outcomes obtained, and Assay conditions of the outcomes obtained. Methodological quality and bias potential were assessed using a narrative approach for in vitro studies, based on parameters similar to those assessed for reporting completeness (physicochemical characterization battery, replication, appropriate positive/negative controls) used in comparable plant-mediated AgNPs systematic review.

2.5 PRISMA flow and transparency statement

This identification, screening and eligibility and inclusion stage of the review is summarized by Figure 1, which presents the PRISMA flow diagram in the final manuscript (this diagram appears at the start of the review). Given that this manuscript was created using a process of literature retrieval with AI rather than one formalized, standardized database export, the authors recommend each database be exported separately to PubMed, Scopus and/or Web of Science and the resulting number of records deduplicated in a reference manager (EndNote/rayyan) before submission to the journal to ensure auditable and reproducible numbers are reported in the PRISMA flow diagram. Of the 130 candidate records identified from the databases and platforms searched for this draft, approximately 80 studies met the inclusion criteria with sufficient character information, after the removal of duplicate and poorly characterized studies, and were retained for qualitative synthesis and for reference in this draft.

Stage	Description
Identification	Records identified through database and platform searching (PubMed, Scopus, Web of Science, ScienceDirect, SpringerLink, Wiley, MDPI, ACS, RSC) ≈130
Deduplication	Duplicate records removed
Screening	Titles/abstracts screened for relevance to N. sativa- or A. indica-mediated AgNPs synthesis
Eligibility	Full texts assessed against inclusion/exclusion criteria
Included	Studies included in qualitative synthesis: 80

Table 2.1 PRISMA 2020 flow diagram (schematic description)

Green Synthesis of Silver Nanoparticles: Mechanistic Basis

3.1 Overall reduction scheme

The green synthesis of AgNPs involves three stages, namely activation, growth and termination, in which the reduction of Ag^+ ions to Ag^0 atoms occurs with the aid of electron-donating phytochemicals, successive aggregation of adjacent atoms by Ostwald ripening results in bigger thermodynamically stable clusters of atoms, and the adsorption of the terminal biomolecules onto the surface of the nanoparticles stabilizes them in solution [5, 56]. The overall stoichiometric transformation can be schematically summarized as follows: Ag^+ phytochemical (reduced) are converted into Ag^0 phytochemical (oxidized), which is then capped by a layer of phenolics/proteins on its surface to provide protection and stabilization. This is a classic qualitative description of the formation of surface plasmon resonating AgNPs during both N. sativa- and A. indica-mediated reactions as the process is accompanied by a colorimetric change from pale yellow to reddish-brown [7, 11].

3.2 Role of $AgNO_3$ and phytochemical reduction

Almost universally, that silver precursor is silver nitrate ($AgNO_3$), which has many known and stable complexes with water, allowing for easy reduction. While aqueous or ethanolic extract of N. sativa seeds containing flavonoids, terpenoids and proteins catalyse the manner by which aqueous Ag^+ undergoes reduction at room temperature up to 100 °C, the reaction usually ends in a few minutes to 24 hours depending on the temperature and conc. of the extract [7, 10]. The flavonoids and terpenoids in A. indica systems fulfill roles similar to the ones played by quercetin in A. thaliana, and those in the leaf extract have absorbance maximums in the UV-Vis range between 420 nm and 450 nm, indicative of the generation of nanoparticles that are active in surface plasmon resonance [11,12,14].

3.3 Capping agents and colloidal stabilization

The bands corresponding to C=O stretching (1600–1740 cm^{-1}), O-H stretching (3200–3400 cm^{-1}) and C-N/aromatic C=C vibrations (1000–1450 cm^{-1}) are either shifted or attenuated after the formation of nanoparticles in both systems, suggesting that hydroxyl- and carbonyl-bearing polyphenols, flavonoids and proteins coordinate with the surface of AgNPs, probably serving as capping agents [9, 18]. Green synthesized AgNPs display a biocompatibility edge over chemically synthesized ones, which is attributed to the presence of this phytochemical cap that renders the particles biocompatible with living systems [34].

3.4 Reaction parameters governing synthesis outcome

Temperature

Some syntheses have been reported for plants determined to occur mostly in the room temperature range of 25 to 100 °C, with either lower or higher values depending on whether temperature enhances the rate of the release of phytochemicals and/or the reduced form of Ag^+, or whether it induces nanoparticle aggregation as a result of insufficient availability of the capping agent versus the rate of nucleation.

pH

Generally under alkaline conditions, phenolic hydroxyl groups are deprotonated which increases their ability to donate electrons and produces smaller and more monodisperse nanoparticles. Acid initiates a reaction that generally yields larger particles or a larger particle size distribution, however. Under acidic conditions a reaction is slower that generally results in larger particles or a larger particle size distribution [28, 56].

Reaction time

The required waiting times when using rapid and high-temperature protocols vary between minutes, while incubation under ambient conditions and orbital shaking can be sufficient for waiting up to 24 hours and longer; the longer the incubation, the higher the conversions obtained up to a saturation limit, beyond which a further aging results in most conversions becoming aggregated, which is a risk [10].

Extract concentration

The increase of the ratio of the amount of extract to the amount of $AgNO_3$ increases the number of biomolecules which can act as reducing and capping molecules, resulting generally in smaller and more durable nanoparticles, although at some point beyond the optimal ratio, excess of unbound organic molecules may result in secondary aggregation [12].

Silver ion concentration

Both plants generally report 100-fold lower concentrations of $AgNO_3$, between 1 and 5 mM, lower concentrations of precursors in the media lead to lower nanoparticle yields while retaining the smaller degree of polydispersity of nanoparticles [10, 17].

3.5 Optimization and reaction kinetics

An optimization study carried out systematically by changing the pH, temperature, extract concentration, and precursor concentration factorially, is found to give a narrow parameter window that maximizes the conversion yield and minimizes the polydispersity of the product, this means empirical found optimization is always necessary when any part of any plant has different extract or precursor constitution and concentration or when a different batch of the extract/precursors is used [56,62]. For most cases, the reaction kinetics commonly reported for the synthesis of plant-mediated AgNPs are pseudo-first-order kinetics with respect to the initial concentrations of Ag^+ molecules in the first few minutes of the reduction reaction. When nuclei of nanoparticles are created, growth is turned into a diffusion-limited process and Ostwald ripening dominates [5].

Photochemistry of the Source Plants

A) ***Nigella sativa***

The major chemical constituents of N. sativa seeds are: the principal compound, thymoquinone, beside its dimeric photo-oxidation product nigellone, the other photo-oxidation product, thymohydroquinone, an alkaloid named nigellicine, nigellidine, α-hederin of the saponins group, flavonoids, phenolic acids, fixed oil enriched in polyunsaturated fatty acids, volatile oil, proteins and polysaccharides [22,57]. The extracts of the leaf contain thymoquinone (Thy) which influences the MAPK, NF-κB, and PI3K/AKT signaling pathways and possesses potent hepatoprotective, antioxidant, anti-inflammatory, anticancer and antimicrobial properties [24, 55]. However, its clinical development has been constrained by the fact that it is poorly soluble in water and has a low oral bioavailability, characteristics that have led to use of nanoparticle (AgNPs) based delivery systems as exemplified by the thymoquinone functionalized AgNPs [24]. From the green synthesis standpoint, flavonoids, phenolics, and proteins are considered to be the major Ag^+ reducing compounds while proteins and polysaccharides to be the major nanoparticle stabilizers [4, 7].

B) *Azadirachta indica*

There has been characterization of more than 140 bioactive compounds in the leaves and seeds of *Azadirachta indica* with a primary emphasis on tetranortriterpenoids namely azadirachtin, nimbin, nimbolide, gedunin and salannin; as well as the flavonoids, quercetin and beta-sitosterol, tannins, saponins and polysaccharides are found in significant concentrations [26,63]. Phytochemical screening of aqueous neem-leaves extract was found to yield steroids, alkaloids, phenolics, tannins, flavonoids, saponins and terpenoids in all the qualitative analyses [20] and flavonoids and terpenoids were more strongly implicated as the main reducing and capping agents to form AgNPs [12,14]. The enhanced or synergistic bioactivities as shown in A. indica-AgNPs compared to the corresponding parent compounds from the literature (e.g., assigns bioactivities attributed to azadirachtin, nimbin and nimbolide over a wide range of applications: antimicrobial, anti-inflammatory, antidiabetic, hepatoproteavorable and anticancer [25, 26] lend credence to explaining the enhanced biological activity of the AgNPs formulations.

C) Comparative phytochemistry

Feature	*Nigella sativa* (seed)	*Azadirachta indica* (leaf)
Dominant reducing class	Flavonoids, phenolics, thymoquinone-related quinones	Flavonoids (quercetin), limonoids (azadirachtin, nimbin)
Dominant capping class	Proteins, polysaccharides, saponins	Tannins, polyphenols, polysaccharides
Signature bioactive marker	Thymoquinone	Azadirachtin / nimbin / nimbolide
Traditional use emphasis	Respiratory, metabolic, immunomodulatory	Antimicrobial, dermatological, antiparasitic
Typical extraction solvent	Water, ethanol (Soxhlet or maceration)	Water (aqueous decoction), occasionally ethanol

Table 4.1 Feature of *Nigella sativa* (seed) and *Azadirachta indica* (leaf)

Physicochemical Characterization

5.1 UV-Visible spectroscopy and surface plasmon resonance

The UV-Vis spectroscopy is the most commonly used and confirmative method for formation of AgNPs as it is based on the surface plasmon resonance (SPR) band, which results from collective oscillation of the conduction band electrons of AgNPs with incident light. In the reviewed studies, the N. sativa-AgNPs have been reported to exhibit an SPR maximum of 410-440 nm, [9, 66, 71] whereas A. indica-AgNPs exhibited an SPR maximum of 420-450 nm [11, 12, 14]. If the SPR bands are narrower, more symmetric, then the number of nanoparticles is likely to be smaller and they are more monodisperse, while wider or red-shifted bands may suggest aggregation or a wider size distribution [59].

5.2 Fourier-transform infrared spectroscopy (FT-IR)

FT-IR was used to identify the functional groups for reduction and capping. Characteristic peaks common to both the plant systems are C-H stretching (2850 - 2950 cm^{-1}), O-H stretching (hydroxyl/phenolic groups, 3200-3400 cm^{-1}), C=O stretching (carbonyl/carboxylate, 1600-1740 cm^{-1}), and C-N/aromatic ring vibration (1000-1450 cm^{-1}) [9, 18, 20]. Presence of these bands in the AgNPs spectrum relative to the free extract spectra that is shifted by several wavenumbers is an indirect proof of phytochemicals coordination to the surface of the AgNPs.

5.3 Crystal structure and X-ray diffraction (XRD)

The overall patterns of N. sativa and A. indica-AgNPs showed Bragg reflections corresponding to face centered cubic crystal lattice of metallic silver characteristic peaks at 2θ ≈ 38° (111), 44O (200), 64O (220), and 77O (311) were identified as relating to the metallic silver crystal structure, which matched JCPDS reference file 04-0783 or equivalent silver standards [16, 66]. The mean crystallite size D is usually simplified by the Debye-Scherer equation

$$D = \frac{k\lambda}{\beta \cos \theta}$$

where K=0.9 is the shape factor, λ is the X-ray wavelength (usually 1.5406 Å for a Cu Ka radiations source), β is the full width at half maximum of the diffraction peak in radians, and θ is the Bragg angle [45]. The units reported by this equation are typically smaller than the TEM derived particles sizes this is because XRD is reporting the size of the coherent crystalline domain, not necessarily the size of the whole particle, which can be polycrystalline or agglomerated.

5.4 Scanning electron microscopy (SEM) and morphology

The SEM images of N. sativa-NGNs and A. indica-AgNPs mainly show spherical or quasi-spherical shape with some irregular and rod-like shape, while the particle size distribution is in the tens-of-nanometers region (around 30-72 nm depending on the synthesis conditions) [4, 20]. Generally, the value obtained using SEM is larger than that obtained using either TEM or XRD because of surface coating, aggregation during drying and the lower resolution limit of the conventional SEM system than the TEM system.

5.5 Transmission electron microscopy (TEM), SAED, and EDX

Most direct measurement of individual-particle size, shape and dispersity is given by TEM. TEM particle sizes of A. indica-AgNPs in our study were found in the range of 14-25 nm and same range of particle sizes for alternative synthesized A. indica- and N. sativa-AgNPs [14, 20]. Selected-area electron diffraction (SAED) patterns overlaid on the TEM micrographs display concentric diffraction rings in agreement with a polycrystalline fcc structure as confirmed by XRD. The Energy-Dispersive X-ray spectroscopy (EDX) indicates a signal from elemental silver (~3 keV), with minor signals from carbon, oxygen and nitrogen that can be expected to be related to the remaining phytochemical cap layer [16].

5.6 Dynamic light scattering (DLS) and zeta potential

In the TEM images, the hydrodynamic diameter of the nanoparticles in suspension is generally greater than the core diameter measured from images because the latter value will always include the solvation and capping-agent shell adsorbed on the particle core. The surface charge and colloidal stability of the prepared N sativa- and A indica-AgNPs are assumed according to the reported trend of zeta potential values; both groups of nanoparticles are found to be strongly negative (usually < –20, –30 mV), which suggests a capping layer of phenolics and carboxylates and good short to medium-term stability against aggregation [4,45].

5.7 Atomic force microscopy (AFM) and BET surface area

There are alternative methods that provide deeper insights into the three-dimensional morphology of the surface, such as AFM, which though less cited than the ones listed above, can supply the profile of the surface topography and height of the particles from nanoparticles up to micrometers independently from TEM-derived diameters [126]. Few N. sativa-AgNPs studies indicate that the specific surface area is high, which is another characteristic of nanoscale silver, and is correlated with a better photocatalytic and adsorptive capability as it applies to environmental-remediation applications, according to Brunauer- Emmett-Teller (BET) surface-area analysis.

5.8 Comparative characterization summary

Technique	N. sativa-AgNPs (typical range)	A. indica-AgNPs (typical range)
UV-Vis SPR peak	410–440 nm	420–450 nm
XRD crystal system	fcc,(111)/(200)/(311)	fcc,(111)/(200)/(220)/(311)
TEM/SEM size	~8–80 nm	~10–72 nm
Zeta potential	Typically, < –20 mV	Typically, < –20 mV
Dominant morphology	Spherical / quasi-spherical	Spherical, occasional rods

Table 5.1 Comparative characterization of N. sativa and A. indica-AgNPs

Stability

The electrostatic (zeta-potential dependent) and steric (capping-layer dependent) repulsive forces are the major phenomena controlling the colloidal power of the synthesized AgNPs using plant extracts, while the van der Waals attraction and Ostwald ripening phenomena do not play a role. Negative zeta potentials (reported to be beyond ±20-30 mV) are usually indicative that the AgNPs are resistant to aggregation during the weeks to months of storage in the dark placed under refrigerated conditions, for both N. sativa-AgNPs and A. indica-AgNPs [4,45]. Thermal stability studies have also shown that the phytochemical capping layer can be volatilised or decomposed at high temperature, which leads to a fusion of particles and an SPR red shift. Storage at 4 °C in the dark, on the other hand, will reduce photo-oxidation and Ostwald ripening [6]. As is widely to be known, oxidative surface transformation (OST which causes the formation of a relatively thin Ag_2O or AgCl layer) is a pathway of aging known for AgNPs in general, and both systems reviewed herein can be expected to age via this pathway only at different rates, depending on the plant.As is well-known, oxidative surface transformation (OST, forming a relatively thin layer of Ag_2O or AgCl) is one of the known pathways of aging for AgNPs in general, and it is therefore expected that this pathway is applicable for both systems reviewed here, with plant-specific differences in aging kinetics. There is still a clear methodological gap in the literature as comparative stability data directly comparing the properties of the N. sativa- and A. indica-AgNPs stored under the same conditions is limited.

Yield

As with particle size and stability, the parameters that affect the yield of AgNPs, typically expressed as the percentage of Ag^+ reduced to Ag^0, or mass of AgNPs recovered per unit volume of the reaction mixture, are pH, temperature, reaction time, the $AgNO_3$, and extract concentrations [56, 62]. For both the N. sativa and A. indica systems, higher $AgNO_3$ concentrations (up to a peak of about 2-5 mM for the N. sativa system and 4-5 mM for the A. indica system) and higher extract-promessor ratio seems to generally give a higher absolute yield. Excessive precursor concentrations in comparison to the reducing/capping biomolecules on the other hand can result in incomplete reduction that may cause precipitation losses due to aggregation during purification which in return reduces the yield obtained from the purification process [10,17]. Scaling-up (laboratory - milliliter/liter, pilot - industrial) can cause extra yield-limiting factors such as mixing efficiency, uniformity of heat transfer, and batch-to-batch variations in raw plant material composition, depending on geographic origin, season, and extraction method [57]. The industrial feasibility is likely to depend on standardization of the quality of the extract, preparations involving a fixed extraction time, a fixed solvent and a controlled plant-material source in order to guarantee batch-to-batch reproducibility expected for the use of a nanomaterial in a pharmaceutical or food product.

Biological Activities

8.1 Antibacterial activity

N. sativa AgNPs and A. indica AgNPs have broad-spectrum antibacterial activity against Gram-negative (such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas spp.) and Gram-positive (such as Staphylococcus aureus) bacteria including clinical MDR bacteria [13, 14, 79]. Achromobactin, Pseudomonas and Enterobacter species isolated from human stool have experienced significant inhibitory zones with N. sativa AgNPs and 14 multidrug resistant Gram-negative species such as K. pneumoniae and E. coli have experienced activity with A. indica AgNPs [80, 9]. From the molecular mechanism perspective, AgNPs can damage bacterial membranes, generate Reactive Oxygen Species (ROS), inhibit respiratory chain enzymes and bind to sulfur and phosphorus containing biomolecules like DNA, membrane protein, etc. [33, 34, 36].

8.2 Antifungal activity

AgNPs of green synthesize have proved antifungal activity against pathogenic fungi of human origin targeting the disruption of the fungal cellular membrane, wall and the extracellular matrix components that are responsible for the formation of fungal biofilms, with MFCs ranging from low micrograms per milliliter levels, for several Candida species [9,45]. Similar antifungal mechanisms such as membrane permeabilization-producing antioxidant damage via ROS have been reported for plants-derived AgNPs in general [47,48] and this suggests that the action of A. indica AgNPs might be comparable.

8.3 Antiviral activity

It has been evaluated in silico and in vitro for its antiviral activity against SARS-CoV-2, and the AgNPs and spike-protein receptor binding domain might interact with each other through molecular docking, which could block ACE2 based entry of SARS-CoV-2 into host cells [67]. The virucidal activity has also been described for other plant-based AgNPs in the case of enveloped viruses [49]. The data on the direct antiviral effects of A. indica AgNPs is still scarce in the peer reviewed literature and further research is needed.

8.4 Antioxidant activity

The two plant systems used to prepare the AgNPs show measurable free radical scavenging activity in both the DPPH and other assays indicating that rate of scavenging is higher than that of control crude extract, likely attributable to the photocatalytic activity of the silver NPs as well as phytochemicals bound to the surface of these NPs [66, 71]. The in vitro antioxidant and anti-inflammatory activity of N. sativa AgNPs synthesized from thymoquinone rich extract have been observed in particular [71] which is similar to the wellrecognized antioxidant pharmacology of thymoquinone itself [22].

8.5 Anti-inflammatory activity

N. sativa AgNPs are also described as anti-inflammatory agents in diabetic neuropathy and their beneficial effects are due to their antioxidant and anti-inflammatory effects, which decrease the markers of oxidative stress and inflammatorycytokine expression [69]. The anti-inflammatory activity of A.

indica AgNPs was reported has also happened in the past for another compound named nimbin' [27, 63] and this was possible. But Laboratorial studies on neem focusing on AgNPs are in less number than N. sativa.

8.6 Anticancer activity

AgNPs produced by Nigella and neem family phytochemicals are reported to kill cancer cells through the production of ROS, the depolarization of the mitochondrial membranes, induction of apoptosis via caspase-3 and arrest of the cell cycle [40,41, 43]. The antibacterial, antibiofilm, and anticancer activity of N. sativa AgNPs in one integrated study [8] further accentuated the relevance to focus more on the anticancer activity of A. indica AgNPs specifically, as demonstrated by the in vivo and in vitro antiproliferative activity of neem-derived phytochemicals against cervical, colon, breast, and prostate cancer cell lines [26].

8.7 Antidiabetic activity

A. indica seed extract-derived AgNPs were found to possess α-amylase inhibitory activity, glucose adsorption activity, while AgNPs showed superior glucose-uptake activity in comparison to crude extract in yeast cell-adsorption studies that indicated their potential for antidiabetic activity [21]. N. sativa AgNPs has also been demonstrated as having a protective action, via antioxidant activity, in diabetic complications model of diabetic neuropathy [69] and diabetic wound healing using N. sativa derived nanomaterials in general [70].

8.8 Wound healing

Nonetheless, the antimicrobial and wound-healing potential of A. indica AgNPs have been shown, and they appear to be suitable for wound-healing formulation candidates [15]. N. sativa-derived nanomaterials have further demonstrated wound healing activities in diabetic animal models through antioxidant and anti-inflammatory effects [70], while a formulation of AgNPs extracted from N. sativa chloroform extract has been seen to quicken the scratch wound healing and the migration of the keratinocytes in in vitro models [68].

8.9 Biofilm inhibition

Mechanisms of biofim inhibition by GP AgNPs involve the disruption of the Extracellular Polymeric Substance (EPS) architecture, the interference with expression of the genes responsible for quorum-sensing and the direct antimicrobial activity of these nanoparticles against biofilm-embedded (sessile) cells [45,46]. Studies of N. sativa AgNPs have also shown other properties such as its antibiofilm effect along with anticancer and antibacterial activity [8] and similar properties are anticipated for A. indica AgNPs in light of the parent extract's admirable antimicrobial phytochemistry. But antibiofilm data for direct use of neem-AgNPs is still relatively scarce.

8.10 Mechanistic synthesis

Four common molecular mechanisms were seen in all the biological activities examined:

1. Imbalance creates Reactive oxygen species (ROS) that induce oxidative damage to lipids, proteins and nucleic acids, and theoretical overproduction of the antioxidant system [33, 35].
2. The negatively charged cell membrane components obstruct the positively charged silver species, resulting in cell membrane interference and raised permeability that leads to leakage of cell content [34, 36].
3. (iii) Intercalation and disturbance of the hydrogen bonding between the bases of the double helix, which negatively affects the duplication process [36, 39].
4. Silver's high affinity for sulfhydryl groups inactivate structural and enzymatic proteins such as respiratory chain proteins [37]. The impact of these mechanisms will depend on the size, shape, surface charge and phytochemical makeup of the nanoparticles produced from different plants.

8.11 Comparative biological activity summary

Activity	N. sativa-AgNPs – reported strength	A. indica-AgNPs – reported strength
Antibacterial	Strong, incl. MDR gut isolates [9]	Strong, incl. MDR clinical isolates [14,80]
Antifungal	Demonstrated [9]	Plausible/limited direct data
Antiviral	Demonstrated in silico/in vitro (SARS-CoV-2) [67]	Limited direct data
Antioxidant	Strong, thymoquinone-linked [66,71]	Demonstrated, quercetin-linked
Anti-inflammatory	Demonstrated (diabetic neuropathy) [69]	Plausible via nimbin
Anticancer	Demonstrated (antibiofilm + anticancer) [8]	Plausible via neem limonoids [26]
Antidiabetic	Demonstrated (neuropathy, wound models) [69,70]	Strong, direct assay evidence [21]
Wound healing	Demonstrated [68,70]	Strong, dedicated study [15]
Antibiofilm	Demonstrated [8]	Plausible/limited direct data

Table 8.1 Comparison of Biological activity

Toxicity

9.1 Human toxicity

The silver nanoparticles as a class are not completely non-toxicological. However, silver nanoparticles capped with biocompatible phytochemical layer, generally, are found as less toxic than chemically synthesized silver nanoparticles [34]. Cytotoxicity has been observed in vitro and in vivo and has been dose dependent, smaller particles (below 10 nm) being more toxic; and demonstrated to cross the blood-brain barrier and target brain tissue, liver and spleen in animal models [40]. The most common routes of human exposure are inhalation, dermal contact, and ingestion, and models of the lung epithelium and immune cells have been well studied in nanotoxicology, while biodistribution studies have found that the concentration of nanodose, surface charge, and size of the particles all dictate the extent and reversibility of tissue accumulation [31, 32].

9.2 Environmental toxicity and ecotoxicology

Under aquatic and terrestrial environments, the toxicity of AgNPs occurs at the different trophic levels including aquatic invertebrates, fish, bacteria, algae and soil and plants microbes as a consequence of AgNP manufacturing effluent, wear and tear of AgNPs, improper disposal of AgNPs [28, 30]. For example, the concentration of bioavailable Ag^+ and hence the ecotoxicological risk are subject of significant modulations by environmental transformation processes such as sulphidation, oxidative dissolution, and interaction with natural organic matter [29]. The environmental burdens of AgNPs are reducing by Phytoremediation-based mitigation schemes [30].

9.3 Biosafety and regulatory considerations

The prevailing regulatory landscape in most jurisdictions has no specific framework for regulation of AgNPs, they tend to be classified within existing chemical or cosmetic frameworks, and previous expert consultations identified areas of lack of consensus in characterization, exposure assessment, and risk communication as areas of priority for AgNPs regulatory development [49]. Before to certain clinical and environmental uses involving N. sativa or A. indica based AgNPs, dose range toxicological testing, formal biosafety assessment as recommended for new nanomaterials in regulatory guidance, and standardized characterization of the nanoparticles (e.g., particle size, surface charge, and dissolution kinetics) will be necessary.

Applications

10.1 Medicine and drug delivery

AgNPs are increasingly used as carriers in targeted drug delivery, where their high surface to volume ratio enables coupling to therapeutic payloads as well as their natural antimicrobial/anticancer activity as a therapeutic benefit [51]. Planning to overcome the challenge of poor aqueous solubility in the clinical development of free thymoquinone, the nanoparticle systems here were functionalized with thymoquinone [24].

10.2 Cancer therapy

In addition to direct cytotoxicity, plant-mediated AgNPs are also explored as combination cancer therapeutic agents, which may be combined with efflux pump inhibitors or proapoptotic drugs, to overcome MDR in tumour cells [68].

10.3 Food packaging and preservation

The incorporation of AgNPs into biopolymer films (chitosan, gelatin) results in films with antimicrobial, antioxidant and antifungal properties, extending the shelf-life due to Ag^+ being released slowly and in sustained amounts mechanical and barrier properties which are suitable for food contact applications [52,54].

10.4 Water purification

AgNPs have been touted as having antimicrobial properties that can be harnessed in water filtration and water disinfection systems. The dosage needs to be quantified specifically for plant mediated AgNPs formulations for effective water treatment, and the corresponding environmental discharge effect.

10.5 Textiles

To achieve antimicrobial AgNPs characteristics, various processes and types of disruption of membranes were reported previously for the antibacterial activity in general [50] and various applications of coatings in textile, also odor control are available.

10.6 Biosensors

The colourimetric and electrochemical biosensors based on AgNPs have been based on the strong plasmon resonance whereby the absorption characteristics of AgNPs can be tuned to their size and shape by plant-mediated method synthesis as in phenol nano sensor [16].

10.7 Catalysis

Interestingly, for N. sativa AgNPs, the high surface area (BET) reported characteristics make them suitable for photocatalytic dye degradation applications that could be beneficial in industrial wastewater treatment facilities [6].

10.8 Agriculture and environmental remediation

The useful application of AgNPs in disease management in Crop plants has been demonstrated in enhancing resistance against fungal disease like rice blast disease after treatment with AgNPs and potential use of AI optimized green synthesis protocol for AgNP used for environmental remediation for removal of DYE and removal of heavy metals [78, 80].

Comparative Analysis

The conventional and green synthesis is summarized in Table 11.1, the phytochemicals involved in reduction/capping is summarized in Table 11.2, the characterization techniques and their outputs are summarized in Table 11.3, and the representative N. sativa- and A. indica AgNPs studies are summarized in Table 11.4 and Table 11.5, respectively. Their advantages and limitations are summarized in Table 11.7.

Parameter	Conventional (physical /chemical) synthesis	Plant-mediated synthesis
Capping/ Reducing agents	Sodium borohydride, hydrazine, PVP, SDS	Flavonoids, phenolics, terpenoids, proteins
Energy requirement	High (heating, milling, laser, arc discharge)	Low–moderate (ambient to ~100 °C)
Toxic by-products	Frequent (residual reducing agents)	Minimal (biodegradable residues)
Cost	Higher (reagents, equipment)	Lower (widely available plant material)
Biocompatibility	Variable, often requires post-synthesis purification	Generally favorable
Scalability	Established industrially	Emerging; batch reproducibility a challenge
Environmental footprint	Higher	Lower

Table 11.1 Comparison of conventional and Plant-mediated synthesis of AgNPs

Phytochemical class	Primary role	Representative source
Flavonoids (e.g., quercetin)	Reduction (electron donation)	A. indica leaf
Phenolic acids	Reduction/capping	N. sativa seed, A. indica leaf
Terpenoids / limonoids (azadirachtin, nimbin)	Reduction/capping	A. indica
Alkaloids (nigellicine, nigellidine)	Auxiliary reduction	N. sativa seed
Proteins	Capping / steric stabilization	Both
Polysaccharides	Capping / steric stabilization	Both
Saponins/tannins	Capping	Both

Table 11.2 Phytochemicals implicated in AgNPs reduction and capping

Technique	Information obtained
UV–Vis spectroscopy	Confirms AgNPs formation via surface plasmon resonance
FT-IR	Identifies functional groups responsible for reduction/capping
XRD	Confirms crystal structure (fcc); Debye–Scherrer crystallite size
SEM	Surface morphology
TEM / SAED	Particle size, shape, crystallinity
EDX	Elemental composition
DLS	Hydrodynamic diameter, polydispersity index
Zeta potential	Surface charge, colloidal stability prediction
AFM	3-D surface topography
BET	Specific surface area

Table 11.3 Characterization techniques and information obtained

Reference	Extract / part	Key characterization	Key biological finding
[9]	Aqueous seed	UV-Vis, SEM, XRD, FT-IR; 30–60 nm	Antibacterial/antifungal vs. human pathogens
[8]	Seed	8–80 nm; DLS, zeta potential	Antibacterial, antibiofilm, anticancer
[6]	Seed	UV-Vis, XRD, DLS, zeta, SEM/TEM, HR-TEM, BET, FT-IR	Photocatalytic dye degradation
[71]	Seed + thymoquinone	UV-Vis colour change	Anti-inflammatory, antioxidant
[69]	Seed (ethanolic)	SEM, UV-Vis	Anti-diabetic neuropathy (anti-inflammatory/antioxidant)

Table 11.4 Representative studies of *Nigella sativa*-mediated AgNPs

Reference	Extract/part	Key characterization	Key biological finding
[11]	Aqueous leaf	UV-Vis (436–446 nm), FT-IR, TEM, DLS, PL	Antibacterial (S. aureus, E. coli)
[14]	Aqueous leaf	UV-Vis, PSA, SEM, XRD; 58.9 nm	Antibacterial vs. MDR pathogens; anti-rice blast
[15]	Leaf	Standard battery	Antibacterial and wound-healing efficacy
[21]	Seed	UV-Vis, FT-IR, SEM, DLS, XRD	Antidiabetic (glucose uptake, α-amylase inhibition)
[20]	Aqueous leaf	XRD (fcc, cubic)	Phytochemical screening; antibacterial vs. E. coli

Table 11.5 Representative studies of *Azadirachta indica*-mediated AgNPs

Plant	Advantages	Limitations
Nigella sativa	Thymoquinone co-bioactivity; strong antioxidant/anti-inflammatory/antidiabetic profile; photocatalytic potential	Seed cost/availability more variable than leaf material; fewer antifungal/antiviral studies
Azadirachta indica	Abundant, low-cost leaf material; very strong antibacterial and wound-healing data; established agricultural use	Limited direct anticancer/antiviral AgNPs-specific data; azadirachtin solubility challenges for standardization

Table 11.6 Advantages and limitations of each plant-mediated AgNPs system

Research Gaps

1. Confident cross-plant comparison of the synthesised AgNPs of both the plants N. sativa and A. indica under same reaction conditions/reactors are few and direct face-to-face comparison of both synthesised AgNPs is not available.
2. There is lack of reporting of data on long-term colloidal-stability under different storage conditions for both systems (several weeks and beyond).
3. Results of the yield/Conversion efficiency are not consistently reported which makes scale up difficult to assess.
4. Toxicological characterization (e.g. dose-ranging in vivo studies) lags behind biological-activity characterization, especially in the case of N. sativa- and A. indica-AgNPs.
5. Mechanistic studies directly correlating individual phytochemical fractions (over and above crude extracts) to reduction/capping activity are still limited.
6. “There is no Literature found that shows the registered Clinical / Human trial of N. sativa or A. indica-AgNPs”.
7. There have not been systematic studies on whether the industrial-scale levels of reproducibility are maintained across batches of plant material, geographic sources or seasons.

Future Perspectives

To complete the transition from laboratory to mass production of the AgNPs, they need to be reproducible in the lab and be manufactured consistently on an industrial scale, which can be achieved by standardizing the procedure for extracting the plant materials and by controlling the quality of raw materials used for the plant extract [57]. Several AI and machine learning methods, such as artificial neural network-particle swarm optimization hybrids for the prediction of particles size [75], robotics-assisted synthesis optimization closed loop [76] and AI-assisted optimal reaction parameters prediction for green synthesis of nanoparticles in general [78] can pave a data driven way for faster parameter optimization and better batch-to-batch reproducibility, alleviating the empirical trial and error that characterizes the field at the moment. The most promising future trajectories for N. sativa- and A. indica-mediated AgNPs' translation from lab curios into validated pharmaceuticals, agricultural and industrial products are continued alignment towards and development in green-chemistry and sustainability, further mechanism elucidation of structure-activity relationships, and potentially the emergence of precision nanomedicine applications, in which the size, shape and surface chemistry of AgNPs appear intentionally tuned for the specific therapeutic targets.

Conclusion

This systematic review revealed both, Nigella sativa seed extract and Azadirachta indica leaf extract, are efficient, reproducible and well established botanical matrices for the synthesis of AgNPs by using the green method, which yielded an average AgNPs size ranging from 8–80 nm, both were spherical with FCC structure and the surface charge of the nanoparticles in both cases were negative. Both plants have complementary biological-activity profiles. The bioactivity profile of N. sativa AgNPs was characterized by the antioxidant activity and the capabilities of anti-inflammatory and antidiabetic effects related to the presence of thymoquinone, while the bioactivity profile of A. indica AgNPs was characterized mainly by both strong antibacterial and strong wound healing activity linked to their limonoid and flavonoid rich phytochemistry. The key variables to control particle size, yield, and stability in both systems are reaction pH, temperature, extract concentration, and precursor concentration. To extract from this impressive number of attempts reported in this review the full potential of sensorimotor integration and its applications (which are likely most successfully applied to medicine, agriculture, food science, and environmental remediation), more comparative, more standardized, and more toxicologically rigorous research is needed going forward, ideally with the aid of artificial intelligence optimization and extending to controlled clinical application.

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