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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING AZADIRACHTA INDICA AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract

Plant extracts are widely used as combined reducing and capping agents for silver nanoparticles, and neem (Azadirachta indica) leaf broth is among the most frequently cited examples. This review summarises published work on how neem-mediated silver nanoparticles are made, how they are characterised and how their antibacterial action is tested and explained. It also considers factors that govern particle size, the mechanisms proposed for bacterial killing, resistance and toxicity concerns. The literature agrees that activity against Gram-negative and Gram-positive bacteria is real but dose dependent, while reporting of synthesis conditions, controls and cytotoxicity is uneven. The article reports no new experimental data.

1. INTRODUCTION

Antibiotic resistance has made old ideas look new again. Silver has been used against infection for centuries, in vessels for storing water, in dressings for burns and in eye drops for newborns, and the interest in it has returned as multidrug-resistant bacteria spread [1, 2]. In nanoparticle form, silver offers a very large surface area, a steady release of ions and effects on several bacterial targets at once, which is why it is reported to be active against a broad range of organisms [3, 4]. Most nanoparticles are still made by chemical reduction with borohydride or citrate, and those routes bring toxic reagents, costly stabilisers and waste that must be handled with care [5].

Green synthesis proposes a gentler alternative. Plant extracts contain sugars, flavonoids, terpenoids, phenolic acids and proteins that can reduce silver ions and then cover the particles that form, so one extract may do the work of both a reductant and a stabiliser [6, 7]. Leaf broth of *Azadirachta indica* (neem) was among the first to be tested in this way, and its low cost, availability and medicinal history have made it a favourite [8, 9]. This review looks at what the published literature reports on silver nanoparticles made with neem: how they are prepared, characterised and tested against bacteria, how they might work, and where the evidence is weak. It reports no new experimental data. Neem is also abundant and cheap across South Asia, so the cost of the starting material is close to nothing, a point that appeals to laboratories with limited budgets.

Plants are not the only biological route. Silver nanoparticles were reported from the fungus *Fusarium oxysporum* in an early study that stressed how the particles were held in the mycelial matrix, and later work traced part of the reduction to a nitrate-dependent reductase and quinone shuttle [10, 11]. Bacteria such as *E. coli* have been used in the same way [12]. Plant leaf extracts have the practical advantage of speed, since a reaction can be complete within minutes to hours at ordinary temperature, and they avoid the sterile culture work that microbial routes demand [7, 13]. That convenience explains why leaf broths now outnumber every other biological reductant in the literature.

2. NEEM-MEDIATED SYNTHESIS

The basic procedure is simple, which partly explains how many papers exist. Fresh or dried neem leaves are boiled or soaked in water, the extract is filtered and a silver nitrate solution is added. A colour change from pale yellow to brown within minutes to hours signals particle formation. In the early report by Shankar and co-workers, neem leaf broth reduced silver and gold ions rapidly, and the authors pointed to flavanones and terpenoids adsorbed on the surface as the likely stabilising agents [8]. Later groups repeated the approach with variations in leaf age, extract strength, silver concentration, temperature and pH, usually reporting that the product was spherical or near spherical and a few tens of nanometres across [14, 15].

Conditions matter more than the simple recipe suggests. Higher extract-to-silver ratios tend to give smaller particles in many plant systems, because more capping molecules are available at the moment nuclei form, whereas higher temperature speeds reduction and often narrows the size distribution [6, 7]. Alkaline pH favours faster reduction and smaller, more stable particles. Verma and Mehata reported that particle size could be steered by changing neem leaf extract concentration, which suggests a degree of control that early papers did not claim [15]. Even so, extract composition varies with leaf origin, season and drying method, so a recipe that works in one laboratory can fail to reproduce in another [7, 9].

Monitoring the reaction is easy but easy to misread. Colour appears within minutes in hot, concentrated mixtures and over several hours at room temperature, and the absorbance at the plasmon maximum is often plotted against time to show kinetics [7, 16]. A rapid initial rise suggests burst nucleation, which tends to give a narrow population, whereas a slow climb usually accompanies growth of larger and more varied particles [6]. Light exposure, stirring and the order in which reagents are mixed affect the outcome, but they are seldom reported. Scale-up is rarely attempted: nearly all published batches are a few tens of millilitres, so claims of industrial convenience rest on extrapolation [5, 17].

Which neem constituent does the reducing is not settled. Candidates include nimbin and other terpenoids, flavonoids such as quercetin glycosides, phenolic acids, ascorbic acid and proteins [8, 18]. Infrared spectra of washed particles usually show bands for hydroxyl, carbonyl and amide groups, which are taken to indicate bound organic molecules, but such spectra cannot assign the molecule responsible. Isolating single compounds and testing them for reduction ability, a rather basic experiment, has seldom been attempted. Until it is, 'neem-mediated' remains a description of the source of the extract and not an explanation of the chemistry [6, 7].

3. CHARACTERISATION

Spherical silver particles absorb strongly near 400 to 450 nm owing to surface plasmon resonance (SPR), so UV-visible spectroscopy is the first and simplest check, and it is cheap enough to follow synthesis in real time [16, 19]. Band position and width shift with size, shape and the surrounding medium. A single narrow peak points to a fairly uniform population, while a broad or double band suggests aggregation or anisotropic shapes [19]. The technique does not give size directly, though, and many papers over-read it. Transmission electron microscopy (TEM) provides direct images and a size distribution, X-ray diffraction (XRD) confirms the face-centred cubic crystal structure of metallic silver, and energy-dispersive spectroscopy gives elemental evidence, which is summarised in table 1.

Table 1: Published methods and findings on silver nanoparticles made with neem and related systems

Topic or method	Reported observation	Ref.
Neem leaf broth synthesis	Rapid reduction of silver and gold ions; terpenoids and flavanones proposed as capping agents	[8]
Neem leaf extract antibacterial testing	Particles active against E. coli and S. aureus in comparison with extract	[14]
Neem extract concentration	Particle size steered by extract strength and reaction conditions	[15]
UV-visible spectroscopy	Plasmon band near 400 to 450 nm indicates spherical silver particles	[16, 19]
Dynamic light scattering	Hydrodynamic size and polydispersity index reflect the capping layer	[18]
Zeta potential	High magnitude of surface charge predicts colloidal stability	[20]
Membrane accumulation	Particles localise in the envelope of E. coli and form pits	[21, 22]
Silver ion release	Ion release explains most activity when oxidation is possible	[23]
Particle shape	Truncated triangular plates more active than spheres and rods	[24]
Human cell cytotoxicity	DNA damage and oxidative stress in cultured human cells	[25, 26]

Hydrodynamic size measured by dynamic light scattering (DLS) is always larger than the core seen under the electron microscope, because it includes the capping layer and solvent shell. The polydispersity index (PDI) shows how broad the population is, and values below about 0.3 are conventionally taken as acceptable for many nanosystems [18]. Zeta potential indicates surface charge and thus colloidal stability, with strongly negative or positive values (beyond roughly 30 mV in magnitude) generally predicting good stability against aggregation [20]. Neem-capped silver particles are usually reported to be negatively charged. Authors sometimes quote the electron microscopy size and the light scattering size as if they were interchangeable, which they are not.

Crystallinity and surface chemistry add detail. In X-ray diffraction patterns of silver nanoparticles, reflections assigned to the (111), (200), (220) and (311) planes of face-centred cubic silver are usually seen, and peak broadening is used with the Scherrer equation to estimate crystallite size [7, 19]. Fourier-transform infrared spectra of neem-capped products show broad hydroxyl and amide bands, taken to indicate bound phytochemicals [8, 17]. These tools tell different stories. Crystallite size, particle size by microscopy and hydrodynamic size can differ several-fold, and a paper that reports only one of them gives an incomplete picture of the material that was tested [18]. Reviewers can usually tell within a paragraph whether a paper measured these quantities or simply assumed them from the colour of the sol.

Purification receives too little attention. Unbound extract components inflate dry weight, scatter light and may carry their own antibacterial effect, so particles should be centrifuged, washed and resuspended before testing [17, 23]. Silver content is best measured by atomic absorption or inductively coupled plasma methods and not inferred from the amount of silver nitrate added, since conversion is rarely complete. Reporting dose as silver mass per millilitre, and stating how much is present as released ions, makes results comparable between studies. Many neem papers specify concentration only as

microlitres of colloid, which cannot be translated into a dose [17].

Stability on storage is a further question. Dispersions can darken, aggregate or lose activity over weeks, particularly at room temperature or when exposed to light, and chloride and protein in biological media can precipitate silver or alter its surface [17, 23]. Testing the same sample immediately after synthesis and again after some weeks, in water and in broth, would reveal how realistic the activity data are for use in a product.

4. ANTIBACTERIAL ACTIVITY AND MECHANISMS

Neem-derived silver nanoparticles have been reported to inhibit both Gram-negative and Gram-positive bacteria, including *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus* species, by disc diffusion and by dilution methods [14, 15]. Tripathi and colleagues found that particles from aqueous neem leaf extract had antibacterial activity against both pathogens and compared them against the extract itself [14]. The usual finding is a clear zone of a few millimetres to a little over a centimetre at doses in the microgram range per disc, with larger zones against Gram-negative organisms. That result is often explained by the thicker peptidoglycan of Gram-positive cells, although the evidence is mixed and some work finds the reverse [27, 28].

The mechanism proposed most often has several parts. Particles attach to the cell envelope and accumulate in the membrane, producing pits and increased permeability, as seen in electron micrographs of *E. coli* [21, 22]. Silver ions released at the surface bind thiol groups in respiratory enzymes and membrane proteins, disturb electron transport, and trigger reactive oxygen species that damage lipids, proteins and DNA [27, 29]. DNA condensation after silver exposure was noted in an early study and is regarded as a sign of stress in the cell [27]. Taken in order, these steps give a coherent account, yet each has been shown mainly in model systems, and the contribution of each in a given experiment is hard to separate.

A longstanding argument concerns whether the particle or the ion does the killing. Xiu and colleagues showed under anaerobic conditions that silver nanoparticles had negligible activity unless oxidation released ions, and they concluded that the effect belongs largely to Ag^{+} [23]. Other authors found that smaller particles of 1 to 10 nm interact directly with bacteria and that shape influences potency, with truncated triangular plates outperforming spheres and rods in one comparison [21, 24]. Particle size, surface chemistry and oxygen availability probably decide the balance. For neem-capped particles, essentially no study has measured the ion fraction, so the answer is unknown.

Size and shape effects, summarised in the second part of table 1, support a simple expectation: smaller particles release ions faster and cross the envelope more readily, so potency per unit mass rises as size falls [21, 28]. Reported neem particles seldom fall below 10 nm, which may be one reason their zones of inhibition are moderate. Few papers compare a neem product with a chemically synthesised particle of the same size, the experiment needed to show any benefit from the green route, and the distribution of activity across the literature is shown in fig. 1.

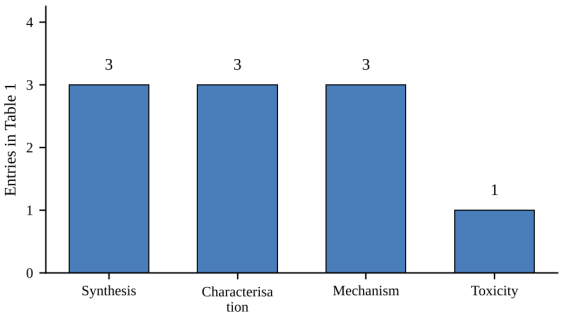


Fig. 1: Number of entries in table 1 by topic group

Assay choice affects what the results mean. Disc and well diffusion are quick, but silver colloids diffuse poorly through agar and stick to the medium, so zones understate activity and rank particles unreliably [30]. Broth microdilution under reference methods such as that of EUCAST yields an MIC and allows a minimum bactericidal concentration to be set alongside [31]. Time-kill curves show whether killing is rapid and concentration dependent. Because chloride, thiols and proteins in culture medium bind silver, the same particles can appear far more active in water than in rich broth, which is another

reason to state the medium used [23].

Choice of bacterial panel limits how far conclusions can be taken. Most neem studies use laboratory reference strains, which are generally more susceptible than clinical isolates carrying efflux pumps, altered porins or protective capsules [1, 2]. International definitions of multidrug-resistant and extensively drug-resistant organisms exist and could structure sampling, yet they are rarely used in nanoparticle work [1]. Resistant isolates matter because the whole case for silver rests on its value where antibiotics fail. A particle that kills only susceptible reference strains offers little that existing drugs do not already provide [2].

Biofilm-forming bacteria are a more demanding target than planktonic cells, because the matrix limits penetration and cells within it are metabolically slow. Silver nanoparticles have been reported to reduce biofilm formation by several species, mostly in the early stages of attachment, whereas mature biofilms often tolerate much higher doses [3, 29]. For wound and catheter applications this distinction would decide whether a product is useful. Neem-derived particles have been tested against biofilms in only a few reports, and those mostly used crystal violet staining, which measures biomass rather than viability.

Combination with antibiotics is often reported to give additive or synergistic effects, perhaps because membrane damage raises intracellular antibiotic levels. For neem-synthesised particles, such results come from a handful of studies, and they typically use disc diffusion and a single fixed ratio. Checkerboard assays that calculate fractional inhibitory concentration indices would give firmer evidence [28].

5. RESISTANCE AND TOXICITY

Resistance to silver exists. Plasmid-borne genes in *Salmonella* and other enterobacteria code for periplasmic silver-binding proteins and efflux pumps, and these were identified from a burns unit in which silver salts had been in heavy use [32]. Bacteria can also respond to nanoparticles by producing flagellin, which makes the particles aggregate and lose potency. Wide use of silver in textiles, wound dressings and consumer goods raises the possibility of selection and of cross-resistance with antibiotics, so the notion that silver sidesteps resistance should be treated with caution [2, 29]. Neem-derived particles have not been investigated for resistance development, a gap that matters if they are to be positioned as antibiotic alternatives.

Toxicity to human cells is the other limit. Silver nanoparticles reduce mitochondrial function and induce DNA damage, chromosomal aberrations and cell death in cultured human fibroblasts and glioblastoma cells [25]. In human hepatoma cells they produce oxidative stress that depends on dose and particle size [26]. Reviews of silver nanoparticles in medicine note accumulation in liver, spleen and other organs after systemic exposure and recommend caution with chronic use [17, 33]. Argyria, the bluish-grey skin discolouration after long exposure to silver compounds, is a recognised though cosmetic consequence of prolonged use on the body [34].

Dose and route define risk. A coating on a wound dressing or catheter delivers silver locally at a low and falling rate, while injection or ingestion of colloidal silver puts much more of the metal into the circulation [33, 34]. Cell culture studies typically expose cells to concentrations that bracket the antibacterial range, which means the therapeutic window is narrow for some particles and may be absent for others [25, 26]. For that reason, authors who propose oral or injectable use of neem-derived silver have to supply pharmacokinetic and organ toxicity data, and none has done so. Topical and surface applications are the more credible near-term uses, and even there skin sensitisation and argyria need evaluating [4, 34].

Regulatory expectations add a further hurdle. Medical products containing silver nanoparticles are assessed on particle characterisation, release of ions, biological safety and manufacturing consistency, and a batch-to-batch variable plant extract makes consistency hard to demonstrate [17, 33]. This does not rule out a green route, but it means standardisation of the extract,

validated analytical methods and stability data would have to accompany any claim. At present the neem literature supplies none of these in a form a regulator could use, though the approach may suit surface disinfection or water treatment, where requirements are lighter [4, 6].

Green origin does not remove these concerns, and here the literature overreaches. Plant capping agents may lower toxicity in some systems by blocking direct contact between metal and cell, yet equally may bring their own pharmacology. A particle that is sold as natural must still be assessed on dose, size, surface and release of ions, the properties that govern biological effect [17, 33]. Wherever neem particles are reported as harmless, one should look for the cytotoxicity assay, the cell type and the comparison with a standard silver preparation. Often none is present.

6. LIMITATIONS AND FUTURE DIRECTIONS

The reliability of this body of work is limited by several recurring weaknesses. Synthesis conditions are described incompletely, extract is not standardised, silver concentration is not measured, and controls with extract alone or with silver nitrate alone are often missing. Antibacterial testing relies mostly on diffusion assays with small panels of reference strains, and strains from the multidrug-resistant groups defined by clinical microbiologists are rarely included [1]. Cytotoxicity is mentioned more often than measured. Because positive results are more likely to be published, the literature probably overstates the effect [6, 7]. A modest dose of scepticism is therefore reasonable when a paper claims strong activity from a single batch.

Terminology and reporting standards deserve attention. Terms such as 'eco-friendly' and 'non-toxic' appear in titles of papers that never measured toxicity or environmental effect, and 'antibacterial' is used for results that show only a zone of inhibition [7]. A minimum reporting list would help: extract preparation and yield, silver source and final concentration, reaction conditions, purification, size by at least two methods, zeta potential, medium used for testing, controls, and dose expressed as silver mass [17, 18, 20]. Journals could ask for it as a checklist. It would cost authors little and would greatly raise the reuse value of published results.

Practical progress would come from a more disciplined design: an extract characterised by chromatographic fingerprint, particles purified and quantified as silver, a panel that includes resistant clinical isolates, MIC and bactericidal values by reference method, and cytotoxicity measured in parallel in relevant human cells [17, 25, 31]. Pairing neem particles with chemically made particles of matched size would test whether the green route adds anything beyond convenience. Only after that would animal wound-infection or topical models be justified.

There are also unexplored scientific questions. The role of specific neem constituents in reduction and capping could be probed with purified compounds. Whether surface coating alters protein corona formation, uptake by macrophages or retention in tissue is unknown for neem-capped particles [17, 33]. Long-term stability in realistic formulations such as gels, hydrogels and coated fabrics remains untested, as does release kinetics of silver ions in wound fluid. Each of these questions is experimentally tractable and would move the field from synthesis reports towards product-relevant science [6, 7]. Multi-centre ring tests, in which several laboratories prepare particles from the same defined extract, would show quickly how reproducible the green route is in practice [6, 7].

7. CONCLUSION

The published evidence shows that neem leaf extract can reduce silver ions to nanoparticles under mild conditions, and that the particles inhibit several pathogenic bacteria in the laboratory [8, 14]. Size, shape and surface chemistry are controlled to a degree by reaction conditions, though control is far from routine [7, 15]. The antibacterial mechanism seems to combine membrane damage, ion release and oxidative stress, and the share of each is still debated [21, 23].

For the field to mature, reporting must improve. Particles should be purified and

dosed as silver, tested by reference methods against resistant isolates, compared with chemically made controls and screened for cytotoxicity [25, 31]. Resistance, environmental fate and long-term safety deserve as much attention as potency [4, 32]. Until such data exist, neem-mediated silver nanoparticles are a promising and inexpensive research material, but not a proven answer to antibiotic resistance.

8. ABBREVIATIONS

CLSI-Clinical and Laboratory Standards Institute, DLS-Dynamic light scattering, DNA-Deoxyribonucleic acid, MIC-Minimum inhibitory concentration, PDI-Polydispersity index, SPR-Surface plasmon resonance, TEM-Transmission electron microscopy, XRD-X-ray diffraction

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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