



Plant-Mediated Nanoparticles from Indigofera: A Critical Review of Green Synthesis, Characterization, and Biomedical Potential

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ABSTRACT :

Green synthesis uses plant extracts as reducing and capping agents to make metal and metal oxide nanoparticles without harsh reagents. The genus *Indigofera* (Fabaceae) is rich in flavonoids, terpenoids and other metabolites and has been used to prepare silver, gold, copper oxide and zinc oxide nanoparticles. This review summarizes the phytochemical basis of bio-reduction, the reaction parameters that control nanoparticle formation, the characterization methods used, and the reported pharmaceutical activities, including antimicrobial, antioxidant, anticancer and drug-release behaviour. Limitations such as extract variability, toxicity and scale-up are discussed, and research gaps for *Indigofera*-mediated nanoparticles are identified.

Keywords: green synthesis; *Indigofera tinctoria*; silver nanoparticles; plant extract; phytochemicals; pharmaceutical applications

1. Introduction

Nanoparticles are widely used because of their size-dependent physical and chemical properties, but conventional physical and chemical synthesis often needs harsh conditions and hazardous reagents [2]. Green biosynthesis using plants, microbes or their extracts is presented as an environmentally friendly alternative [1,3]. Plant extracts are attractive because their secondary metabolites can reduce metal ions in a one-step process and often remove the need for separate stabilizing agents [2]. Reviews of plant-mediated synthesis exist for silver nanoparticles [3,20,21], for metal and metal oxide nanoparticles with antimicrobial activity [4], and for pharmaceutical nanocarriers in general [5]. However, reviews focused on a single medicinally important genus appear scarce. This article addresses that gap for *Indigofera*.

2. The Genus *Indigofera*: Phytochemistry and Pharmacology

Indigofera is the third-largest genus of the Fabaceae, with about 750 species distributed across tropical regions, and many species are used in traditional medicine [7]. A review of the genus reported 65 isolated compounds, mainly terpenoids, flavonoids and nitro-group-containing compounds, together with steroids [6]. Reported bioactivities include antimicrobial, insecticidal, cytotoxic and lipoxygenase-inhibitory effects [6]. A later review added phylogeny and traditional-use information [7], and a recent narrative review covers the ethnopharmacology of *I. tinctoria* specifically [14]. These metabolites are the likely reducing and capping agents in nanoparticle synthesis.

3. Mechanism of Plant-Mediated Nanoparticle Formation

Phytochemicals act as redox mediators that donate electrons to metal ions, and the general procedure is to mix an extract containing reducing agents with a metal salt solution [1]. Flavonoids, alkaloids, terpenoids, phenolic compounds and enzymes in extracts serve as both reducing and stabilizing agents and limit aggregation [2]. The bio-reduction process is described in three phases in the literature [2], and the reduction mechanisms of green-synthesized silver nanoparticles are discussed in [20].

4. *Indigofera*-Mediated Nanoparticles: Reported Studies

4.1 Silver and gold nanoparticles

Silver nanoparticles were prepared from *I. tinctoria* extract, and the effects of pH and temperature on formation were studied. They were characterized by UV-Vis, FTIR, zeta potential, SEM and AFM [8]. The particles inhibited *E. coli*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *A. niger* and *P. chrysogenum*, showed antioxidant activity with an IC₅₀ of about 55.72 µg/mL, and followed zero-order drug-release kinetics at pH 4.6 and 7.4 [8]. Silver and gold nanoparticles from *I. tinctoria* and *Aristolochia indica* stem extracts were characterized by UV-Vis, FTIR, TEM, XRD and EDAX and assessed for antioxidant activity, cytotoxicity on HeLa cells (MTT) and calf thymus DNA binding [9]. Silver nanoparticles from *I. macrophylla* leaf and stem extracts

showed surface plasmon resonance at 430 and 426 nm and degraded methylene blue with rate constants of 0.0204 and 0.0182 min⁻¹ [10]. Silver nanoparticles from *I. aspalathoides* leaf extract were reported to be square-shaped by SEM and were evaluated for antibacterial, antioxidant and anticancer activity [11].

4.2 Copper oxide and zinc oxide nanoparticles

CuO nanoparticles from *I. linnaei* leaf extract showed UV peaks at 269 and 337 nm, were crystalline by XRD, inhibited four bacterial species, and showed larvicidal activity against *Culex quinquefasciatus* (LC50 55.716 µg/mL) [12]. ZnO nanoparticles from a methanolic *I. tinctoria* leaf extract had a hexagonal wurtzite structure, and crystallite size fell from 16.55 to 13.75 nm as the extract amount rose from 1 g to 10 g [13].

4.3 Summary of reported studies

Table 1. Reported Indigofera-mediated nanoparticles.

Species	Nanoparticle	Key reported findings	Ref.
<i>I. tinctoria</i>	Ag	Antimicrobial, antioxidant, zero-order release	[8]
<i>I. tinctoria</i> , <i>A. indica</i>	Ag, Au	Antioxidant, HeLa cytotoxicity, DNA binding	[9]
<i>I. macrophylla</i>	Ag	SPR 430/426 nm, methylene blue degradation	[10]
<i>I. aspalathoides</i>	Ag	Antibacterial, antioxidant, anticancer	[11]
<i>I. linnaei</i>	CuO	Antibacterial, larvicidal	[12]
<i>I. tinctoria</i>	ZnO	Wurtzite; size falls with extract amount	[13]

5. Characterization Techniques

The Indigofera studies above rely on UV-Vis spectroscopy for plasmon bands, FTIR for capping functional groups, XRD for crystallinity, SEM/TEM/AFM for morphology, EDAX/XRF for composition, and zeta potential for stability [8,9,12,13]. More broadly, green-synthesized nanoparticles are routinely examined by UV-Vis, TEM, SEM, EDX, XRD, XPS, DLS, Raman and FTIR, each giving different information: UV-Vis reports optical properties, SEM adds surface morphology, XRD gives crystalline structure and phase purity, and FTIR and Raman identify chemical bonding [16]. The use of UV-Vis spectroscopy and localised surface plasmon resonance for green-synthesized nanomaterials is reviewed in [17]. A systematic review of green nanoparticles reported UV-Vis bands of 360–460 nm for silver, 524–540 nm for gold and 300–400 nm for ZnO nanoparticles [18], and the characterization of biogenic ZnO nanoparticles is reviewed in [19]. A weakness across the field is incomplete characterization of the plant extract itself, which hampers reproducibility; LC-MS, FTIR and NMR profiling of the extract are recommended [15].

6. Pharmaceutical Applications

Reported activities of Indigofera nanoparticles include antimicrobial, antioxidant, anticancer and DNA-binding effects, and sustained drug release [8,9,11]. General mechanisms of antibacterial action for metal and metal oxide nanoparticles are reviewed in [4]. For green-synthesized silver nanoparticles, reviews describe wound healing, antibacterial, antifungal, anti-tumour and antiviral activity, as well as targeted drug delivery, drug-release mechanisms and toxic effects [20]. Antimicrobial, antifungal, antiviral and anticancer properties, especially against breast, prostate and lung cancer, are covered in [21], and the use of plant-synthesized silver nanoparticles in wound care, including clinical studies, is reviewed in [22]. Applications such as drug delivery and wound healing are also summarized in [3]. These reports indicate directions that Indigofera nanoparticles have not yet been tested for, notably wound healing and in vivo anticancer evaluation.

7. Challenges and Future Perspectives

Key challenges are scale-up and control of nanoparticle properties [1], toxicity and the lack of standardization [3,20], and the variability of plant extract composition [15]. Most Indigofera studies are in vitro, so in vivo safety, pharmacokinetics and extract standardization are priority research areas. Comparisons across species and plant parts are also lacking.

8. Conclusion

Indigofera species are promising bio-reductants for silver, gold, copper oxide and zinc oxide nanoparticles with antimicrobial, antioxidant and anticancer activity. Standardized extracts, full characterization and in vivo evaluation are needed before pharmaceutical translation.

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