

NANO-ENCAPSULATION OF AZADIRACHTA INDICA EXTRACTS FOR IMPROVED BIOAVAILABILITY AND ANTI-INFLAMMATORY ACTIVITY

Renish.C¹, Siva Devi S², Saroj Kumari S³, Gunasekar Ramanan⁴, Krishnapriya B⁵, Anbuselvan G⁶, Karolin Acnes N⁷, Sudha Murugesan^{*8}

¹Research scholar, Department of Pharmacology, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Science (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India
Email: Pharmacology@rajasdentalcollege.edu.in; ORCID iD: 0009-0008-7522-0258

²Research Scholar Department of Pharmacology, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India;

³Department of Pharmacology, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India;

⁴Department of Pharmacology, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India;

⁵Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India ;

⁶Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India;

⁷Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India.

⁸Associate Professor, Department of Pharmacology, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India.

Email. sudham.scop@saveetha.com and kaviyasudha7@gmail.com

orcid id: <https://orcid.org/0009-0003-2987-2531>

Corresponding author: Dr. Sudha Murugesan, Associate Professor, Department of Pharmacology, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences (SIMATS), Deemed to be University, Thandalam, Chennai, Tamil Nadu, India; Email. sudham.scop@saveetha.com and kaviyasudha7@gmail.com; orcid id: <https://orcid.org/0009-0003-2987-2531>

Abstract

Azadirachta indica A. Juss. (neem), a member of the Meliaceae family, is one of the most extensively documented medicinal plants of the Indian subcontinent, historically valued for its antimicrobial, antioxidant, antidiabetic, and anti-inflammatory properties. The therapeutic potential of neem is attributed to a diverse pool of secondary metabolites, most notably the limonoids azadirachtin, nimbin, nimbidin, nimbolide, and gedunin, together with flavonoids such as quercetin and phenolic acids. Despite strong preclinical evidence supporting the anti-inflammatory efficacy of neem extracts, clinical translation has been hindered by poor aqueous solubility, chemical instability under light and heat, rapid first-pass metabolism, and low systemic bioavailability of the active constituents. Nano-encapsulation has emerged as a promising strategy to overcome these pharmacokinetic barriers by protecting labile phytoconstituents from degradation, enhancing membrane permeability, enabling sustained and targeted release, and improving cellular uptake at inflamed tissue sites. This paper reviews the phytochemistry and anti-inflammatory pharmacology of *Azadirachta indica*, critically examines the bioavailability limitations of conventional neem extracts, and evaluates nanocarrier platforms including liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions, and niosomes as delivery vehicles for neem phytochemicals. A representative experimental framework is presented, encompassing extraction, nanoparticle fabrication, physicochemical characterization, in vitro release kinetics, and in vitro and in vivo anti-inflammatory evaluation. Findings synthesized from the literature indicate that nano-encapsulated neem formulations consistently demonstrate smaller and more uniform particle sizes, encapsulation efficiencies exceeding 70%, sustained release profiles, and markedly enhanced suppression of pro-inflammatory mediators (TNF- α , IL-6, IL-1 β , COX-2, and nitric oxide) relative to free extracts, along with superior efficacy in carrageenan-induced paw oedema models. The review concludes that nano-encapsulation represents a viable and scalable approach to unlock the full therapeutic potential of neem as an anti-inflammatory phytopharmaceutical, while identifying scale-up, regulatory standardization, and long-term toxicity assessment as priority areas for future research.

Keywords: *Azadirachta indica*; neem; nano-encapsulation; bioavailability; anti-inflammatory activity; nanoemulsion; polymeric nanoparticles; drug delivery

How to cite this article: Renish.C1 Siva Devi S2 Saroj Kumari S3 Gunasekar Ramanan4 Krishnapriya B5 Anbuselvan G6 Karolin Acnes N7 Sudha Murugesan*8. NANO-ENCAPSULATION OF AZADIRACHTA INDICA EXTRACTS FOR IMPROVED BIOAVAILABILITY AND ANTI-INFLAMMATORY ACTIVITY. Int J Drug Deliv Technol. 2026;16(85s):1780-1790. DOI: 10.25258/ijddt.16.85s.180.

1. Introduction

Inflammation is a fundamental biological response to tissue injury, infection, or chemical irritation, orchestrated by a complex network of cellular and molecular mediators including cytokines, chemokines, eicosanoids, and reactive oxygen species. While acute inflammation is a protective and self-limiting process, chronic and dysregulated inflammation underlies the pathophysiology of numerous non-communicable diseases, including rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, neurodegenerative disorders, and certain cancers. Current pharmacological management relies heavily on non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, both of which are associated with significant adverse effects on prolonged use, including gastrointestinal ulceration, cardiovascular risk, and immunosuppression. This has intensified interest in plant-derived anti-inflammatory agents that may offer comparable efficacy with a more favourable safety profile.

Azadirachta indica A. Juss., commonly known as neem, is a fast-growing tree indigenous to the Indian subcontinent and widely naturalized across South Asia, Africa, and parts of Latin America. Nearly every part of the neem tree — leaves, bark, seeds, flowers, and roots — has been used in Ayurvedic, Unani, and traditional folk medicine for the management of fever, skin disorders, dental ailments, and inflammatory conditions. Modern pharmacological investigations have substantiated many of these traditional claims, demonstrating that neem extracts and their isolated constituents exhibit antimicrobial, antioxidant, antidiabetic, hepatoprotective, immunomodulatory, and notably anti-inflammatory activities [1][2].

Despite this substantial body of preclinical evidence, the clinical translation of neem-based anti-inflammatory therapeutics remains limited. The principal barrier is not a lack of intrinsic bioactivity but rather unfavourable biopharmaceutical properties of the key phytoconstituents. Azadirachtin and related limonoids are highly lipophilic yet exhibit poor aqueous solubility, are chemically unstable in the presence of light, heat, and alkaline pH, and undergo rapid hepatic metabolism, collectively resulting in low and erratic oral bioavailability. These limitations substantially undermine the therapeutic index of conventional neem extracts and formulations such as decoctions, tinctures, and simple capsule preparations. Nanotechnology-based drug delivery offers a rational solution to these challenges. By encapsulating

bioactive phytoconstituents within nanoscale carriers — including liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions, and niosomes — it becomes possible to protect labile molecules from degradation, improve their apparent aqueous solubility, prolong systemic circulation, and achieve site-specific or sustained release at inflamed tissues. This review synthesizes current understanding of neem phytochemistry and anti-inflammatory pharmacology, critically appraises the bioavailability constraints of conventional extracts, and evaluates the comparative performance of nanocarrier platforms developed for neem-derived actives. A representative research framework for the formulation and evaluation of nano-encapsulated neem extracts is also presented, integrating physicochemical characterization with in vitro and in vivo anti-inflammatory assessment.

2. Phytochemistry of *Azadirachta indica*

Neem is a rich reservoir of structurally diverse secondary metabolites, predominantly tetranortriterpenoids (limonoids), flavonoids, and phenolic compounds. Over 300 distinct phytochemicals have been isolated from various parts of the tree, of which a subset has been specifically implicated in anti-inflammatory activity through inhibition of pro-inflammatory enzymes and transcription factors.

2.1 Major Bioactive Constituents

Compound class	Representative constituents	Plant part (principal source)	Reported bioactivity
Limonoids (tetranortriterpenoids)	Azadirachtin, nimbin, nimbidin, nimbolide,	Seed kernel, leaf	Anti-inflammatory, antimalarial, insecticidal, anticancer

Compound class	Representative constituents	Plant part (principal source)	Reported bioactivity
	gedunin, salannin		
Flavonoids	Quercetin, kaempferol, rutin	Leaf	Antioxidant, COX/LOX inhibition, cytokine modulation
Phenolic acids	Gallic acid, chlorogenic acid, ellagic acid	Leaf, bark	Free-radical scavenging, membrane stabilization
Triterpenes	Nimbolidin, nimbinin, nimbandiol	Bark, seed	NF- κ B inhibition, pro-apoptotic in inflamed cells
Polysaccharides	Neem gum polysaccharides	Bark exudate	Immunomodulatory, mucoadhesive carrier potential

2.2 Traditional and Pharmacological Evidence of Anti-inflammatory Activity

Ethnopharmacological use of neem leaf paste and bark decoctions for the topical and oral management of swelling, arthritic pain, and skin inflammation is well documented across South Asian traditional medicine

systems [3]. These traditional applications have been substantiated by a growing body of preclinical research. Aqueous and ethanolic leaf extracts have demonstrated significant reduction of carrageenan-induced paw oedema in rodent models, inhibition of cotton-pellet-induced granuloma formation, and suppression of croton-oil-induced ear oedema, indicating activity against both acute exudative and chronic proliferative phases of inflammation [4][5]. Nimbolide and gedunin, in particular, have been reported to suppress lipopolysaccharide (LPS)-induced production of nitric oxide and pro-inflammatory cytokines in murine macrophage cell lines, supporting a mechanistic basis for the whole-extract effects observed in vivo.

3. Mechanisms of Anti-inflammatory Action

The anti-inflammatory activity of neem phytoconstituents is mediated through multiple, partially overlapping molecular pathways rather than a single target, which is characteristic of complex phytopharmaceutical mixtures.

- Inhibition of cyclooxygenase (COX-1/COX-2) and 5-lipoxygenase (5-LOX) enzymes, thereby reducing the synthesis of pro-inflammatory prostaglandins (PGE₂) and leukotrienes (LTB₄).
- Suppression of nuclear factor-kappa B (NF- κ B) nuclear translocation, downregulating transcription of TNF- α , IL-1 β , IL-6, and inducible nitric oxide synthase (iNOS).
- Membrane stabilization, reducing lysosomal enzyme release from neutrophils and preventing erythrocyte membrane lysis under hypotonic stress, a widely used in vitro surrogate for anti-inflammatory potential.
- Antioxidant and free-radical scavenging activity, limiting oxidative amplification of the inflammatory cascade via reactive oxygen and nitrogen species.
- Modulation of mitogen-activated protein kinase (MAPK) signalling, including p38 and JNK pathways implicated in cytokine gene expression.

Collectively, these mechanisms position neem phytoconstituents as multi-target anti-inflammatory agents; however, their pharmacological expression in vivo is critically dependent on achieving and sustaining adequate concentrations of active molecules at the site of inflammation — a requirement that conventional extract formulations frequently fail to meet.

4. Bioavailability Limitations of Conventional Neem Extracts

The disconnect between the demonstrable in vitro potency of neem constituents and their comparatively modest in vivo and clinical efficacy is largely

attributable to unfavourable biopharmaceutical characteristics.

4.1 Physicochemical Constraints

Azadirachtin and related limonoids possess high molecular weight, extensive oxygenation, and pronounced lipophilicity, resulting in very low aqueous solubility (often below 50 µg/mL). This poor solubility directly limits dissolution rate in gastrointestinal fluids and, by extension, the fraction of dose available for absorption following oral administration.

4.2 Chemical Instability

Azadirachtin is notably photolabile and hydrolytically unstable, undergoing significant degradation on exposure to UV light, elevated temperature, and alkaline pH — conditions encountered during storage, processing, and gastrointestinal transit. Studies report half-lives of only a few days for unprotected azadirachtin under ambient light, substantially reducing the effective dose delivered from unencapsulated preparations.

4.3 Pre-systemic and First-Pass Metabolism

Following absorption, neem limonoids are subject to extensive hepatic phase I and phase II metabolism, generating polar metabolites that are rapidly eliminated. Combined with efflux transporter activity (e.g., P-glycoprotein) in the intestinal epithelium, this results in low absolute oral bioavailability, frequently reported at well below 10% for isolated limonoids in animal pharmacokinetic studies.

4.4 Formulation-Related Limitations

Conventional dosage forms — aqueous decoctions, hydroalcoholic tinctures, and simple powder-filled capsules — provide no protection against these degradation pathways and no mechanism for sustained or targeted release. Consequently, therapeutic plasma and tissue concentrations are difficult to achieve and maintain, necessitating frequent high-dose administration that increases the likelihood of gastrointestinal intolerance and formulation cost.

5. Nano-encapsulation Strategies for Neem Extracts

Nano-encapsulation refers to the incorporation of an active compound within or onto a nanoscale carrier matrix (typically 10–1000 nm), conferring protection from the external environment while modulating release kinetics and biological interactions. Several nanocarrier platforms have been investigated for neem phytoconstituents, each offering distinct advantages relevant to anti-inflammatory delivery.

5.1 Liposomes

Liposomes are spherical vesicles composed of one or more phospholipid bilayers enclosing an aqueous core, capable of encapsulating both hydrophilic (in the core) and lipophilic (within the bilayer) constituents. Phosphatidylcholine-cholesterol liposomal systems

loaded with neem leaf extract have demonstrated improved encapsulation of nimbin and quercetin, enhanced skin permeation in topical anti-inflammatory applications, and protection of azadirachtin from photodegradation [6]. Their principal limitations include physical instability on storage and comparatively low drug-loading capacity for highly lipophilic limonoids.

5.2 Polymeric Nanoparticles

Polymeric nanoparticles, fabricated from biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), chitosan, and sodium alginate, offer tunable particle size, high encapsulation efficiency, and controlled release governed by polymer degradation kinetics. Chitosan nanoparticles, in particular, exploit mucoadhesive and permeation-enhancing properties advantageous for oral and topical anti-inflammatory delivery, while PLGA systems allow sustained release over several days, reducing dosing frequency [7][8].

5.3 Solid Lipid Nanoparticles and Nanostructured Lipid Carriers

Solid lipid nanoparticles (SLNs) and their second-generation counterpart, nanostructured lipid carriers (NLCs), employ physiologically compatible lipid matrices (e.g., glyceryl monostearate, stearic acid) to encapsulate lipophilic actives. These carriers are particularly well suited to neem limonoids owing to their high lipophilicity, offering good encapsulation efficiency, enhanced lymphatic uptake bypassing first-pass hepatic metabolism, and improved physical stability relative to liposomes.

5.4 Nanoemulsions

Oil-in-water nanoemulsions, typically formed by high-energy (ultrasonication, high-pressure homogenization) or low-energy (spontaneous emulsification) methods, provide a thermodynamically stabilized system with droplet sizes below 200 nm. Nanoemulsified neem seed oil formulations have shown markedly improved in vitro dissolution and enhanced anti-inflammatory activity in carrageenan-induced oedema models compared with unformulated oil, attributed to increased interfacial surface area and improved gastrointestinal permeability [9].

5.5 Niosomes and Other Vesicular Systems

Niosomes, formed from non-ionic surfactants and cholesterol, present a cost-effective and chemically stable alternative to phospholipid liposomes, with demonstrated capacity to enhance transdermal delivery of neem flavonoids for localized anti-inflammatory effect in topical formulations.

5.6 Comparative Summary of Nanocarrier Platforms

Nanocarrier	Typical size range	Encapsulation efficiency*	Key advantage	Key limitation
Liposomes	80–300 nm	40–65%	Dual hydrophilic/lipophilic loading	Storage instability, oxidation
PLGA nanoparticles	100–300 nm	60–85%	Sustained, tunable release	Organic solvent residues
Chitosan nanoparticles	150–350 nm	55–80%	Mucoadhesion, permeation enhancement	Batch-to-batch variability
Solid lipid nanoparticles	100–250 nm	65–90%	High lipophilic loading, stability	Drug expulsion on storage
Nanostuctured lipid carriers	80–220 nm	70–92%	Reduced expulsion vs. SLN	Complex formulation optimization
Nanemulsions	20–200 nm	60–78% (apparent)	Rapid dissolution, ease of scale-up	Thermodynamic/kinetic instability

Nanocarrier	Typical size range	Encapsulation efficiency*	Key advantage	Key limitation
Niosomes	100–300 nm	50–75%	Low cost, chemical stability	Aggregation on prolonged storage

*Encapsulation efficiency ranges are representative of values reported across the neem nano-delivery literature and may vary with formulation composition and process parameters.

6. Materials and Methods

The following section outlines a representative experimental framework suitable for the formulation and evaluation of nano-encapsulated Azadirachta indica extract, integrating standard practices reported in the phytonanotechnology literature.

6.1 Plant Material and Extraction

Fresh, disease-free Azadirachta indica leaves (or seed kernels, depending on target constituent) are collected, authenticated by a qualified botanist/taxonomist, shade-dried at room temperature to constant weight, and pulverized to a coarse powder. Extraction is performed by Soxhlet extraction or maceration using a graded solvent series (petroleum ether, followed by ethanol or hydroalcoholic mixtures, 70:30 v/v) to sequentially remove non-polar and then polar constituents. Extracts are concentrated under reduced pressure using a rotary evaporator (temperature maintained below 45 °C to minimize thermal degradation of azadirachtin) and stored at 4 °C, protected from light, until further use. Preliminary phytochemical screening and quantification of marker compounds (azadirachtin, nimbin, quercetin) by high-performance liquid chromatography (HPLC) are performed to standardize the extract prior to encapsulation.

6.2 Fabrication of Nano-encapsulated Formulations

Depending on the selected carrier system, one or more of the following standard fabrication methods is employed:

- Solvent displacement/nanoprecipitation — for PLGA and other polymeric nanoparticles, wherein the polymer–extract solution in a water-miscible organic solvent is injected into an

aqueous phase containing a stabilizer under continuous stirring.

- Ionic gelation — for chitosan nanoparticles, based on electrostatic crosslinking between cationic chitosan and anionic sodium tripolyphosphate (TPP) in the presence of the extract.
- Hot homogenization–ultrasonication — for solid lipid nanoparticles and nanostructured lipid carriers, involving melt-emulsification of the lipid phase containing the extract followed by high-shear homogenization and probe sonication.
- Spontaneous emulsification / high-pressure homogenization — for nanoemulsions, combining the oil phase (neem oil/extract), surfactant, co-surfactant, and aqueous phase under controlled energy input.
- Thin-film hydration — for liposomes, involving deposition of a phospholipid–cholesterol–extract film followed by hydration and extrusion through polycarbonate membranes to achieve the target size.

6.3 Physicochemical Characterization

Parameter	Technique	Purpose
Particle size and polydispersity index (PDI)	Dynamic light scattering (DLS)	Assess size distribution and homogeneity
Zeta potential	Electrophoretic light scattering	Predict colloidal stability
Morphology	TEM / SEM / AFM	Visualize shape and surface characteristics
Encapsulation efficiency and drug loading	HPLC / UV-Vis spectrophotometry after ultracentrifugation	Quantify entrapped active content

Parameter	Technique	Purpose
Chemical interaction / compatibility	FTIR spectroscopy	Detect drug–excipient interactions
Thermal behaviour	Differential scanning calorimetry (DSC)	Assess crystallinity and polymorphic changes
Storage stability	Accelerated stability testing (25 °C/60% RH, 40 °C/75% RH)	Predict shelf life

6.4 In Vitro Drug Release Studies

Release kinetics are evaluated using the dialysis bag diffusion method in simulated gastrointestinal fluids (pH 1.2 and pH 6.8) or phosphate-buffered saline (pH 7.4) maintained at 37 ± 0.5 °C under constant agitation. Aliquots withdrawn at predetermined intervals are analyzed by HPLC or UV-spectrophotometry, and cumulative percentage release is fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to elucidate the predominant release mechanism (diffusion-, erosion-, or swelling-controlled).

6.5 In Vitro Anti-inflammatory Assays

- Protein denaturation inhibition assay — using bovine serum albumin (BSA) or egg albumin heated at 70–72 °C; percentage inhibition of denaturation is compared between free extract, nanoformulation, and a diclofenac sodium reference standard.
- Human red blood cell (HRBC) membrane stabilization assay — evaluating protection against hypotonicity-induced haemolysis as a surrogate for lysosomal membrane stabilization.
- Nitric oxide and pro-inflammatory cytokine assays — LPS-stimulated RAW 264.7 murine macrophages are treated with test formulations, and nitrite accumulation (Griess assay) together with TNF- α , IL-6, and IL-1 β levels (ELISA) are quantified in culture supernatants.
- COX-2 inhibition assay — using a validated colorimetric or fluorometric COX-2 inhibitor screening kit to determine percentage enzyme inhibition and IC₅₀ values.

6.6 In Vivo Anti-inflammatory Evaluation

Acute anti-inflammatory activity is assessed using the carrageenan-induced hind paw oedema model in healthy adult rats, randomized into control, free-extract, nanoformulation, blank-carrier, and standard-drug (diclofenac/indomethacin) groups following institutional animal ethics committee approval. Paw volume is measured plethysmographically at 0, 1, 2, 3, 4, and 6 hours post-carrageenan injection, and percentage inhibition of oedema relative to control is calculated. Chronic anti-inflammatory activity may additionally be assessed via the cotton-pellet granuloma model over 7 days. Serum and tissue levels of TNF- α , IL-6, and C-reactive protein, along with histopathological examination of paw tissue, provide corroborative mechanistic and safety data.

6.7 Statistical Analysis

All quantitative data are expressed as mean $\pm$ standard deviation (SD) of at least three independent experiments or animals per group. Statistical comparisons are performed using one-way analysis of variance (ANOVA) followed by Tukey's or Dunnett's post-hoc test, with $p < 0.05$ considered statistically significant. Release kinetics model fitting is evaluated using coefficient of determination (R^2).

7. Results (Representative Synthesis)

The following section synthesizes representative quantitative trends consistently reported across the nano-encapsulated neem literature, illustrating the magnitude of improvement typically achieved relative to free (non-encapsulated) extract.

7.1 Particle Characterization and Encapsulation Efficiency

Formulation	Mean particle size (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)
Neem-loaded liposomes	185 $\pm$ 12	0.21 $\pm$ 0.03	-32 $\pm$ 3	58.4 $\pm$ 3.1
Neem-loaded PLGA nanoparticles	162 $\pm$ 9	0.17 $\pm$ 0.02	-24 $\pm$ 2	76.9 $\pm$ 2.6

Formulation	Mean particle size (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)
Neem-loaded chitosan nanoparticles	208 $\pm$ 15	0.24 $\pm$ 0.04	+28 $\pm$ 3	71.2 $\pm$ 3.4
Neem-loaded SLN	142 $\pm$ 8	0.19 $\pm$ 0.02	-19 $\pm$ 2	82.5 $\pm$ 2.2
Neem nanoemulsion	94 $\pm$ 6	0.16 $\pm$ 0.01	-15 $\pm$ 2	68.7 $\pm$ 3.8

Across carrier types, encapsulation efficiency correlated positively with the lipophilicity match between the extract's dominant constituents and the carrier matrix, with solid lipid nanoparticles and PLGA systems consistently outperforming liposomal and emulsion-based systems for azadirachtin-rich extracts.

7.2 In Vitro Release Behaviour

Free neem extract exhibited rapid, near-complete release (>90%) within the first 2 hours under simulated gastric and intestinal conditions, consistent with immediate dissolution of unprotected constituents. In contrast, nano-encapsulated formulations demonstrated biphasic release profiles, characterized by an initial burst release (20–35% within 1–2 hours, attributable to surface-adsorbed drug) followed by sustained release extending to 24–48 hours. Korsmeyer–Peppas modelling indicated release exponents (n) in the range of 0.45–0.89 across formulations, suggesting a combination of Fickian diffusion and polymer/lipid matrix erosion as the governing release mechanism, most notably for PLGA and SLN systems.

7.3 In Vitro Anti-inflammatory Activity

Test system	Free extract (% inhibition / IC50)	Nanoformulation (% inhibition / IC50)	Standard drug
Protein denaturation inhibition (at 500 µg/mL)	52.3 ± 2.9%	78.6 ± 3.2%	Diclofenac: 84.1 ± 2.5%
HRBC membrane stabilization (at 500 µg/mL)	48.7 ± 3.1%	74.2 ± 2.8%	Diclofenac: 81.5 ± 2.1%
Nitric oxide inhibition (RAW 264.7, IC50)	146 µg/mL	58 µg/mL	L-NAME: 22 µg/mL
COX-2 inhibition (IC50)	112 µg/mL	41 µg/mL	Celecoxib: 0.8 µg/mL

Nano-encapsulation consistently improved apparent potency by 1.5- to 3-fold across all in vitro assays, most likely reflecting enhanced cellular uptake and improved solubilization of lipophilic limonoids rather than an intrinsic change in molecular mechanism of action.

7.4 In Vivo Anti-inflammatory Efficacy

In the carrageenan-induced paw oedema model, nano-encapsulated neem formulations produced significantly greater and more sustained inhibition of paw oedema compared with equivalent doses of free extract at all measured time points beyond 2 hours (p

< 0.05). Peak oedema inhibition for nanoformulations typically reached 65–72% at the 4–6 hour interval, compared with 38–45% for free extract at equivalent dose, approaching but generally not exceeding the efficacy of standard NSAID comparators. Corroborating ELISA data demonstrated significantly greater suppression of serum TNF- α and IL-6 in animals receiving nano-encapsulated formulations, consistent with enhanced systemic exposure to active constituents.

8. Discussion

The synthesized findings support the central hypothesis that the anti-inflammatory potential of *Azadirachta indica* extracts is substantially constrained by unfavourable biopharmaceutical properties rather than by intrinsic pharmacological weakness, and that nano-encapsulation meaningfully addresses this gap. Across nanocarrier platforms, improvements in particle size uniformity, encapsulation efficiency, and sustained release translated into consistent and reproducible enhancement of anti-inflammatory activity in both cell-based and animal models.

Solid lipid nanoparticles and nanostructured lipid carriers emerged as particularly well-suited platforms for lipophilic limonoids such as azadirachtin and nimbolide, combining high encapsulation efficiency with physical stability superior to liposomal systems. Polymeric nanoparticles, particularly chitosan-based systems, offered the additional benefit of mucoadhesion, which may prolong intestinal residence time and enhance absorption for orally administered formulations — a property of particular relevance for inflammatory bowel conditions where localized gut delivery is desirable. Nanoemulsions, while offering the simplest and most scalable manufacturing process, showed comparatively greater sensitivity to long-term physical instability (creaming, Ostwald ripening), suggesting that formulation optimization and appropriate surfactant selection are critical for translational success.

Mechanistically, the enhanced in vitro and in vivo activity of nano-encapsulated formulations is best explained by a combination of (i) protection of labile constituents such as azadirachtin from premature degradation, preserving a larger fraction of intact active molecule for absorption; (ii) increased effective surface area and improved wetting/dissolution of otherwise poorly soluble limonoids; (iii) enhanced cellular internalization via endocytic pathways, particularly relevant for macrophage-targeted anti-inflammatory effects; and (iv) sustained release kinetics that maintain therapeutic concentrations over a longer interval, reducing the peak-and-trough exposure pattern characteristic of free extract administration.

These findings are broadly consistent with the wider phytonanotechnology literature, in which nano-encapsulation of other poorly bioavailable phytoconstituents — curcumin, resveratrol, quercetin — has produced comparable relative improvements in bioavailability and pharmacodynamic response, lending external validity to the neem-specific findings discussed here.

9. Challenges and Future Perspectives

9.1 Manufacturing and Scale-up

Many nano-encapsulation techniques described in laboratory-scale studies rely on process parameters (e.g., probe sonication energy, solvent removal rates) that are difficult to reproduce at pilot or industrial scale without loss of critical quality attributes. Continuous manufacturing approaches, such as microfluidic nanoprecipitation, warrant further investigation for consistent large-batch production.

9.2 Standardization of Plant Extract

Batch-to-batch variability in raw neem material, arising from geographic origin, harvest season, and post-harvest handling, presents a persistent challenge to formulation reproducibility. Robust extract standardization against validated marker compounds (azadirachtin, nimbin) prior to encapsulation is essential for regulatory acceptance.

9.3 Toxicological and Safety Evaluation

While neem itself has a long history of traditional use, the nanoscale reformulation of its constituents necessitates dedicated toxicological evaluation, as altered biodistribution and cellular uptake kinetics may modify the safety profile relative to conventional extract. Comprehensive acute and sub-chronic toxicity studies, genotoxicity screening, and reproductive toxicity assessment (particularly relevant given historical data on azadirachtin's antifertility effects in some animal models) are necessary prerequisites for clinical development.

9.4 Regulatory Pathway

Nano-phytopharmaceuticals occupy a regulatory grey zone in most jurisdictions, situated between botanical drug and nanomedicine frameworks. Clear guidance on characterization requirements, stability data, and bioequivalence standards specific to nano-encapsulated herbal extracts would substantially accelerate translational progress.

9.5 Clinical Translation

To date, clinical trial data for nano-encapsulated neem formulations remain sparse. Well-designed, adequately powered randomized controlled trials evaluating pharmacokinetics, tolerability, and clinical anti-inflammatory endpoints (e.g., in osteoarthritis or inflammatory dermatoses) are needed to substantiate preclinical promise and support regulatory approval.

10. Conclusion

Azadirachta indica remains a pharmacologically rich source of anti-inflammatory phytoconstituents whose full therapeutic potential has been constrained by poor aqueous solubility, chemical instability, and low systemic bioavailability. Nano-encapsulation across a range of carrier platforms — including liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, and nanoemulsions — has been consistently shown to overcome these limitations, yielding formulations with favourable particle characteristics, high encapsulation efficiency, sustained release kinetics, and significantly enhanced *in vitro* and *in vivo* anti-inflammatory activity relative to conventional extracts. Solid lipid nanoparticles and nanostructured lipid carriers appear particularly well matched to the lipophilic limonoid fraction of neem, while polymeric and mucoadhesive systems offer complementary advantages for oral and localized delivery. Realizing the clinical potential of these formulations will require continued investment in standardized extraction protocols, scalable and reproducible manufacturing processes, comprehensive toxicological characterization, and rigorously designed clinical trials. With these advances, nano-encapsulated *Azadirachta indica* formulations represent a promising and scientifically well-grounded strategy for the development of next-generation, plant-derived anti-inflammatory therapeutics.

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