Journal of Molecular Science

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1182-1188
DoI-10.004687/1000-9035.2025.159

Journal of Molecular Science
www.jmolecularsci.com ISSN:1000-9035

Preparation, Optimization, and Characterization of Eugenol Oil-Based Phytosomes as Therapeutic Agent

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Article Information
Received: 27-10-2025
Revised: 16-11-2025
Accepted: 04-12-2025
Published: 24-12-2025

Keywords
Eugenol phytosomes, Nanoformulation, Soya lecithin, Antioxidant, Colon cancer, Drug delivery.

ABSTRACT
Colon cancer represents a significant global health threat, with an estimated 10 million deaths annually. While conventional chemotherapeutic agents demonstrate efficacy in cancer treatment, they are accompanied by severe adverse effects and cellular toxicity. Eugenol, a naturally occurring phenolic compound with established antioxidant and anti-inflammatory properties, has emerged as a promising phytopharmaceutical for cancer chemoprevention. However, eugenol's poor bioavailability and aqueous solubility limit its therapeutic potential. This study presents the optimization and characterization of eugenol oil-based phytosomes using soya lecithin as a biocompatible phospholipid carrier to enhance bioavailability and cellular uptake. Eugenol oil granules were prepared through adsorption granulation methodology, followed by phytosome formulation at three drug-to-lipid ratios (1:0.5, 1:1, 1:2) using both N-hexane and acetone as solvent systems. Particle size analysis revealed optimal nanoparticle dimensions ranging from 92.9 nm to 177.2 nm depending on the solvent and formulation ratio employed. Zeta potential analysis demonstrated sufficient electrostatic stability across all formulations (ranging from -21.7 mV to -31.2 mV), indicating colloidal stability and reduced aggregation tendency. The acetone-based formulations exhibited superior particle size reduction, with the 1:2 ratio achieving the smallest particle size (92.9 nm) and highest zeta potential magnitude (-31.2 mV). These optimized phytosomes possess enhanced physicochemical characteristics favorable for cellular uptake and bioavailability, positioning them as promising carriers for eugenol delivery in colon cancer prevention and therapeutic applications.

1. INTRODUCTION:

Colorectal cancer, commonly referred to as colon cancer, represents the third most prevalent malignancy worldwide, with an estimated incidence of 1.9 million new cases and 935,000 deaths annually[1]. The disease is characterized by malignant transformation in the epithelial cells of the large intestine, driven by a complex interplay of genetic, environmental, and nutritional factors[1]. Approximately 60% of patients diagnosed with colon cancer require systemic therapy for metastatic disease at diagnosis or following disease recurrence[2]. The propensity of colon cancer to metastasize to vital organs including the liver, lungs, and ovaries significantly impacts patient prognosis and overall survival rates[3].

Current therapeutic approaches for colon cancer management encompass surgery, chemotherapy, and radiation therapy. While these modalities have advanced significantly in recent decades with the development of novel cytotoxic agents and targeted monoclonal antibodies, they remain associated with substantial treatment-related toxicity and adverse effects[2]. Conventional chemotherapeutic agents frequently induce severe side effects that

significantly compromise quality of life, including gastrointestinal disturbances, hematologic toxicity, immunosuppression, and organ damage[4].

In response to the limitations inherent to synthetic chemotherapeutic agents, there has been heightened scientific interest in naturally occurring phytochemicals as adjuvant therapeutic agents[4]. Plant-based compounds have demonstrated the capacity to suppress carcinogenesis through multiple mechanisms including apoptosis induction, cell cycle arrest, inhibition of cellular proliferation, and modulation of inflammatory signaling pathways[5]. Notably, these naturally derived compounds typically exhibit reduced toxicity profiles compared to conventional chemotherapeutic agents, rendering them attractive candidates for integration into comprehensive cancer management strategies[5].

Eugenol (1-allyl-4-hydroxy-3-methoxybenzene, 4-allyl-2-methoxyphenol) is a simple phenolic compound predominantly extracted from *Syzygium aromaticum* (clove) and other aromatic plants[6,16]. This bioactive molecule has been traditionally employed in Ayurvedic and Chinese traditional medicine systems for management of diverse health conditions[6]. Recent scientific evidence has established that eugenol possesses a broad spectrum of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties[6,15]. Specifically, eugenol has been demonstrated to induce apoptosis in colon cancer cells through multiple mechanisms, including p53 activation, promotion of sub-G1 cell cycle arrest, and regulation of matrix metalloproteinases (MMP) and non-protein thiols[7,17].

Herbosomes and probiotics both represent innovative approaches in the field of pharmacology and nutritional science. Their relationship, particularly in the context of enhancing bioavailability and therapeutic effectiveness, highlights their significant potential when combined.

Herbosomes, a novel herbal formulation, are essentially lipid-based vesicles that encapsulate herbal bioactive compounds. This advanced technology improves the solubility, stability, and bioavailability of herbal ingredients, thus overcoming the typical challenges such as poor water solubility and rapid degradation of these compounds in the body. By improving the delivery of herbal drugs to their target sites, herbosomes increase the therapeutic potential of plant-based compounds[7,18-21].

Probiotics, on the other hand, are live microorganisms that confer health benefits when consumed in adequate amounts. They play a pivotal role in maintaining gut health, modulating the immune system, and influencing the metabolism of various drugs and nutrients. Probiotics are often used to support the gut microbiota, which can, in turn, enhance the absorption of nutrients and drugs. The relationship between herbosomes and probiotics becomes evident in the context of drug delivery and bioavailability. While herbosomes ensure the effective transport and release of bioactive compounds, probiotics can improve the overall absorption and metabolism of these compounds in the gut. By working synergistically, herbosomes can protect herbal compounds from degradation in the gastrointestinal tract, while probiotics can facilitate the breakdown and uptake of the active ingredients [7, 22].

In the case of eugenol, a potent bioactive compound with substantial pharmacological potential but limited clinical utility due to its poor aqueous solubility and rapid metabolism, combining herbosomes with probiotics could significantly enhance its therapeutic efficacy. [8, 23-25]. The herbosome technology would improve the solubility and stability of eugenol, while probiotics might support its absorption in the intestines and mitigate its rapid hepatic metabolism, leading to better bioavailability and enhanced clinical outcomes. Thus, the synergy between herbosomes and probiotics offers a promising strategy for overcoming the barriers associated with the pharmacological use of compounds like eugenol, facilitating their improved therapeutic efficacy and broader clinical applications. Phytosomes represent an emerging drug delivery system combining plant-derived phytochemicals with biocompatible phospholipids in a molecular complex[8, 26-31]. Unlike conventional liposomal formulations, phytosomes facilitate formation of stable complexes between the active phytochemical and phospholipid moieties at the molecular level, resulting in enhanced membrane permeability and sustained tissue distribution. Soya lecithin, a naturally derived phospholipid rich in phosphatidylcholine, is commonly employed in phytosome formulation due to its biocompatibility, biodegradability, and capacity to facilitate cellular uptake through interaction with cell membrane phospholipids[9, 32-34].

The present research was undertaken to optimize and characterize eugenol oil-based phytosomes utilizing soya lecithin as a carrier system. Our objectives encompassed the development of optimized formulations at varying drug-to-lipid ratios, comprehensive characterization of physicochemical properties, and evaluation of

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formulation parameters affecting nanoparticle dimensions and colloidal stability [9,36-37].

2. MATERIALS AND METHODS:

2.1 Materials:

Eugenol oil (pharmaceutical grade) was procured from commercial pharmaceutical suppliers. Microcrystalline cellulose (MCC), lactose monohydrate, and colloidal silicon dioxide (Aerosil) were obtained from Loba Chemie Pvt. Ltd. (Maharashtra, India). Soya lecithin (purified, batch no. 238329014) was acquired from pharmaceutical grade suppliers. N-hexane (95%, analytical grade) and acetone (analytical grade) were utilized as solvent systems. Ethanol (95% pharmaceutical grade) and magnesium stearate (precipitated, extra pure) were obtained from Loba Chemie Pvt. Ltd. All chemicals and reagents were of pharmaceutical or analytical grade and used without further purification.

2.2 Preparation of Eugenol Oil Granules:

Eugenol oil granules were prepared utilizing the adsorption granulation methodology as previously described[10]. The adsorbent mixture was prepared by combining microcrystalline cellulose (25 g), lactose monohydrate (10 g), and colloidal silicon dioxide (2.5 g) in a dry mixing vessel and blending uniformly. Eugenol oil (10 mL) was added dropwise to the adsorbent mixture while continuously stirring to ensure complete adsorption onto carrier materials, forming a free-flowing powder.

For granulation, ethanol 95% (10 mL) was gradually added to the adsorbed mixture under continuous stirring until moist granules formed. The granules were subsequently dried in an oven at 50°C for 2 hours. Following drying, the granules were sieved through a mesh screen to obtain uniform particle size. Magnesium stearate (1 g) was finally incorporated into the dried granules to enhance flow properties. The resulting eugenol oil granules were stored in an airtight container at room temperature until further use.

2.3 Phytosome Preparation:

Phytosomes were prepared at three different drug-to-soya lecithin molar ratios (1:0.5, 1:1, and 1:2) using dual solvent systems (N-hexane and acetone) to optimize formulation parameters and assess the influence of solvent selection on phytosome characteristics.

Procedure: Soya lecithin (150 mg, 300 mg, or 600 mg depending on the desired ratio) was dissolved in either N-hexane or acetone (20 mL) in a round-bottom flask at room temperature. Eugenol oil granules (300 mg) were gradually added to the soya lecithin solution. The resulting mixture was subjected to sonication for 20 minutes at 40 kHz frequency to ensure homogeneous distribution and complex formation.

Following sonication, the solution was transferred to a 100 mL round-bottom flask equipped with a heating mantle and condensed at 50-60°C until 50% of the solvent had evaporated. The condensed mixture was subsequently filtered using Whatman filter paper (Grade 40) to remove any particulate matter. The phytosome residue retained on the filter paper was dried under ambient conditions for 24 hours.

The dried phytosome was reconstituted with distilled water until a transparent appearance was achieved, rendering it suitable for particle size analysis and zeta potential measurement. All phytosome preparations were conducted in triplicate to ensure reproducibility.

2.4 Characterization of Phytosomes:

2.4.1 Particle Size Analysis:

Particle size determination was performed using dynamic light scattering (DLS) methodology employing a HORIBA SZ-100 particle size analyzer (HORIBA, Inc., Japan). Phytosome samples (previously diluted with distilled water to achieve transparency) were analyzed at a scattering angle of 90° at room temperature (25.0°C). The measurement was conducted using a standard distribution form for monodisperse systems. Multiple measurements (n=3) were performed for each formulation batch, and results were expressed as mean particle size in nanometers with standard deviation.

2.4.2 Zeta Potential Analysis:

Zeta potential measurements were conducted using the same HORIBA SZ-100 instrument equipped with electrophoretic light scattering capabilities. Phytosome dispersions were subjected to zeta potential analysis at 25.0°C with measurement parameters optimized for the charged nanoparticulate system. The electrostatic potential was recorded and expressed in millivolts (mV). Three independent measurements were performed for each formulation, and values were reported as mean ± standard deviation.

2.4.3 Monodispersity Index (PDI):

The polydispersity index was calculated from the DLS measurements to assess the uniformity of particle size distribution. PDI values closer to zero indicate more monodisperse (uniform) systems, while values approaching 1.0 suggest broader size distributions.

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3. RESULTS:

3.1 Eugenol Oil Granule Preparation:

Eugenol oil granules were successfully prepared through the adsorption granulation method with high efficiency. The resulting granules exhibited a uniform, free-flowing appearance following the incorporation of magnesium stearate. The adsorption capacity of the carrier system efficiently accommodated the entire eugenol oil content without evidence of leakage or product degradation.

3.2 Phytosome Characterization Data:

3.2.1 Particle Size Analysis Results:

Particle size analysis revealed significant differences in nanoparticle dimensions based on solvent selection and drug-to-lipid ratio employed during formulation. Comprehensive characterization data are presented in Table 1.

Table 1: Particle size analysis of eugenol oil phytosomes prepared using different solvents and drug-to-lipid ratios

Formulation	Drug:Lipid Ratio	Particle Size (nm)	Polydispersity Index
N-hexane batch 1	1:0.5	171.1 ± 8.9	0.333
N-hexane batch 2	1:1	173.9 ± 12.3	—
N-hexane batch 3	1:2	177.2 ± 10.8	—
Acetone batch 1	1:0.5	97.3 ± 6.2	—
Acetone batch 2	1:2	92.9 ± 5.1	—

The N-hexane formulation series demonstrated particle sizes in the range of 171.1-177.2 nm, with the smallest particles observed in the 1:0.5 ratio formulation (171.1 nm). Notably, the N-hexane (1:0.5) batch exhibited a polydispersity index of 0.333, indicating a relatively monodisperse distribution of nanoparticles. Progressive increase in lipid concentration (moving from 1:0.5 to 1:2 ratios) resulted in marginal increases in mean particle size, suggesting that excessive lipid incorporation may facilitate aggregation or altered complex geometry.

The acetone-based formulations demonstrated superior particle size reduction, achieving markedly smaller dimensions compared to N-hexane counterparts. The acetone (1:0.5) formulation yielded particles of 97.3 nm, while the acetone (1:2) ratio produced the smallest particles observed across all formulations at 92.9 nm. This substantial reduction (approximately 50% smaller than N-hexane equivalents) represents a clinically significant achievement in nanoparticle dimensionality optimization.

3.2.2 Zeta Potential Analysis Results:

Electrostatic characterization of the formulated phytosomes revealed negative zeta potential values across all batches, ranging from -21.7 mV to -31.2 mV (Table 1). The negative charge arises from the dissociation of phosphate groups on the soya lecithin component, imparting anionic character to the nanoparticles.

Table 2: Zeta potential analysis of eugenol oil phytosomes demonstrating colloidal stability characteristics

Formulation	Drug:Lipid Ratio	Zeta Potential (mV)	Solvent
Phytosome-1	1:0.5	-21.7 ± 1.2	N-hexane
Phytosome-2	1:1	-29.0 ± 1.8	N-hexane
Phytosome-3	1:2	-26.9 ± 1.5	N-hexane
Phytosome-4	1:0.5	-30.3 ± 2.1	Acetone
Phytosome-5	1:2	-31.2 ± 1.9	Acetone

The N-hexane formulation series exhibited zeta potential values of -21.7 mV (1:0.5 ratio), -29.0 mV (1:1 ratio), and -26.9 mV (1:2 ratio), respectively. The 1:1 ratio demonstrated the maximum electrostatic charge magnitude within this series, suggesting optimal phospholipid surface coverage at this specific molar ratio.

Acetone-based formulations demonstrated higher zeta potential magnitudes, with values of -30.3 mV and -31.2 mV for the 1:0.5 and 1:2 ratios respectively. These elevated negative charges indicate enhanced electrostatic stability and reduced aggregation tendency compared to their N-hexane counterparts. All recorded zeta potential values exceeded the ±20 mV threshold generally considered adequate for colloidal stability, with acetone formulations particularly demonstrating robust electrostatic stabilization suitable for in-vivo applications.

4. DISCUSSION:

The successful preparation and comprehensive characterization of eugenol oil-based phytosomes represents a significant advancement in delivery system design for natural bioactive compounds targeting colon cancer pathology. The multifactorial optimization approach employed in this investigation, encompassing systematic variation of both solvent systems and drug-to-lipid ratios, has yielded valuable insights into the formulation variables affecting nanoparticle characteristics and colloidal stability[11].

4.1 Formulation Methodology and Eugenol Incorporation:

The adsorption granulation approach employed for eugenol oil incorporation into solid carriers proved highly efficacious for maintaining eugenol stability and bioactivity. This methodology capitalizes on

the physical adsorption of eugenol onto hydrophilic carrier materials (microcrystalline cellulose, lactose monohydrate, and colloidal silicon dioxide) rather than relying on chemical modification or derivatization. The preservation of eugenol's structural integrity through this approach is advantageous, as chemical modification may alter pharmacological properties[12]. The subsequent incorporation of magnesium stearate as a flow-promoting agent enhanced processability without introducing potential incompatibilities with the phospholipid system.

4.2 Influence of Solvent Selection on Phytosome Characteristics:

A key finding of this investigation concerns the profound influence of solvent selection on the resulting phytosome nanoparticle dimensions and electrostatic properties. The acetone-based formulations consistently yielded particles approximately 45-50% smaller than their N-hexane counterparts, representing a clinically substantial improvement in nanoparticle size optimization. This differential response to solvent systems may be attributed to several factors.

N-hexane, a nonpolar solvent with limited capacity for solvation of polar phospholipid head groups, may result in less favorable complexation geometry with the eugenol-lecithin system. The reduced solvent polarity may constrain the spatial orientation of phospholipid molecules, resulting in loose complex formation with accompanying particle enlargement[13]. Conversely, acetone's intermediate polarity (dielectric constant approximately 20.7) may facilitate superior interaction between the eugenol molecule and phospholipid acyl chains while maintaining adequate solvation of the phosphate head group, promoting formation of more compact and organized complexes[13].

The zeta potential magnitudes were consistently higher (more negative) in acetone formulations, indicating superior electrostatic stabilization. This enhanced charge development may result from improved accessibility of phosphate groups to the aqueous phase following acetone evaporation, facilitating ionization and electrostatic charge accumulation[14].

4.3 Drug-to-Lipid Ratio Optimization:

The influence of drug-to-lipid molar ratio on phytosome characteristics revealed interesting trends with important implications for formulation optimization. Within the N-hexane series, progressive increase in lipid concentration (1:0.5 to 1:2) resulted in marginal increases in particle size, suggesting that excess phospholipid may not uniformly distribute across the eugenol surface but instead accumulates in the complex periphery, leading to particle enlargement[11].

Conversely, acetone formulations demonstrated increased particle size reduction with elevated lipid ratios. The acetone (1:2) formulation achieved the optimal particle size (92.9 nm) among all tested conditions. This divergent behavior between solvent systems suggests that acetone facilitates more efficient organization of excess phospholipid in the complex structure, potentially through formation of bilayer regions or micelle-like domains that maintain small overall particle dimensions while accommodating increased lipid content[15].

4.4 Colloidal Stability and In-Vivo Implications:

The zeta potential values recorded across all formulations consistently demonstrated electrostatic stability suitable for in-vivo application. According to established pharmaceutical nanoscience principles, nanoparticulate systems with zeta potential magnitudes exceeding ±20 mV generally demonstrate resistance to aggregation through electrostatic repulsion[16]. All formulations in this study exceeded this threshold, with acetone formulations demonstrating particularly robust electrostatic stabilization (-30 to -31 mV).

These stability characteristics are crucial for maintaining uniform particle dimensions and preventing aggregation-related issues such as reduced cellular uptake, altered biodistribution, and potential vascular occlusion[16]. The acetone (1:2) formulation, combining the smallest particle size (92.9 nm) with the highest zeta potential magnitude (-31.2 mV), emerges as the optimally balanced formulation for further development.

4.5 Bioavailability Enhancement Through Nanoformulation:

The nanoparticulate dimensions achieved in this investigation hold significant implications for bioavailability enhancement. Particles in the 90-200 nm size range have been extensively documented to facilitate preferential uptake by specialized gut-associated lymphoid tissue (GALT) cells and mucosal dendritic cells through various endocytic pathways[17]. The 92.9 nm particles achieved with acetone (1:2) formulation fall within this optimal size range for enhanced mucosal absorption and lymphatic uptake.

Furthermore, the phytosome-phospholipid complex structure facilitates enhanced cell membrane interaction and penetration through mechanism of lipid exchange and incorporation into enterocyte

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membranes[18]. This membrane-mimetic property represents a substantial advantage over conventional particulate delivery systems, potentially explaining the enhanced bioavailability observed with phytosomes compared to free active components or simple particulate formulations[18].

4.6 Mechanistic Considerations for Anti-Colon Cancer Activity:

Eugenol has been established through extensive preclinical investigation to exert anti-colon cancer effects through multiple complementary mechanisms[7]. The phytosomal delivery system developed in this research is hypothesized to enhance these pharmacological effects through improved bioavailability and cellular uptake. Enhanced intestinal permeability via specialized epithelial pathways and potential M-cell targeting through optimal particle sizing may facilitate superior tissue accumulation in the colon, amplifying local eugenol concentration at the site of carcinogenic lesions[17].

The negative electrostatic charge on the phytosomal surface may additionally facilitate interaction with positively charged regions on neoplastic cell membranes, potentially promoting selective uptake by transformed cells relative to normal epithelial cells[19]. This differential cellular uptake would theoretically concentrate the therapeutic agent preferentially in the target tissue, minimizing systemic exposure and associated toxicity[19].

The monodisperse nature of the optimized formulations (particularly evident in N-hexane 1:0.5 ratio with PDI 0.333) ensures uniform cellular exposure and reproducible pharmacological responses, advantageous characteristics for both preclinical research and clinical application development[16].

4.7 Comparison with Literature and Technological Advances:

The particle sizes and zeta potential values achieved in this investigation are comparable to or superior to those reported in contemporary phytosome formulation studies utilizing similar active components[20]. The 92.9 nm dimensions of the optimized formulation fall within the emerging consensus regarding optimal nanoparticle size for mucosal drug delivery, and the zeta potential magnitudes (-30 to -31 mV) align with specifications for colloidal systems designed for extended circulation and lymphatic uptake[20].

5. CONCLUSION:

This investigation successfully demonstrated the optimization and comprehensive characterization of eugenol oil-based phytosomes utilizing soya lecithin as a biocompatible phospholipid carrier. The systematic evaluation of formulation variables revealed that acetone-based solvent systems, particularly at a 1:2 drug-to-lipid molar ratio, yield optimally characterized phytosomes combining minimal particle dimensions (92.9 nm), robust electrostatic stability (zeta potential -31.2 mV), and physicochemical properties favorable for enhanced bioavailability.

The phytosomal formulations developed through this research represent significant advancement toward addressing the bioavailability limitations that currently restrict the clinical utility of eugenol despite its well-established antioxidant and anti-neoplastic properties. The nanoparticulate dimensions and electrostatic characteristics achieved align with evidence-based design principles for oral mucosal absorption and lymphatic targeting, suggesting high probability of enhanced therapeutic efficacy in targeted colon cancer prevention and treatment applications.

Future investigation should encompass in-vitro cytotoxicity and antioxidant activity assessment employing colon carcinoma cell lines (HT-29, Caco-2) to validate the hypothesis that phytosomal delivery enhances cellular uptake and bioactivity compared to unconjugated eugenol. Comparative antioxidant analysis utilizing established methodologies (DPPH radical scavenging assay) will provide quantitative evaluation of pharmacological efficacy. Additionally, in-vivo biodistribution studies employing animal models of chemically-induced colon carcinogenesis would establish tissue accumulation patterns and therapeutic effectiveness of the optimized phytosomal formulation in cancer prevention models.

The optimized eugenol phytosomes produced through this research constitute a promising lead formulation for development into a therapeutic entity addressing a significant global health need in colorectal cancer prevention and adjuvant treatment strategies.

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