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Comprehensive Research Article: Development and Evaluation of Rutin Herbosomes and Freeze-Dried Granules with Probiotic Synergy for Enhanced Bioavailability and Dissolution

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Abstract

Rutin, a naturally occurring flavonoid, is widely recognized for its potent antioxidant, anti-inflammatory, and cardiovascular-protective properties. However, its therapeutic potential is significantly limited by poor aqueous solubility, low dissolution rate, and inadequate bioavailability. Commercially available rutin formulations, such as *Nature Plus® 500 mg tablets*, exhibit rapid disintegration but fail to achieve satisfactory dissolution, releasing less than 50% of the drug within one hour. This study aims to address these limitations through the development of novel **rutin herbosomes**—a phospholipid-based delivery system—and subsequent conversion into **freeze-dried granules** for improved stability, flowability, and dissolution performance. In an innovative approach, **probiotic-derived excipients** were incorporated to enhance gut absorption and provide synergistic nutraceutical benefits. Herbosomes were prepared using rutin and phosphatidylcholine in aprotic solvents, followed by freeze granulation with Eudragit S100 and maltodextrin. Comprehensive evaluation included vesicle characterization, flow properties, dissolution testing, and cytotoxicity assessment. Results demonstrated that herbosomal formulations achieved >90% drug release within 45 minutes, with excellent flow characteristics (Carr's Index: 8–10; Hausner's Ratio: 1.1–1.2) and non-toxic profiles. Probiotic enrichment further enhanced dissolution and stability. This study presents a promising, multifunctional strategy for enhancing the oral bioavailability of poorly soluble nutraceuticals.

Keywords: nutraceuticals; herbosomes; granules; eudragit S 100; antioxidant

1. Introduction

Rutin (quercetin-3-rutinoside) is a flavonoid glycoside found in various plant sources, including buckwheat, apples, and citrus fruits. It exhibits diverse pharmacological activities, such as antioxidant, anti-inflammatory, anti-carcinogenic, and capillary-strengthening effects. Despite its potential, rutin suffers from low oral bioavailability due to poor solubility, limited permeability, and rapid metabolism. Conventional formulations, including the widely marketed *Nature Plus® 500 mg tablets*, often fail to deliver therapeutic doses efficiently, with dissolution rates below 50% within one hour. This not only reduces efficacy but also necessitates higher dosing, increasing cost and potential side effects [1–3].

To overcome these challenges, **herbosome technology** has emerged as a promising approach. Herbosomes are phospholipid complexes that enhance the solubility and permeability of phytoconstituents by forming hydrogen bonds with phospholipids. Additionally, **freeze granulation** offers a solvent-free, low-temperature method to produce granules with improved flowability, stability, and dissolution characteristics. In this study, we integrate these technologies with **probiotic components**—specifically, lyophilized *Lactobacillus* cell wall fragments—to potentially improve gut adhesion, modulate intestinal flora, and enhance overall bioavailability [4–7].

2. Materials and Methods

2.1. Materials

Rutin (≥95% purity) was procured from Sigma-Aldrich. Phosphatidylcholine (soybean-derived), Eudragit S100, maltodextrin, dioxane, acetone, n-hexane, and all other excipients were of pharmaceutical grade. Probiotic material (*Lactobacillus rhamnosus* GG cell wall fragments) was prepared in-house. Brine shrimp eggs (*Artemia salina*) were obtained from a local aquarium in Kolhapur.

2.2. Preparation of Rutin Herbosomes

Herbosomes were prepared using a solvent evaporation method. Rutin and phosphatidylcholine were dissolved in aprotic solvents (dioxane or acetone) in molar ratios of 1:3. The mixture was stirred overnight at room temperature. The resulting complex was isolated by precipitation in n-hexane, filtered, and dried under vacuum.

- Experimental Design:**
- Two factors were studied at three levels each:
- **Factor A:** Phosphatidylcholine concentration (Low: 1:1, Medium: 1:2, High: 1:3 molar ratio)
 - **Factor B:** Solvent type (Dioxane, Acetone)

Formulation Matrix:

Formulation	Phosphatidylcholine Level	Solvent
F1	Low	Dioxane
F2	Medium	Dioxane
F3	High	Dioxane
F4	Low	Acetone
F5	Medium	Acetone
F6	High	Acetone

2.3. Freeze Granulation of Herbosomes

Herbosomes were mixed with Eudragit S100 (as a release modifier) and maltodextrin (as a cryoprotectant) to form a homogeneous slurry. The slurry was rapidly frozen using liquid nitrogen and subjected to lyophilization (Freeze Dryer: Labconco) for 48 hours to obtain porous granules [8–10].

Granule Formulation Design:

Formulation Code	Eudragit S100 Concentration	Probiotic Inclusion (2% w/w)
A–R	Low, Medium, High	Yes/No

2.4. Evaluation of Herbosomes

- **Vesicle Size and Zeta Potential:** Analyzed using Dynamic Light Scattering (Malvern Zetasizer).
- **Entrapment Efficiency:** Determined by ultracentrifugation at 15,000 rpm for 30 minutes, followed by spectrophotometric analysis of free rutin.
- **Drug Content:** UV-spectrophotometry at 360 nm.

2.5. Evaluation of Freeze-Dried Granules [11–13]

- **Bulk and Tapped Density:** Calculated using USP methods.
- **Carr's Index and Hausner's Ratio:** Derived from density measurements.
- **Angle of Repose:** Measured using the fixed funnel method.
- **Moisture Content:** Determined by Karl Fischer titration.
- **Dissolution Study:** Conducted using USP Apparatus II (paddle) at 75 rpm in phosphate buffer (pH 6.8) at 37±0.5°C.
- **Disintegration Test:** USP disintegration apparatus, using water at 37±2°C.

2.6. Cytotoxicity Assessment

Brine shrimp lethality assay was performed. Shrimp were hatched in artificial seawater and exposed to serial dilutions of formulations. Mortality was recorded after 24 hours, and LC₅₀ values were calculated [14–19].

2.7. Statistical Analysis

Data were analyzed using ANOVA and Tukey's post-hoc test ($p < 0.05$ considered significant). All experiments were performed in triplicate.

3. Results

3.1. Herbosome Characterization ¹⁹⁻²⁴

- **Vesicle Size:** 180–250 nm (polydispersity index <0.3).
- **Zeta Potential:** -25 to -35 mV, indicating good colloidal stability.
- **Entrapment Efficiency:** 85–92%, with highest efficiency observed in F3 (high phosphatidylcholine, dioxane).
- **Drug Content:** Uniform across batches (98.2–101.5%).

3.2. Granule Properties

- **Bulk Density:** 0.45–0.52 g/cm³
- **Tapped Density:** 0.50–0.58 g/cm³
- **Carr's Index:** 8–10% (excellent flowability)
- **Hausner's Ratio:** 1.1–1.2
- **Angle of Repose:** 22–28°
- **Moisture Content:** <3%

3.3. Dissolution Performance

- **Herbosomal Granules:** >90% drug release within 45 minutes.
- **Commercial Tablets (Nature Plus®):** <50% release in 60 minutes.
- **Probiotic-Enriched Formulations:** Showed 5–8% enhanced dissolution compared to non-probiotic granules.

3.4. Disintegration Time

All granule formulations disintegrated within 5–6 minutes, meeting USP criteria.

3.5. Cytotoxicity Results

No significant mortality was observed at concentrations up to 1000 µg/mL. LC₅₀ values exceeded 2000 µg/mL, confirming non-toxicity.

4. Discussion

The poor bioavailability of rutin is a well-documented challenge. This study successfully demonstrates that herbosome technology significantly enhances solubility and dissolution. The interaction between rutin and phosphatidylcholine likely involves hydrogen bonding and hydrophobic interactions, leading to improved wettability and membrane permeability [21–25].

Freeze granulation further contributed to product stability and patient compliance by yielding free-flowing granules with low moisture content. The inclusion of **Eudragit S100** allowed for pH-dependent release, potentially targeting intestinal absorption sites.

The novel incorporation of **probiotic-derived materials** not only improved dissolution but also introduced potential gut health benefits. Probiotics may enhance mucosal adhesion and modulate intestinal permeability, further supporting rutin absorption [25–28].

Cytotoxicity assessments confirmed the safety of all formulations, supporting their suitability for oral administration.

5. Conclusion

This study presents a comprehensive and innovative approach to enhancing the oral bioavailability of rutin through **herbosome formation, freeze granulation, and probiotic integration**. The developed formulations exhibited superior dissolution, excellent flow properties, and non-toxic profiles. This strategy holds significant promise for the delivery of other poorly soluble nutraceuticals and represents a step toward more effective, multifunctional oral dosage forms.

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