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Unleashing the Power of Rutin-Loaded Nanophytosomes: Enhancing Antioxidant Potential for Improved Health Outcomes

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ABSTRACT

Plant-derived materials are increasingly recognized for their nutritional and medicinal benefits. Rutin (RN), a bioflavonoid with phenolic antioxidant properties, is known for scavenging superoxide radicals and enhancing blood vessel permeability. Despite these benefits, rutin's oral bioavailability is low due to poor absorption. To improve rutin's antioxidant qualities and bioavailability, this study sought to create a stable nanophytosomal formulation filled with rutin. The thin-layer hydration approach was used to create rutin-loaded nanophytosomes utilizing phosphatidylcholine (PC) and cholesterol (CH). Using differential scanning calorimetry (DSC), zeta potential, scanning electron microscopy, IR spectroscopy, and particle size analysis, the physicochemical characteristics of the nanophytosomes were evaluated. For a duration of three weeks during storage, the nanophytosomes' stability was assessed. As per the findings, the lowest particle size was obtained with an RN: PC: CH molar ratio of 1:2:0.5 (F3), and the physical stability of the nanophytosomes was considerably enhanced by the addition of cholesterol. Analytical techniques confirmed the formation of Rutin-Nanophytosomes. The formulation of Rutin nanophytosomes markedly improved the antioxidant activity of Rutin by increasing its bioavailability and stability.

Keywords: *Rutin- infused nanophytosomes, Antioxidant-packed nanocarriers, Enhanced drug delivery, Nanotechnology in healthcare, Bioavailability of antioxidants*

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INTRODUCTION

Rutin, also chemically known as 3, 3′, 4′, 5, 7-pentahydroflavone-3-rhamnoglucoside, is a well-known flavonoid that is present in a wide range of plants, including the Marantaceae family member Ruta graveolens. It has a variety of pharmacological properties, including anti-inflammatory, antithrombotic, antioxidant, and antineoplastic actions (Amjadi *et al*., 2021; Mahmood *et al*., 2023; Omidfar *et al*., 2023). Additionally, rutin is effective in mitigating ultraviolet radiation-induced oxidative stress and inflammation, treating capillary fragility, reducing hypertension, and lowering both hepatic and blood cholesterol levels. Its anti-platelet properties further enhance its therapeutic profile (Alharbi *et al*., 2021; Shriram *et al*., 2022; Moghaddam *et al*., 2023; Dehnad *et al*., 2024).

Typically made from soy, phospholipids and naturally occurring water-soluble phytoconstituents combine to form phytosomes, which are sophisticated delivery mechanisms. Lipid-compatible molecular complexes that considerably improve absorption and bioavailability are produced when certain concentrations of phospholipids combine with phytoconstituents in a solvent (Alharbi *et al*., 2021; Shriram *et al*., 2022; Ibrahim *et al*., 2023; Pozos-Nonato *et al*., 2023; Dehnad *et al*., 2024). Unlike conventional herbal extracts, phytosomes offer superior bioavailability due to their enhanced absorption and systemic circulation reach. This makes phytosomes particularly advantageous for the delivery of herbal medicines, nutraceuticals, and topical skin care products (Alharbi *et al*., 2021; Barani *et al*., 2021; Susilawati *et al*., 2021; Kumar *et al*., 2023).

Rutin, also known as quercetin-3-rutinoside or sophorin, is a flavanol glycoside consisting of the flavanol quercetin and the disaccharide rutinose. It is extracted from sources like the Japanese pagoda tree, buckwheat seed, and citrus fruits such as oranges, grapefruits, and lemons (Rathee & Kamboj, 2018; Lu *et al*., 2019; Islam *et al*., 2022; Shriram *et al*., 2022; Tiwari *et al*., 2023). One innovative drug delivery system for transdermal application is the nanophytosome, a vesicular complex formed between phytoconstituents and phospholipids, resembling cell membranes with polar heads and nonpolar tails. Phytoconstituents bind to the polar head of phospholipids, typically phosphatidylcholine, to form a stable complex that improves absorption and bioavailability (Zhang *et al*., 2013;

Sabzichi *et al*., 2014; Telange *et al*., 2017; Alhakamy *et al*., 2020; Bhargav *et al*., 2021; Jain *et al*., 2021; Gaikwad *et al*., 2023).

Phytosomes are distinct from liposomes in their structural formation; while liposomes encapsulate the active ingredient within their cavity or membrane layers, phytosomes integrate the active ingredient into the membrane itself, anchoring it through chemical bonds to the phospholipid's polar head. This structural difference grants phytosomes superior physical stability, enhancing the absorption and bioavailability of hydrophilic polar phytoconstituents and leading to greater therapeutic benefits (Huang *et al*., 2020; Nandhini & Ilango, 2020; Alharbi *et al*., 2021).

Rutin's pharmacological activities extend beyond its antioxidant properties, including anti-inflammatory, neuroprotective, cardioprotective, anti-arthritic, anti-psoriasis, antimicrobial, antiallergic, antiviral, hepatoprotective, anticancer, and gastroprotective effects. Its notable antioxidative and radical-scavenging abilities make it effective against hydroxyl, superoxide, and peroxyl radicals (Riva *et al*., 2019; Shriram *et al*., 2022). Foods originating from plants and animals both include phospholipids, which are essential parts of all cell membranes. An excellent source of choline for dietary supplements is soy lecithin, a natural phospholipid combination (Naik *et al*., 2006; Gnananath *et al*., 2017; H Shariare *et al*., 2020).

The objective of this research is to create and assess rutin-loaded phytosomes as a possible topical treatment for inflammatory disorders, with the goal of long-term therapeutic advantages.

MATERIALS AND METHODS

Rutin (RN) was procured from Loba Chemie, Mumbai, India. Phosphatidylcholine (PC) was acquired from Labogen, Gujarat, India, while cholesterol (CH) was sourced from Labogen, Punjab, India. All additional chemicals and solvents required for the research were obtained from Himedia Laboratories Research Lab, Mumbai, India.

Preparation of rutin nanophytosomes (RN-NPs)

RN and PC were diluted in different molar ratios (1:1, 1:2, and 1:4) to create phytosomes by the thin layer hydration technique. Dichloromethane was used to dissolve cholesterol, but ethanol was used to dissolve RN and PC. A rotary evaporator (Heidolph, Germany) was used to evaporate the solvents from the mixture and create a thin, dry layer. The mixture was put in a flask with a circular bottom. The film was exposed to nitrogen gas flow and allowed to sit at room temperature for the whole night before being hydrated to guarantee total elimination of organic solvents. Next, using a rotary evaporator set at 45°C, the film was hydrated with distilled water. Three techniques—bath sonication (Model 8852, Cole-Parmer Instrument, Chicago, IL) at 45°C, homogenization (Heidolph, Germany) at 20,000 rpm, and probe sonication (Sonix, Vibracell)—were used to decrease the size of the phytosomes (Nagpal *et al*., 2016; Matias *et al*., 2017; Deleanu *et al*., 2023). The graphical approach of rutin-nanophytosome production is shown in **Figure 1**.

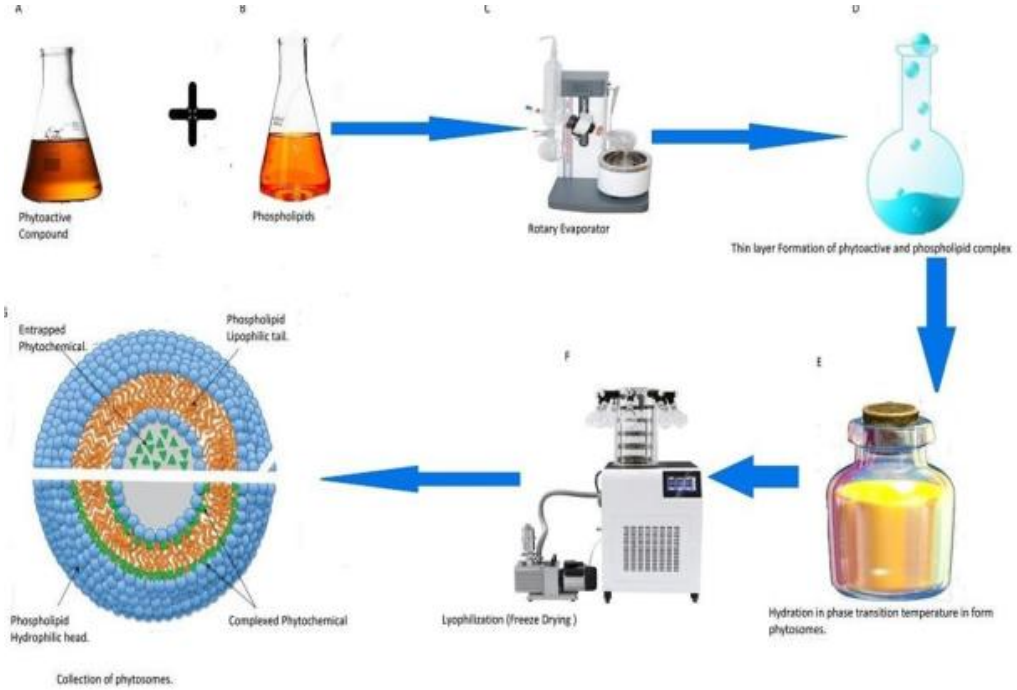

Figure 1. Schematic Illustration of the Preparation Method of Rutin Nanophytosomes (RN-NPs)

Characterization of nanophytosomes

IR spectroscopy: Using an FT-IR spectrometer (Brucker Alpha2, Germany), FT-IR spectra were captured. Potassium bromide was added to physical mixes, rutin pure, cholesterol, phosphatidylcholine, and lyophilized nanophytosomal formulations. The particles were compacted in a hydraulic press for 10 minutes at 15 tons of pressure (Direito *et al*., 2019). Between 4000 and 400 cm^{-1}, scans were conducted at a resolution of 2 cm^{-1}.

Differential scanning calorimetry

Samples containing phospholipids and phytosomes were placed in aluminum crimp cells and heated at a rate of 100°C/min from 0 to 4000°C under a nitrogen atmosphere using a Perkin Elmer 4000 (Germany). The onset temperatures of peak transitions were recorded with an analyzer.

Particle size

The particle size was measured using photon correlation spectroscopy (Horiba SZ-100, Japan) through the dynamic light scattering method (Abdelkader *et al*., 2016; El-Menshawe et al., 2018; Permana *et al*., 2020). Samples were diluted in distilled water and sonicated for 5 minutes. The analysis was performed three times, and the average hydrodynamic particle size was reported as the z-average size ± SD.

Zeta potential

A Malvern Zetasizer (Horiba SZ-100) was used to estimate the surface charge of rutin nanophytosomes. Experiments were conducted at 25 °C and 149 watts after samples were diluted 50 times with distilled water. Three measurements were used to determine the average zeta potential of the nanophytosomes.

Encapsulation efficiency

Using the following formula, the rutin encapsulation efficiency in nanophytosomes was determined

$$EE\ \% = \text{Total drug added} - \text{free non-entrapped drug} / \text{total drug added} \quad (1)$$

W [added drug] is the amount of free drug found in the lower chamber of an Amicon Ultra-15 tube (Merck Millipore Ltd., Ireland) with a 100 kDa molecular weight cutoff following centrifugation. W [added drug] is the amount of added drug used in the creation of nanophytosomes. One milliliter of the sample was diluted with one milliliter of ethanol to dissolve any unentrapped rutin in order to separate the drug from the nanoparticles. The nanophytosomes remained in the upper chamber after the mixture was centrifuged at 5000 rpm for 10 minutes in a Hettich EBA 20 centrifugal filter in Germany. The fact that the nanophytosomes retained their stability in a 50:50 hydroethanolic solution is noteworthy. With the use of a Shimadzu 8400 S (Japan) spectrophotometer, the amount of unentrapped Rutin in the lower chamber was measured.

Scanning electron microscopy [SEM]

About 5 µL of the phytosomal suspension was placed onto a coverslip and mounted on a specimen stub. The samples were then allowed to dry, and the particle size of the formulation was examined using scanning electron microscopy (SEM) with a Hitachi 4000plus (Japan).

Transmission electron microscopy [TEM]

Prior to testing, the newly made nanoparticle solution was diluted 1:5 in ethanol and sonicated for five minutes. Samples were seen in transmission electron microscopy (TEM; JEM-2000 EX; JEOL, Japan) at magnifications ranging from 50 to 200 nm after being arranged on a copper grid covered with carbon. The form and surface morphology of the particles were ascertained using TEM. Particle size was determined by measuring the diameter of individual nanoparticles in images that were taken using a digital camera.

Anti-oxidant activity

The assay measures antioxidants by their reaction with stable free picrylhydrazyl (DPPH) (Sikarwar *et al*., 2008; Kim *et al*., 2019; Rondanelli *et al*., 2022). The method involves mixing the sample with DPPH in methanol/water, which helps extract antioxidant compounds. 1.0 mL of the 0.4 mM DPPH solution and 1.0 mL of each test solution concentration series were combined for the test. After a certain amount of time, the mixtures were vortexed for thirty seconds. After that, absorbance at 516 nm was measured. Pure rutin and rutin nanophytosome samples were both subjected to absorbance tests.

RESULTS AND DISCUSSION

IR spectroscopy

Spectroscopic sleuthing was employed to uncover the intricate dance between phosphatidylcholine (PC) and Rutin. Using FTIR spectroscopy, the unique functional groups and their frequencies were revealed, showcasing the key chemical components of Rutin and PC, and highlighting the emergence of novel interactions between them during the nanophytosome creation. The FTIR spectra of pure Rutin, PC, and cholesterol are shown in **Figure 2**, along with physical mixes and the resultant Rutin, PC, and cholesterol nanophytosomes.

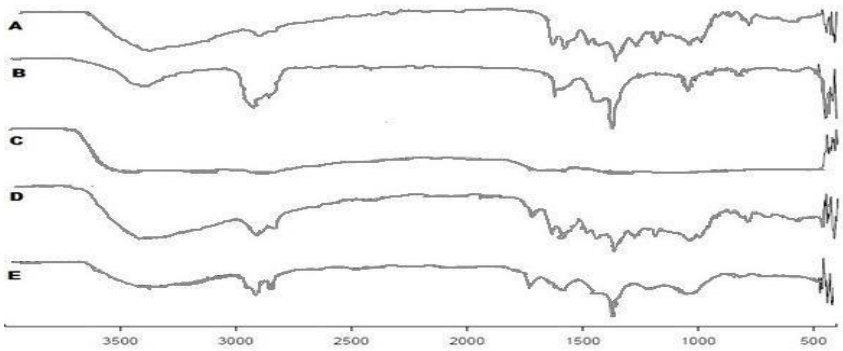

Figure 2. IR Spectrum of a) Rutin, b) Cholesterol, c) Phosphatidylcholine, d) Physical Mixture, E) RN-NPs

Differential scanning calorimetry [DSC]

DSC thermograms were inspected in order to confirm that a complex had formed between phospholipid and rutin (Martins-Gomes *et al*., 2022). **Figure 3** shows the rutin-loaded nanophytosomes, their physical mixing, and the DSC thermograms of pure rutin, cholesterol, and phosphatidylcholine (PC).

Cholesterol's DSC thermogram exhibited endothermic peaks at 150°C, likely indicating the melting of its non-polar hydrocarbon tail. This phase transition produced a sharp peak. The physical mixture's DSC thermogram showed a similar peak for cholesterol at around 149°C. But there were no endothermic peaks for PC or Rutin. Both compounds' crystalline states changed when PC and rutin were combined.

The melting point of pure rutin, or 192 °C, was shown as an endothermic peak on the DSC thermogram. Remarkably, the thermogram of the nanophytosomes loaded with rutin showed that this peak had vanished, indicating that rutin was completely incorporated into the matrix of the nanophytosome, which had distinct thermal characteristics from the physical mixture. The polar portion of phosphatidylcholine and the -OH group of rutin most likely formed a hydrogen bond, which is responsible for this embedding, as **Figure 3** shows.

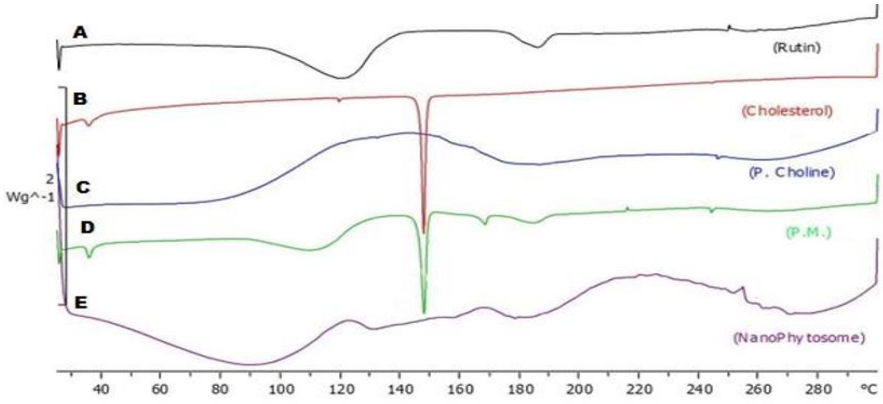

Figure 3. DSC Spectra of a) Rutin, b) Cholesterol, c)Phosphatidylcholine, d) Physical Mixture, e) RN-NPs

Zeta potential

Zeta potential, the electric potential at a particle's surface, is a crucial indicator of colloidal system stability (Palachai *et al*., 2020; Hajizadeh Moghaddam *et al*., 2021; Costa *et al*., 2022). Colloids with high absolute Zeta potential values (typically above 30 mV), whether positive or negative, are considered electrically stable. Conversely, those with low Zeta potential values are prone to coagulation or flocculation, indicating instability. Generally, higher Zeta potential values correlate with greater and more enduring particle stability.

Various factors, such as pH, ionic strength, and the type and concentration of biopolymers used, can influence a particle's Zeta potential. The surface charge analysis results, depicted in **Figure 4**, indicate a Zeta potential of 3.3 mV for Rutin phytosomes, pointing to their high physical stability.

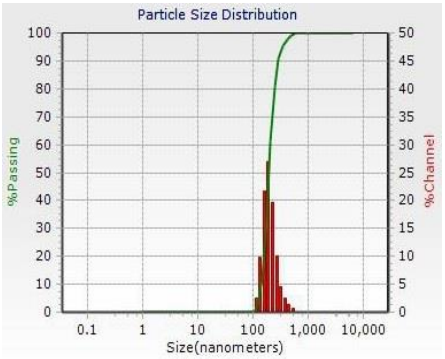

Figure 4. Zeta potential of Rutin Nanophytosomes

Particle size and encapsulation efficiency: **Table 1** below details the encapsulation effectiveness, polydispersity index (PDI) value, mean particle size, antioxidant activity, and composition of Rutin nanophytosomes made with varying molar ratios of Rutin (RN), phosphatidylcholine (PC), and cholesterol (CH). Three experiments' mean ± standard deviation are displayed as the data.

Table 1. Composition, Mean Particle Size, PDI Value, Encapsulation Efficiency, and Antioxidant Activity of Rutin Nanophytosomes

Formulations	RN:PC: CH	Particle size	Encapsulation efficiency [%]	PDI	IC50
F1	1:2:0.1	373.31 ± 1.51	96.00 ± 1.00	0.394	20.34
F2	1:2:0.3	177.57 ± 1.54	96.65 ± 0.57	0.456.	30.45
F3	1:2:0.5	112.32 ± 2.87	95.64 ± 0.57	0.463	21.63
F4	1:2:0.7	201.65 ± 6.49	95.64± 1.13	0.489	28.67
F5	1:2:0.9	391.64 ± 2.87	96.30 ± 0.57	0.762	29.89

Scanning electron microscopy

Scanning Electron Microscopy (SEM) offers valuable insights into the solid-state characteristics and surface morphology of drugs and their complexes. The SEM analysis confirms the vesicle size, as measured by the size analyzer, to be 477 nm. The drug particles are observed to be associated with the phospholipids, forming spherical, uniform, and rigid vesicles. **Figure 5** displays the SEM image of Rutin, illustrating these observations.

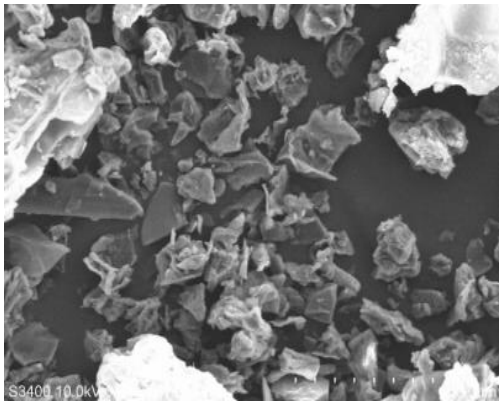

Figure 5. SEM of Rutin nanophytosomes

TEM

The Rutin nanophytosome sample utilized for the TEM analysis corresponds to the Rutin formula 2 nanophytosome, which has a composition of Rutin, Phosphatidylcholine, and Cholesterol in a ratio of 1:2:0.3. This specific formulation, incorporating 1 mole of phosphatidylcholine, helps to prevent particle agglomeration, ensuring that the particles remain small. TEM observations of the Rutin formula 2 nanophytosome reveal spherical nanoparticles. The TEM micrograph, depicted in **Figure 6**, shows that the polar regions of the vesicles appear black, whereas the non-polar regions are indicated by transparent or colorless areas.

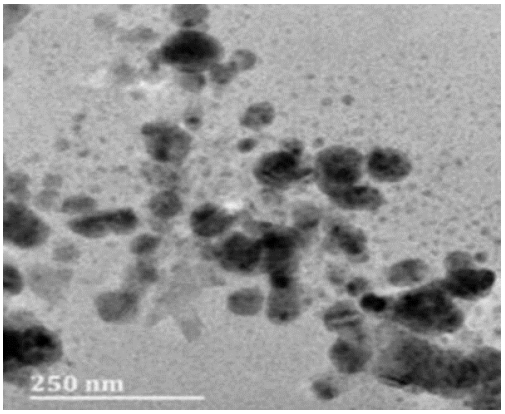

Figure 6. TEM of Rutin Nanophytosomes

Anti-oxidant activity

The IC50 values in **Table 1** indicate the antioxidant capability of Rutin nanophytosomes in each formula, which range from 20 to 32 µg/ml. In particular, rutin nanophytosomes made with RN:PC:CH molar ratios of 1:2:0.5 (F3) showed IC50 values of 21.63 µg/ml. The IC50 value of pure rutin powder is 25.28 µg/ml, which is higher than these values. This finding demonstrates that rutin nanophytosomes are more effective in scavenging free radicals than coarse rutin powder. As opposed to micronized products, a substantial improvement in free radical scavenging is obtained when particle size is reduced to the nanorange, which improves surface area and concentration gradient (Omidfar *et al*., 2023). So, by creating its nanophytosomes, rutin's antioxidant activity is enhanced. Rutin's antioxidant capacity was found to be efficient even after being encapsulated in the formulation of the nanophytosome.

The enhancement of antioxidant activity in Rutin nanophytosomes can be attributed to several key factors. Firstly, the encapsulation of Rutin in nanophytosomes significantly increases its solubility and bioavailability. Rutin, being lipophilic, typically exhibits poor solubility in aqueous environments, limiting its effectiveness. However, when encapsulated in nanophytosomes, the phospholipid bilayer facilitates better dispersion and interaction with biological membranes, enhancing absorption and cellular uptake.

Additionally, the small particle size of the nanophytosomes ensures a larger surface area for interaction with free radicals, leading to more efficient scavenging activity. This increased surface area also allows for a more uniform distribution of Rutin in biological systems, promoting consistent antioxidant activity throughout.

Moreover, the encapsulation process protects Rutin from degradation and oxidation, preserving its antioxidant properties over time. The stability provided by the nanophytosome structure ensures that Rutin remains active and effective until it reaches its target site within the body.

The high encapsulation efficiency of Rutin in the nanophytosomes, confirmed by FTIR and DSC analyses, further supports the enhanced antioxidant activity. The formation of a stable Rutin-Phospholipid complex ensures that a substantial amount of Rutin is delivered effectively, maximizing its therapeutic potential.

Overall, the improved solubility, increased surface area, enhanced stability, and efficient delivery provided by the nanophytosome formulation collectively contribute to the significantly enhanced antioxidant activity of Rutin, making it a promising approach for various therapeutic applications.

CONCLUSION

Because rutin has so many health advantages, industrial food science is interested in incorporating rutin into food items. Rutin's lipophilic properties, however, restrict its use in the treatment of diseases including cancer, COVID-19, bacterial infections, and viral infections. Our research on preparing nanostructures loaded with high amounts of Rutin (using a rutin-to-phosphatidylcholine ratio of 1:2) advances the understanding of incorporating lipophilic herbal supplements into food and beverages. The low particle size, excellent encapsulation effectiveness, and stability of the rutin-loaded nanophytosomes were observed upon physicochemical evaluation. Rutin was successfully loaded into the phytosomes, as shown by the FTIR and DSC tests that verified the development of a rutin-phospholipid complex in the nanophytosomes. In conclusion, the development of Rutin nanophytosomes significantly enhanced the antioxidant activity

of Rutin, demonstrating improved bioavailability and stability compared to its conventional form.

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