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Research Article

Preparation and Characterization of Rutin-loaded Nanophytosomes

Zahra Hooresfand[1], Saeed Ghanbarzadeh[2], Hamed Hamishehkar[3*]

[1]*Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
[2]*Department of Pharmaceutics, Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.*
[3]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

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ABSTRACT

Background: Plant-derived materials are increasingly gaining attention as dietary supplements and due to their role in medicinal application. Rutin, a phenolic antioxidant, is a member of bioflavonoids which has been demonstrated to scavenge superoxide radicals and is believed to be a vital nourishing supplement due to its ability to strengthen and modulation of the permeability of the blood vessels walls. However, Rutin shows poor absorption when administered orally and its bioavailability is an important restrictive factor. ***Purpose:*** The present study was aimed to prepare and characterize a stable Rutin-loaded nanophytosomal formulation to improve its antioxidant property and bioavailability. ***Methods:*** Rutin-loaded nanophytosomes were prepared by phosphatidylcholine (PC) and cholesterol by thin layer hydration method. The physicochemical properties of prepared nanophytosomes were evaluated using particle size analyses, Furrier transformation infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Stability of the prepared nanophytosomes was also investigated during three weeks of storage period. ***Results:*** Results showed that formulation with the Rutin: PC molar ratio of 1:2 possess lowest particle size and the incorporation of cholesterol improved the physical stability of nanophytosome for over three weeks. FTIR and DSC analysis showed the formation of Rutin-phospholipid complex during the formulation development process. ***Conclusion:*** Results of the present study showed that nanophytosome can be introduced as a useful carrier for fortifying herbal medicinal compounds for application the formulation of various food and pharmaceutical products.

Introduction

The most of the biologically active compounds of plants are water soluble or polar molecules. Water soluble phytoconstituents (such as flavonoids, tannins, etc.) are poorly absorbed, either because of their large molecular size (which cannot absorb by passive diffusion) or due to their poor lipid solubility (which limits their ability to pass across the lipid-rich biological membranes), which resulted in poor bioavailability. On the other hand, some constituents of extracts when taken orally are destroyed in the gastric environment, resulting poor bioavailability and limitation in their clinical utility.[1-3] Phytosomes are a complex between natural water soluble phytoconstituents and natural phospholipids (like soy phospholipids) which are prepared by reaction of stoichiometric amounts of phospholipid and the phytoconstituents in a solvent to achieve lipid compatible molecular complexes and improve their absorption and bioavailability.[4,5] Phytosomes show more bioavailability as compared to conventional herbal extracts, because of the being much better absorbed (enhanced capacity to cross the lipoidal biomembrane) than liposomes, showing better bioavailability and reaching the systemic circulation. Therefore, phytosomes have been found superior benificial compared to the liposomes in delivery of herbal medicines and nutraceuticals as well as using in topical and skin care (cosmetic) products.[1,6] Phenolic compounds have attracted considerable attention due to their biological activities including antioxidants properties.[7,8] Flavonoids are a class of phenolic compounds that exhibit powerful antioxidant effects in biological systems (including free radical scavenging) however, their efficiency considerably depends on chemical features.[9,10] Flavonoids are classified, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Flavonoid are hardly absorbed from the small intestine because of the sugar moieties which increases their hydrophilicity. Other reasons for this poor absorption are due to the bacterial degradation of the phenol moiety of these molecules and formation of complex with other substances in the

***Corresponding Author:** Hamed Hamishehkar, Tel: (+98) 41 33363231, Fax: (+98) 41 33363231, E-mail: hamishehkarh@tbzmed.ac.ir

gastrointestinal tract, which prevent their absorption.[11,12] Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside), also known as quercetin-3-rutinoside or sophorin and be composed of the flavonol quercetin and the disaccharide rutinose, is a flavanol glycoside plant metabolite extracted from Japanese pagoda tree, buckwheat seed, fruits and fruit rinds (especially in citrus fruits such as orange, grapefruit, lemon etc). Rutin was isolated during studying of citrus fruits by Ruszinak and Scent-Györgi in 1936 and was suggested to be included into a group of vitamins (vitamin P).[13,14] Rutin could antagonize the increase of capillary fragility associated with hemorrhagic disease or hypertension and usually used for the therapy of lymphatic and chronic venous insufficiency. Rutin is also possess several other pharmacological activities including anti-inflammatory, neuroprotective, cardioprotective, antiarthritis, antipsoriasis, antimicrobial, antiallergic, antiviral, hepatoprotective, anticancer and gastroprotective effects. The most important properties of Rutin are antioxidative and radical-scavenging properties on oxidizing species such as hydroxyl radical, superoxide radical, and peroxyl radical.[15-17] Phospholipids are components of all cell membranes and are present in food derived from plant and animal sources. Soy lecithin is a combination of natural phospholipids, phosphatidylcholine, phosphatidylethanolamine as well as phosphatidylinositol, and is considered as an excellent source of choline for nutritional supplement.[6,18] In recent times, the phytosomal formulations have been applied to several popular herbal extracts of ginkgo biloba, grape seed, hawthorn, green tea, milk thistle and ginseng root with increasing their biological effect. Without using phytosomal formulations, a reduced quantity of the non-lipophylic herbal extracts and their active ingredients were permeated from the intestinal lumen.[1,19] Therefore, in the present study we aimed to produce a stable nanophytosomal formulation of Rutin to be used in the dietary supplements and food stuffs.

Material and Methods

Materials

Soybean phosphatidylcholine (PC), cholesterol (Chol), methanol and chloroform were purchased from Merck Company (Darmstadt, Germany). Rutin was obtained from Sigma Aldrich Company (St Louis, USA).

Preparation of Rutin nanophytosomes

Phytosomes were prepared by using thin layer hydration method with different molar ratio of Rutin and PC (1:1, 1:2 and 1:4). Rutin, PC and Chol were dissolved in a mixture of methanol and chloroform (1:4), taken in a round bottom flask. Solvents was evaporated in a rotary evaporator (Heidolph, Germany) at 45 °C until producing thin dry film in the round bottom flask. Finally, the film was hydrated with distilled water in rotary at the same temperature.[20]

Characterization of Rutin-loaded nanophytosomes

Size distribution

Particle size of prepared nanophytosomes was analyzed by photon correlation spectroscopy using a Shimadzu particle size analyzer (SALD 2101, Japan). Diluted nanophytosomal suspension was placed into the sample dispersion unit while stirring at room temperature (in order to reduce the inter particle aggregation). All analyses has been performed in triplicate.[21,22]

Encapsulation efficiency

The encapsulation efficiency of Rutin in nanophytosomes was calculated by using following equation:

$$EE\ (\%) = \frac{W_{(Added\ \ drug\ )} - W_{(free\ \ drug\ )}}{W_{(Added\ \ drug\ )}} \times 100 \quad \text{Eq.(1)}$$

Where, $W_{(added\ drug)}$ is the amount of added drug used for the preparation of the nanophytosomes and $W_{(free\ drug)}$ is the amount of free drug measured in the lower chamber of Amicon® Ultra-15 tube (Merck Millipore Ltd., Ireland) with the molecular weight cutoff of 100 KDa after centrifugation.[23,24]

To separate unentrapped drug from nanoparticles, 1 mL of sample was diluted with 1 mL of ethanol to make sure that the unentrapped Rutin was dissolved. Then the sample was placed into a centrifugal filter (Hettich EBA 20, Germany) and centrifuged at 5000 rpm for 10 min. The nanophytosomes remained in the upper chamber. It should be noted that the nanophytosomes remained unchanged in the hydroethanolic solution (50:50). The amount of unentrapped Rutin in the lower chamber was determined spectrophotometrically at 270 nm (Shimadzu 8400 S, Japan).[25,26]

Zeta potential determination

Surface charge of Rutin-loaded nanophytosomes was determined using a Malvern Zetasizer (Nano-ZS, UK). Samples were diluted (50 folds) using distilled water and then analysis was performed at 25 ºC and 149 watt. The average three zeta potential determination of the nanophytosomes was calculated.

Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (DSC 60, Shimadzu, Japan) was used to determine enthalpy and melting point of all materials used in the present study. The equipment was calibrated using indium and zinc. Generally, 5 mg of the sample were weighed into an aluminum pan, which was crimped non-hermetically, and heated in the range of 30-300 °C at a heating rate of 10 °C /min.

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra were obtained using a FT-IR spectrometer (Shimadzu 4300, Japan). Pure drug, cholesterol, phosphatidylcholine, physical mixtures and nanophytosomal formulation (after liophilization) were mixed with potassium bromide, separately. The potassium bromide discs were prepared by

compressing the powders at pressure of 15 tons for 10 min in hydraulic press. Scans were obtained at a resolution of 2 cm^{-1}, from 4000 to 400 cm^{-1}.

Result and Discussion

Particle size

Particle size plays an important role in the stability, availability and organoleptic properties of the solution and the particles with smaller size is desirable. Results of particle size analysis indicated that nanophytosomes prepared with Rutin and PC possess the particle size in the range of 99-123 nm, and increasing the ratio of PC did not change the size of nanophytosomes significantly (P>0.05). However, the particle size of these nanophytosomes was not stable and nanophytosomes size was increased up to 14 µm over seven days of storage period. Although incorporation of Chol (up to the Rutin: PC: Chol ratio of 1:2:0.5) in nanophytosomes formulation increased the particle size in the preparation stage, the particle sizes of nanophytosomes were not increased higher than 850 nm after 21 days. However, higher cholesterol content resulted in increasing of particle size of nanophytosomes.

Stability studies

Nanophytosomal formulations of Rutin without (Table 1) and with Cholesterol (Table 2) were stored at 25 °C and physical stability of prepared nanophytosomes was evaluated at 7, 14 and 21 days. As shown, formulation with Rutin: PC ratio of 1:2 showed the most particle size stability. Furthermore, addition of cholesterol with Rutin: PC: Chol ratio of 1:2:0.2 caused highest stability.

Table 1. Particles size of prepared nanophytosomes without cholesterol during 7 days storage period at 25 ºC.

Formulation code	Composition (Rutin: PC)	Particles size (nm)		
		Day 1	Day 3	Day 7
F1	1:1	99 ± 6	222 ± 15	14610 ± 326
F2	1:2	119 ± 7	312 ± 21	403 ± 30
F3	1:4	123 ± 10	222 ± 20	14651 ± 538

Results are expressed as mean ± SD of three experiments.
PC: Phosphatidylcholine

Table 2. Particles size of prepared nanophytosomes with cholesterol during 21 days storage period at 25 ºC.

Formulation Code	Composition	Particle size (nm)				
	(Rutin: PC: Chol)	Day 1	Day 2	Day 7	Day 14	Day 21
F2a	1:2:0.2	164.5 ± 11	282.0 ±22	479.5 ± 43	430.0 ± 37	582.5 ± 43
F2b	1:2:0.5	476.5 ± 27	326.0 ± 31	468.0 ± 45	4606.5 ± 202	8441.0 ± 547
F2c	1:2:1.0	307.0 ± 21	653.0 ± 43	389.0 ± 39	7320.5 ± 340	9805.0 ± 594

Results are expressed as mean ± SD of three experiments.
PC: Phosphatidylcholine and Chol: Cholesterol

Zeta potential

The Zeta potential is the electric potential in the interface or particle surface and is used to predict the stability of colloidal systems. Colloids with high absolute Zeta potential values (normally above 30 mV), regardless of their positivity or negativity, are electrically stabilized and those with low Zeta potential values are not stable and tend to coagulate or flocculate. In general, higher Zeta potential values indicate a higher and longer-term stability of the particles. Several factors such as pH, ionic strength, type and concentration of the used biopolymers are effective on the Zeta potential of the particles.

The surface charge analysis results (-45.2 mv) are shown in Figure 1 and point to the high physical stability of Rutin nanophytosomes.

Encapsulation efficiency

Encapsulation efficiency of three prepared formulations are shown in Table 3. Results indicated that all prepared formulations showed high encapsulation efficiency. On the other hand, although formulation with lower cholesterol value poses higher encapsulation efficiency, the difference was not significant (P> 0.05). The importance of the presented data in Table 3 is that Rutin was loaded in high value by using low amounts of lipid.

Table 3. Encapsulation efficiency percent of prepared formulations.

Formulation code	F2a	F2b	F2c
Encapsulation efficiency (%)	80.4 ± 1.3	72.5 ± 1.4	72.3 ± 1.1

Results are expressed as mean ± SD of three experiments.

FTIR Spectroscopy

Spectroscopic analysis was used in order to identify and diagnose of complex formation between PC and Rutin. In FTIR spectroscopy, functional groups and their numbers were identified from the frequency of radiation that absorbs infrared spectra which showed the main chemical groups in Rutin and PC as well as the formation of new interactions between them in the nanophytosomes preparation process. FTIR spectrum of pure Rutin, PC and cholesterol as well as physical mixtures and nanophytosomes containing Rutin, PC and cholesterol are shown in Figure 2.

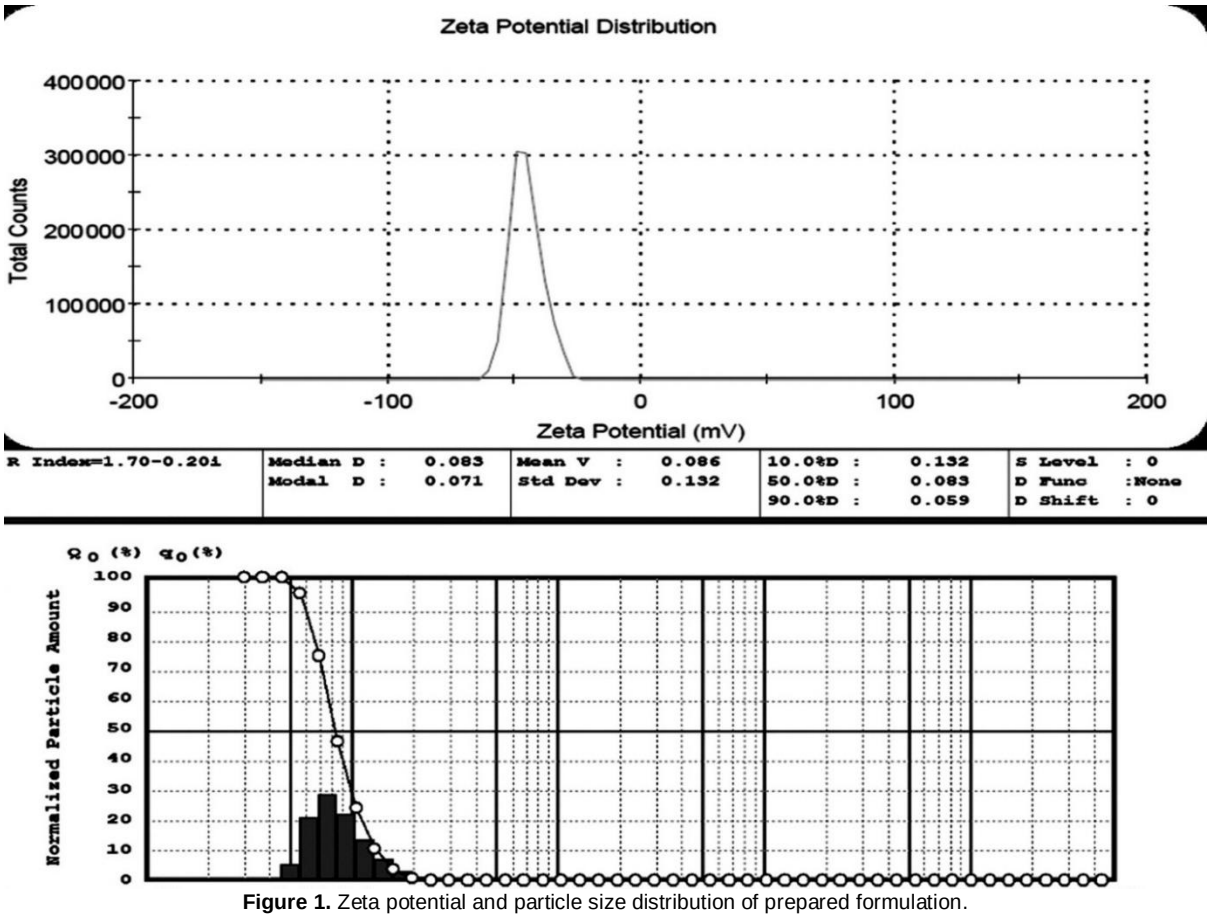

Figure 1. Zeta potential and particle size distribution of prepared formulation.

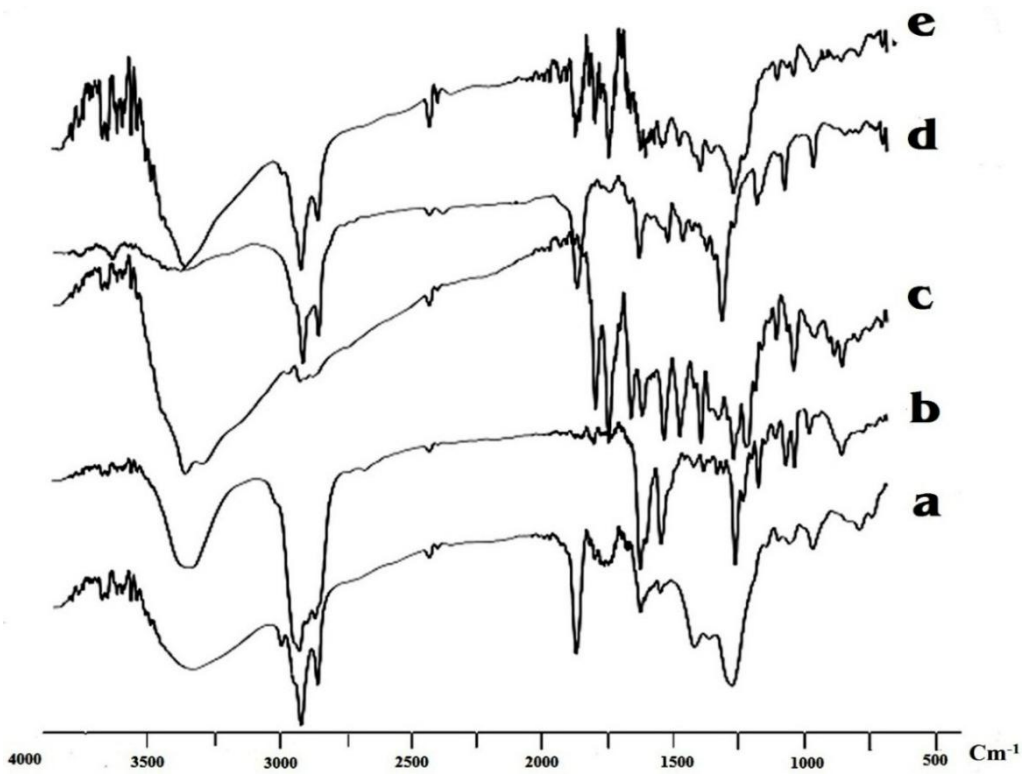

Figure 2. FTIR spectrum of pure cholesterol (a), phosphatidylcholine (b) Rutin (c), physical mixture (d) and Rutin-loaded nanophytosome (e).

Rutin showed the characteristic peaks in 3285.79, 3400, 3035.80, 1750, 1629.76 and 1602.09 cm^{-1}. Phosphatidylcholine showed a broad peak at 3437.05, sharp peaks at 2917.93 and 2849.91, as well as several sharp peaks below than 1800 cm^{-1}. FT-IR spectra of Rutin–loaded nanophytosomes exhibited disappearance of phenolic-OH of Rutin peaks which indicates to the interaction of PC and Rutin through these phenolic-OH groups in the process of nanophytosome preparation. This interaction interprets the observed high loading value of Rutin. On the other hand, the physical mixture of PC and Rutin showed all peaks of PC and all of the sharp peaks of Rutin at about the same positions as those pure substances.

Differential Scanning Calorimetry

Differential scanning calorimetry was carried out to ensure formation of complex between Rutin and phospholipid. DSC thermograms of the pure Rutin, cholesterol, PC, physical mixture of them and Rutin-loaded nanophytosomes are displayed in Figure 3. DSC thermogram of PC and cholesterol showed endothermic peaks at 205 °C and 152.45 °C, respectively. They appear due to phase transition from gel to liquid crystalline state. The non-polar hydrocarbon tail of phospholipids may be melted during this phase transition, resulting a sharp peak. This melting might have occurred in two phases which subsequently gave another peak (250 ºC) which was relatively less sharp. DSC thermogram of the physical mixture showed the corresponding peak of cholesterol at about 249.47 ºC. However, the endothermic peaks of PC and Rutin were eliminated. Mixing Rutin with PC resulted in changes in crystalline state of both compounds. The endothermic peak of Rutin was observed at 205.65 ºC corresponding to its melting point. On the other hand, in the Rutin–PC complex, the major endothermic peak was observed at 170.7 °C, which was different from the peaks of the individual components of the complex. Thermogram of Rutin-loaded nanophytosomes interestingly showed disappearance of the endothermic melting peak of Rutin, indicating that Rutin was completely embedded inside the matrix of nanophytosomes which had different thermal properties as compared to the physical mixture. Rutin and phosphatidylcholine interact by Hydrogen bonding between-OH group of Rutin and polar part of phosphatidylcholine.

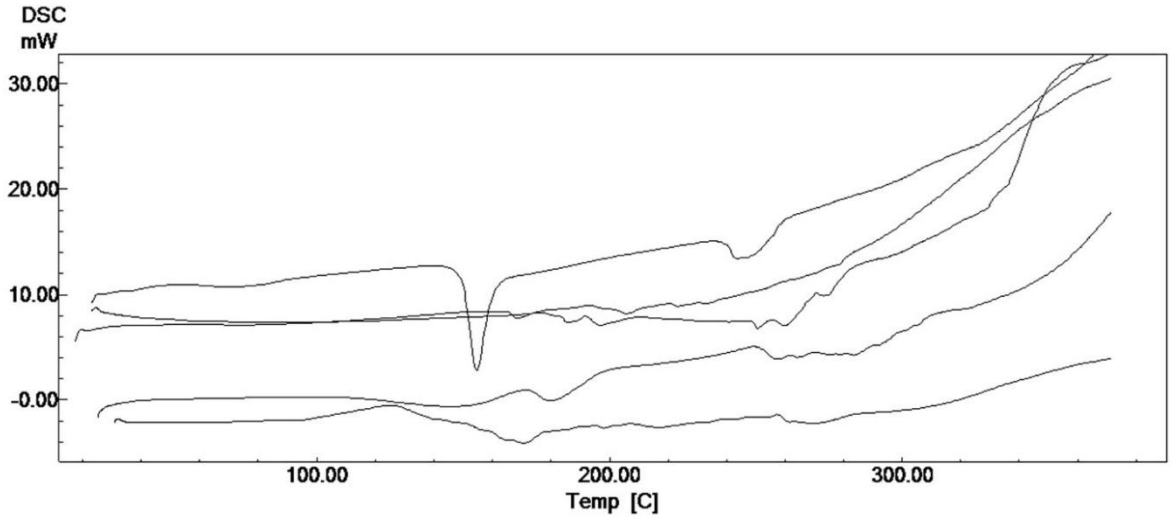

Figure 3. DCS thermograms of pure cholesterol (a), Rutin (b), phosphatidylcholine (c), physical mixture (d) and Rutin-loaded nanophytosome (e).

Conclusion

Several benefits have been corresponded to Rutin. Therefore, the fortification of food products with Rutin would be an interesting topic in industrial food sciences. However, the lipophilic nature of Rutin restricts its application in beverages. Our findings in the preparation of nanotrsuctures loaded with high amounts of Rutin (the ratio of 1 to 2 of Rutin to PC) paves the way for growing the knowledge for fortification of food beverages with lipophilic herbal supplements. The Rutin-loaded nanophytosomes were evaluated for its physicochemical properties and the obtained results demonstrated low particle size as well as high encapsulation efficiency and stability. FTIR and DSC results confirmed the formation of Rutin-Phospholipid complex in the nanophytosomes interpreting high loading of Rutin into phytosomes.

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Conflict of Interest

The authors report no conflicts of interest.

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