Formulation, Characterization and Antioxidant Myricetin Nanophytosome for Topical Delivery

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A B S T R A C T

Background: Phytosomes are recently introduced drug delivery system and novel botanical formulation to produce lipophilic molecular complex to improve absorption and bioavailability of phytoconstituent. Myricetin is a well-known flavonoid with different biological effects and contributed in food preserving by free radical scavenging activity. However, bioavailability of myricetin is an important limiting factor for its antioxidant activities.

Purpose: To overcome this limitation, in the present study we aimed to produce myricetin-loaded nanophytosomes to improve its physicochemical stability and bioavailability.

Methods: myricetin-loaded nano phytosome was prepared by using phosphatidylcholine (PC) and cholesterol (CH). Myricetin nanophytosomes system was characterized by particle size analyzer, particle size distribution (PDI), encapsulation efficiency percent and potential antioxidant activity.

Results: Results showed that formulation with the myricetin: PC: CH molar ratio of 1:2:0.8 had lower particle size (291.11 nm) and higher encapsulation efficiency percent (93%). Morphology analysis showed that myricetin nanophytosome spherical shape. The potential antioxidant data showed that incorporation myricetin in the phospholipid myricetin remained unchanged even after encapsulation of myricetin in binarynanophytosome formulation.

Conclusion: Nanophytosomal formulation of myricetin showed promising potential in fortification of nutraceutical with water insoluble antioxidants.

Keywords: Nanophytosome, Formulation, Myricetin, Antioxidant, Topical Delivery.

INTRODUCTION

Encapsulation techniques are most widely used in the development and production of improved delivery systems1. One of the phospholipid-based drug delivery systems that has the potential to be developed is the nanophytosome2. Nanophytosome is an advanced delivery system and a novel botanical formulation to produce lipophilic molecular complex which improves absorption and bioavailability of phytoconstituent specially polyphenolics3. 4 The phytosome consists of two terms: phyto and some. Phyto indicates that the bioactive portion of the complex originates from plants and some implies that the final structure of complex have similarity to the cells5,6. Phytosome complexes are compatible with lipophilic mediums. They have chemical structures similar to cell membranes and are considered as phyto-lipid delivery system7,8. Some studies have reported that phospholipids exhibit a marked affinity for some classes of flavonoids. It has been developed by complexation with polar botanical derivatives such as catechin, quercetin, escin and glycyrrhetinic acid. Phytosomes are complexes between a pure phospholipid and pure active principles from the chemical perspective9,10. The soothing activity of silymarin has shown to be increased by six fold in silymarin phytosomes compared to
free active principles, which is proposed to be due to higher affinity of complexes for skin phospholipids. The green tea (polyphenol), grape seed, silybum marianum, hawthorn extracts and olive polyphenols were successfully commercialized as phytosomes for antioxidant, free radical scavenger, uv protectant actions. The phytosome structures have general similarity to liposomes when exposed to water. Mixing active ingredients with PC in specific ratios is the major step in the production of phytosome and liposomes.

Although several herbal extracts and phyto-constituents have high in vitro bio-activity, they reveal less in vivo activities due to their poor lipophilic nature or large molecular structures or both, causing lower absorption and bioavailability. Most of the biologically active constituents of plants, including many of flavonoids, are polar compounds or have affinity to the aqueous phase. Their poor lipid solubility restricts their aptitude to pass across lipid rich biological membranes.

Myricetin is a group of flavonoid compounds with chemical name (MYR, 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromene, Fig 1). It has activities, as antioxidant, anti-inflammatory, antiallergic, antiaging activity and anticancer, antiviral, antiatheroscerotic and antidiabetic activity. Myricetin is considered a nutritional supplement that increases energy level and vitality of the body. The major drawbacks of many of this natural compounds is the low water solubility and bioavailability, it has strongly limiting their development as active pharmaceutical ingredients.

Several methods have been carried out to improve myricetin solubility and bioavailability for the oral and dermal delivery system, include nanosuspension, cocrystal, solid dispersion, liposomal formulation dan self nanoemulsion.

Previously, myricetin nanophytosome was successfully developed and inhibited GBM cell growth through the SIRT3/p53-mediated PI3K/Akt-ERK and mitochondrial pathways using a combination poloxamer 188 and PEG2000-DPSE as aqueous phase, cholesterol and glyceryl behenate as oil phase.

The aim of the present study was to prepare a physically stable phytosomal formulation of Myricetin with higher encapsulation efficiency and potential antioxidant activity using photon correlation spectroscopy (PCS) in term of particle size distribution, polydispersity index, encapsulation efficiency and physical stability to improve myricetin solubility and bioavailability for the oral and dermal delivery system, include nanosuspension, cocrystal, solid dispersion, liposomal formulation dan self nanoemulsion.

**METHODS**

**Preparation of Nanophytosome Myricetin**

Phytosomes were prepared by using thin layer hydration method with different molar ratio of Myricetin, PC and cholesterol. Myricetin and PC was dissolved in methanol, while cholesterol was dissolved in dichloromethane. The mixture was taken in a round bottom flask and evaporated in a rotary evaporator (Heidolph, Germany) at 45 °C until evaporation of all solvents and producing thin dry film in the round bottom flask. The vacuum drying evaporate the organic solvents completely. Moreover, the prepared lipid thin layer had been exposed to nitrogen gas flow and kept an overnight in the room temperature before hydration to ensure the complete removal of the organic solvents. The film was hydrated with distilled water in a rotary at 45 °C. Three methods were used to decrease phytosomes size, including bath sonication (Model 8852, cole- Palmer Instrument, Chicago, IL) at 45 °C, homogenization (Heidolph, Germany) with 20,000 rpm and probe sonication method (Sonix, Vibracell).

**Particle size analyses**

The mean particle size of distribution, polydispersity index and zeta potential were determined by a Zetasizer Nano ZS (Malvern Instrument UK). The samples were diluted using purified water until the appropriate scattering intensity and placed in an electrophoretic cell.
Table: 1 Composition of MYR Nanophytosome

| Formulation | MYR | PC | CH |
|-------------|-----|----|----|
| F1          | 1   | 1  | 0.1|
| F2          | 1   | 1  | 0.2|
| F3          | 1   | 1  | 0.4|
| F4          | 1   | 1  | 0.8|
| F5          | 1   | 1  | 1  |

Determining Encapsulation Efficiency

The encapsulation efficiency of MYR was determined by calculating the amount of entrapped MYR in the phytosomes. To determine the encapsulation efficiency of MYR in phytosome, an appropriate amount of dispersion was transferred in Millipore Amicon® Ultrafiltration tube (Ultracel, cut off 30 kDa). The dispersion was centrifuged (Sigma3k-30, Germany) for 5 min at 5000 rpm. After centrifugation the supernatant was collected and amount of free MYR was determined spectrophotometrically (λmax=210 nm). The encapsulation efficiency has been determined according to the following equation.

$$EE\,\% = \frac{W(\text{added drug}) - W(\text{free drug})}{W(\text{added drug})} \times 100\%$$

Where, W (added drug) is the amount of drug added during the preparation of phytosomes, W (free drug) is the amount of free drug measured in the lower chamber of the Millipore Amicon® after centrifugation.

Morphology Particle

The morphology particle was analyzed by transmission electron microscopy at an accelerating voltage of 200 kV, equipped an digital camera (JEOL 2100, Tokyo, Japan). A drop of nanophytosome solution was deposited on a TEM copper grid coated with a carbon film.

Physical stability studies

Phytosomal MYR dispersions were stored at 4 °C and samples were regularly withdrawn and physical stability of prepared phytosomes was evaluated at 7, 14, 21 and 28 days.

Determination of Antioxidant Activity using DPPH(2, 2-diphenyl-1-picryl-hydrazyl) assay

The sample (0.5 ml) was at first dissolved in 3 ml methanol and treated with 0.3 ml of DPPH methanolic solution (0.5 mM). The change in colour of reaction mixture was taken as a measure to assess antioxidant potential of samples due to its hydrogen donating capability and the change in colour was assessed after 100 minutes of reaction at dark room by estimating the absorbance of reaction mixture at 517 nm. The blank solution was consisted of 3.3 ml methanol and 0.3 ml of sample while control solution was composed of methanol and DPPH solution taken at 3.5 ml and 0.3 ml respectively (Garcia et al., 2012). The free radical scavenging potential of samples was expressed as antioxidant activity (%) as per Mensor et al (Mensor et al., 2001).

RESULT AND DISCUSSION

Particle Size and Encapsulation Efficiency

The particle size of nanophytosomes is extremely important as it can affect the stability and bioavailability of phytosome encapsulated systems. Smaller particles possess a large surface area and have faster release as well as higher stability. Table 2, shows the effects of changing molar ratio of phosphatidylcholine to cholesterol on the characteristics of the phytosome, including particle sizes, particle size distribution index (PDI) and encapsulation efficiency of myricetin.

Average particle size of myricetin phytosomes when prepared by molar ratios of 1: 1: 0.1; 1: 1: 0.2 and 1:1:0.4 of MYR: PC: CH, were ± 300 nm and ratio of 1:1: 0.8 has the smallest particle size 291.1 nm. Considering the mean particle size and size distribution, the optimum ratio of PC to CH was found 1: 0.8. It can be seen from the data in Table.1, the phytosomes size was enhanced with increasing cholesterol content. This might be due to the interaction between cholesterol and phosphatidylcholine which induces a tighter packing of PC in membrane resulting increase in the mechanical stiffness of the membranes. Cholesterol also increases the thickness of phospholipid bilayer. Myricetin exhibits a high affinity for phytosomes which is due to the its planar configuration, that can easily located into the organized structure of the phospholipids within the phytosomes membranes. As it was shown in Table 1, encapsulation efficiency of myricetin in the phytosomes was in the range of 92-95% which did not change by different molar ratios of MYR : PC: CH. Myricetin phytosomes with the molar ratio of 1:1:0.8 (F1), which has the smallest particle size then used for physical stability testing.
Table 2 Mean particle size, PDI value and encapsulation efficiency of prepared myricetin nanophytosomes with different molar ratio of MYR, phosphatidyl choline (PC) and cholesterol (CH). Data were expressed as mean ± SD of three experiments

| Formulation | MYR : PC : CH | Particle size (nm) | PDI    | Encapsulation Efficiency (%) |
|-------------|---------------|--------------------|--------|-------------------------------|
| F1          | 1 : 1 : 0.1   | 334.2              | 0.353  | 95.65                         |
| F2          | 1 : 1 : 0.2   | 393.5              | 0.439  | 94.46                         |
| F3          | 1 : 1 : 0.4   | 323.9              | 0.495  | 93.54                         |
| F4          | 1 : 1 : 0.8   | 291.1              | 0.470  | 93.58                         |
| F5          | 1 : 1 : 1     | 1527.5             | 0.416  | 92.25                         |

**Physical Stability Study**

Phytosomes must be stable during the storage period and remain at the appropriate size range before reaching their targeted tissues when used as a drug delivery system. Physical stability of formulation 1 has been studied for seven days and results indicated to instability due to the size increasing. Results showed that, particle size of nano phytosomes was increased up to 6 folds after 7 days. However, addition of cholesterol into the nanophytosomes formulation resulted in physical stability of particle size over a 21 days period.

Previous studies also showed that the physical stability of liposome can be enhanced by cholesterol addition. The formation of the lipid bilayer and its fluidity is influenced by the amount of cholesterol introduced between the phosphatidylcholine molecules. Presence of cholesterol is advantageous as it makes the bilayer sufficiently flexible. The molecular structure of cholesterol includes a tetracyclic hydrocarbons rings, a single hydroxyl group at carbon 3 and an isooctyl hydrocarbon side chain at carbon 17. Where cholesterol is incorporated into phospholipid bilayers, hydroxyl polar group of cholesterol is placed next to the phosphatidylcholine carbonyl groups by formation hydrogen bond. Therefore, this bonding between cholesterol and phosphatidylcholine can enhance electrostatic repulsion between phospholipids bilayer and finally increase its stability by limiting the movement of acyl chains of phosphatidylcholine.

**Morphology Particle**

The morphology analysis showed that myricetin nanophytosome spherical shape with size ranging mostly from 50-300 nm.

**Determination of Antioxidant Activity using DPPH(2, 2-diphenyl-1-picryl-hydrayl) assay**

Antioxidant potential of MYR Nanophytosome in each formula has an IC<sub>50</sub> value that ranges between 20 – 30 µg/ml (Table 3). Myricetin nanophytosomes when prepared by molar ratios of 1: 1: 0.8(F4) and 1: 1: 1 (F5) of MYR: PC: CH, have value IC<sub>50</sub>21.73 µm/ml and 20.56 µg/ml, respectively. They have a smaller IC<sub>50</sub> value when compared with pure myricetin powder (24.28 µg/ml). This observation confirms the antioxidant potential of myricetin nanophytosome showed more scavenging of free radicals than myricetin coarse powder. When particle size is reduced up to nano range, not only surface area but concentration gradient is also increased which results in dramatic increase of the scavenging of free radicals as compared to a micronised product and antioxidant activity of myricetin was improved by formulating their nanophytosome. It was concluded that the antioxidant potential of myricetin remained unchanged even after encapsulation of myricetin in binary nanophytosome formulation.

**CONCLUSION**

Myricetin exhibited a high affinity for the phytosomes that resulted from its planar configuration, which can easily introduce into the structured phosphatidylcholine within the phytosome membranes. The decrease in the melting point of cholesterol in nano phytosome compared to the pure cholesterol can be attributed to its incorporation into the bilayer of the phytosome leading to the formation of less ordered structure.
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