Available online on 15.06.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access

Research Article

Formulation, Characterization and Antioxidant Myricetin Nanophytosome for Topical Delivery

Purnamasari Nur Aini*[1], Dzakwan Muhammad[1], Pramukantoro Ganet Eko[2], Mauludin Rachmat[2], El Fahmi[2]

[1]Faculty of Pharmacy, University Setia Budi, Surakarta, Central Java, Indonesia

[2]School of Pharmacy, Institute Technology Bandung Labtek VII Ganesha 10 Bandung, Indonesia

ABSTRACT

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 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.

ARTICLE INFO: Received 07 March 2020; Review Completed 03 May 2020; Accepted 08 May 2020; Available online 15 June. 2020

Cite this article as:

Purnamasari N A D, Dzakwan M, Pramukantoro G E, Mauludin R, El Fahmi, Formulation, Characterization and Antioxidant Myricetin Nanophytosome for Topical Delivery , Asian Journal of Pharmaceutical Research and Development. 2020; 8(3):09-13.DOI: http://dx.doi.org/10.22270/ajprd.v8i3.718

***Address for Correspondence:**

Nur Aini Dewi Purnamasari,Faculty of Pharmacy, University Setia Budi, Surakarta, Central Java, Indonesia

INTRODUCTION

Encapsulation techniques are most widely used in the development and production of improved delivery systems[1]. One of the phospholipid-based drug delivery systems that has the potential to be developed is the nanophytosome[2]. Nanophytosome is an advanced delivery system and a novel botanical formulation to produce lipophilic molecular complex which improves absorption and bioavailability of phytoconstituent specially polyphenolics[3, 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 cells[5,6]. Phytosome complexes are compatible with lipophilic mediums. They have chemical structures similar to cell membranes and are considered as phyto-lipid delivery system[7,8].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 perspective[9,10].

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[11,12,13].

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[14].

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[15,16.17]. Most of the biologically active constituents of plants, including most 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[18,19].

Myricetin is a group of flavonoid compounds with chemical name (MYR, 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenil)-4-chromenone, Fig 1). It has activities, as antioxidant, anti-inflammatory, antiallergic, antiageing activity and anticancer[20], antimutagenic, antiviral, antiartheroscerotic and antidiabetic activity[21]. Myricetin is considered a nutritional supplement that increases energy level and vitality of the body[18]. 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 [22,23].

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[24,25,26,27,28].

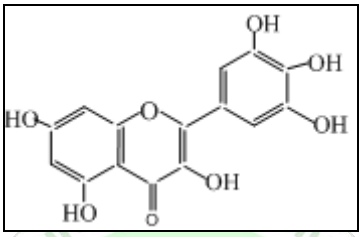

Figure: 1 –Chemical structure of myricetin

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[29,30].

The aim of the present study was to prepare a physically stable phytosomal formulation of Myricetin with higher encapsulation efficiency and physical stability to improve its efficacy and its preservation from oxidation in food stuffs. Nanophytosome produced was then characterized using photon correlation spectroscopy (PCS) in term of particle size distribution, polydispersity index, encapsulation efficiency and potential antioxidant activity

MATERIALS AND METHODS

MATERIALS

Myricetin (MYR) was obtained from Shaanxi Dideu Medichem Co. Ltd (Xi'an, China). Soybean phosphatidylcholine (Lipoid) was obtained from Evonik (German) as a gift sample. Cholesterol, methanol and dichloromethane were purchased from Merck Company (Darmstadt, Germany. Distilled water was obtained from a Milli-Q Plus purification system and all other used chemicals and solvents were of analytical grade.

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 scaterring intensity and placed in an electrophoretic cell.

Table: 1 Composition of MYR Nanophytosome

Formulation	Composition		
	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

Determination of 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® Ultra filtration 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 (ʎmax= 210 nm). The encapsulation efficiency has been determined according to the following equation.

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

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 be affect the stability and bioavailability of phytoconstituent 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 particles 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 1:1:08 (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[36].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[37]. 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[38].

Morphology Particle

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

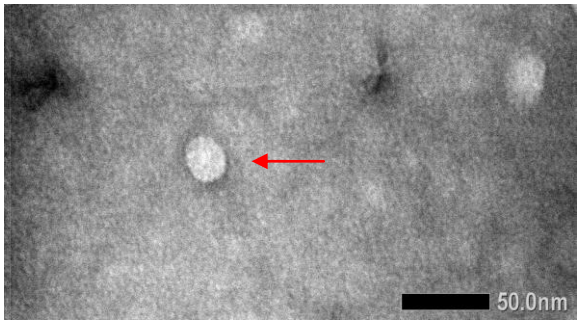

Figure: 1. TEM analysis myricetin nanophytosome, magnification 500 x

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

Antioxidant potential of MYR Nanophytosome in each formula has an IC_{50} 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_{50}21.73 µm/ml and20.56 µg/ml, respectively. They have a smaller IC_{50} 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.

Table: 3. Comparation antioxidant activity myricetin nanophytosome

Formulation	IC_{50}
F1	27.36
F2	30.49
F3	31.18
F4	21.73
F5	20.56
MYR powder	24.28

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.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of PKAPT RESEARCH from DRPM, Ministry of Research, Technology and Higher Education Republic of Indonesia.

REFERENCES

1. Kohlmeier M. Free radicals and antioxidants. In: Kohlmeier M, editor. Nutrient Metabolism. Lond: Academic Press; 2003. p. 457-464.
2. Gacche RN. Shegokar. HD. Gond DS. Yang Z. Jadhav AD. Evaluation of selected flavonoids as antiangiogenic, anticancer, and radical scavenging agents: an experimental and in silico analysis. Cell Biochem Biophys.2011; 61:651–663.
3. Sindhi V. Gupta V. Sharma K. Bhatnagar S. Kumari R. Dhaka N. Potential applications of antioxidants- a review. Journal of Pharmacy Research.2013; 7:828-835.
4. Leonarduzzi G. Testa G. Sottero B. Gamba P and Poli G. Design and development of nano vehicle-based delivery systems for preventive or therapeutic supplementation with flavonoids. Current Medicinal Chemistry. 2010; 17:74-95.
5. OdehF.Al-JaberH and KhaterD.Application of nanotechnology in drug delivery. New York: InTech; 2014:344-368.
6. ProcházkováD.BoušováI.WilhelmováN. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011; 82:513–523
7. Hu-JunX.Wang-ShuM.Fu-RongL.Jie-HuiX. Lei. Qun-FangL.Wen-JunF.Radical scavenging activity of fisetin, Acta Phys. -Chim. Sin.2013; 29(7):1421-1432.
8. Hong C. Dang Y. Lin G. YaoY. LiG. Ji G. Shen H. XieY. Effects of stabilizingagents on the development of myricetinnanosuspension and its characterization: an in vitro and in vivo evaluation, International Journal of Pharmaceutics.2014; 477:251–260.
9. Guzzo MR.Semi M.Donate PM. Nikolaou S.Antonio Eduardo H. Machado AEH.Okano LTA. Study of the complexation of fisetin with cyclodextrins. Journal PhysChem.2006;110 (36):10545-1055.
10. Sowa M. Slepokura K. Matczak -Jon E. Improving solubility of fisetin by cocrystallization. CrysEngCom. 2014; 16:10592-10601.
11. Seguin J. Brulle L. Boyer R. Lu YM. Ramos Romano M. Touil YS. Liposomal encapsulation of the natural flavonoid fisetin improves bioavailability and antitumor efficacy. International journal of pharmaceutics. 2013; 444(1-2):146-54.
12. Wang L. Zhang DZ. Wang YX. Bioflavonoid fisetin loaded alpha-tocopherol-poly(lactic acid)-based polymeric micelles for enhanced anticancer efficacy in breast cancers. Pharmaceutical Research. 2017; 34(2):453-61.
13. Bothiraja C. Yojana BD, Pawar AP. Shaikh KS and Thorat UH. Fisetin-loadednanocochleates: formulation and
14. Characterization, *in vitro* anticancer testing, bioavailability and biodistribution study. Expert Opin. Drug Deliv. 2014; 11(1):17-29.
15. Al-Kassas R. Bansal M. Shaw J.Nanosizing techniques for improving bioavailability of drugs. Journal Control Release. 2017; 260:202 – 2012.
16. Junghanns AH and Müller RH. Nanocrystal technology, drug delivery and clinical applications. International Journal of Nanomedicines. 2008; 3(3):295-309.
17. KeckCM. and MullerRH. Drug nanocrystal of poorly soluble drugs produced by high-pressure homogenization. Eur J Pharm Biopharm. 2008; 62(1)3-16.
18. Sinha B. Muller RH. Moschwitzer JP. Bottom-up approaches for preparing drug nanocrystals: formulations and factors affecting particle size. Int J Pharm. 2013;453(1):126-141.
19. Junyaprasert VB. and MorakulB. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian Journal of Pharmaceutical Science. 2015; 10:10-23.
20. De Waard H. Frijlink HW. Hinrichs WLJ. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharmaceutical Research. 2011; 28(5):1220-1223.
21. Möschwitzer JP. Drug nanocrystals in the commercial pharmaceutical development process. International Journal of Pharmaceutics. 2013; 453(1):142-156.
22. Rautenbach F, Venter I. Hydrophilic and lipophilic antioxidant capacity of commonly consumed South African fruits, vegetables, grains, legumes, fats/oils and beverages. J Food Compost Anal 2010; 23:753761.
23. Santos NW, Santos GTD, Silva-Kazama DC, Grande PA, Pintro PM, De Marchi FE, et al. Production, composition and antioxidants in milk of dairy cows fed diets containing soybean oil and grape residue silage. Livest Sci 2014;159:37-45
24. Contini C, Álvarez R, O'sullivan M, Dowling DP, Gargan SÓ, Monahan FJ. Effect of an active packaging with citrus extract on lipid oxidation and sensory quality of cooked turkey meat. Meat Sci 2014; 96:1171-1176.
25. Kerry JP. 20 - Application of smart packaging systems for conventionally packaged muscle-based food products. In: Kerry JP, editor. Advances in Meat, Poultry and Seafood Packaging: Woodhead Publ 2012. p.522-564.
26. Mahajan T, M C. A novel approach towards phytosomal flavonoids. Pharma Sci 2012; 4:20792121.
27. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 1983; 32:1141-1148.
28. Hollman PCH, Trijp JMP, Buysman M, Gaag M, Mengelers M, Vries J, et al. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett 1997; 418:152-156.
29. Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Antiinflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Il Farmaco 2001; 56:683-687.
30. Wang L, Wang B, Li H, Lu H, Qiu F, Xiong L, et al. Quercetin, a flavonoid with anti-inflammatory activity, suppresses the development of abdominal aortic aneurysms in mice. Eur J Pharmacol 2012; 690:133-141.
31. Bose S, Michniak-Kohn B. Preparation and characterization of lipid based nanosystems for topical delivery of quercetin. Eur J Pharm Sci 2013; 48:442-452
32. Li H, Zhao X, Ma Y, Zhai G, Li L, Lou H. Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. J Controlled Release 2009; 133:238-244.
33. Liu D, Hu H, Lin Z, Chen D, Zhu Y, Hou S, et al. Quercetin deformable liposome: Preparation and efficacy against ultraviolet B induced skin damages in vitro and in vivo. J Photochem Photobiol B: Biology 2013; 127:8-17
34. Xie Y, Luo H, Duan J, Hong C, Ma P, Li G, et al. Phytic acid enhances the oral absorption of isorhamnetin, quercetin, and kaempferol in total flavones of Hippophae rhamnoides L. Fitoterapia 2014; 93:216-225.
35. Yang B, Chen F, Hua Y, Huang S-S, Lin S, Wen L, et al. Prooxidant activities of quercetin, p-courmaric acid and their derivatives analysed by quantitative structure–activity relationship. Food Chem 2012; 131:508-512.
36. Pandey R, Ahmad Z, Sharma S, Khuller GK. Nanoencapsulation of azole antifungals: Potential applications to improve oral drug delivery. Int J Pharm 2005; 301:268-276.
37. Dian L, Yang Z, Li F, Wang Z, Pan X, Peng X, et al. Cubic phase nanoparticles for sustained release of ibuprofen: formulation, characterization, and enhanced bioavailability study. Int J Nanomed 2013;8:845-854
38. Guo L, Hamilton R, Goerke J, Weinstein J. Interaction of unilamellar liposomes with serum lipoproteins and apolipoproteins. J Lipid Res 1980; 20:993-1003.
39. Vandijk C, Driessen A, Recourt K. The uncoupling efficiency and affinity of flavonoids for vesicles. Biochem Pharmacol 2000; 60:1593-1600.