ATLANTIS PRESS

Advances in Health Sciences Research, volume 26

2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019)

Evaluation Nano-Phytosome of Myricetin with Thin Layer Film Hydration-Sonication Method

Yuli Edy Saputra[1], Muhammad Dzakwan[2], Nur Aini Dewi[3]
Fakultas Farmasi, Universitas Setia Budi, Surakarta
E-mail: yulled089@gmail.com
Corresponding author: aini_farmasi2008@yahoo.com

Abstract - Nano-phytosome is a nano technology that's use to improve the bioavailability of active compound in plants by binding with phospholipids which have similar characteristic with cell membranes. In this study, Myricetin is the main ingredient that we used in nano-phytosome formulation. Myrisetin is a natural flavonoid compound with antioxidant activitiy and has low bioavailability and permeability values. The purpose of this study is to knowing characterization nano-phytosomes of myricetin.

Nano-phytosomes is made using thin-sonication hydration method with a variation ratio of myrisetin: phosphatidylcholine: cholesterol 1: 1: 0.4 (F1), 1: 2: 0.4 (F2) and 1: 3: 0.4 (F3). Characterization of nano-phytosome includes particle size, polydispers index, stability, adsorption efficiency and antioxidant activity.

The results showed that particle size in F1, F2 and F3 were 233.6 nm, 250.0 nm, and 242.7 nm with polydispersion indexes were 0.260, 0.260 and 0.447, respectively. Potential zeta values (F1) -21.7 mV, (F2) -15.7 mV and (F3) -11.3 mV. Entrapment efficiency (F1) 91.94%, (F2) 91.39% and (F3) 91.39%. The antioxidant activity of the formulas have values (F1) 41,13 ppm; (F2) 25.46 ppm; and (F3) 20.64 ppm. Based on the evaluation results, that the more the concentration of phosphatidylcholine added, the more particle size, polydispersion index, adsorption efficiency and antioxidant activity were increases.

Keywords: nano-phytosome, myricetin, thin layer film hydration

I. INTRODUCTION

Flavonoid is a common group of polyphenol and have many benefits such as antioxidants, antimicrobials, anticancer and anti-inflammatory effects [1]. Mirycetin is a natural polyphenol flavonoid that is widely distributed in fruits, vegetables and herbs that are being studied use for treatment such as antioxidants. Mirycetin like other flavonoids has limitations in bioavailability and absorption [2-4]. Low absorption due to its solubility in lipids is so bad and it need to make a new formulations for mirysetin.

Drug delivery system of the latest generation has the advantage to improve the nature of penetration of the skin. Recent research in nanotechnology have enabled the manufacture of nano-sized particles used for various biomedical applications [5,6].

One of the developments in the Drug Delivery System in transdermal delivery is the vesicular system, it is known as phytosomes. Phytosomes are a combination of phospholipids, such as phosphatidylcholine in nonpolar solvents such as acetone. In case, phytosomes are made up of complex micellar structures of nature – phospholipids [7].

The composition of phytosomes is safe and its components are accepted use for pharmaceutical, phytosome can increasing the absorption and bioavailability of water-soluble natural ingredients. This delivers a better therapeutic effect [8].

Nano-phytosomes are made by mixing phytoconstituents with phosphatidylcholine at certain molar ratios (1: 1 to 1: 3), it will produce a complex with stronger bonds because phytoconstituent molecule will be bound by phosphatidylcholine. The methods used in making nano-fitosomes include solvent evaporation, reflux, salting out, and lyophylization methods [9]

The purpose of this study was to create a Drug Delivery System nanoparticles with a vesicular system of phytosomes from mirysetin powder using three phosphatidylcholine comparisons.

II. MATERIAL AND METHOD

UV-Vis Spectrophotometer (Genesys 10s, Thermo scientific), rotary evaporator (Heidolph), probe sonicator (QSonica, Newtown, USA), particle size analyzer (Malvern Panalytical, USA), magnetic stirer (Thermo Scientific Scientific) , China), centrifuges (SPLC Series, Gemmy 8 Hole, Taiwan), analytical scales (Ohaus), glassware (Pyrex, Japan) and non-glass found in the laboratory.

The sample were myricetin (Tocris, China), Phospholipon 90 G (Lipoid, Germany), cholesterol pa (Sigma, USA), acetone pa (Merck), ethanol pa (Merck), dichloromethan pa (Merck), aqua pro injection (PT. Ikapharmindo Putramas).

A. Nano-phytosome Formulation

Nano-phytosomes are formulated by making three different variations using the sonication thin layer film hydration method. The nano-phytosome formula can be seen in table.

TABLE 1. NANO-PHYTOSOME FORMULA OF MYRICETIN

Materials	Myricetin : Phosphatidylcholine: Cholesterol		
	F1 (1:1:0.4)	F2 (1:2:0.4)	F3 (1:3:0.4)
Myricetin (mg)	10	10	10
Phosphatidylcholine (mg)	24	48	71
Cholesterol (mg)	2	2	2
Acetone (ml)	20	20	20
Dichloromethane (ml)	5	5	5
Aqua pro Injection (ml)	25	25	25

*In molar comparison

Nano-phytosome is made by dissolving myricetin and phosphatidylcholine in acetone p.a, 10 ml each ingredient, and cholesterol is dissolved in dichloromethane p.a. Phytoactive and phospholipid solutions were mixed using a magnetic stirrer at 35° C with rotation of 2000 rpm in 10 minutes, the nano-phytosome complex was made a thin layer film on a rotary evaporator at 55° C and speed 50 rpm until the solvents evaporated. The thin layer film formed on the walls of the round flask then hydrated with aqua pro injection characterized by the formation of colloidal dispersions. Colloidal dispersions formed were sonicated using probe sonication for 10 minutes with an amplitude of 60%.

B. Characterization of nano-phytosome

1. Determination of Zeta Potential and Particle Size Distribution

To find out the size of nano-phytosome, particle size analysed and particle size distribution were carried out using a Particle Size Analyzer (PSA). To find out the zeta potential value were using the zeta potential analyzer.

2. Determination of Entrapment efficiency

Nano-phytosome myricetin was centrifuged at 3000 rpm at room temperature (27 ° C) for 50 minutes in order to separate the not absorbed active substance. The supernatant from centrifugation from formulas 1, 2 and 3 was taken 0.5 ml each, then diluted with aqua pro injection up to 10 ml, then the absorption was read three times using UV-Vis spectrophotometry at 369 nm wavelength. The entrapment efficiency (% EE) is calculated by the formula:

$$\%\ EE = \frac{TD-FD}{TD} \times 100\% \quad \text{..... (1)}$$

TD is the total number of myricetin contained in the formula and FD is the number of myricetin detected in the supernatant (not absorbed).

3. Nano-phytosome Stability

Nano-phytosome of myricetin was evaluated after storage at 3 weeks of storage at 27° C. During storage, we observed of separation phase, physical and chemical changes of the preparations were made.

4. Antioxidant Activity

0.1 mL of 0.4 M DPPH solution was mixed with 1.0 mL of each concentration series of the test solution. Then each mixture was vortexed for 30 seconds and left for OT (Operating Time (45 minutes)). Then measured the solution for its absorbance at a maximum wavelength (516 nm). Absorbance measurements were performed on pure myricetin and nano-phytosome of myricetin samples.

III. RESULT AND DISCUSSION

A. Particle Size Analysis

Particle size is the most important characteristic in a nanoparticle system because it determines the speed and ease of the drug to optimally absorbed. The phospholipids will influences the particle size and the stability of the nanoparticle dispersion. The results nano-phytosome of myricetin particle size analysis showed that formula 1 to 3 had fulfilled the nanoparticle size range of 10-1000 nm [10]. The results of the particle size analysis are shown in table 2.

TABLE 2. RESULTS OF MYRICETIN NANO-PHYTOSOME CHARACTERIZATION

Evaluation	Formula		
	1	2	3
Particle size (nm)	233,6 ±1,209	250,0 ±3,552	242,7 ±1,792
Polydispersity Index	0,260 ±0,001	0,260 ±0,008	0,447 ±0,006
Potential Zeta (mV)	-21,7 ±1,209	-15,7 ±0,750	-11,3 ±0,624

The results showed that formula 1 with a comparison of the concentration of myricetin: phosphatidylcholine: cholesterol (1: 1: 0.4) had the smallest average particle size that is 233.6 nm. The concentration of phosphatidylcholine is bigger than myricetin can bind myricetin perfectly because 1

phytoconstituent molecule will be bound by 1 phosphatidylcholine molecule so the bond is stronger and the particle size can increase. Adding cholesterol to the formula can increase the physical stability of nano-phytosome for more than 21 days.

B. Polydispersity Index (IP)

IP is a value that explain the extent of particle size distribution in a preparation. IP for monodispersion particles has a value of ≤0.5, while IP >0.5 represents a nanoparticle system with a very wide particle size distribution (polydispersion). The best polydispersity index value is <0.5 because the smaller the IP value, the better the stability of nano-phytosome. The polydipersity index results are shown in table 2.

The smallest polydispersity index value is shown in formulas 1 and 2 that is 0.260. The smaller polydispersity index value said to say the preparation has a homogeneous distribution of particles with other particles, this shows that the myricetin nano-phytosome is homogeneous and has a monodispersion particle system.

C. Potential Zeta

Zeta potential is a measure of the magnitude of the electrostatic charge of particles in dispersion. Zeta potential is measured to determine colloidal stability. The colloidal solution system is stabilized by the electrostatic repulsion force, where the greater the repulsive force between particles will cause the particles to be difficult to close together to form aggregates. Zeta potential value ± 30 mV has good colloidal stability. The zeta potential results are shown in table 2.

The best measurement results of zeta on myricetin nano-phytosome in formula 1 of three replications has an average value of -21.7 mV, negative results indicate that the phosphatidylcholine used is negatively charged.

D. Nano-phytosome Stability

Nano-phytosome of myricetin is stored at 27°C for more than 3 weeks. The color nano-phytosome of myricetin from fisrt week until third week has the same color, it is yellowish. The smell that is owned is the typical odor of myricetin. The results nano-phytosome of myricetin stability are shown in table 3.

TABLE 3. STABILITY NANO-PHYTOSOMES OF MYRICETIN AT ROOM TEMPERATURE.

Formula	1st week	2nd week	3rd week
1	No Sediment	No Sediment	No Sediment
2	No Sediment	No Sediment	Sediment
3	No Sediment	Sediment	Sediment

There is sediment in 2 and 3 formula, the sediment is reversible because it can be dispersed quickly again after shaking. Formula 1 does not undergo precipitation and remains clear until 3rd weeks.

E. Entrapment Efficiency

The aims of entrapment efficiency to determine the amount of myricetin that absorbed in the nano-phytosome carrier system. Determination of levels of active substances that are not absorbed is calculated using the equation:

$$Y = 1{,}057x10^{-3} + 0{,}0603 \; . \; x \; ..... \quad (2)$$

The results of the adsorption efficiency are shown in table 4.

TABLE 4. RESULTS NANO-PHYTOSOM OF MYRICETIN SAMPLE ANALYSIS

Analysis Result	Formula		
	1	2	3
Entrapment Efficiency (%)	91,94 %,	91,39%	91,39%
Antioxidant activity (ppm)	41,13	25,46	20,64

The results of the adsorption efficiency in formula 1 were 91.94%, it means that myricetin was 91,94% absorbed in the phospholipid component, formula 2 and 3 were 91.39% myricetin was absorbed in the phospholipid component. From these results, each formula have a good entrapment efficiency range more than 80%. The difference in entrapment efficiency of each formula is due to differences in the amount of phospholipids used. The more phosphatidylcholine added into the formula, the efficiency of nano-phytosome absorption was decrease. The highest value of nano-phytosome of myricetin formula entrapment efficiency is F1 which has 24 mg of phosphatidylcholine has a particle size at 233.6 nm and can absorb myricetin quite large at 91.94%.

F. Antioxidant Activity test

The antioxidant activity test was carried out using the DPPH 1,1-diphenyl-2pikrilhidrazil method (α, α-diphenyl-β-picrilhidrazil). DPPH is a

free radical that is stable and does not form dimers due to the delocalisation of free electrons in all molecules. Using this method, Antioxidant activity testing can observed based on the loss of purple color due to the reduction of DPPH by active substances that contain antioxidant activity. The color intensity of the test solution was measured through UV-Vis spectrophotometry at a wavelength of 516 nm. The percent (%) yield of the inhibition is substituted in a linear equation. IC_{50} is defined as the amount of antioxidants needed to reduce the initial DPPH concentration by 50%. A substance has antioxidant properties if the IC_{50} value obtained ranges from 200-1000 µg / mL[11].

The test results showed a very strong antioxidant activity on myricetin because it has 22.69 ppm of IC_{50} value. IC_{50} value of myricetin F1 nano-phytosome sample is 41.13 ppm, F2 is 25.46 ppm, and F3 is 20.64 ppm. Antioxidant activity results are shown at table 3. The content in myricetin that provides the greatest antioxidant effect is flavonoids. Flavonoids act as antioxidants by donating hydrogen atoms or by their ability to chew metal, in the form of glucosides (containing glucose side chains) or in a free form called aglycones [12].

IV. CONCLUSION

Characterization and evaluation nano-phytosome of myricetin seen from the particle size of all formulas having a size between 10-1000 nm, the use of phosphatidylcholine at a concentration of 24 mg was able to produce the smallest particle size is 233.6 nm and the lowest polydipersity index is 0.260 with the highest entrapment efficiency is 91.94 %. Nano-phytosome of myricetin F2 and F4 are unstable for 3 weeks of storage. F1 has a zeta potential value -21.7 mV, so F1 with the ratio of myricetin: phosphstidylcholine : cholesterol (1 : 1 : 0.4) is the best formula in the production nano-phytosome of myricetin.

ACKNOWLEDGMENT

The researchers say thanks to the Ministry of for Research, Technology, and Higher Education of the Republic of Indonesia who had funded this research.

REFERENCES

1. Mahajan T, M C. A novel approach towards phytosomal flavonoids. Pharma Sci 2012;4:20792121.
2. 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.
3. Vijayalakshmi A, Ravichandiran V, Velraj M, Nirmala S, Jayakumari S. Screening of flavonoid "quercetin" from the rhizome of Smilax china Linn. for anti–psoriatic activity. Asian Pac J Trop Biomed 2012;2:269-275.
4. 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.
5. Papakostas D., Rancan F., Sterry W., Peytavi U.B., Vogt A. Review Article : Nanoparticles in Dermatology. Archives of Dermatological Research. Springer-Verlag. Berlin, Germany. Vol 303. 2011. pp 533– 550
6. Kumaresh S., Tejraj, M., Aminabhavi, Anandrao R., Kulkarni Walter E.R. Biodegradable polymeric nanoparticles as drug delivery devices. Review Journal of Controlled Release. 2001. 1 – 20.
7. Ramadon et al. Pemanfaatan Nanoteknologi dalam Sistem Penghantaran Obat Baru untuk Produk Bahan Alam. Jurnal Ilmu Kefarmasian Indonesia, 2016, vol 14 hlm. 118-127
8. Jain N., Gupta B.P., Thakur N., Jain R., Banweer J., Jain D., Jain S. Phytosome: A Novel Drug Delivery System for Herbal Medicine. International Journal of Pharmaceutical Sciences and Drug Research. 2010. 2:224- 228.
9. Freag MS, et al. Lyophilized Phytosomal Nanocarriers as Platforms for Enhanced Diosmin Delivery: Optimization and Ex Vivo Permeation. International Journal of Nanomedicine. 2013; 8:2385-2397
10. Jonassen H. 2014. Polysaccharide based nanoparticle for drug delivery application [Thesis School of Pharmacy]. Faculty of Matematics and Natural Science, University of Oslo.
11. Molyneux P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 26(2): 211-219.
12. Redha A. 2010. Flavonoid: Struktur, sifat antioksidatif dan peranannya dalam sistem biologis. Jurnal Belian. 9(2): 196-202.