ATLANTIS PRESS

Advances in Health Sciences Research, volume 26

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

Development of the Myricetin Nano-Phytosome Formula with Phosphatidylcholine Variations

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Abstract—Objectives: Myricetin is a flavonoid compound with natural antioxidant activity. Solubility, permeability and absorption rate is very low makes myricetin's bioavailability very limited. To address this problem, myricetin made in nano-phytosome technology. Nano-phytosome was made by mixing phytoconstituents and phosphatidylcholine using a specific molar ratio. This study aims to develop the Myricetin Nano-phytosome formula with variations of phosphatidylcholine. Myricetin Nano-phytosome were formulated by making three variations of phosphatidylcholine, the comparisons Myricetin:Phosphatidylcholine:Cholesterol were 1:1:0,4 (F1), 1:2:0,4 (F2), and 1:3:0,4 (F3), using thin layer film hydration-sonication method. Evaluation of nano-phytosome included measurement particle size, absorption efficiency and antioxidant activity. The result showed that particle size of nano-phytosome were F1 (198,1 nm), F2 (276,1 nm), F3 (313,2 nm). Entrapment efficiency F1 (90,28%), F2 (88,82%), F3 (86,91%). The antioxidant activity of Myricetin Nano-phytosome formulas is included as a high antioxidant. Based on the evaluation results, every increasing phosphatidylcholine concentration, the particle size increase and decrease entrapment efficiency.

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

I. INTRODUCTION

The effects of drugs in the body are greatly influenced by the delivery system. Drug delivery systems to target organs, often experience several obstacles that result in reduced effectiveness of the drug. Therefore we need a drug delivery system that can deliver drugs directly to the target, whether in the form of receptors, tissues or organs in the body [1].

One of the drug delivery systems in transdermal delivery is the vesicular system, one of which is known as nano-phytosome. Nano-phytosome are a complex formed between phytoconstituents and phospholipids which have properties similar to cell membranes where phospholipids have polar heads and nonpolar tails. Phytoconstituents bind to the head part of phospholipids. Phospholipids that are often used in making phytosomes are phosphatidylcholine. Nano-phytosome also contain other elements such as cholesterol, the addition of cholesterol has been largely used to improve the characteristics of the phytosome bilayer [2]. The use of adding cholesterol can reduce fluidity and microviscosity, thereby preventing leakage, reducing membrane permeability in water-soluble molecules, maintaining stability in biological fluids, such as plasma [3]. In addition, cholesterol can increase the efficiency of drug absorption in preparations. The efficiency of the absorption of this drug is an important component in the formulation of phytosomes, because it is related to the level of bioavailability and concentration of the drug that is useful in determining the dose in therapy [4].

The phytoconstituent used in Nano-phytosome is myricetin. Myricetin is a flavonoid compound that has activities as a natural antioxidant, anti-inflammatory and anticancer. Solubility, permeability and very low absorption rate cause the bioavailability of myricetin in topical dosage forms is very limited. Thus, the creation of Nano-phytosome myricetin can improve the weaknesses of myricetin.

Nano-phytosome myricetin is made with the ratio of myricetin: phosphatidylcholine: cholesterol into 3 formulas namely 1: 1: 0.4 (F1), 1: 2: 0.4 (F2), and 1: 3: 0.4 (F3). The making of Nano-phytosome myricetin uses thin layer hydration and sonication methods. This study aims to determine the effect of variations of phosphatidylcholine on the characterization of myricetin Nano-phytosome.

II. MATERIAL & METHOD

A. Material

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

B. Equipment

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.

C. Myricetin Nano-phytosome Formulation

Nano-phytosome myricetin is formulated into 3 formulas by comparison of myricetin : phosphatidylcholine: cholesterol using the thin-layer sonication hydration method. Nano-phytosome myricetin is prepared by dissolving myricetin in 10 ml of ethanol p.a, phosphatidylcholine is dissolved in 10 ml of ethanol p.a and cholesterol is dissolved in dichloromethane p.a. Then the three solutions are mixed together, using a magnetic stirrer at 35° C, a speed of 2000 rpm for 10 minutes, the complex formed is inserted a rotary evaporator until the solvent used evaporates. Then do hydration with aqua pro injection. The resulting hydration solution was sonicated using probe sonication for 10 minutes with an amplitude of 60%. The Nano-phytosome myricetin formula can be seen in table 1.

TABLE 1. MYRICETIN NANO-PHYTOSOME FORMULA

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

III. EVALUATION OF MYRICETIN NANO-PHYTOSOME

A. Zeta Potential and Particle Size Determination

Nano-phytosome myricetin was analyzed for particle size using the Particle Size Analyzer (PSA). The sample solution was put into the cuvette and then carried out particle size analysis.

B. Determination of absorption efficiency

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

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

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

C. Antioxidant Activity Test

1.0 mL of 0.4 mM DPPH solution was mixed with 1.0 mL of each concentration series of the test solution. Then each mixture is vortexed for 30 seconds and left for OT. Next, the absorbance was measured at a maximum wavelength of 516 nm. Absorbance measurements were performed on pure myricetin and myricetin Nano-phytosome samples.

IV. RESULTS AND DISCUSSION

Nano-phytosome myricetin is made using the thin-sonication hydration method. This method is a simple method compared to other methods [5]. To get myricetin in the form of Nano-phytosome, a sonication process was carried out where previously the thin layer hydration process was carried out with aqua pro injection solvent. The process for obtaining thin layer hydration, the ingredients of myricetin, phosphatidikolin, and cholesterol are dissolved in organic solvents. The use of organic solvents to dissolve ingredients aims to make it easier to evaporate the solvent when a Nano-phytosome thin film is formed [6]. Then the sonication process continues, after sonication the solution is observed physically, where a clear yellow solution is produced in the absence of particles. This shows that myricetin can be made Nano-phytosome. Then the Nano-phytosome myricetin produced was evaluated including, particle size, entrapment efficiency and antioxidant activity. Evaluation results are presented in table II.

TABLE II. RESULT OF MYRICETIN NANO-PHYTOSOME EVALUATION

Evaluation	F1 (1:1:0.4)	F2 (1:2:0.4)	F3 (1:3:0.4)
Particle Size (nm)	198,10	276,10	313,20
Absorption Efficiency (%)	90,28	88,82	86,91
Antioxidant Activity (ppm)	22,08	21,79	21,29

Based on the data in table II, the particle size obtained from the three Nano-phytosome myricetin formulas has particle sizes in the nano range and in the classification of fine particles. According to Tiwari (2013), that structural and functional units of nanotechnology are known as nanoparticles. Coarse particles range in size between 2,500 - 10,000 nanometers, fine particles range between 100 - 2,500 nanometers and ultra-fine particles range between 1 - 100 nanometers [7]. The smallest size is in formula one, with a particle size of 198.10 nm with the smallest phosphatidylcholine composition, Formula 3 has the largest particle size among the three formulas. A greater concentration of phosphatidylcholine than myricetin can bind myricetin completely because one phytoconstituent molecule will be bound by 1 phosphatidylcholine molecule so that the bond is stronger and can increase particle size.

Evaluation of the determination of the efficiency of nano-phytosome absorption is done to find out how much the ability of Nano-phytosome made to absorb myricetin. In this evaluation, Nano-phytosome samples that have been hydrated are centrifuged at a speed of 3000 rpm for 30 minutes. This centrifugation process aims to precipitate the myricetin that is absorbed in the nano-phytosome complex so that the supernatant will only contain myricetin which is not absorbed. Based on the results listed in the table above, it can be seen that the highest efficiency of Nano-phytosome absorption is found in formula 1, which is 90.28%. From these results, each formula entered a good absorption efficiency range of > 80%. The difference in entrapment efficiency of each formula is due to differences in the amount of phospholipids used. The more phosphatidylcholine added to the formula, the lower the efficiency of Nano-phytosome absorption.

The nano-phytosome myricetin sample has a strong antioxidant activity that is IC_{50} less than 50 ppm. Where the results of sequential antioxidant activity are formula 1 (22.08 ppm), formula 2 (21.79 ppm), formula 3 (21.29 ppm). The content in myricetin that provides the greatest antioxidant effect is flavonoids. Flavonoids act as antioxidants by donating hydrogen atoms or through their ability to chew metal, in the form of glucosides (containing glucose side chains) or in a free form called aglycones [8-12edy]. Determination of antioxidant activity methods with DPPH 1,1-diphenyl-2 -pikrilhidrazil 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. Antioxidant activity testing using this method can be 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 200-1000µg/mL [9-11edy].

V. CONCLUSION

Based on the results of this study it can be concluded that:

1. Myricetin can be made in the form of Nano-phytosome by the thin-sonication hydration method with phosphatidylcholine ratio between formulas 1, 2 and 3.
2. The best formula is formula 1 where the smallest particle size, adsorption efficiency and highest activity of antioxidants.

VI. RECOMMENDATION

Based on the potential of the results of this study, it is hoped that the results of making Nano-phytosome can be continued with cosmetic formulations.

ACKNOWLEDGMENT

Thank you to the honorable Ministry of Research and Technology of the Republic of Indonesia who have funded this research so that this research can be carried out properly and smoothly.

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