International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 12, Issue 5, 2020

Original Article

EVALUATION OF MYRICETIN NANOPHYTOSOME WITH THIN-SONICATION LAYER HYDRATION METHOD USING ETHANOL AND ACETONE SOLVENTS

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Received: 30 Nov 2019, Revised and Accepted: 16 Jun 2020

ABSTRACT

Objective: Nano-phytosome is a nanotechnology that is used to improve the bioavailability of active ingredients contained in plants by binding to active ingredients with phospholipids which have properties that resemble cell membranes. The active ingredient used in the nano-phytosome formulation is myricetin. Myricetin is a natural flavonoid compound that has antioxidant properties with low bioavailability and permeability. The purpose of this study was to determine the characterization of the nano-phytosome myricetin formulation with different solvents using ethanol and acetone.

Methods: Nano-phytosome was made using a thin-sonication hydration method by comparing the acetone and ethanol solvents as well as the variation of the myricetin: phosphatidylcholine: cholesterol ratio. Characterization of nano-phytosome includes particle size, polydisperse index, zeta potential, absorption efficiency and antioxidant activity, and TEM test.

Results: Characterization and evaluation of myricetin nano-phytosome using two different solvents, acetone and ethanol. The particle size of all formulas has a size between 10-1000 nm, the use of ethanol solvent produces the smallest particle size of 198.1 ± 1,74 nm and the lowest polydispersity index of 0.175 ± 0,020 in ethanol solvent.

Conclusion: The ethanol solvent is better compared to the acetone solvent, and the best formula is formula 4 with the ratio of myricetin: phosphatidylcholine: cholesterol (1: 1: 0.4).

Keywords: Nano-phytosome, Myricetin, Thin layer hydration, Sonication

DOI: http://dx.doi.org/10.22159/ijap.2020v12i5.36520. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Flavonoids are a general group of polyphenols that have many benefits such as antioxidants, antimicrobials, anticancer, and anti-inflammatory effects [1]. Myricetin is one of the natural polyphenol flavonoid compositions that is widely distributed in fruits, vegetables, and herbs available under close supervision for widespread use as an antioxidant treatment. Myricetin, like other flavonoids, has significant limitations in terms of bioavailability and absorption [2, 3]. Myricetin shows low oral bioavailability (<10%), due to its poor solubility in water. Low absorption due to fat solubility is needed for the development of new formulations for myricetin [4].

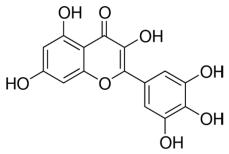

Fig. 1: Chemical structure of myricetin

Currently, modern drug delivery systems can control the release or modification of drug release routes, resulting in better pharmacological activity. In the latest generation of drug delivery systems, there is an advantage in terms of increasing the penetration properties of the skin. Recent developments in the field of nanotechnology have enabled the manufacture of nano-sized particles used for a variety of biomedical applications [5].

There are various developments in the Drug Delivery System, one of which is in transdermal delivery, namely the vesicular system, for example, the phytosomes. The phytosomes are an integral part of the membrane, where molecules are anchored through chemical bonds to the head of phospholipids [6]. Phytosomes are a combination of phospholipids, one of which is phosphatidylcholine in nonpolar solvents such as acetone and ethanol. Phytosomes are made up of those which are complex micellar structures of nature–phospholipids [7]. The composition of phytosomes is safe, its components are accepted for use in the pharmaceutical field, and the absorption and bioavailability of water-soluble natural materials are increasing. This results in a better therapeutic effect [8].

One approach to improve solubility, permeability, and bioavailability, especially for topical drugs, is nano-phytosome technology. Nano-phytosome drugs are nanoscopic crystals with dimensions of size less than 1 µm, which contain 100% drugs and stabilizers (polymers/surfactants) without a carrier [9]. The advantage of the nano-phytosome is that it can deliver drugs better to small units in the body, overcoming resistance caused by physiological barriers in the body caused by drug delivery systems that are directly affected by particle size [10].

Nano-phytosomes are one of the latest lipid-based nano-carriers [11]. Nano-phytosomes are made by mixing phytoconstituents with phosphatidylcholine at certain molar ratios (1: 1 to 1: 3), because 1 phytoconstituent molecule will be bound by 1 phosphatidylcholine molecule so that it will produce a complex with stronger bonds. Methods that can be used in making nano-phytosomes include solvent evaporation, reflux, salting out, and lyophilization methods [12]. The method used in this study is the thin layer hydration method using two different solvents, namely ethanol, and acetone. The thin layer hydration method is simple.

This study aims to make myricetin nano-phytosomes by thin layer hydration method and using two solvents, ethanol, and aceton. The

nano-phytosome results were obtained and evaluated the particle size, polydisperse index, zeta potential, entrapment efficiency, and antioxidant activity.

MATERIALS AND METHODS

Reagents and chemical

Myricetin (Tocris, China), Phospholipon 90G (Lipoid, Germany), cholesterol (Proanalisys sigma-grade,≥99%) were obtained from Sigma Chemicals, SigmaAldrich Corporation, St. Louis, MO, acetone, ethanol (proanalysis, Merck). Chemical used in this work were of analytical grade (AR).

Formulation of nano-phytosome

Nano-phytosomes were formulated using the sonication thin layer hydration method by making three different variations using two different solvents. The nano-phytosome formula are shown in table 1.

Table 1: The nano-phytosome formulation

Materials	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
Myricetin (mg)	10	10	10	10	10	10
Phosphatidilcoline (mg)	24	48	71	24	48	71
Cholestrol (mg)	4	4	4	4	4	4
Aceton (ml)	20	20	20	-	-	-
Ethanol (ml)	-	-	-	20	20	20
Dichlorometan (ml)	5	5	5	5	5	5
Aqua pro Injection (ml)	25	25	25	25	25	25

Information: *comparison in molar, Formula 1: Myricetin: Phosphatidylcholine: Cholesterol (1: 1: 0.4), Formula 2: Myricetin: Phosphatidylcholine: Cholesterol (1: 2: 0.4), Formula 3: Myricetin: Phosphatidylcholine: Cholesterol (1: 3: 0.4), Formula 4: Myricetin: Phosphatidylcholine: Cholesterol (1: 1: 0.4), Formula 5: Myricetin: Phosphatidylcholine: Cholesterol (1: 2: 0.4), Formula 6: Myricetin: Phosphatidylcholine: Cholesterol (1: 3: 0.4)

The formulation was made by dissolving nano-phytosome, myricetin, phosphatidylcholine, and cholesterol in their respective solvents. Phytoactive and phospholipid solutions were mixed using a magnetic stirrer (Thermo Scientific, China) at a temperature of 35 °C at 2000 rpm for 10 min. The nano-phytosome complex was made a thin layer on a rotary evaporator at a temperature of 55 ° C at a speed of 50 rpm until the solvent phase evaporated. The thin layer formed on the walls of the round bottom flask was hydrated with a 20 ml aqua pro injection marked by colloidal dispersion. Colloidal dispersions formed were sonicated using probe sonication (QSonica, newtown, USA) for 10 min with an amplitude of 60%.

Characterization of nanophytosomes

Determination of particle size distribution

Particle size analysis and particle size distribution were carried out using the Particle Size Analyzer (Malvern Panalytical, USA), The vesicle size and PDI of the resultant nano-phytosome were measured by dynamic light scattering (DLS) using a photon correlation spectrometer (Zetasizer, Malvern Instruments LTD, UK) which analyzes the fluctuations in light scattering due to the Brownian motion of the particles. Light scattering was monitored at 25 °C at a scattering angle of 90° [13]. The zeta potential of the formed niosomaldispersions was determined using Zetasizer (Malvern Instruments, UK). Samples were placed in clear disposable zeta cells, and results were noted. Charges on the vesicular surface and their corresponding zeta potential values were obtained [14].

Determination of absorption efficiency

Purification of PTL formulation was done by the ultracentrifugation method [15]. Myricetin nano-phytosome was initially centrifuged for 50 min at a speed of 3000 rpm at room temperature (27 °C) in order to separate the active substance, which is not absorbed. Each of supernatant results from centrifugation from formula 1 to formula 6 was taken as much as 0.5 ml, then diluted with aqua pro injection up to 10 ml, then the absorption was read at three times replication using UV-Vis spectrophotometry (Shimadzu 1800, Thermo Scientific, Japan) at a wavelength of 369 nm. The entrapment efficiency (% EE) is calculated by the formula:

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

TD is the total number of myricetin contained in the formula and FD is the number of myricetin detected in the supernatant (free form).

Nano-phytosome stability

The nano-phytosome stability test was carried out in storage at room temperature (27 °C) for three weeks. During storage, observations of phase separation, physical and chemical changes of the preparations were done.

Antioxidant activity test

The assay is based on the measurement of the scavenging capacity of antioxidants towards a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH). The method is unique in carrying out the reaction of the sample with DPPH in methanol/water, which facilitates the extraction of antioxidant compounds from the sample [16]. In the antioxidant activity test, as much as 1.0 ml of 0.4 mmol 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 operating time. The solution was then measured for absorbance at a maximum wavelength of 516 nm. Absorbance measurements were performed on pure myricetin as blanks and myricetin nano-phytosome samples.

Transmission electron microscope

The particle morphology was observed using the Transmission Electron Microscope (JEOL JEM-1400, Germany). The stages of TEM work are the preparation of samples at room temperature using 1% phosphotungstic acid (pH 6.0) staining. After that, the sample is dropped on one layer of the carbon-coated copper grid and then dried at room temperature; after drying it the stained films were photographed using TEM [17].

RESULTS AND DISCUSSION

Particle size analysis

Particle size and size distribution characteristics are critical in nanoparticle systems. Particle size and size distribution determined *in vivo* distribution, toxicity, and targeting ability in nanoparticle systems. In addition, particle size and size distribution can also affect drug delivery, drug release, and nanoparticle stability [18]. Also, particle size is the most important characteristic in a nanoparticle system because it determines the speed and ease of the drug to be absorbed optimally. Myricetin nano-phytosome particle size analysis results show that formula 1 to formula 6 has fulfilled the nanoparticle size range of 10-1000 nm [19]. The use of different solvents will definitely affect the particle size and the stability of the dispersion of the nanoparticles produced. The results of the particle size analysis are shown in table 2.

Table 2: Results of myricetin nanophytosome characterization

Characteristics	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
Particle size (nm)*	233.63 ± 1.21	250.00 ± 3.55	242.70 ± 1.79	198.1 ± 1.74	276.1 ± 1.55	313.2 ± 1.87
Polydispersity Index *	0.260 ± 0.002	0.260 ± 0.008	0.447 ± 0.006	0.175 ± 0.020	0.285 ± 0.009	0.504 ± 0.012
Zeta Potential (mV) *	-21.70 ± 1.21	-15.70 ± 0.75	-11.30 ± 0.62	-16.47 ± 0.67	-12.70 ± 0.36	-6.44 ± 0.37

*The data are written the average value and the SD value of each formula.

The results of research that has been done for the characteristics of myricetin nano-phytosomes show that formula 1 in the acetone solvent has the smallest average particle size of 233.6 ± 1.21 nm, whereas the ethanol solvent shows formula 4 which has the smallest particle size that is 198.1 ± 1.74 nm. With differences in the number of moles of phosphatidylcholine affect the particle size. Ethanol solvents show better particle size than Acetone solvents, while ethanol solvents are also safer than acetone solvents.

Polydispersity index

Values that show the breadth of particle size distribution in preparation are called the Polydispers or IP Index. IP>0.5 represents a nanoparticle system with a very broad particle size distribution (polydispersion), whereas IP has a value of 50.5 for monodispersed particles. The best polydispersity index value is<0.5 because the smaller the IP value, the better the stability of the nano-phytosome. The polydispersity index results are shown in table 2.

In acetone formula 1 and 2, the smallest polydispersity index was 0.260 ± 0.008. Meanwhile, the ethanol solvent of formula 4 shows the smallest polydispersity index value that is 0.175 ± 0.020. It is said that the preparation has a homogeneous distribution of particles with other particles if the polydispersity index value is getting smaller, this shows that the myricetin nanophytosome is homogeneous and has a mono dispersion particle system. The best polydispersity index value obtained by Ethanol is 0.175 ± 0.020, because it is smaller than Acetone which is 0.260 ± 0.008.

Potential zeta

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

The measurement of zeta potential produced in the nano-phytosome myricetin in the best acetone in Formula 1 is-21.7 ± 1.21 mV, while in ethanol, the best in Formula 1 is-16.47± 0.67 mV. Of the two, acetone has Zeta Potential, which is better than ethanol because it shows a value of close to±30 mV, which is-21.7 ± 1.21 mV. Negative results indicate that the phosphatidylcholine used is negatively charged.

Nanophytosome stability

During storage, myricetin nano-phytosomes are stored at room temperature (27 °C) for more than three weeks. The smell that is owned is the typical odor of myricetin. The color of myricetin nano-phytosome from week 0 to week 3 has the same color, which is yellowish. The results of myricetin nano-phytosome stability are shown in table 3.

Table 3: Stability of myricetin nanophytosomees at room temperature

Solvent	Formula	1st week	2nd week	3rd week
Acetone	1	no sediment	no sediment	no sediment
	2	no sediment	no sediment	sediment
	3	no sediment	sediment	sediment
Ethanol	4	no sediment	no sediment	no sediment
	5	no sediment	no sediment	no sediment
	6	nearly sediment	sediment	sediment

In the acetone solvent shows that Formula 2 and 3 are deposited, the sediment that occurs is reversible because it can be dispersed again quickly after shaking. Formula 1 does not undergo precipitation and remains clear for up to 3 w, whereas the ethanol solvent shows that formula 6 has sedimentation. The higher the concentration of phosphatidylcholine, the more concentrated the phospholipid nano-phytosome. This can cause the poor stability of formula 3. Adding cholesterol to the formula can increase the physical stability of nano-phytosomes for more than 21 d.

Absorption efficiency

Adsorption efficiency test was carried out to investigate the amount of myricetin that was absorbed in the nano-phytosome carrier system, adsorption efficiency calculation. Determination of levels of active substances that are not absorbed is calculated using the equation:

$$y= 1.057x10^{-3}+0, 0603. X. \ldots . (2)$$

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

Table 4: Results of analysis of myricetin nanophytosome samples

Evaluation result	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
Absorption efficiency (%)	91.94%	91.39%	91.39%	90.28%	88.82%	86.91%
Antioxidant activity (ppm)	41.13	25.46	20.64	22.08	21.79	21.29

In the Acetone Solvent, the adsorption efficiency results in formula 1 of 91.94%, which means that 90.94% of myricetin is absorbed in the phospholipid component, both formula 2 and 3 are 91.39% myricetin is absorbed in the phospholipid component. In the ethanol solvent showed the efficiency of absorption in Formula 4 was 90.28% myricetin was absorbed in the phospholipid component, then formula 5 was 88.82% myricetin was absorbed in the phospholipid component, and Formula 6 was 86.91% myricetin was absorbed in the component phospholipids. From these results, each formula entered a good absorption efficiency range of>80%. However, Acetone shows better efficiency compared to Ethanol.

Test antioxidant activity

The method of determining antioxidant activity with the DPPH 1,1-diphenyl-2pikrilhidrazil method (α, α-diphenyl-β-picrilhidrazil) is called the Antioxidant Activity Test. 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. DPPH is a free radical that is stable and does not form dimers due to the delocalization of free electrons in all molecules. 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 number of antioxidants needed to reduce the initial DPPH concentration by 50%. A substance would have antioxidant properties if the IC_{50} value obtained ranges from 200-1000 µg/ml [20].

In the Acetone solvent, the results of the antioxidant activity test showed IC_{50} value of nano-phytosome myricetin formula 1 sample was 41.13 ppm, formula 2 was 25.46 ppm, and formula 3 was 20.64 ppm can be seen in table 3. Then, the ethanol solvent showed the value IC_{50} myricetin formula 4 nano-phytosome sample of 22.08 ppm, formula 5 of 21.79 ppm, and formula 6 of 21.29 ppm can be seen in table 4. This shows the presence of very strong antioxidant activity in myricetin because it has an IC_{50} value of 22.69 ppm. In this case, the myricetin nano-phytosome samples of the three formulas from these two different solvents have strong antioxidant activity. 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 [21].

TEM test

The myricetin nanophytosome sample used for the TEM test is the myricetin formula 6 nanophytosome sample with the Myricetin: Phosphatidylcholine: Cholesterol (1: 1: 0.4) test used to see the morphology. The use of 1-mole phosphatidylcholine in the formula can prevent agglomeration so that the particles do not grow bigger. Observations with TEM nanophytosome formula 6 show irregularly shaped round nanoparticles vesicles. The micrograph of the TEM results can be seen in fig. 2. The polar portion of the vesicles is shown in black, while the transparent or colorless portion indicates the presence of a non-polar compound. The method of manufacture influences the shape of vesicles, in some previous studies, the shape of particles produced was spherical and uniform, but in this study, the shape of the resulting vesicles was irregularly rounded. This needs to be developed in further research to find other manufacturing methods to produce better vesicle shapes.

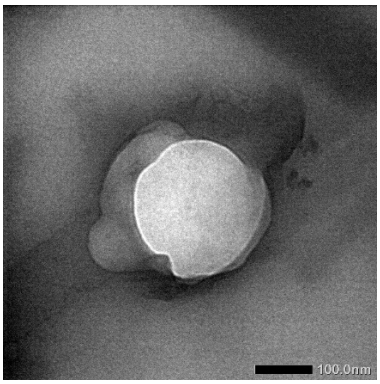

Fig. 2: TEM formula 6 test results

CONCLUSION

Characterization and evaluation of myricetin nano-phytosome were carried out using two different solvents, namely Acetone and Ethanol in terms of particle size, all formulas have sizes between 10-1000 nm, the use of ethanol solvent produces the smallest particle size of 198.1± 1.74 nm and the lowest polydioners index of 0.175± 0.020 in solvents Ethanol. Thus, it can be concluded that the ethanol solvent is better compared to the acetone solvent, and the best formula is formula 1 with the ratio of Myricetin: Phospatidilcolin: Cholesterol (1: 1: 0.4).

ACKNOWLEDGMENT

This research was supported and funded by the Ministry of Research and Technology of the Republic of Indonesia and this research was also supported by PKPT team.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

There is not a conflict of interest from this works.

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