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

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ABSTRACT

Objective: An example of nanotechnology is the nano-phytosome, which binds active plant components to phospholipids with characteristics similar to cell membranes in order to increase the bioavailability of these components. Myricetin is the main component of the nano-phytosome. An antioxidant with poor bioavailability and permeability, myricetin is a flavonoid chemical found in nature. This research set out to characterise the nano-phytosome myricetin formulation utilising ethanol and acetone as solvents of choice.

INTRODUCTION

Flavonoids are a class of polyphenols with a wide range of useful properties, including anti-inflammatory, anticancer, antibacterial, and antioxidant properties [1]. One of the naturally occurring polyphenol flavonoid compositions found in many fruits, vegetables, and herbs is myricetin, which is being carefully monitored for its potential use as an antioxidant therapy. Bioavailability and absorption are major issues for myricetin and other flavonoids [2, 3]. The reason why myricetin has a low oral bioavailability of less than 10% is because it is poorly soluble in water. New myricetin formulations should have low absorption owing to fat solubility [4].

as an example, phytosomes. Phytosomes are a crucial component of cell membranes, where chemicals are bound to phospholipid heads chemically [6]. Phospholipids, including phosphatidylcholine, in nonpolar solvents like ethanol and acetone make up phytosomes. The micelles that make up phytosomes are phospholipids, which are complex structures found in nature [7]. Phytosomes are a

promising new way to increase the absorption and bioavailability of water-soluble natural ingredients, and their composition is acceptable for use in pharmaceuticals. The therapeutic impact is improved as a consequence [8].

A method for increasing solubility, permeability, and an nano-phytosomes enhance bioavailability, particularly for topical medicines.

modern technology. The term "nano-phytosome drug" refers to crystals that are less than 1 μm in size and include just the

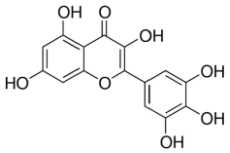

Fig. 1: Chemical structure of myricetin

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 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

drug plus stabilisers (polymers/surfactants) [9]. The nano-phytosome has the potential to improve medication delivery.

to subcellular levels throughout the body, overcoming obstacles brought on by

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**	**2**	**3**	**4**	**5**
		For mula	**For mula**	**For mula**	**For mula**	**Form ula 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

Nano-phytosome stability

The nano-phytosome stability test was carried out in storage at

solvents. Phytoactive and phospholipid solutions were mixed using a magnetic stirrer (Thermo Scientific, China) at a

room temperature (27 °C) for three weeks. During storage, observations of phase separation, physical and chemical changes of

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

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

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 f rmula 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). 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 □ 0.008	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:

$y= 1.057x10^{-3}+0, 0603. X$ (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% 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 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

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 IC50 value obtained ranges from 200- 1000 μg/ml [20].

In the Acetone solvent, the results of the antioxidant activity test showed IC50 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 IC50 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 IC50 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.

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).

CONCLUSION

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