

Preparation and Characterization of Ethosomes Loading Petai Pods Extract (*Parkia speciosa* Hassk.)

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Abstract

Preparation of petai pods extract (*Parkia speciosa*) into ethosome aim to increased penetration through the skin. The method of cold is used in preparing petai pods extract encapsulation by ethosome with variation in concentration of soya lecithin, propylene glycol, and ethanol. The proportion of optimum formula ethosome consisted were 0.2 g soya lecithin, 1 ml propylene glycol, and 4 ml ethanol that response values obtained pH of 4.74, viscosity of 0.950 cP, %EE of 74.326%, and stability of 7.288%. The resulted of optimum formula obtained were PDI of 0.23, zeta potential of -7.5 mV, and particle size of 818.7 nm. Ethosome showed spheric particle using Transmissin Electron Microscopy. The diffusion analysis showed highest on ethosome of petai pods extract (9.525%) than petai pods extract (5.466%). The interaction study used FTIR show no chemical interaction extract pods and ethosome components.

Keywords

ethosome, petai pods, extract, soya lechitin, propylene glycol, ethanol

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1. INTRODUCTION

Petai (*Parkia speciosa* Hassk.) has been long used by public especially in Indonesia as food ingredients but petai pods not used and only being waste. Petai pods have phytochemical compounds such as phenolics and flavonoids compound (Fithri et al., 2019). Phenolics and flavonoids compound have high antioxidants activity that needed by human body (Ko et al., 2014). Flavonoids can protect cell from free radicals and increase cell integrity. Besides having high antioxidants activity, flavonoids also have activity as anti-inflammatory, wound healing, and skin aging. Flavonoids have poor oral bioavailability and absorption due to its poor aqueous solubility. The stability of flavonoids in gastrointestinal tract is poor because its can go through enzymatic hydrolysis (Ramadon et al., 2018). The penetration mechanism of flavonoids into cell also can be limited due to its poor aqueous solubility and lipophilicity partition coefficient ($\log P = 1.82$) as the cause of nonpolar group in its structure (Vickers, 2017).

The limitation of penetration in the delivery flavonoids compound into the skin requires a novel delivery system. Transdermal delivery system have high potentially for resolve this limitation. One of transdermal delivery system known as nanovesicles, its a method that used increase the penetration of active substances into the skin. Ethosomes are a nanovesicles system that have high potential as penetration enhancer of flavonoids into skin

(Ramadon et al., 2018). Ethosomes are phospholipid-based elastic nanovesicles containing a high content of ethanol (20–45%) and water. Ethanol can be used as penetration enhancer in vesicles system and make the ethosomes more elastic, its can disturb the lipid bilayer on skin (Verma and Pathak, 2010). Propylene glycol used as penetration enhancer. The concentration of propylene glycol mainly used for ethosomes is 5-20% and it influenced the size, stability, encapsulation efficiency, and penetration of ethosomes into the skin (Abdulbaqi et al., 2016).

In this study, petai pods extract (*Parkia speciosa*) loaded ethosomes were formulated, then it performed in vitro penetration test using Franz diffusion cells. Chemical interaction between material for making ethosomes and petai pods extract also analyzed.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used in this research were petai pods extract (*Parkia speciosa* Hassk.) obtained from the UNSRI pharmacy department, Soya Lecithin (PT. Lansida), Ethanol (PT. Dira Sonita), Propylene glycol (PT. Bratachem), Cellophane Membrane (Merck), Quercetine (Sigma-Aldrich®), $AlCl_3$ (PT. Bratachem) and solvents with technical quality and p.a obtained from PT. Bratachem and Merck.

2.2 Extraction Petai Pods (*Parkia speciosa* Hassk.)

Extract preparation was carried out based on previous research. The 4 kg of dry simplisia petai pods (*Parkia speciosa*), crushed using a blender. Petai pods powder is macerated with 6 L of 70% ethanol, stirred, and let stand for 48 hours. The resulting maserate is filtered with filter paper to obtain the filtrate. The resulting filtrate is remacerated to obtain a clear filtrate. The resulting filtrate was concentrated using a rotary evaporator at a temperature of 60°C and a speed of 35 rpm to remove the solvent and a thick extract was obtained then the percent yield was calculated. The thick extract was identified and the levels of flavonoids were calculated using quercetin as a marker compound.

2.3 Formulation of Petai Pods Extract (*Parkia speciosa* Hassk.) Loaded Ethosomes

Formulation of petai pods extract (*Parkia speciosa*) loaded ethosomes in this research varied the amount of lecithin soybean (0.2 g; 0.6g), propylene glycol (1 ml; 2 ml), and 96% ethanol (4 ml; 8 ml). The formula used can be seen in Table 1.

Petai pods extract (*Parkia speciosa*) loaded ethosomes was prepared using the cold method. The petai pods extract was dissolved in 96% ethanol, stirred using a magnetic stirrer at a speed of 700 rpm for 10 minutes. The homogeneous extract was added with soy lecithin and then homogenized using a magnetic stirrer with a speed of 700 rpm for 5 minutes. Propylene glycol is added to the mixture and homogenized using a magnetic stirrer for 20 minutes at room temperature. Distilled water was added to the mixture while stirring with a magnetic stirrer at 30°C. The ethosome was homogenized with an ultrasonic bath (GT SONIC® VGT-1620QTD 50 KHz) with frequency of 50 kHz for 30 minutes. Petai pods extract (*Parkia speciosa*) loaded ethosomes was stored at 4°C.

2.4 Analysis of Response Petai Pods Extract (*Parkia speciosa* Hassk.) Loaded Ethosomes

2.4.1 Determination of pH

pH test for each formula was carried out using a pH meter (Lutron® pH Electrode PE-03) dipped into the petai pods extract (*Parkia speciosa*) loaded ethosomes. The pH result of each formula is displayed on the pH meter screen.

2.4.2 Determination of Viscosity

The viscosity test was carried out using an Ostwald viscometer (PTC Chemical Equipment). The viscosity test is carried out by measuring the time of flow velocity in the Ostwald viscometer tube.

2.4.3 Determination of Percent of Encapsulation Efficiency (%EE)

The %EE test was started with petai pods extract loaded ethosomes centrifuged (DLAB ©: D2012 PLUS) at a speed of 10,000 rpm for 90 minutes. The supernatant was taken and measured the levels of flavonoids that were not absorbed in the ethosomes vesicles using a UV-Vis spectrophotometer with a length of 436 nm where quercetin was used as a marker compound.

2.4.4 Stability Testing of Petai Pods Extract Loaded Ethosomes

The stability test of the petai pods extract loaded ethosomes was determined using the cycling test method for 6 cycles. Stability testing was carried out by placing each ethosome formula at 4°C for 24 hours then transferred at 40°C for 24 hours. Measurement of petai pods extract levels was carried out in the last cycle (6th cycle) using a UV-Vis spectrophotometer with a wavelength of 436 nm (quercetin).

2.5 Formula Optimization

Determination of the optimum formula of petai pods extract loaded ethosomes is determined from the relationship between the components of soy lecithin, propylene glycol, and ethanol with the interaction of each response, namely pH, viscosity, % EE, and stability. The optimum formula analysis uses the DX® 10 program.

2.6 Optimal Formula Characterization

Measurement of particle diameter, polydispersion index, and zeta potential was carried out using the Particle Size Analyzer (PSA) (Horiba Scientific® SZ-100) with the dynamic light scattering (DLS) method. A total of 50 µl of the petai pods extract loaded ethosomes was added to the PSA cuvette.

2.7 Determination of Surface Morphology

The surface morphology particles of petai pods extract loaded ethosomes was determined using a transmission electron microscopy (TEM) (Jeol® JEM 1400) device. The ethosomes optimum formula of petai pods extract as much as 50 µl was diluted 100 times with distilled water. The ethosomes formula sample was observed under a digital focusing microscope using a voltage of 10 kV and 30 kV.

2.8 In Vitro Diffusion of Ethosomes

Testing In-vitro diffusion testing was carried out using Franz Diffusion Cells (FDC) and PBS solution pH 7.4. A total of 2 ml of petai pods extract loaded ethosomes was evenly placed on the cellophane membrane and stirred with a magnetic stirrer at a speed of 400 rpm at 35°C. Sampling was done at minute: 0; 5; 10; 15; 30; 45; 60; 120; 180; 240; 300; 360; 420; 480; 540; 600; 660; 720. The absorbance is measured at a maximum wavelength of 436 nm. In-vitro diffusion was carried out on the petai pods extract and petai pods extract loaded ethosomes.

2.9 Chemical Interaction with FTIR

The determination of the chemical interactions of petai pods extract loaded ethosomes, placebo ethosomes, and petai pods extracts was carried out using FTIR. The FTIR spectrum will produce an interpretation of chemical interactions and functional groups.

3. RESULTS AND DISCUSSION

The petai peel extraction process produced a thick extract of 1.029 kg with 25.72% yield. The yield percentage indicated the

Table 1. Formulation of Petai Pods Extract (*Parkia speciosa*) Loaded Ethosomes

Formulation	Extract of petai pods (mg)	Soya lecithin (g)	Propylene glycol (mL)	Ethanol (mL)	Aquadest (mL)
F1	440	0.2	1	4	ad 20
F2	440	0.6	1	4	ad 20
F3	440	0.2	2	4	ad 20
F4	440	0.6	2	4	ad 20
F5	440	0.2	1	8	ad 20
F6	440	0.6	1	8	ad 20
F7	440	0.2	2	8	ad 20
F8	440	0.6	2	8	ad 20

number of components or secondary metabolites extracted during the maceration process and the effectiveness of the extraction process.

3.1 Determination of Total Flavonoid

Determination of total flavonoid in petai pods extract using quercetin compound as a marker. The maximum wavelength is determined by the addition of $AlCl_3$ which will react with quercetin. The maximum wavelength resulting from the scanning process is 436 nm. The maximum wavelength obtained was used to test the percent efficiency of encapsulation and to test the stability of the petai pods extract content in the ethosomes. The total of flavonoids contained in the extract was 35.706 mg/g.

3.2 Formulation of Petai Pods Extract Loaded Ethosomes

The Petai Pods Extract Loaded Ethosomes is formulated using components consisting of soya lecithin, propylene glycol, and 96% ethanol. Soya lecithin is used in ethosomes formulas because it contains unsaturated fatty acids that have compatibility in the body, good penetration into the skin, and a relatively cheap price. Propylene glycol can increase the flexibility of the ethosomes vesicles, thereby increasing diffusion into the skin. Meanwhile, the addition of ethanol can increase the penetration of ethosomes vesicles through the stratum corneum. The mechanism of penetration of the ethosomes in the skin is by disrupting the lipid structure of the stratum corneum and having good flexibility so it can deform when it passes through the stratum corneum to penetrate the stratum cornea. This can occur because of the high concentration of ethanol in the ethosomes formulation (Korade et al., 2016).

The selection of methods making ethosomes used the cold method because flavonoids are compounds that are unstable to heat. The results of the preparation of the ethosomes formula can be seen in Figure 1. Ethosome preparations with a low concentration of soya lecithin have a lighter color than those with a high concentration of soy lecithin. The high concentration of soy lecithin can cause the size of the ethosomes vesicles to get bigger.

Table 2. pH Determination of Petai Pods Extract (*Parkia speciosa*) Loaded Ethosomes

Formulation	Mean \pm SD	CV (%)
F1	4.74 \pm 0.008	0.211
F2	4.84 \pm 0.005	0.119
F3	4.70 \pm 0.012	0.324
F4	4.82 \pm 0.015	0.315
F5	4.83 \pm 0.012	0.317
F6	5.13 \pm 0.011	0.225
F7	4.86 \pm 0.014	0.355
F8	4.92 \pm 0.010	0.204

3.3 Determination of pH Analysis

The results of pH test Petai Pods Extract Loaded Ethosomes were obtained in the range 4.71-5.14 into the desired criteria, namely 4.5-7.0. Data from pH testing results can be seen in Table 2. pH preparations that are too acidic or alkaline can irritate because the skin becomes dry. Observation of the pH response on each factor has a significant effect with a p-value <0.0001. Based on the pH response equation, soy lecithin and ethanol can increase the response value while propylene glycol decreases the response value because it has a pH that tends to be acidic. Based on the pH response equation, the interaction between soy lecithin, propylene glycol, and ethanol can reduce pH. This is influenced by the propylene glycol and petai pods extract components, which have a pH that tends to be acidic. The following is the equation of the factorial design of the combination of ethosomes-forming components to pH:

$$pH = 4,86 + 0,073A - 0,073B + 0,078C - 0,036AB + 0,017AC - 0,019BC - 0,039AB \quad (1)$$

Description:

A: Soya lecithin concentration

B: Propylene glycol concentration

C: Ethanol concentration
 AB: Interaction of soy lecithin with propylene glycol
 AC: Interaction of soy lecithin with ethanol
 BC: Interaction of propylene glycol with ethanol

3.4 Viscosity Analysis

Table 3. Viscosity Determination of Petai Pods Extract (*Parkia speciosa*) Loaded Ethosomes

Formulation	Mean \pm SD(cP)	CV (%)
F1	0.950 \pm 0,009	0.969
F2	1.287 \pm 0,020	1.593
F3	1.427 \pm 0,016	1.152
F4	1.817 \pm 0,036	2.042
F5	1.255 \pm 0,026	2.135
F6	1.564 \pm 0,017	1.13
F7	1.088 \pm 0,013	1.268
F8	1.145 \pm 0,026	2.281

Viscosity analyze was carried out to determine the viscosity properties of the prepared ethosome. Observation of the viscosity response on each factor has a significant effect with a p-value <0.0001. Based on Table 3, ethosomes with high soy lecithin concentrations produce an increased viscosity compared to low soy lecithin concentrations. This is because the viscosity of soy lecithin is high so that it can significantly affect the viscosity of the ethosome. Based on the viscosity response equation, the concentration of soy lecithin can increase the viscosity response. The interaction of soy lecithin, propylene glycol, and ethanol can reduce the viscosity response.

$$Y = 1,31 + 0,14A + 0,05B - 0,059C - 0,029AB - 0,045AC - 0,019BC$$

$$- 0,032ABC \quad (2)$$

Description:
 Y: Viscosity response
 A: Soya lecithin concentration
 B: Propylene glycol concentration
 C: Ethanol concentration
 AB: Interaction of soy lecithin with propylene glycol
 AC: Interaction of soy lecithin with ethanol
 BC: Interaction of propylene glycol with ethanol

3.5 Encapsulation Efficiency Analysis

The percentage of encapsulation efficiency aims to determine the amount of extract absorbed in the ethosomes vesicles. The determination of % EE was carried out by purification of the ethosomes through centrifugation at a speed of 10,000 rpm for 90 minutes. The supernatant obtained was measured using a UV-Vis spectrophotometer with a wavelength of 436 nm using the quercetin standard.

The results of the %EE test show that formula 1 has the highest %EE while formula 7 has the lowest %EE. Based on the %EE response, the concentration of soy lecithin, propylene glycol and ethanol can significantly reduce %EE with a p-value <0.0001. Soya lecithin is a former of ethosome vesicles so that the addition of high concentrations can cause the vesicles to be more flexible and cause leaks so that the %EE of active substances decreases (Estanqueiro et al., 2014). The interaction of propylene glycol and ethanol can significantly reduce the %EE of the active substance which can show a synergy effect to increase the permeability of ethosome vesicles (Barupal et al., 2010).

$$\%EE = 63,43 - 0,21A - 5,6B - 4,29C - 0,96AB + 4,11AC - 0,75BC$$

$$+ 1,66ABC \quad (3)$$

Description:

A: Soya lecithin concentration
 B: propylene glycol concentration
 C: ethanol concentration
 AB: interaction of soy lecithin with propylene glycol
 AC: interaction of soy lecithin with ethanol
 BC: interaction of propylene glycol with ethanol

3.6 Stability of Ethosomes Analysis

Ethosomes stability was performed to determine the resistance of ethosomes preparations to temperature changes. Ethosomes have vesicles that are tight, compact, and do not leak before testing. Giving heat treatment causes the vesicles to stretch because of the breaking of the bonds between the lipid constituents so that heat exposure can cause the vesicles to leak. Treatment at cold temperatures will cause the components that form the ethosome to form tightly back into aggregates, but the vesicles that have leaked and separated cannot return to the form of a complete ethosome. The extracts that had come out of these ethosome vesicles were measured for the ethosome stability of the petai pods. The results of decreasing levels of petai pods extract can be seen in Table 4.

Table 4. %EE Determination of Petai Pods Extract (*Parkia speciosa*) Loaded Ethosomes

Formulation	Mean \pm SD(%)	CV (%)
F1	74.326 \pm 0,079	0.107
F2	70.940 \pm 0,079	0.112
F3	69.755 \pm 0,079	0.114
F4	55.874 \pm 0,079	0.142
F5	62.363 \pm 0,079	0.127
F6	68.739 \pm 0,079	0.116
F7	48.144 \pm 0,079	0.165
F8	57.341 \pm 0,079	0.241

Table 5. Determination of percent reduction in flavonoid levels in stability test

Formula	Mean (%) ± SD	CV (%)
F1	7.288 ± 0.079	0.107
F2	4.295 ± 0.079	0.112
F3	11.891 ± 0.079	1.765
F4	11.412 ± 0.079	1.187
F5	19.634 ± 0.079	0.598
F6	10.918 ± 0.079	1.126
F7	15.237 ± 0.079	2.262
F8	7.970 ± 0.079	0.241

Based on the results of the response of all factors to the stability of the petai pods extract levels, it shows that soy lecithin can significantly increase ethosome stability. This is because soy lecithin is a component of ethosomes vesicles and does not leak easily. Propylene glycol and ethanol can increase the percentage reduction in the content of petai bark extract in the ethosome vesicles. This is because ethanol can increase permeability so that the petai pods extract can come out of the ethosome vesicles.

$$Y = 11,08 - 2,43A + 0,55B + 2,36C + 0,5AB - 1,56AC - 2,38BC$$

$$-0,13ABC \quad (4)$$

Description:

Y: decreased level response

A: Soya lecithin concentration

B: propylene glycol concentration

C: ethanol concentration

AB: interaction of soy lecithin with propylene glycol

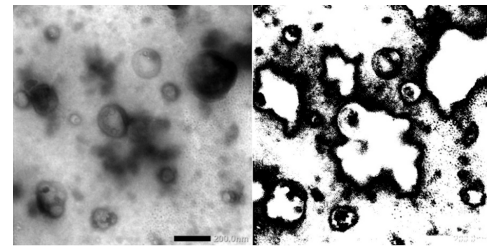
AC: interaction of soy lecithin with ethanol

BC: interaction of propylene glycol with ethanol

Table 6. The results of statistical analysis of predictive value of DX® 10 of formula ethosome

Evaluation	DX® 10 prediction	Experiment ± SD	p-value
pH	4.729	5.023±0.016	0.001
Viscosity	1	0.959±0.0015	0.008
%EE	73.816	73.761±0.138	0
Stability	7.801	7.200±0.104	0.389

Optimization of the optimum formula for petai pods extract loaded ethosomes was carried out using the DX® 10 program showed Table 6. The optimum formula for ethosomes were determined based on the analysis of the results of the response to pH, viscosity, %EE, and the stability of levels of petai pods extract in the ethosomes. There are 4 responses to determine the optimum formula, namely pH, viscosity, %EE, and the stability

**Figure 1.** Morphology Particles of Petai Pods Extract (*Parkia speciosa*) Loaded Ethosomes using TEM

of extract content. %EE has the criteria for determining the maximum optimum formula because of the amount of extract that can be absorbed into the vesicles. The higher the %EE, the more active substances are absorbed so that it can provide maximum activity. The highest %EE value is shown in formula 1, namely 74.326%. This is the use of soy lecithin with a concentration of 1% can form vesicles that are more stable in absorbing the extract. The stability of the decrease in extract content has a criterion of 5, the lower %value of decreased stability then more stable the preparation is. Formula 1 has a decreased percentage of extract content of 7,288%. The desired stability value is in a low range.

Particles diameter and distribution are important parameters of the ethosome vesicles. This parameter will affect the ability of the ethosomes to enter or penetrate into the skin (Limsuwan et al., 2017). The particles diameter resulted from the ethosome of the petai skin extract was 818.7 nm. Vesicles with a diameter of 810 can pass through the stratum corneum and deposit as much as 39.952% (Verma and Pathak, 2010). This indicates that ethosomal vesicles can accumulate in the skin.

The particle size distribution is determined by the polydispersity index value which indicates the uniformity of particle size. The polydispersity index value of petai pods extract loaded ethosomes was 0.231 indicating that there were 76.9% of the total number of particles in the ethosome formula of petai pods extract loaded ethosomes which had a homogeneous size. This ethosomes have a PDI value <0.5 which indicates that the diameter distribution of the ethosome particles tends to be homogeneous.

Zeta potential will show the charge size of a particle which that show an effect on the stability of the particles during storage. Stable zeta potential is represented by values greater than +25 mV or more than -25 mV. The zeta potential value of the petai pods extract loaded ethosomes is -7.5mV, the negative value obtained is influenced by the ethosome-forming components, which is contributed by the phosphate group of lecithin and oxygen atoms which are electronegative from the hydroxy group in the propylene glycol and ethanol components. The zeta potential value obtained indicates that ethosome particles have a tendency to form aggregates because of the low repulsion between particles.

The particle morphology produced by the petai pods extract loaded ethosomes shows a spheric (round) shape (Figure 1). The spheric particle form has the ability to enter the body's cells better than the rod shape (Chithrani and Chan, 2007). The spheric

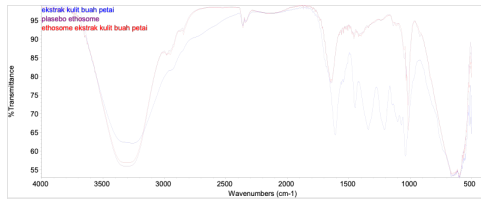


Figure 2. Expectation was physical interaction between extract and ethosomes using FTIR

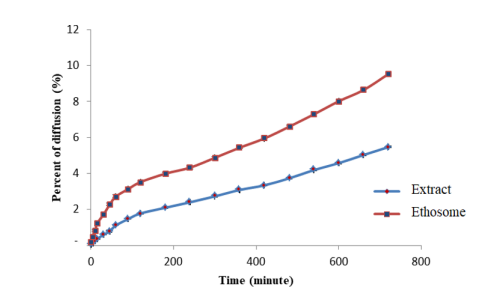


Figure 3. Graph Mean Percentage of Diffusion Extract Petai Pods dan Petai Pods Extract (*Parkia speciosa*) Loaded Ethosomes

shape is easier to penetrate into the body because the surface area of the particles in contact is wider than that of the rod.

The diffusion test was carried out to determine the amount of petai pods extract in ethosomes that could penetrate into the skin compared to the petai pods extract. The parameter used to determine the diffusion test is the percent diffusion. A high percent diffuse value may indicate that the extract can penetrate more deeply into the skin. The average result percent diffusion of petai pods extract loaded ethosomes was more than the petai pods extract result. This is due to the presence of ethosome vesicles which have deformability properties that can help the extract penetrate into the skin. Percent diffusion was analyzed using one way ANOVA with p -value < 0.05 , which indicates that there is a significant difference between the diffused percent of the extract and the petai pods extract loaded ethosomes (Figure 2).

The chemical interactions that occur between the ethosome formers and the petai skin extract were analyzed using FTIR. The intensity that appears in the spectra is influenced by the concentration of the sample. Based on the FTIR results, there was no interaction between placebo and extract, it can be seen that no new peaks were formed on the spectrum (Figure 3).

4. CONCLUSIONS

The vesicle-forming components of the ethosomes affect the response of the percent encapsulation and the stability of the extract which is seen with the percent decrease in levels. Ethosomes-forming compositions that can produce an optimal formula are

0.2 g of soy lecithin, 1 ml of propylene glycol, and 4 ml of ethanol. The ethosomes optimum formula produces a higher diffuse percent compared to petai pods extract.

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