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Ethanol extract of *Parkia speciosa* Hassk. loaded transfersome: Characterization and optimization

[Transfersoma cargado con extracto etanólico de *Parkia speciosa* Hassk.: caracterización y optimización]

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Abstract

Context: *Parkia speciosa* is a species which contains hydrophilic compounds such as flavonoids and phenolics. These compounds are difficult to penetrate lipophilic biological membranes. Transfersome is a targeted dosage form that can overcome the limitations of phytomedicine.

Aims: To improve the ability of extracts to penetrate biological membranes.

Methods: Transfersome was formulated with soya lecithin and Tween-80 as independent variables using factorial design 3^2. The influence of factor compositions and factor interactions on transfersome characteristics was observed based on efficiency absorption percentage (%EE), polydispersity index (PDI), stability, and pH.

Results: Analysis with DX®10 produced optimum formula with 0.85 g of lecithin and 0.15 g of tween-80. The optimum formula characteristics' results analysis was as follows: %EE (91.6884 ± 0.0261%), particle size (495.6 nm), PDI (0.484), and zeta potential (-21.4 mV) and spherical vesicle shape. Stability testing showed that transfersome was more stable compared to the pure extracts. Diffused transfersome percentage (6.6253 ± 0.5817 %) on the minute 360 was better than the pure extracts (1.8800 ± 0.0187 %). Compartmental transfersome analysis with WinSAAM™ software followed lag compartment model ($p<0.05$). Interaction test using FTIR showed that there was no chemical interaction between extract and excipients.

Conclusions: The finding result in this investigation shows that transfersome loaded with ethanol extract of *Parkia speciosa* is more stable and more easily diffused compare to the pure extract.

Keywords: factorial design; *Parkia speciosa* Hassk; soya lecithin; transfersome; Tween-80.

Resumen

Contexto: *Parkia speciosa* es una especie que contiene compuestos hidrofílicos como flavonoides y fenoles. Estos compuestos son difíciles de penetrar en las membranas biofilofílicas. Transfersoma es una forma de dosificación dirigida que puede superar las limitaciones de la fitomedicina.

Objetivos: Mejorar la capacidad de los extractos para penetrar las membranas biológicas.

Métodos: Transfersoma se formuló con lecitina de soja y Tween-80 como variables independientes utilizando el diseño factorial 3^2. La influencia de las composiciones de factores y las interacciones de los factores en las características de los transfersomas se observó en función del porcentaje de absorción de eficiencia (%EE), índice de polidispersidad (PDI), estabilidad y pH.

Resultados: El análisis con DX® 10 produjo una fórmula óptima con 0,85 g de lecitina y 0,15 g de Tween-80. El análisis de resultados de las características óptimas de la fórmula fue el siguiente: %EE (91,6884 ± 0,0261%), tamaño de partícula (495,6 nm), PDI (0,484) y potencial zeta (-21,4 mV) y forma de vesícula esférica. Las pruebas de estabilidad mostraron que el transfersoma era más estable en comparación con los extractos puros. El porcentaje transferido difuso (6,6253 ± 0,5817%) en el minuto 360 fue mejor que los extractos puros (1,8800 ± 0,0187%). El análisis de transferencia de compartimentos con el software WinSAAM ™ siguió el modelo de compartimiento de retraso ($p<0,05$). La prueba de interacción con FTIR mostró que no había interacción química entre el extracto y los excipientes.

Conclusiones El resultado del hallazgo en esta investigación muestra que el transfersoma cargado con extracto etanólico de *Parkia speciosa* es más estable y se difunde más fácilmente en comparación con el extracto puro.

Palabras Clave: diseño factorial; lecitina de soya; *Parkia spaciosa*; transfersoma; Tween-80.

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INTRODUCTION

Herbal medicines have been used for generations on treating and curing illness. The important role of herbal medicine is recognized in the health community (Calixto, 2000). *Parkia speciosa* (local name: petai) is known herbal medicine plant which grow in Indonesia, Malaysia, Philippines and Thailand (Zaini and Mustaffa, 2017). Petai pod is mostly wasted although it has great potential due to flavonoid and phenolic content, which can be used as free radical antidote and as medication of some common disease. Petai was reported to have hypoglycemic effect (Jin and Noor, 2014), antioxidant and antiangiogenic (Aisha et al., 2012; Kamisah et al., 2013), antimicrobial, antitumor and antihypertension (Siow and Gan, 2013) and antiulcer (Al-Batran et al., 2013). Pharmacological activity of petai pod extract presumably originated from secondary metabolite such as flavonoid, phenolic and tannin (Kamisah et al., 2013). However, herbal medicine has limitation of a low solubility in lipid which make it hard to penetrate biological membrane. This limitation can be solved by converting the extract into transfersome (Giriraj, 2011).

Transfersome is a flexible vesicular consist of aqueous compartment surrounded by lipid bilayer. Transfersome has both hydrophobic and hydrophilic part which can be used to deliver drug in various solubility condition. The high deformability of transfersome make it able to pass through pore or slit and back to initial shape without changing its size. Transfersome can be used to deliver drug both topical and systemic (Singh, 2013).

The objective of this study was to prepare transfersome containing ethanol extract of *Parkia speciosa* Hassk. (*Fabaceae*) pod in various Tween-80 and soya lecithin weight. Transfersome was formulated by preparing phospholipid as vesicle precursor, surfactant to improve flexibility, alcohol as solvent and buffering agent as hydration medium of transfersome (Giriraj, 2011).

The optimum condition of formula was determined based on entrapment efficiency (%EE), thermodynamic stability test, pH and polydispersity index (PDI) using Design Expert®10. Transfersome made by optimum formula was characterized to determine its particle size, PDI, zeta potential, stability and diffused percentage.

MATERIAL AND METHODS

Chemicals and reagents

Materials used in this research were pod of *Parkia speciosa* Hassk. fruit (harvested from Semeteh village, Musi Rawas). All chemicals used in this study were analytical grade.

Extract preparation

Petai pod was collected in Musi Rawas districts (2.90°S, 103.28°E), South Sumatera, Indonesia. This vegetation (petai) has been identified by Indonesian Institute of Science with Register No. 218/IPH.06/HM/I/2018. Fresh petai pod was washed and dried under the sun. The sample (1 kg) was grinded and then extracted in maceration process by using 6 L ethanol 70% for 48 hours. The maceration process was repeated twice for the residue at same duration (48 hours). The macerated liquids from the process was mixed, filtered and evaporated in rotary evaporator (Yamato®) at 70°C. The ethanol extract of petai pod was collected after all solvent was evaporated and labelled as EEPP.

Preparation of transfersome

Transfersome EEPP formula was design base on the weight of lecithin and Tween-80. The transfersome formula was designed with factorial design 3^2 using the DX®10 software. This formula was referred to the research by Wonghirudencha et al.(2014) and Tejaswini et al.(2016) as shown on Table 1.

Transfersome of EEPP was prepared by thin layer hydration method (Tejaswini et al., 2016; Venkantesh et al., 2014). Lecithin, Tween-80 and EEPP were mixed in ethanol solvent at 60°C in water bath. Phosphate buffer 25 mL, pH 7.4 was added into the mixture and then homogenized

Table 1. Transfersome formula.

Composition Formula	F1	F2	F3	F4	F5	F6	F7	F8	F9
EEPP (%)	4	4	4	4	4	4	4	4	4
Lecithin (mg)	0.80	0.80	0.85	0.85	0.90	0.80	0.90	0.90	0.85
Tween-80 (mg)	0.10	0.15	0.15	0.20	0.15	0.20	0.20	0.10	0.10
Ethanol 96 % (mL)	8	8	8	8	8	8	8	8	8
Phosphate buffer pH 7.4 (mL)	ad 25	ad 25	ad 25	ad 25	ad 25	ad 25	ad 25	ad 25	ad 25

using magnetic stirrer (IKA®C-MAG HS 4) for 1 hour at 60 rpm. Transfersome preparation was left for 24 hours at room temperature and then reduce its particles size by using sonicator (GT SONIC®) for 30 minutes at 40°C.

Transfersome was separated and purified using centrifugation technique (Centrifuge DLAB©: D2012 PLUS) at 10,000 rpm for 90 minutes and 25°C. Precipitate and supernatant was separated. The resulting precipitate was stored in refrigerator (SANYO®) while supernatant was used to determine the active ingredient percentage within transfersome (Aukunuru et al., 2009).

Transfersome characteristics

Encapsulation efficiency percentage (%EE)

Flavonoids were identified by thin layer chromatography. Quercetin compounds was used as marker. The encapsulation efficiency percentage (%EE) was determined by measuring transfersome supernatant with spectrophotometer UV-Vis (Biobase®BK-UV1900PC) at 434 nm wavelength. The value of absorbed drugs was measured using flavonoid quercetin as marker and calculated by following formula [1] (Singh, 2013).

$$\%EE = \frac{\sum \text{flavonoid in formula} - \sum \text{flavonoid in supernatant}}{\sum \text{flavonoid in formula}} \times 100\% \qquad [1]$$

Accelerated stability test

The stability test was referred to (Venkantesh et al., 2014; Loong et al., 2014) with a modification. The test was carried out by storing transfersome preparation at 4°C for 24 hours and then at 40°C for another 24 hours (1 cycle), for at least 6 cycles. At the initial and the end of cycles, flavonoid within transfersome preparation was measured. The decrease content of flavonoid was calculated by using following formula [2]:

$$\%\text{Content decrease} = \frac{\text{initial flavonoid} - \text{final flavonoid}}{\text{initial flavonoid}} \times 100\% \qquad [2]$$

pH measurement of transfersome preparation

The pH of transfersome suspension was measured by using digital pH-meter (Lutron® pH electrode PE-03) at room temperature (Tejaswini et al., 2016).

Optimization of transfersome formula

Transfersome optimum formula was determined by using software DX®10 (Stat-Ease Inc.) according to desired response criteria and importance i.e. %EE, pH and stability (% content decrease). The desired response criteria are displayed on Table 2. Importance was set on the highest %EE because it's a vital criterion to find out how much of active ingredient (flavonoid) was encapsulated in transfersome.

Table 2. Response criteria.

Response	Target	*Low limit*	*Upper limit*	*Importance*
EE (%)	*Maximize*	93.5732	95.1439	+ + + + +
PDI	*Minimize*	0.1000	0.6000	+ + + +
pH	*In range*	4	7	+ + +
% content decrease	*Minimize*	3.5659	11.1931	+ + +

Characterization of transfersome optimum formula

Determination of diameter, PDI and zeta potential

Particle size and particle size distribution (PDI) were measured by using dynamic light scattering method and particle size analyzer/PSA (Horiba Scientific® SZ-100).

Identification of Interaction using FTIR spectrophotometer

Possible chemical interaction between excipients and EEPP within transfersome was detected by FTIR spectrophotometer (Thermo Scientific™ Nicolet™ iS™ 10) (Tejaswini et al., 2016).

In vitro diffusion test

Franz diffusion cell (Prima Medicha®) was used in transfersome *in vitro* diffusion test. The receptor compartment consisted of 14.5 mL phosphate buffer pH 7.4 (Merck®) at 37 ± 0.50°C stirred in 100 rpm on magnetic stirrer (IKA®C-MAG HS4). Cellophane membrane (Merck®) was placed between donor and receptor compartment. Donor compartment was filled with EEPP transfersome, 2 mL phosphate buffer (pH 7.4) was added and stirred continuously using magnetic stirrer. Samples were collected from receptor compartment after certain duration i.e. 0, 5, 10, 15, 30, 60, 90, 120, 180, 240, 300 and 360 minutes, then 4 mL phosphate buffer (pH 7.4) was added on each sample collection to maintain sink condition (Singh, 2013). The number of flavonoids diffused per unit of time was measured by taking aliquot from sample solution and analyzed by using spectrophotometer UV-Vis (Biobase® BK-UV 1900PC).

Transferosome morphology

Transfersome morphology was determined by using the Transmission Electron Microscope (TEM) (Jeol® JEM 1400, USA). The transfersome solution (50 µL) was diluted with aquadest 100 times. A total of 50 µL of liquid solution was applied to the slide. Analysis of the sample was by using focusing digital microscope with 10-30 kV voltage.

Data analysis

Data was analyzed by using software DX®10(Stat-Ease Inc.), Minitab®17 and SPSS®17 (one sample t-test). The results of $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Petai pod extraction was carried out by maceration method to prevent damage of active ingredient due to heating process. The extract produced from 1 kg of petai pod sample was 257.100 g (25.71% w/w). This extract was used as active ingredient in transfersome formula. The EEPP transfersome formula was design by using software DX®10 (Stat-Ease Inc.) in 3^2 factorial design. There were nine formulas and their characteristics were shown on Table 3.

Analysis of variance results on the 9 formulas were conducted using DX®10 (Stat-Ease Inc.) and shown on following equations [3-6].

$$Y = +94.35 - 0.30(A1) + 0.36(A2) - 0.0077(B1) + 0.35(B2) + 0.0860(A1B1) - 0.11(A2B1) - 0.40(A1B2) + 0.060(A2B2) \quad [3]$$

$$Y = +6.66 - 0.068(A1) + 0.0077(A2) + 0.021(B1) - 0.00556(B2) + 0.0022(A1B1) - 0.073(A2B1) - 0.018(A1B2) + 0.047(A2B2) \quad [4]$$

$$Y = +6.67 + 0.81(A1) + 0.93(A2) - 0.063(B1) + 1.93(B2) + 0.22(A1B1) + 0.48(A2B1) + 1.75(A2B1) - 1.38(A2B2) \quad [5]$$

$$Y = +0.41 - 0.0566(A1) + 0.011(A2) - 0.0222(B1) + 0.011(B2) - 0.033(A1B1) + 0.067(A2B1) - 0.033(A1B2) + 0.000(A2B2) \quad [6]$$

Remark:

Y	= response %EE (equation 3)
	= pH (equation 4)
	= stability (drug content decrease) (equation 5)
	= PDI (equation 6)
A	= number of Tween-80
B	= number of soya lecithin

Table 3. Analysis results of physical properties of transfersome.

Formula	% EE	% CV	pH	% CV	% content decrease	%CV	PDI (%) ± SD	% CV
1	94.123 ± 0.026	0.027	6.620 ± 0.010	0.151	7.629 ± 0.062	0.817	0.300 ± 0.173	57.735
2	94.594 ± 0.026	0.027	6.620 ± 0.010	0.151	8.007 ± 0.030	0.375	0.466 ± 0.057	12.371
3	95.126 ± 0.015	0.015	6.713 ± 0.011	0.172	8.136 ± 0.318	3.913	0.433 ± 0.288	66.617
4	94.986 ± 0.026	0.027	6.690 ± 0.026	0.395	6.494 ± 0.040	0.621	0.5000± 0.100	20.000
5	94.419 ± 0.015	0.016	6.683 ± 0.011	0.172	6.635 ± 0.031	0.474	0.366 ± 0.251	68.634
6	94.315 ± 0.039	0.042	6.816 ± 0.005	0.084	4.172 ± 0.062	1.495	0.400 ± 0.200	50.000
7	93.590 ± 0.015	0.016	6.66 ± 0.015	0.229	4.130 ± 0.042	1.019	0.466 ± 0.230	49.487
8	94.009 ± 0.054	0.057	6.596 ± 0.035	0.532	3.638 ± 0.082	2.279	0.433 ± 0.152	35.250
9	94.000 ± 0.015	0.016	6.573 ± 0.005	0.087	11.148 ± 0.040	0.366	0.333 ± 0.057	17.320

Values are written as mean ± SD of percent entrapment, pH, percent depletion and PDI value. There were no significant differences between formulas in pH ($p = 0.002$), percent entrapment ($p = 0.000$) and percent reduction in levels ($p = 0.000$) but there were significant differences in PDI values ($p = 0.999$).

Equation 3 through 6 indicates Tween-80 factor reduced %EE, pH, PDI and increased the stability. Meanwhile soya lecithin factor increased %EE, stability and PDI but reduced pH. The correlation of all responses was analysis by SPSS®17 using Spearman correlation and the results were shown on Table 4.

Table 4. Spearman correlation analysis.

Relation	Spearman correlation	*p-value*
pH – PDI	0.255	0.199
pH – stability	-0.260	0.191
pH – %EE	0.502	0.008
PDI – stability	-0.129	0.521
PDI – %EE	0.153	0.446
Stability – %EE	0.361	0.064

Correlation analysis showed that pH and PDI response to stability response was negative, which means the increase in pH and PDI will reduce the transfersome stability. On contrary, if the response correlation was positive, the increase of one response would increase other response as well.

Optimum formula obtained from DX®10 (Stat-Ease Inc.) was 0.85 g lecithin and 0.15 g Tween-80 and result of response test against optimum formula of EEPP transfersome was shown on Table 5.

Comparative analysis one sample t-test was carried out against predicted data and research data analyzed by SPSS®17.

Test result of optimum formula suggested a high percentage of entrapment efficiency (Kaur and Saraf, 2011). PDI value showed the sufficiently distributed EEPP transfersome dispersion indicated by PDI value less than 0.5 (Shah et al., 2014). The percentage of content decreased was small (3.655%), which suggest transfersome formula was stable. This fact was supported by analysis result of zeta potential of vesicle quite large (-21.4 mV). pH value was found 6.713.

Calculation on residual standard error (RSE) which represent error level was conducted to make sure the accuracy of predicted data with research data using equation as follows [7].

$$\% \text{ RSE} = \frac{\text{research data} - \text{predict data}}{\text{research data}} \times 100\% \quad [7]$$

Comparison of predictive data and research data are shown in Table 5. The smaller the value of %RSE suggesting the more accurate the result because it is closer to the predicted value (Loong et al., 2014). The highest %RSE value was shown by stability percentage of content decreased. This finding reflects nonconformity between predicted data and research data, but percentage of content decreased from research data was smaller than

predicted data. Response of %EE, PDI and pH confirmed that the research prediction given by DX®10 (Stat-Ease Inc.) program was proven.

Table 5. Comparison of predictive data with research data.

Response	Predictive	Research ± SD	%RSE
%EE	95.126	91.688 ± 0.026	3.613
%Content decrease	8.136	3.655 ± 0.017	55.066
PDI	0.433	0.484	0.000
pH	6.713	6.896 ± 0.011	0.027

Diameter analysis, polydispersity index, zeta potential and particle morphology

The average particle size from PSA analysis was 495.6 nm, this size was within interval for liposome modified at nanometer to micrometer. PDI value for transfersome optimum formula was 0.484 which was below 0.5. According to (Shah et al., 2014) this value represents homogeneous distribution of transfersome particle size. Surface charge of a particle is measured by zeta potential. Zeta potential value +25 or -25 reflects surface stability within suspension which prevent particles from aggregation (Mohanraj and Chen, 2006). Zeta potential for EEPP transfersome was -21.4 mV. The spectra of zeta potential are shown in Fig. 1. This value indicated good stability of EEPP transfersome. Negative charge was resulted from soya lecithin, with high proportion of isoelectric point between 6 and 7 whereas preparation environment had pH 7.4. The low isoelectric point of soya lecithin compares to environment resulted on the negative charge of transfersome surface. This negative charge improved transfersome dermal penetration because keratin component also had negative charge as indicated by isoelectric point 5.3 (Duangjit et al., 2011). Analysis of transfersome particle was carried out using TEM at 100 kV voltage which produced magnification up to 150,000 times. TEM image showed particle of transfersome forms spherical shape. According to (Pal et al., 2007), spherical particle has round edge hence penetrates membrane easily. Fig. 2 depicts transfersome preparation has dark edge layer represent lipid bilayer consist of soya lecithin and Tween-80 with petai pod extract encapsulated within the sphere.

Chemical interaction study analysis by FTIR

The possibility of chemical interaction can be detected by evaluating FTIR spectra of soya lecithin, Tween-80 and extract. Chemical interaction occurred if new chemical bond forms and confirmed by new functional group on FTIR spectra. Fig. 3 shows no indication of chemical interaction between extract and excipient confirm by similar peaks on spectra.

Accelerated stability of transfersome optimum formula

Transfersome stability was tested in heating-cooling cycle method. The test would confirm whether the transfersome withstand against extreme change of temperature (Anusha et al., 2014). Decrease in flavonoid content was calculated at each cycle so that transfersome stability can be evaluated. Analysis was conducted using Spectrophotometer UV-Vis at 434 nm wavelength.

The decrease in flavonoid content due to temperature change occurred since cycle 0 to cycle 6 as shown in Fig. 4. Overall, there was significant decrease in flavonoid content between transfersome and pure extract ($p<0.05$). It was first observed in cycle 1 ($p=0.022$). In cycle 2, there was no significant difference in flavonoid content between transfersome and pure extract ($p=0.055$). In cycle 3 to cycle 6, there were significant decrease in flavonoid content ($p<0.05$). Pure extract showed bigger decrease in flavonoid content compare to transfersome which indicated better stability of transfersome.

In vitro diffusion test

Penetration ability of EEPP transfersome was evaluated and compare to pure extract through diffusion test. Synthetic cellophane membrane was used during the test due to its similarity with skin membrane (Patel et al., 2009; Parveen et al., 2014). Difference in diffusion profile between pure extract and transfersome within buffer phosphate pH 7.4 condition is displayed on Fig. 5.

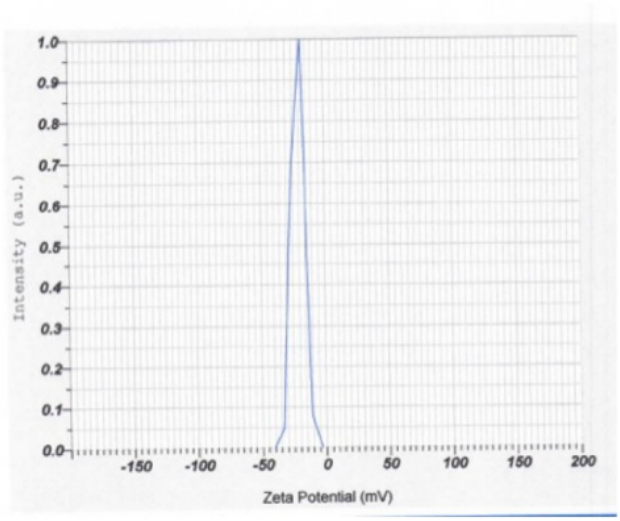

Figure 1. Spectra of Zeta potential.

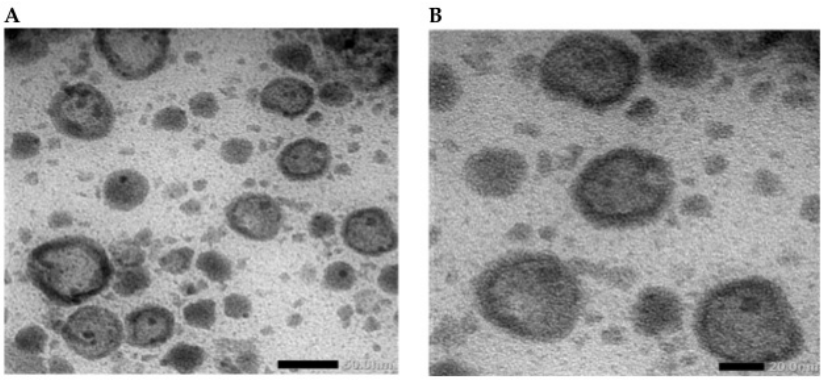

Figure 2. Transfersome morphology image created by TEM at 80,000× **(A)** and 150,000× **(B)**.

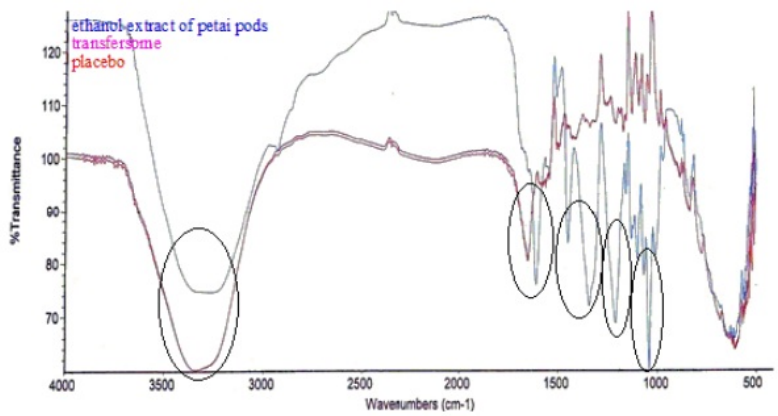

Figure 3. FTIR Spectra of transfersome and pure extract.

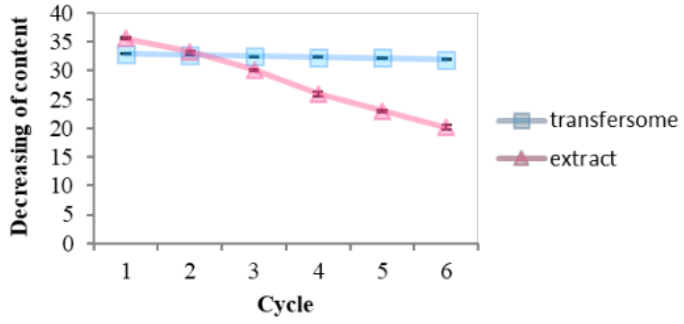

Figure 4. Content decrease within transfersome loaded with EEPP.

Value showed as mean ± SD with p value cycle 1 (p=0.002), cycle 2 (p=0.055), cycle 3 (p=0.003), cycle 4 (p=0.001), cycle 5 (p= 0.000) , cycle 6(p=0.000).

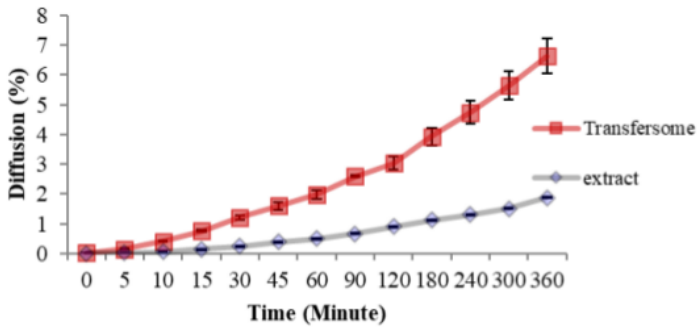

Figure 5. Diffusion profile of optimum formula transfersome EEPP and pure extract.

Value showed as mean ± SD with p value=0.000.

The total of transfersome of ethanol extract of petai pod diffused after 6 hours provided higher penetration ability (6.6253%) compare to pure extract (1.8800%). Transfersome's small particle size i.e. 495.6 nm along with its vesicular deformability resulted in the ability of transfersome to decrease its size down to tenth of original size hence gave better penetration ability (Jain et al., 2014).

Compartmental analysis of diffusion result

Compartmental analysis of diffusion result was conducted using software WinSAAM™ to evaluate pharmacokinetic parameter. The compartmental model of pharmacokinetic parameter was predicted in two models i.e. two-compartments (non-lag time) and three-compartments (lag time).

Compartmental analysis conducted on pure extract and EEPP transfersome was aimed to compare each of compartmental models. Correlation analysis was implemented on Qo data (research data) and Qc (predicted data) using SPSS®17. Correlation analysis was to compare the data Qo and Qc. Correlation analysis was done by Pearson correlation analysis. Transfersome correlation analysis showed that the Pearson correlation value for two-compartments was 0.929 and three-compartments was 0.989. While the Pearson correlation value for extract two-compartments was 0.895 and three-compartments was 0.993. The highest correlation value indicated the stock compartment model. Therefore, it was concluded that the extract and transfersome to follow the three-compartments model (lag time model).

Three-compartments model were pharmacokinetic compartment model which experienced lag time during absorption process and passed three compartments throughout diffusion. Lag time was a delay time between drug administration and

drug presence in the systemic circulation (Ikasari et al., 2015). Three-compartment model of EEPP transfersome and extract means both preparations had lag time on its penetration process. The lag time was required for absorption process in penetration and then entering the systemic circulation. Topical preparation generally must be pass through three compartments: surface of skin, viable epidermis layer and capillary vessel (Ikasari et al., 2015).

CONCLUSIONS

Ethanol extract of petai pod loaded transfersome made with composition of lecithin (0.85 g) and Tween-80 (0.15 g) shows high entrapment efficiency percentage (91.688%) and better stability compare to pure extract. Vesicular transfersome forms spherical shape with enhanced diffusion percentage of pure extract. The diffusion result compartment model analysis showed that the transfersome followed the three-compartments model (lag time model).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Fitrya F	Fithri NA	Winda M	Muharni M
Concepts or ideas	x			
Design	x	x		
Definition of intellectual content	x	x		
Literature search	x	x	x	x
Experimental studies	x	x	x	
Data acquisition	x	x	x	
Data analysis	x	x	x	
Statistical analysis	x		x	x
Manuscript preparation	x			
Manuscript editing	x			x
Manuscript review	x	x	x	x

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