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Physical Evaluation of Transfersome that Contains Pandan Leaves Extract (*Pandanus amaryllifolius* R.)

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Abstract

Pandan leaves have been researched and have effectiveness in the treatment of burns. The process of healing burns takes a long time and cause a hard tissue because it loses its elasticity, making it difficult to penetrate. In this study, pandanus leaves were formulated into the nanovesicle carrier system, namely trasfersom. Transfersomes have the ability to deform, namely the ability to reduce the particle size 5-10 times from the original size when passing through the gaps between cells so that transfersom can increase the penetration of active substances. The three formulas used are based on the ratio of concentrations of trasfersome vesicles, namely phospholipids and span 80. Formula 1 is (90:10), Formula 2 (85:15) and Formula 3 (80:20). The best formula is determined based on transfersom characterization, including particle size and PDI (solidispersity index), zeta potential, entrapment efficiency, deformability, and TEM particle morphology. The results showed that Formula 3 (80:20) is the most stable formula with an average particle size of 730.1 ± 4.9 nm, PDI value <0.7, zeta potential - 9.94 ± 1.02 mV, efficiency absorption 80.23%, and the deformability value 6.225.

Keywords: Pandan Leaves, Transfersome, Nanoparticle, burns, Span 80

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1 Introduction

Pandan leaves are usually used as a spice, flavoring agent, natural green dye and fragrance in foods and used as a raw material for making perfume [1]. Pandan leaves have effectiveness in healing burns [2]. The content of compounds in pandan leaves that play a role in healing burns includes flavonoids, saponins, tannins and alkaloids.

Burned skin requires a long healing time due to tissue damage that makes it more difficult for active substances to penetrate [3]. Skin that is affected by burns in the healing process will experience hardening due to decreased elasticity [4], [5]. So, in order to penetrate into the skin which has a different structure because it is undergoing healing, it is necessary to use the Transfersome carrier system.

Transfersome is a nanovesicle carrier system which has a wide solubility range because it is composed of hydrophilic and hydrophobic groups [6]. Transfersome constituent components are phospholipids, surfactants and water [7]. Transfersome vesicles are more elastic than liposomes and are therefore suitable as skin penetrating agents [8]. Transfersome has a deformability capability that is able to reduce its size 5 to 10 times without losing its shape so that it can deliver active substances to penetrate through the stratum corneum, or reach target sites including the dermis and blood circulation [9].

The deformability of transfersome can be related to the composition of the vesicles used. This study aims to characterize the transferome formula for pandan leaf extract based on the concentration ratio of the constituent trasfersome vesicles, namely phospholipids and span 80.

2 Materials and Methods

2.1 Instruments

TEM (JEOL JEM-1010), maceration bottles, micro pipettes, refrigerators (Samsung), membrane filters, ovens, Particle size analyzer / Zetasyzer (Malvern), pH meter (Ohaus), refrigerator, Rotary evaporator (IKA), sonicator bath (Branson), centrifugator (Cole Parmer), spatels, UV-Vis Spectrophotometer (Jasco V-730), syringes, analytical scales (LabPRO), and Vacuum drying.

2.2 Materials

Pandan leaves, Phospolipon 90H (Giving GmbH German Lipoid), Span 80, Ethanol p.a, methanol p.a, Chloroform p.a, Aqua pro injection, Sodium Hydroxide (NaOH), Potassium dihydrogen phosphate (KH₂PO₄).

2.3 Extraction

Pandan leaves extract was made by maceration method. A total of 250 g of simplicia powder was put into the chocolate flask using 10 times the 70% ethanol extractor of the amount of simplicia to be macerated. The simplicia was soaked and stirred for 30 minutes then left for 24 hours, then filtered the filtrate. Remaceration was carried out for 3 days. The resulting filtrate was poured for 24 hours then vacuum dried until a dry extract was obtained.

2.4 Preparation of Transfersome Containing Pandan Leaf Extract

The transfersome was made into three formulas with variations in the concentration of phospholipon 90H and span 80. The transfersome was made using a thin layer hydration method. A number of phospholipids (phospholipon 90H) and span 80 and dissolved with a mixture of chloroform methanol (2:1), then to the mixture is added 50 mg of dry extract of pandan leaves. Furthermore, the solution is evaporated using a rotary evaporator at a temperature of 56°C at a speed of 60-150 rpm to remove organic solvents, the flask is left to stand for 24 hours to complete the formation of vesicles. The thin film formed is hydrated using a 100 mL phosphate buffer solution pH 7.4 at 60 rpm for 30 minutes. The suspension formed was collected into the vial and sonicated for 30 minutes using a sonicator bath.

Table 1. The formula for transfersome		
Formula	Fosfolipid:Span 80	
1	90:10	_
2	85:15	
3	80:20	

2.5 Characterization of the Transfersome Particle Size Distribution, Polydispersity Index and Zeta Potential

The particle size distribution uses a PSA (Particle Size Analyzer) based on the principle of light scattering at 25°C to determine the particle size and zeta potential of the transfersome formed. The base line used is 10 mL of aquadest solution, the sample is inserted into the cuvette for measuring particles and the polydispersity index. Furthermore, for zeta potential measurement, the sample is inserted dropwise into the fluid tank to the expected concentration. After that, transferome globules will be measured.

2.6 Entrapment Efficiency

The measurement of the entrapment efficiency was carried out using the UV-Vis Spectrophotometric calibration curve method. Previously, a quercetin calibration curve was made first with a series of 2,4,6,8, and 10 ppm using pure quercetin. The absorption of each concentration series was measured using a UV-Vis spectrophotometer at the maximum wavelength of quercetin which was obtained from the determination of the maximum wavelength, then the value obtained was entered into the linear regression equation. The regression curve is considered linear if the value of r is close to 1. The entrapment efficiency (EE) test was carried out using a centrifugator at a speed of 3,400 rpm with the aim of separating the transfersome suspension of pandan leaf extract into two parts, namely the supernatant and sediment. The free pandan leaf extract will be in the supernatant, the absorption of the supernatant was measured using a UV-Vis Spectrophotometer. The content of the active substance was determined through the absorption data obtained [10].

2.7 Deformability

The deformability test was measured by the extrusion method. The filter membrane was used for the extraction of 450 nm pore-sized transfers for 5 minutes. Furthermore, the instructed volume was measured [11].

2.8 Morphology

The instrument used to see particle morphology is the Transmission Electron Microscope (TEM). The work was carried out by dropping one drop of the sample on a carbon coated copper grid and waiting for 30 seconds, dropping the sample with uranyl acetate and waiting for 5-10 minutes at room temperature to dry, then observing it with a TEM tool.

3 Results and Discussion

3.1 Extract Preparation

The pandan leaf simplicia powder obtained was 825 grams with a yield value of 16.5%. The simplicia powder of wangi pandan leaves was made into extracts by maceration method using 70% ethanol solvent [2]. The macerated filtrate is made into dry extract by evaporating using a vacuum dry tool. The dry extract obtained was 41.20 grams so that the dry extract yield value was 16.48%.

3.2 Transfersome Characterization Particle Size Distribution and Polydispersity Index

Transfersome particle size is measured using a Particle Size Analyzer (PSA) with the Dynamic Light Scattering (DLS) type. The DLS method is a method for measuring particle size in liquids based on the principle of brown particle motion, where each in the suspension/solution will move randomly due to collisions with the surrounding liquid molecules. Dynamic Light Scattering can determine the average particle size distribution followed by the polydispersity index (PDI) value.

The Polydispersity Index (PDI) is an index of the heterogeneity of a collection of particles. The average PDI value obtained at F2 and F3 <0.7. The PDI value of F2 and F3 can be said to be good because it falls within the middle range of the polydispersity index, while F1 has a PDI value> 0.7 which indicates that the sample has a broad and inhomogeneous particle size distribution.

In the PSA (Particle Size Analyzer) test, readings were carried out 3 times for each formula and then the average was taken from each formula. Table 2 shows the results of the particle size distribution and the polydispersity index.

Table 2. Particle size and polydispersity index oftransfersome suspension

transfersonie suspension				
Parameter	Formula 1	Formula 2	Formula 3	
	(90:10)	(85:15)	(80:20)	
Particle Size	893.33±36.7 nm	753.87±24.5 nm	730.1±4.9nm	
PDI	0.827±0.1838	0.4543±0.0211	0.46±0.0635	

The average value of the three formulas is still in the nanoparticle size range, namely 1-1000 nm. Based on the results obtained, Formula 1 has a larger particle size than Formula 2 and Formula 3 so that the order of the transfersom formula from the smallest particle size is F3<F2<F1. Formula 1 has a larger particle size than other formulas because the concentration of phospholipids used in formula 1 is more, besides formula 1 has a PDI value> 0.7 which indicates that formula 1 has an inhomogeneous distribution.

3.3 Zeta Potesial

The purpose of zeta potential measurement is to characterize the surface charge properties of a particle. Particles are considered stable if they have a zeta potential value greater than ± 30 mV, because the repulsive force between particles with the same charge can avoid aggregation so that they will not combine into larger particles [12].

The measurement results obtained show that F1, F2, and F3 have a negative zeta potential value with a range -5.51 mV to -9.94 mV which indicates that the sample is not stable.

Table 3. Zeta Potential Suspension Transfersom

Formula	Zeta Potensial (mV)
1 (90:10)	-5,51 ± 0,69
2 (85:15)	-8,803 ± 0,78
3 (80:20)	$-9,94 \pm 1,02$

3.4 Entrapment Efficiency

3.4.1 Preparation quercetin calibration curve

Quercetin calibration curve was made using the UV-Vis spectrophotometric method. The maximum wavelength of quercetin obtained is 434.5 nm. The calibration curve is made a series and the obtained absorption results are made a linear regression equation, namely y=0.054045x+0.07413 with a correlation percent of 0.988.

3.4.2 Determination of flavonoid levels of pandan leaf dry extract

Determination of flavonoid levels was carried out first before the absorption efficiency test was carried out, which aims to determine the levels of flavonoids in pandan leaf extract. The results obtained in 50 mg of pandan leaf dry extract containing 58.34% flavonoids or 29.17 mg.

3.4.3 Etrapment Efficiency

The absorption efficiency is measured to determine the amount of active substance that has been trapped or absorbed in the transfersom vesicle. Table 4 shows the results of the absorption efficiency of the transfersome suspension.

Table 4. The absorption efficiency of transfersomsuspension

Formula	Entrapment Efficiency (%)
1 (90:10)	87,88
2 (85:15)	82,76
3 (80:20)	80,23

Results of measuring the absorption efficiency of pandan leaves transfersome with various concentrations of phospholipids and span 80 showed good results. Based on these data it is known that formula 1 with a concentration of 90:10 between vesicles and surfactants has a higher trapping efficiency value because formula 1 contains more phospholipids, which is 90% of the total vesicles. The increase in the amount of phospholipid will make the transfersom membrane stiffer which will increase the encapsulation [13]. The surfactant does have a role in flexibility in transfersom but if the phospholipid composition is less, the increase in the amount of surfactant can decrease the absorption efficiency.

3.4.4 Deformability

The deformability test is an important and unique parameter in the characterization of transfersome because it is this parameter that distinguishes transfersome from other nanovesicles. Deformability test was used to measure the flexibility value of transfersome [14]. Table 5 shows the results of the deformability test

Table 5. Deformability Index

Formula	Deformability Index
1	5.301
2	5.792
3	6.225

Formula 3 has a deformability index that is greater than formula 1 and formula 2 because the concentration of surfactants used in formula 3 is higher. The greater the surfactant used, the greater the deformability index [11]. The higher the deformability index value, the better the flexibility of transfersome to pass through smaller pores.

3.4.5 Morfologi Vesikel Transfersome

The results of observations with the TEM tool were carried out to show an overview of the vesicle shape of the transfersome and through this observation, the aggregation between transfersome nanovesicles will also be seen [15]. The sample for which the TEM test was carried out was formula 3 with a particle size of 730.1±4.9 nm, a PDI value <0.7, an absorption efficiency of 80.23% and a deformability value of 6.225.



Figure 1. Observation with TEM at 40,000 magnification

The results of observations with TEM showed that the vesicles were spherical as shown in Figure 1. However, the vesicles obtained were not perfectly round or referred to as irregular shapes. This phenomenon is thought to occur because there is Span 80 which does not fill the gaps between the phospholipids, so that the formation and arrangement of the lipid bilayer of the vesicles is imperfect [16].

4 Conclusions

The transfersome formula for pandan leaf ethanol extract which is considered the most stable is formula 3 with a ratio of 80:20 phospholipid concentration and span 80 because the results of the characterization of transfersom formula 3 have the smallest particle size, namely 730.1 ± 4.9 nm, PDI value <0, 7, the zeta potential value is -9.94 ± 1.02 mV, the absorption efficiency value is 80.23% and the deformability value is 6.225.

5 References

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