

Formulation and Penetration Study of Liposome Xanthone of Mangosteen Pericarp Methanol Extract (*Garcinia mangostana* L.)

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Abstract- Liposome is a drug carrier system that can enhance the effectiveness of drug delivery which is made from the lipid that easily penetrated into the skin. The methanol extract of mangosteen pericarp (*Garcinia mangostana* L.) has been proved rich in xanthone compounds that have very high potential of antioxidant activity, especially the fractionation of dichloromethane (FD). The aim of this study to test the penetration ability of liposome cream throughout mouse's skin. The FD has been used in making liposome as triploid. The precipitate of liposome with the best entrapment efficiency of liposome (77,09%) is used in making liposome cream (LC) with 5%, 10% and 15% concentration. The liposome had been made as triploid and the precipitate of the liposome with the best entrapment efficiency will be used in LC. The three dosage forms and FD cream was examined their physical stability and penetration ability via in vitro Franz Diffusion Cell test using *Sprague-Dawley* rat abdomen skin as diffusion membrane. Total cumulative penetration of α -mangostin from 5%, 10% and 15% (LC) and FDC were $1,65 \pm 2,22$; $3,95 \pm 0,13$; $8,27 \pm 0,14$; and $3,44 \pm 0,27 \mu\text{g}/\text{cm}^2$. The percentage of penetrated α -mangostin from 5%, 10% and 15% LCs and DFC were $1,43 \pm 1,92 \%$; $1,72 \pm 0,06 \%$; $2,4 \pm 0,04 \%$; and $0,24 \pm 0,02 \%$ respectively. Flux of α -mangostin from 5%, 10% and 15% LCs and DFC were $0,058 \pm 0,07$; $0,088 \pm 0,04$; $0,349 \pm 0,25$; $0,22 \pm 0,046 \mu\text{g}/\text{cm}^2/\text{hour}$, respectively. Penetration ability of 10% LC is higher than FDC, 5% and 15% LCs.

Index Terms- liposome cream; mangosteen pericarp; penetration

I. INTRODUCTION

Human will aging soon or later and our skin will clearly shown the sign of aging [1]. Aging skin often shown as dry, because the water content in upper skin layer is reduced, while sebaceous and sweat glands are reduced. Skin surface becomes rough and dull, appears wrinkles and fine line skin, and loss its elasticity [2].

Aging skin can be treated using cosmetics that contain antioxidant. The usage of the cosmetics are based on free radical theory. Free radical theory describes that aging happens as accumulation oxidative damages caused by Reactive Oxygen Species (ROS) as result of aerobic metabolism process. Increasing ROS formation on aging skin [3]. Antioxidant can reduce free radical into less reactive molecule, so that avoids and reduces oxidative damages (Yaar & Gilchrest, 2007). Usage of

antioxidant on antiaging skin treatment is important to prevent skin damage more further (Graf, 2005).

Antioxidants are substances that may protect cells (including skin cells) from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals. Human body does not make enough their own antioxidant [5], so when free radical exposure happen, body will need antioxidant from outside the body. There is concern about unknown side effect of synthetic, that is why natural antioxidant becomes an alternative needed for it [6,7]

Mangosteen fruit is one of the Indonesian's favorite fruit and often exported to abroad. The pericarp of mangosteen fruit is known contains xanthone compounds as antioxidant and antimicrobe [8]. Alpha-mangostin is on of main component of mangosteen pericarp. Beside can induces the death of apoptotic cell on cancer cells, α -mangostin is also prevent toxicity to oxidative pressure with protecting mitochondria from peroxidative damage [9].

Alpha-mangostin as xanthone compound of mangosteen fruit pericarp is hydrophilic, so it need a way to be penetrated easily into human skin. Liposome can be a topical dermal drug delivery system can pass through corneum stratum and can deliver hydrophilic drugs. It occurs because liposome can trap various drug polarity, such as hydrophilic as well as lipophilic. Hydrophilic drug can be trapped inside the compartment centre, on the other hand lipophilic drug can be trapped in lipid membrane [10]. Liposome is a dermal topical delivery system which can pass through corneum stratum and has optimum absorption ability. With these advantages, liposome formulation can be used to increase herbal drug delivery [11] into the skin. Liposome is a small spheric vesicle that consists of two phospholipid layers [12] which is the same properties as human skin which also bilayer phospholipid. Liposome as drug carrier in cosmetics has advantages because penetrated lipid can reduce skin hydration which is the main cause of aging, that is why liposome is suitable for making antiaging product [13]. The application of liposome technology for topical can be proved effectively on drug delivery into the skin [14]. The researches about antioxidant activities of α -mangostin have been done [15, 16, 17], but none of research about the penetration ability of dichloromethane fraction cream and liposome cream that contains dichloromethane fraction, that is why it should be done. Liposome cream formulation that contains mangosteen pericarp extracts (*Garcinia mangostana* L.) is been made in this research,

and their penetration ability are tested via *in vitro* using Franz diffusion cell technique.

II. THEORY

Liposome is a microscopic vesicle which consists of one or more lipid encapsulated double layer sphere. The double layer is formed from lipid such as cholesterol and lecithine. Lecithine has hydrophilic and hydrophobic molecule part which has different solubility and spontaneously forms single or double layer, which make closed vesicle with water solution. Liposome size is ranged 0.025 μm to more than 5 μm . Liposome may appear as unilamellar (a bilayer that liquid centre) or multilamellar (few bilayer oriented concentrically liquid centre). The choice of bilayer material generates the bilayer fluidity and charges. Adding the positive or negative charges lipid will give charges to liposome surface. Ability liposome to absorb and maintain drug widely and structural flexibility are the main element to control the drug action. Entrapment effectivity and be shown by encapsulation aqueous solution in lipid. The ability of liposome to entrap drug materials depends on phytochemistry, liposome composition, charges and aqueous environment. Works as slow release carrier, liposome can prolong drug exposure duration. Liposome also can protect drug from degradation process and protect patients from side effects of encapsulated drugs. Liposome can solubilise lipophilic compounds [18,19].

Mangosteen pericarp contains mangosteen pericarp consists of xanthone compounds [20]. Xanthone is a polyphenol compound as tricyclic aromatic ring that is substituted with variation from isoprene, phenolic and methoxy are mainly found in mangosteen [21]. Xanthone can be found in mangosteen pericarp, such as alpha-mangostin, beta-mangostin, gamma-mangostin, [22; 23]. (Chairungsrilerd *et al.* 1996; Pothitirat *et al.* 2009). Methanol extracts from mangosteen fruit pericarp has a potential antioxidant activity [24;25]. Some research showed that α -mangostin has antioxidant activity. α -mangostin compound has antiperoxidative effect on mouse brain tissue as free radical scavenger, dengan terbukti dapat reduce lipoperoxidation significantly and to prevent reduction of mitochondria ability [26]. The evaluation result of antioxidant activity on 14 xanthone compound, isolation result of mangosteen pericarp is α -mangostin has the greatest peroxynitrite antioxidant activity by inhibit preneoplastic lesion, shows that α -mangostin has a very strong antioxidant activity dengan compare to other 13 xanthone compound in mangosteen pericarp [15].

Topical dosage form is used to give localisation drug effect on applied site according to drug penetration into the lower layer of skin or mucose membrane [27] and also to get systemic effects. The systemic effect of a drug can be achieved by transdermal delivery systems. The advantage of giving drug transdermal is to prevent first cross metabolism and to prevent risks and other effects on intravenous therapy and various conditions that can affect drug absorption per oral such as pH changes, gastrointestinal enzyme presence, and the period of stomach [28].

III. RESEARCH METHODS

3.1. Extraction of Mangosteen Pericarp

Mangosteen pericarp was cut and dried in room temperature for 7 days. After dried, mangosteen pericarp is mashed into powder until gained 1 kg. Mangosteen pericarp powder as much as 1 kg is macerated using methanol 3 x 500 mL for 24 hours in room temperature, is evaporated using rotary evaporator at 50°C and gives out 300 gram crude methanol extracts [15].

3.2. Making Dichloromethane Fraction of

Mangosteen Pericarp Extract

Methanol crude extract of mangosteen pericarp is added with 500 mL aquadestilate, be partised with 400 mL *n*-hexane pa three times, then be partised with 400 mL Dichloromethane (DCM) pa two times. The result of fractionation using dichloromethane is stored in acid room for 48 hours (boiling point DCM 39.8°C) until all solvent is evaporated and gained DCM fraction powder [15].

3.3. Making Liposome

Liposome formulation (Tabel 1) is made using thin layer hydration method. Mangosteen DCM fraction, phosphatidylcholine and cholesterol are weighted, and dissolved in 10 mL DCM. The solution then being evaporated using rotary evaporator (RE) to get rid off the organic solvent for 1-2 hour at 45-50°C with speed 150 rpm in vacuum. The flask then is given with nitrogen gas, and left out for 24 hour with closed flask. Next step is hydration dengan phosphate buffer pH 7,4. Flask is placed at RE in 60°C speed 70 rpm, without vacuum condition. Suspension result is removed from RE and be left until cool at 4°C for 48 hours. Liposome size is reduced using bath-sonication for 20 minutes [29].

Liposome with DCM fraction of mangosteen pericarp extracts was centrifuged 30.000 rpm for 1 hour at 4°C. At the end of this ultrasentrifugation process, supernatant will separated from presipitate. Purified liposome was kept in vial at 4°C [29].

Table 1. Liposome Formulation

Ingredients	Formulation (6:1)
Mangosteen pericarp fraction powder	100 mg
Egg Phosphatidilcholine 60%	515 mg
Cholesterol	43 mg
Dichloromethane (DCM)	10 mL
Phosphate buffer pH 7,4	5 mL

3.4. Entrapment Efficiency

Using ultrasentrifugation technique using 60,000 rpm at 4°C for 60 minutes, the free liposome was separated from entrapped liposome. The supernatant was removed from the liposome precipitate. Total liposome and supernatant concentration is measured using TLC densitometry (TLC Scanner III, CAMAG). The eluent of TLC was chloroform-Etyl Acetate (85:15). After the TLC plat was eluated, the concentration of supernatant determined by TLC Scanner using D2 lamp at 319.0 nm. After getting the results using calibration curve, the entrapment efficiency of liposome is calculated by following equation:

$$EE (\%) = \frac{Cd - Cf}{Cd} \times 100$$

Where EE (%) is the presentage of Entrapment Efficiency, Cd is the concentration detected of total fraction mangosteen pericarp added and Cf is the concentration of supernatant.

3.5. Making the liposome cream

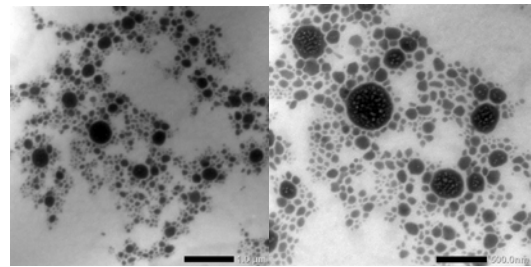
Before making liposome cream, the base cream should be cooled down until 40°C, then liposome presipitate (with DCM fraction of mangosteen pericarp extract) using stiring speed 3000 rpm. Adding liposome into base cream using trituration technique. For DCM fraction cream, base cream is added with fraction of mangosteen pericarp, and being stirring 3000 rpm. Adding liposome presipitate as much as 5%, 10%, and 15% is added into the base cream for making Formula I, II and III creams. The oil part is added to water part with stirring, then being homogenization by homogenizer.

Composition	Formula (%)			
	I	II	III	IV
Liposome	5	10	15	Fraction equal with 15% liposome
Stearic Acid	5	5	5	5
Cetyl Alcohol	3	3	3	3
Glycerin Mono Stearic (GMS)	2	2	2	2
Isoprophyl Myristate (IPM)	3	3	3	3
Triethanolamine (TEA)	0,4	0,4	0,4	0,4
Methyl Paraben	0,2	0,2	0,2	0,2
Prophyl Paraben	0,1	0,1	0,1	0,1
Propylene Glicol	10	10	10	10
Sodium metabisulphite	0,1	0,1	0,1	0,1
Demineralized water	Ad 100	Ad 100	Ad 100	Ad 100

4. Result and Discussion

4.1 Evaluation of Liposome

Presipitate of liposome that has the highest absorbance is evaluated on its vesicle morphology using TEM (Transmission Electron Microscope). The evaluation result using TEM with magnification 5.000 and 10.000, shows that the shape of vesicle round with varies size and shows the lamelar part on liposome. Agregation between precipitate liposome globules can occur as small particles has tendency to aggregate and will form bigger size of particles [30]. Liposome precipitate morphology has a good shape (Picture 1).



(a) (b)

Notes : a. Liposome Morphology 5.000x
b. Liposome Morphology 10.000x

Picture 1. Result of TEM

4.2 Evaluation of Liposome Cream

Liposome cream dengan concentration of 5%, 10% and 15% on storage in low temperature (4±2°C), room temperature (29±2°C) and high temperature (40±2°C) show no phase separation and looks homogenic. Viscosity measurement using Viscometer Brookfield using spindel no.5. Rheogram shows the flow properties of three concentration cream that has stored for 12 weeks in room temperature showed that there were no changes of flow properties. The flor properties of liposome cream has the same features, which is pseudoplastic tixotropic. It can be concluded that 5%, 10% and 15% liposome cream is stable after storing in room temperature for 12 weeks.

There were increased consistency of each concentration of liposome cream. This things is proporsional with the viscosity result of liposome cream which is thicker. Acidity of liposome cream is measured using pH-meter, every 2 weeks for 12 weeks at 3 different storage temperatures, which are low temperature (4±2°C), room temperature (29±2°C) and high temperature (40±2°C). All cream made in this research has pH range 5,3-6,5. These range of pH are acceptable, because the normal skin pH are 4,5-6,5. Skin products with acidic property can make skin irritation, while alkaline skin products can make scally skin, as result of damage of acid isolation on corneum stratum skin layer. The measurement of globules diameter shows that the average sizes of globules diameter changes irregular, especially creams that stored in 40°C. Range of average globul diameter 3 cream formulation on different temperature is 0.396 µm. When stored, globules keep moving so the globules keep meet one with another, as result the spaces between globules is lesser. Particles yang berdekatan tersebut may dapat menyatu and become the bigger size of particles (coalescence) [32]. These particles moves faster in high temperature, therefore the cream globules size that stored in high temperature were bigger than the one stored in room temperature.

This test is used to keep cream in low temperature (4°±2°C) for 24 hour and continued with storing in high temperature (40°±2°C) for 24 hour as much as 6 cycles. The purpose of this *cycling test* is to crystalization and separation of oil and water phase in form [33]. As result, the three formulation are stable and do not have any colour change, and no water and oil phase separation. It shows that liposome cream is stable physically.

Mechanic test on cream to find out whether separation phase after mixing. According to Stokes law, gravitation force can influence the stability of cream. The centrifugation with

speed 3750 rpm for 5 hours is equal with gravitation force that taken by a cream for whole 1 year. As result, there is no separation of two phase (creaming), but still as a single cream emulsion, homogenic.

4.3 In Vitro Penetration Test

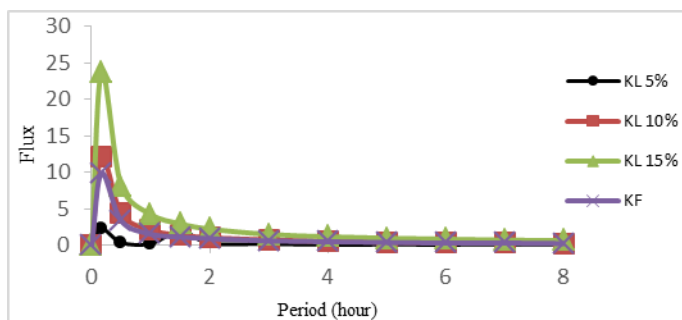
Franz diffusion cell method is used to find out how much α -mangostin of liposome cream being penetrated through skin for certain period. Penetration test *in vitro* is taken by using membrane as model skin. Biological membranes from animals can be used as skin model [34].

Abdominal skin of female white mouse *Sprague Dawley* abdomen skin are used as membranes. The mouse ages 2-3 months, with 150-200 gram weigh and membrane thickness $0,6 \pm 0,1$ mm and membrane area $1,52 \text{ cm}^2$. Hairless mouse skin has the permeability almost the same as human skin permeability. Human skin has permeability coefficient as $92,27 \text{ cm/hour} \times 10^5$, while mouse skin has permeability coefficient as $103,08 \text{ cm/hour} \times 10^5$ [35].

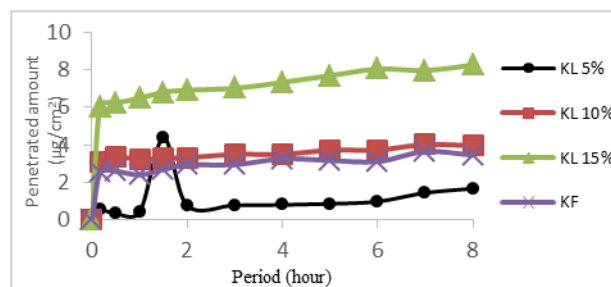
Other thing that should be considered in *in vitro* penetration test is active material (such as liposome cream), should be soluble in receptor compartment solution. The cream formulation is oil in water emulsion, so that the cream base can be soluble in receptor compartment solution. During penetration test, the temperature is keep in 37°C as human body temperature using thermostat.

After 8 hours penetration test with sample taking in 11 time interval, diperoleh hasil bahwa penetrated α -mangostin through mouse membrane skin 5%, 10% and 15% liposome cream are $11,19 \pm 3,15$; $19,24 \pm 0,75$; $18,22 \pm 1,35 \mu\text{g}/\text{cm}^2$. The greatest amount of penetrated α -mangostin is from 10% liposome cream. The percentage of penetrated α -mangostin from each liposome cream can be calculated from the amount cummulative of penetrated α -mangostin. The percentage of penetrated α -mangostin in 5%, 10% and 15% liposome cream are $0,0373 \pm 0,0053$ %; $0,0378 \pm 0,00158$ %; and $0,0227 \pm 0,00178$ %.

Picture 2 shows that the absorption of α -mangostin through skin occurred really fast. In first 10 minute, there is an increasing the amount of penetrated α -mangostin. This stage is an early condition before the condition reaches steady state. The fast absorption is maybe because of the added material in cream, such as stearic acid, propylene glycol, isoprophyl myristate. Propylene glycol as humectant is also a material that can increase skin penetration [36].



Notes: KL : Liposome Cream, KF : Fraction DCM Cream equivalent to the 15% liposome cream
Picture 2. Flux α -mangostin



Notes: KL : Liposome Cream, KF : Fraction DCM Cream equivalent to the 15% liposome cream
Picture 3. The amount cummulative of penetrated α -mangostin

The amount cummulative of penetrated α -mangostin is plot with period of time then the linear regression equation is made so flux α -mangostin can be calculated from each concentration of liposome cream. Flux gathered from linear line shows the flux value is taken from steady state follows Fick law [32]. Flux of 5%, 10%, 15% liposome cream and DCM fraction cream are $0,058 \pm 0,07 \mu\text{g}.\text{cm}^{-2}.\text{hour}^{-1}$; $0,08 \pm 0,04 \mu\text{g}.\text{cm}^{-2}.\text{hour}^{-1}$; $0,35 \pm 0,25 \mu\text{g}.\text{cm}^{-2}.\text{hour}^{-1}$ and $0,22 \pm 0,05 \mu\text{g}.\text{cm}^{-2}.\text{hour}^{-1}$. From this result, it is stated that flux value in liposome cream has no significant difference with flux value in DCM fraction cream (without liposome), because flux value from each four type cream has not achieved $1 \mu\text{g}.\text{cm}^{-2}.\text{hour}^{-1}$. It can be concluded that no difference between the penetration rate between liposome cream and cream without liposome. It maybe happen because of the liposome should be reduced it particle size. Liposome particle size has a important role in penetration into the skin. Smaller size of liposome can penetrate easier into skin layer than bigger size liposome [37].

Cummulative amount of penetrated α -mangostin from 5% liposome cream is increases in beginning, then decreases, and keep the same at the end. It happens because in the first minutes, there are big differences α -mangostin concentration between receptor and donor compartments. It is called nonsteady-state [32]. To find out whether it has good penetration speed, can be defined from flux level and lag time. Lag time is the period need by moving drug through membrane and diffuses into receptor, until achieved diffusion condition called steady state. Steady state will happen when the penetration speed of drug compound is consistent through the membrane [38]. Based on the penetration, there was no steady state, so that the lag time can not be defined. Therefore, the sample taking should be in every minutes for an hour.

The penetration on 5% liposome cream shows instability of the cummulative amount of penetrated α -mangostin. It may caused by mistake during penetration test process, during taking, handling, keeping skin test, taking the sample from receptor compartment in inaccurate time, amount of sample and replacing the amount sample from receptor compartment and concentration/dosage of drug compound to get an optimum penetration result.

Other factors that can influence drug absorption through skin is viscosity, dissolution of a drug in carrier, diffusion of solute from carrier to skin surface, the drug penetration through skin surface, especially corneum stratum [32; 36]. Penetration speed is inversely proporsional with viscosity. The thicker the

skin products, so the the release drug from its carrier would harder [39].

Drug partition coefficient also can influence the speed of drug penetration. Alpha-mangostin is a drug that can not soluble in water. Oil phase from cream in water emulsion is good carrier for drug material that is not soluble in water. It is known that fatty acid such as stearic acid act by disrupting intercellular lipid packing in the stratum corneum, allowing any applied drug to more readily permeate through the layer [40]. Stearic acid can increases skin permeability by desrupt the composition of lipid bilayer stratum corneum, so that it can increase penetration α -mangostin. Other factor is drug diffusion from the carrier into skin surface. Diffusion process of a drug is influenced by drug dissolubility of in carrier. If a drug has low solubility in carrier, then the rate of solubility will slower and will takes more time to reach the skin surfaces.

IV. CONCLUSION

Liposome cream that contains xanthone DCM fraction of mangosteen pericarp methanol extracts can penetrate the mouse skin. Penetration ability of liposome cream and DCM fraction (without liposome) cream did not have any significant different according to flux value and cummulative amount of α -mangostin penetrated into skin. Liposome cream with concentration 5%, 10%, and 15% that stored in low temperature ($4 \pm 2^\circ\text{C}$), room temperature ($29 \pm 2^\circ\text{C}$), and high temperature ($40 \pm 2^\circ\text{C}$) for 12 weeks can be stated as stable physically for its organoleptic, homogeneity, viscosity, consistency, and globule diametre.

V. SUGGESTION

In making liposome, it is needed to use *probe-sonication* to reduce the size of liposome. In Penetration test using method Franz Diffusion Cell, it is needed to have a exact and accurate standardized procedure for period, dosage, concentration to get optimum result of penetration test. HPLC is needed on quantitative analysis to find the amount of α -mangostin from DCM fraction of mangosteen pericarp to get better separation and more accurate result.

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