

Research Article



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

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ABSTRACT

The mangosteen pericarp (*Garcinia mangostana* L.) has been proved rich in compounds of xanthone that have very high potential of antioxidant activity, especially the fractionation of dichloromethane. This study aimed to investigate the *in vitro* penetration by using liposome xanthone gel dichloromethane fraction of mangosteen pericarp compare non-liposome one and investigate the antioxidant activity. Liposomes were made by thin layer hydration method. The antioxidant activity was determined by DPPH method. Alfa mangostin was determined by TLC densitometry. The IC_{50} values of dichloromethane fraction are 37.53 ppm. The concentration of α -mangostin in dichloromethane fraction is $49.059 \pm 0.8\%$. The *in vitro* penetration of α -mangostin of liposome gels had higher penetration ($35.33 \pm 1.208 \mu\text{g cm}^{-2} \text{hour}^{-1}$) than non-liposome gel ($8.398 \pm 0.018 \mu\text{g cm}^{-2} \text{hour}^{-1}$). Liposome xanthone gel dichloromethane fraction of mangosteen pericarp had better penetration than non-liposome one.

Keywords: Fractionation, Franz diffusion cell, Gels, Liposome, Mangosteen pericarp, Skin penetration, α -mangostin.

INTRODUCTION

The mangosteen pericarp has xanthone as an active substance. The used of xanthone as antioxidant was known could block the oxidative stress through neutralize free radicals. Oxidative stress is caused by the accumulation of free radicals in the body is known as one of the causes of chronic and degenerative diseases and it is associated with skin. From the previous researches known that the substances in the mangosteen pericarp were α -mangostin and γ -mangostin as the potent antioxidant. Fraction of mangosteen pericarp has antioxidant effect which can protect the skin from the damage of oxidation from damage caused by oxidation that can prevent premature aging.¹⁻³

In producing the effect of an anti-aging cosmetic, it takes a topical dermal system that can penetrate the stratum corneum of the skin barrier and has an optimal absorption. One of drug carriers for that capability is vesicles technology in the form of liposome gel.

The objective of this research is to develop formulation and investigate the *in vitro* penetration of liposome gel which contain xanthone resulted from fractionation of mangosteen pericarp.

MATERIALS AND METHODS

Chemical and Reagents

α -Mangostin (Sigma-Aldrich), egg phosphatidyl choline 60% (Sigma-Aldrich), cholesterol 99% (Sigma-Aldrich), nitrogen, dichlorometane (Mallincrodt), demineralized water (Brataco Chemical), HPMC (Brataco Chemical), propylene glycol (Brataco Chemical), sodium metabisulphite (Brataco Chemical), methanol (Brataco Chemical, Mallincrodt), n-hexane (Mallincrodt),

chloroform pa (Merck), ethyl acetate pa (Merck), DPPH, vitamin C (Brataco).

Plant Materials

The fruits of *G. mangostana* were purchased from local markets in Jakarta. The samples were identified and deposited by Dr. Joeni Setijo Rahajoe, Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences. The voucher herbarium specimen number is 1143/IPH.1.02/lf.8/VII/2012. The fruits were cleaned and the edible aril part was removed. The fruits rinds were cut into small pieces and dried in the air for 7 days. The dried samples were ground into powder, passed through a sieve (20 meshes). The samples were separately kept in air tight container and protected from light until used.

Extraction and Fractionation

The 5 kgs of dried and milled pericarp *G. mangostana* was extracted with methanol by maceration method three times for three days and evaporated at 50°C. The 166,6 grams thick extract was fractioned with dichloromethane for twice and dried in the air until there was any solvent and resulted powder of dichlormethane fraction.²

Preparation of Liposome

The liposomes were prepared by the hydration thin layer method which were composed of phospholipid and cholesterol. Firstly, fraction of mangosteen pericarp, phospholipid and cholesterol were dissolved using dichlormethane in around bottom flask. The flask was connected to a rotor evaporator (Rotavapor, Buchi, Germany) and immersed in a water bath preheated at temperature equal to or more than the transition temperature of phospholipids about 40°C. The obtained film was hydrated with phosphate buffer of pH 7.4.



Afterwards, all liposome dispersions were sonicated (20 min) with a probe sonicator (Branson 3200) to obtain a small liposome particle size.^{4,6} The liposomes were made in triplo. The composition of liposome is represented in Table 1.

Table 1: The composition of fraction mangosteen pericarp-loaded liposome formulation

Composition	Formulation
Powder of fraction mangosteen pericarp	100 mg
Egg Phosphatidyl choline 60%	515 mg
Cholesterol	43 mg
Dichlorometane	20 mL
Phosphate buffer pH 7,4	20 mL

Entrapment Efficiency

The free liposome was separated from entrapped by using ultracentrifugation technique (Thermo Electron Corporation Sorvall WX Ultra Series WX Ultra 90) at 60,000 rpm at 4°C for 60 min. The supernatant was taken from the liposomal precipitat. The concentration of supernatant and total of liposome were determined by TLC densitometry (TLC scanner III, CAMAG). The eluent of TLC was chloroform-ethyl acetate (85:15). After the plat was eluated than determined the concentration supernatant and total liposomes by TLC scanner CAMAG III using D2 lamp at λ 319.0 nm.

The percentage entrapment efficiency (%EE) of fraction mangosteen pericarp was determined relative to the original substance added, applying the following equation:

$$\% EE = [(Cd - Cf) / Cd] \cdot 100$$

Where %EE is the percentage entrapment efficiency, Cd is concentration detected of total fraction mangosteen pericarp added and Cf is concentration of supernatant.

Preparation of Liposome Gel^{4,7,8}

Preparation of gel

Gel was made by dispersed 3% HPMC in demineralized water (water temperature 80°C) and stirred until homogenous while heated in water heater. The 0,1% methyl paraben was dissolved in 10% propylene glycol, while 0.8% sodium metabisulphite was dissolved in demineralized water. The solution of sodium metabisulphite, methyl paraben and propylene glycol were put in HPMC gel, then homogenized in a homogenizer (1000 rpm).

Preparation of liposome gel

The liposome gel formulations were prepared by incorporation of liposome's containing fraction mangosteen pericarp (separated from the untrapped drug) were mixed in the HPMC gel with a mechanical stirer (500 rpm). The three formulas were made with different concentration of fraction mangosteen pericarp

liposome. The fourth formula was made with fraction mangosteen pericarp equal with 15% fraction mangosteen pericarp liposome. The composition of gel is represented in Table 2.

Table 2: Gel Formula

Composition	Formula			
	I	II	III	IV
Liposome	5%	10%	15%	Fraction equal with 15% liposome
HPMC	3%	3%	3%	3%
Propylene glycol	10%	10%	10%	10%
Methyl paraben	0,1%	0,1%	0,1%	0,1%
Sodium metabisulphite	0,8%	0,8%	0,8%	0,8%
Demineralized water	Ad 100%	Ad 100%	Ad 100%	Ad 100%

Photomicroscopic analysis

The size (Particle size analyzer, Beckman Coulter LS Series 3.19) and the morphology (TEM, JEOL JEM 1400) of fraction mangosteen pericarp liposome and fraction mangosteen pericarp liposome gel were examined.

Peneration studies

Skin preparation

The abdominal skin of rat by a Franz diffusion cell was used for the penetration studies. The using rat on these research had the ethical approval by Health Research Ethics Committee, Faculty Medicine Universitas Indonesia, Cipto Mangunkusumo Hospital. The number of this approval is 740/H2.F1/ETIK/2012. Rat abdominal skin was obtained after the surgery, then the subcutaneous fatty tissue was removed from the skin. After the fatty tissue was completely removed, the surface of the skin was cleaned by the phosphat buffer solution pH 7.4. The skin was stored in the phosphat buffer solution pH 7.4 at 4°C, then used within one day.

Skin penetration experiment

The skin penetration of fraction mangosteen pericarp was measured using a Franz diffusion cell. The nominal surface of the Franz cell was 1.52 cm² and the receptor compartments had a capacity of approximately 13 mL. The abdominal skin was put between the donor and receptor compartments with the stratum corneum side facing the donor compartments. The donor medium consisted of 1 g fraction mangosteen pericarp liposome gel in formula 1, 2, 3 and fraction mangosteen pericarp gel in formula 4. The receptor medium had a pH of 7.4 phosphat buffer solution to mantain the sink condition. The stirring rate and the temperature were kept at 400 rpm and 37°C. At appropriate intervals (10, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min) 0.5 mL of



receptor medium was withdrawn and immediately replaced with an equal volume of fresh medium. The receptor samples were then analyzed for the drug content by TLC densitometry.

TLC densitometry assay

The 0.5 mL of receptor medium was put into volumetric flask and added methanol until 5 mL. Each samples were determined the drug concentration by TLC densitometry and counted the drug contents.

Data analysis of skin penetration

Alpha mangosteen amounts of fraction mangosteen pericarp liposome gels and non liposome gelprepared over with time were used to calculate the transdermal drug flux, which was obtained from the slope of the regression line fitted to the linear portion of the profile. The skin flux can be experimentally determined from the following equation:

$$J = (dQ/dt)/A$$

Where J is the steady-state flux ($\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$), A is the diffusion area of the skin tissue (cm^2) through which drug permeation take place, and dQ/dt is the amount of drug passing through the skin per unit time at a steady-state ($\mu\text{g}\cdot\text{h}^{-1}$).

Drug content studies

Standard preparation

Stock standard solution was always freshly pre-pared by dissolving α -mangosteen (2.5 mg) in 25 mL methanol (100 $\mu\text{g}/\text{mL}$).

Sample preparation

a. Drug content in fraction

The fraction mangosteen pericarp (± 5.0 mg) was weighed and dissolved with methanol (10 mL) in volumetric flask (500 $\mu\text{g}/\text{mL}$).

b. Entrapment efficiency

Entrapment efficiency were prepared by weighed 1.0 g of total liposome and supernatant. Each samples were added methanol (25 mL) into volumetric flask (4000 $\mu\text{g}/\text{mL}$).

c. Drug content in penetration study

The 0.5 mL of receptor medium was put into volumetric flask and added methanol until 5 mL.

Determination α -mangosteen with TLC densitometry

Chromatographic was performed on TLC silica gel GF 254 aluminium-backed sheets (Merck). The chamber (Camag) was saturated for one hour with the mobile phase containing chloroform-ethyl acetate (85:15). After chamber saturated, the plates were developed to a distance of 9 cm. Each spot volume was 10 μL with glass capillaries. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode at

319 nm for all measurements. The chromatograms were integrated using winCATS evaluation soft-ware (Version 1.4.1.8154). The concentration of α -mangosteen was determined that based on calibration curve equation which taken with this equation:

$$y = a + bx$$

where x: counted concentration (μg); y: peak area

After the counted concentration was determined using equation above, the concentration of α -mangosteen was counted in samples using equation below.

Concentration (%) = (Counted concentration / real concentration) x 100

Stability Studies

The ability of vesicles to retain the drug was assessed by keeping the liposome gels and non liposome gel at three different temperature conditions, i.e., $4\pm 2^\circ\text{C}$ (Refrigerator; RF), $27\pm 2^\circ\text{C}$ (Room temperature; RT), and $40\pm 2^\circ\text{C}$ (High temperature, HT) for a period of 3 weeks. Samples were withdrawn periodically and analyzed for physical stability.

Rheologi Studies

Rheological analysis of fraction mangosteen pericarp liposome gels and non liposome gel were performed using a stress control rheometer (Viscotech Rheometer, Rheologica Instruments AB, Lund, Sweden). Rheological analysis was performed at room temperature. The following parameters were carried out for rheology measurement.

RESULTS AND DISCUSSION

From phytochemical analysis has known that fraction mangosteen pericarp contains alkaloids, flavonoids, tannins, Saponins, anthraquinone and terpenoids, and does not contain steroids.

The average of α -mangostin concentration of fraction mangosteen pericarp is $49,059 \pm 0,8\%$

After making liposomes, liposomes were sonicated than analyzed the distribution vesicle with Particle Size Analyzer (PSA) and compared with liposome before sonicated to see the impact of size and homogeneity of liposomes vesicles. A lot of factor that can influence in making liposomes like the process mixing of ingredients liposomes and active ingredient, process of making thin layer, nitrogen process, hydration process, light influence, heat impact when was rotavapored and storage condition. The best liposome is liposome that has small vesicle with high entrapment efficiency.

To determine the entrapment efficiency of liposome, first necessary to determine the concentration of the supernatant and the total concentration of the three liposomes with TLC densitometry method. Total concentrations obtained are respectively 0.081; 0.081, and 0.103% and for supernatant concentrations are



respectively 0.028; 0.020, and 0.035% so the entrapment efficiency of liposomes are respectively 65.432; 75.308, and 66.019%. Thus used for further research are the best liposomes having the smallest size and highest entrapment efficiency that is the second one.

Table 3: The result of Particle Size Analyzer (PSA) for the first liposome

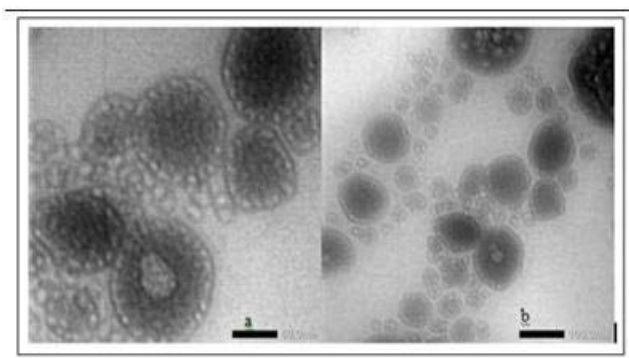
Particle size	Before sonicated (μm)	After sonicated (μm)
Averages	10.70 ± 6.185	10.73 ± 7.889
d_{10}	2.002	1.026
d_{50}	10.29	10.63
d_{90}	18.95	20.89

Table 4: The result of Particle Size Analyzer (PSA) for the second liposome

Particle size	Before sonicated (μm)	After sonicated (μm)
Averages	2.314 ± 2.110	2.085 ± 1.720
d_{10}	0.619	0.608
d_{50}	1.614	1.507
d_{90}	4.873	4.449

Table 5: The result of Particle Size Analyzer (PSA) for the third liposome

Particle size	Before sonicated (μm)	After sonicated (μm)
Averages	7.104 ± 2.825	7.189 ± 2.919
d_{10}	3.595	3.630
d_{50}	7.073	7.089
d_{90}	10.85	11.12



Where, a: The liposomes morphology, 80,000x; b: The liposomes morphology, 40,000x

Figure 1: The result of TEM

The evaluation of morphology liposomes using TEM (Transmission Electron Microscope) with 40,000 and 80,000 times magnification. Visible form spherical vesicles with varying size and looks the liposomes aggregate, it is thought to be the aggregation between each globule liposomes, because the particles have a tendency to aggregate so that it will form a larger particle size. But physically, the morphology of the liposomes has good form with lamellar visible. From the Magnification of 80,000 times is seen that the morphological liposomes

are multi lamellar large vesicle (MVV) that have lamellar more than one with a size of more than $0.5 \mu\text{m}$. The results can be seen in Figure 1.

The Preparation and Evaluation of Liposomes Gel and Fraction Dichloromethane of Mangosteen Pericarp Gel Equivalent to 15% Liposome Gel

For making liposome gels, were used HPMC (hydroxy propyl methyl cellulose). HPMC is a gelling agent. The selection of HPMC because it is a good thickening agent dispersion to combine liposomes used for topical use and is also compatible with the liposome dispersion.⁹ Besides that using HPMC for gel that can easily release the active substances that can be well penetrated into the skin.¹⁰ Concentration of HPMC is used by 3% with the aim of forming a gel with medium viscosity in order to simplify the deployment process as a topical application to the skin.

Evaluation of liposome gels and fraction gel are needed to determine the condition of the liposome gels and fractions gel before and after the stability test using the physical parameters so as to know the physical stability of liposome gel. From the observations cycling test liposome gels for 6 cycles, there were no change in organoleptic and have phosphatidyl choline characteristic odor also not happen syneresis. So is the fraction gel equal to the equivalent liposome gel 15%, there is no change in organoleptic and no syneresis. This suggests that the liposome gels and fraction gels are stable without any physical changes. All liposomes gels and non liposomes gel that were stored at low temperature ($4 \pm 2^\circ\text{C}$) and room temperature ($27 \pm 2^\circ\text{C}$) for 12 weeks showed a physically stable with parameter organoleptic, homogeneity and level of acidity (pH). Gel that was not made liposomes stored at high temperature ($40 \pm 2^\circ\text{C}$) for 12 weeks also stable. While all liposome gels that were stored at high temperature ($40 \pm 2^\circ\text{C}$) for 12 weeks showed a physically unstable with organoleptic parameters, homogeneity and a high acidity level changes. Liposome gels instability at high temperatures the possibility of phosphatidyl choline is degraded by oxidation or hydrolysis.⁷ Liposome gel is indicated better stability at room temperature and cold temperature.⁵

The viscosity measurement of 5, 10 and 15% liposome gels and fraction gel that is equivalent to the 15% liposome gel yield the descending curve is located in the left ascending curve, this suggests that the nature of flow is pseudo plastic thixotropy (Figure 2).¹¹

In Vitro Penetration Study

Receptor medium used for penetration testing α -mangostin in this study was phosphate buffer pH 7.4. Phosphate buffer pH 7.4 was selected as the receptor medium is used as a biological body fluids simulation.⁴

After the penetration study for eight hours with sampling at 11 point intervals, the result that the cumulative number of α -mangostin penetrate through the

membrane of rat skin from 5, 10, 15% liposomes gels and fraction DCM mangosteen pericarp gel equivalent to the 15% liposome gel, respectively, 215.0 ± 3.1 ; 432.4 ± 7.5 , 1218.2 ± 46.2 , and $299.7 \pm 1.6 \mu\text{g}/\text{cm}^2$.

Based on these results, the amount of α -mangostin that the most widely penetrate is the 15% liposome gel. From the cumulative amount penetration of α -mangostin can be calculated percentage of α -mangostin penetrate from each preparation. The percentage of α -mangostin that are penetrated from 5, 10, 15% liposomes gels and fraction DCM mangosteen pericarp gel equivalent to the 15% liposome gel, respectively, 30.54 ± 0.54 ; 30.64 ± 0.80 ; 58.05 ± 2.53 , and $14.15 \pm 0.07\%$.

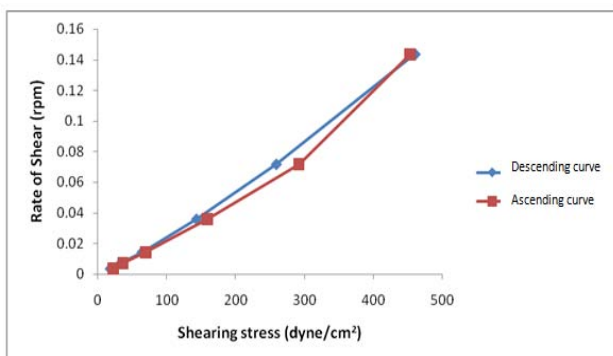


Figure 2: Rheogram of 5% liposome gel at 12th week

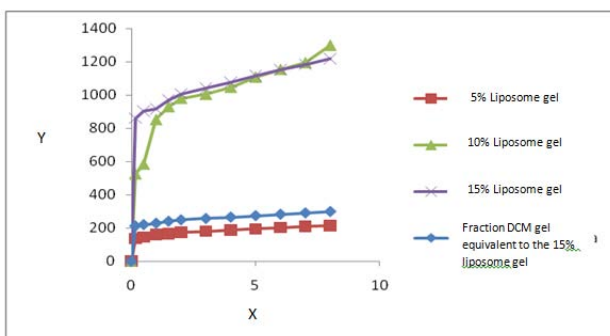


Figure 3: Profile of cumulative amount of α -mangostin that are penetrated From 5, 10, 15% liposome gels and fraction DCM mangosteen pericarp gel equivalent to the 15% liposome gel [Where x: time (hours); y: the amount of penetration ($\mu\text{g}/\text{cm}^2$)]

From Figure 3 can be seen that α -mangostin absorption through the skin occurs very quickly. At 0 minute to the 10th minute there was a big amount of α -mangostin that was penetrated. This stage was the initial condition due to conditions that had not yet reached steady state. That is because the α -mangostin is a compound that is soluble in fat so that percutaneous absorption of α -mangostin pretty good and has maximum cutaneous concentration that can be achieved especially in the form of liposome gel compared to gel that is not made liposomes with the content of the fraction DCM mangosteen pericarp which is equivalent.

Rapid absorption is also suspected due to the additional material in the preparation such as propylene glycol. Although in the formula, propylene glycol as a humectant,

it is also represents a percutaneous penetration enhancer and also plays a role in hydrating the skin.¹² The cumulative amount of α -mangostin penetration plotted against time linear regression equation was then made to determine which α -mangostin flux of each preparation. Flux obtained from the slope of the line indicates that the flux values are taken at steady state following the rules of the law of Fick.¹¹

Penetration curve of α -mangostin from 5, 10, 15% liposome gels and fraction DCM mangosteen pericarp gel equivalent to 15% liposome gel in steady state conditions for flux calculations can be seen in Figure 4.

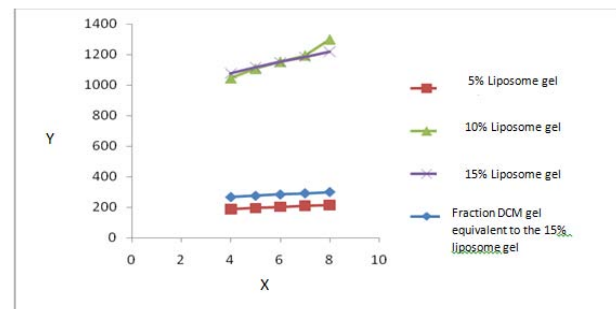


Figure 4: Curve of α -mangostin penetration from 5, 10, 15% liposome gels and Fraction DCM mangosteen pericarp gel equal to 15% liposome gel in steady state condition for flux calculation. [Where X: time (hours); Y: the amount penetration ($\mu\text{g}/\text{cm}^2$)]

Based on Figure 5 can be compared to the flux of each preparation. The flux of 5, 10, 15% liposome gel and fraction DCM mangosteen pericarp gel equivalent to 15% liposome gel, respectively, 7.096 ± 0.039 ; 19.67 ± 0.834 ; 35.33 ± 1.208 , and $8.398 \pm 0.018 \text{ mg cm}^{-2}\text{hr}^{-1}$. From these results it can be seen that higher concentration liposome gels provide a higher flux. It is proved that the rate of penetration of α -mangostin influenced by the amount of fraction DCM mangosteen pericarp is added. Also note that by making a liposome gel gives a higher flux than the fraction DCM mangosteen pericarp gel without liposomes although the number of fractions is equivalent.

Flux must be obtained from the steady state. Steady state can be described as a straight line on the flux curve plotted against time unit. This is due to the early minutes there is a difference in the concentration of α -mangostin significant between receptor and donor compartment. The state of non steady-state conditions. Upon reaching the 4th hour (240 minute) flux into straight illustrating that the situation had reached steady state.¹¹

Other factors that may affect drug absorption through the skin is the viscosity of the preparation, dissolution of a drug in the carrier, the diffusion of the dissolved drug from the carrier to the skin surface, and the penetration of drugs through the skin, especially the stratum corneum layer.^{11,12} It can be attributed to the relationship between viscosity and rate of penetration, the penetration rate is inversely proportional to the viscosity value. The more

viscous a preparation the more difficult drug release from the carrier.

Phospholipids and cholesterol, the substance of liposome gel, are also penetration enhancer preparations by increasing hydration of the stratum corneum that will facilitate the inclusion of the active substance.¹³ So, the liposome gels had higher penetration than non-liposome gel.

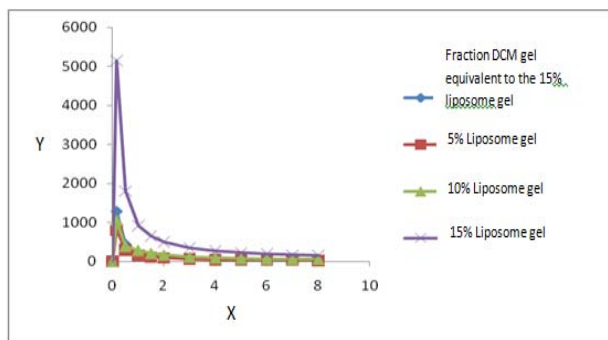


Figure 5: Flux of α -mangostin each time retrieval of 5, 10, 15% liposome gels and fraction DCM mangosteen pericarp gel equivalent to 15% liposome gel. [Where x: time (hours); y: flux ($\mu\text{g cm}^{-2} \text{hour}^{-1}$)]

CONCLUSION

IC_{50} values of fraction DCM mangosteen pericarp is 37.53 ppm, which is a good antioxidant because it has a fairly high antioxidant power. The concentration of α -mangostin in fraction DCM mangosteen pericarp is $49.059\% \pm 0.8\%$. The highest entrapment efficiency is 75.308%, the second liposomes with the smallest particle size is $2.085 \mu\text{m}$. All liposome gel that were stored at high temperature ($40 \pm 2^\circ\text{C}$) for 12 weeks, were showed unstable physically with parameter of organoleptic, homogeneity and high acidity changes level. So need to get the formulation liposome gel that are stable and good consistency gel that can be applied. The in vitro penetration of α -mangostin of liposome gels had higher penetration ($35.33 \pm 1.208 \mu\text{g cm}^{-2} \text{hour}^{-1}$) than non-liposome gel ($8.398 \pm 0.018 \mu\text{g cm}^{-2} \text{hour}^{-1}$). Based on these results it can be concluded that the liposome gel can penetrate through the skin in vitro better than the non-liposome gel.

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Conflict of Interest: None.