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

Desy Muliana Wenas, Mahdi Jufri, Berna Elya

Herbal Medicine Master Programme, Faculty of Farmacy, University of Indonesia

***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 ± 2.22; 3,95 ± 0.13; 8.27 ± 0.14; and 3.44 ± 0.27 μg/cm^2. The percentage of penetrated α-mangostin from 5%, 10% and 15% LCs and DFC were 1.43 ± 1.92 %; 1.72 ± 0.06 %; 2.4 ± 0.04 %; and 0.24 ± 0.02 % respectively. Flux of α-mangostin from 5%, 10% and 15% LCs and DFC were 0.058 ± 0.07; 0.088 ± 0.04; 0.349 ± 0.25; 0.22 ± 0.046 μg/cm^{2}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 show the sign of aging [1]. Aging skin often shown as dry, because the water content in upper skin layer is reduced, while sebaceous and sweet 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 is 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. Usage of antioxidant on antiaging skin treatment is important to prevent skin damage further more [5].

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 compounds, that is why natural antioxidant becomes an alternative option 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 has antioxidant and antimicrobial activities [8]. Alpha-mangostin is one of main component of mangosteen pericarp. Beside it 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 hydrophobic, so it need a way to be penetrated easily into human skin. Liposome can be a topical dermal drug delivery system that can pass through stratum corneum and can deliver hydrophilic drugs. It occurs because liposome can trap various drug polarity, such as hydrophobic as well as hydrophilic. Hydrophobic drug can be trapped in lipid membrane, on the other hand hydrophilic drug can be trapped inside the compartment center [10]. Liposome is a dermal topical delivery system which can pass through stratum corneum 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 is consist of one or more lipid encapsulated double layer sphere. The double layer is form from lipid such as cholesterol and lecithin. Lecithin 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 be appears as unilamellar (a bilayer that liquid center) or multilamellar (few bilayer oriented concentrically liquid center). The choices of bilayer material support 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 phytochemical compounds, 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 solubilize lipophilic compounds [18,19].

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]. 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, was proved that it can reduce lipoperoxidation significantly and to prevent reduction of mitochondria ability [26]. The evaluation result of antioxidant activity on 14 xanthone compounds, 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 compounds in mangosteen pericarp [15].

Topical dosage form is used to give localization drug effect on applied site according to drug penetration into the lower layer of skin or mucous 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 50ºC and gives out 300 g crude methanol extracts [15].

3.2. Making DCM Fraction of Mangosteen Pericarp Extract

Methanol crude extract of mangosteen pericarp is added with 500 mL aquadestilated, 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 of the organic solvent for 1-2 hour at 45-50ºC with speed 150 rpm in vacuum. The flask then is given with nitrogen gas, and left out for 24 hours with closed flask. Next step is hydration with pH 7.4 phosphate buffer. Flask is placed at RE in 60ºC 70 rpm speed, without vacuum condition. Suspension result is removed from RE and be left until cool at 4ºC 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 4ºC. At the end of this ultracentrifugation process, supernatant will separated from presipitate. Purified liposome was kept in vial at 4ºC [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 ultracentrifugation 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-Ethyl Acetate (85:15). After the TLC plate 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 percentage 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

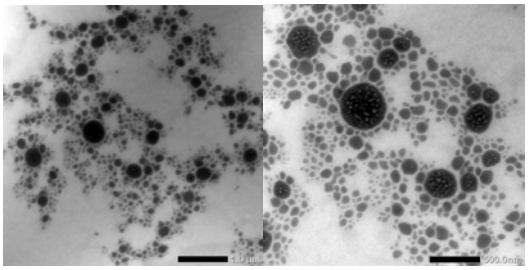
Before making liposome cream, the base cream should be cooled down until 40˚C, then liposome precipitated (with DCM fraction of mangosteen pericarp extract) using stir speed 3000 rpm. Adding liposome into base cream using trituration technique. For DCM fraction cream, base cream is added with fraction 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 lamellar part on liposome. Agregation between precipitate liposome globul 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. The Evaluation of Liposome Cream

Liposome cream dengan concentration of 5%, 10% and 15% on storage in low temperature (4±2ºC), room temperature (29±2ºC) and high temperature (40±2ºC) show no phase separation and looks homogenous. 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 flow properties of liposome cream have 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. These is proportional 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±2ºC), room temperature (29±2ºC) and high temperature (40±2ºC). 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 stratum corneum skin layer. The measurement of globules diameter shows that the average sizes of globule diameter changes irregular, especially creams that stored in 40°C. Range of average globule diameter 3 cream formulation on different temperature is 0.396 µm. When stored, globule 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 move 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 hours and continued with storing in high temperature (40°±2°C) for 24 hours as much as 6 cycles. The purpose of this *cycling test* is to crystallization and separation of oil and water phase in form [33]. As result, the three formulation are stable and do not have any color 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 3,750 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 homogenous cream emulsion.

4.3 In Vitro Penetration Test

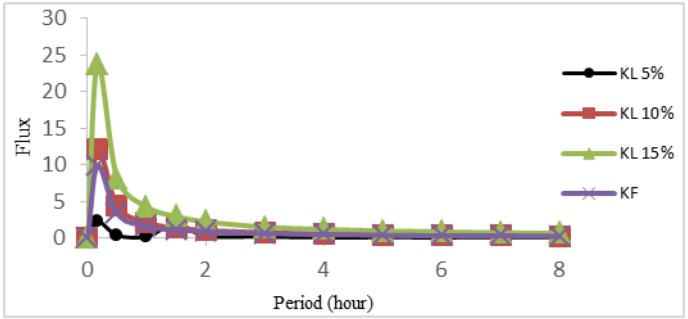
Franz diffusion cell method is used to find out how much α-mangostin of liposome cream being penetrated through skin for certain period. Penetration test *in vitro* is taken by using membrane as model skin. Biological membranes from animals can be used as skin model [34].

Abdominal skin of female white mouse *Spraque Dawley* abdomen skin are used as membranes. The mouse ages 2-3 months, with 150-200 g weigh and membrane thickness 0.6 ± 0.1 mm and membrane area 1.52 cm^2. Hairless mouse skin has the permeability almost the same as human skin permeability. Human skin has permeability coefficient as 92,27 cm/hour x 10^5, while mouse skin has permeability coefficient as 103,08 cm/hour x 10^5 [35].

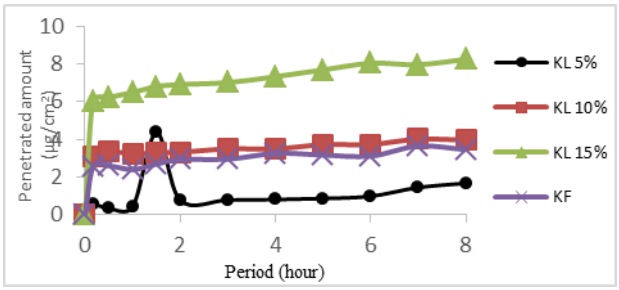
Other thing that should be considered in in vitro penetration test is active material (such as liposome cream), should be soluble in receptor compartment solution. The cream formulation is oil in water emulsion, so that the cream base can be soluble in receptor compartment solution. During penetration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

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 ± 3.15; 19.24 ± 0.75; 18.22 ± 1.35 μg/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 cumulative of penetrated α-mangostin. The percentage of penetrated α-mangostin in 5%, 10% and 15% liposome cream are 0,0373 ± 0,0053 %; 0,0378 ± 0,00158 %; and 0,0227 ± 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. Prophylene 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 cumulative of penetrated α-mangostin

The amount cumulative 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 ± 0.07 μg.cm^{-2}.$hour^{-1}$; 0.08 ± 0.04 μg.cm^{-2}.$hour^{-1}$; 0.35 ± 0.25 μg.cm^{-2}.$hour^{-1}$ and 0.22 ± 0.05 μg.cm^{-2}.$hour^{-1}$. 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 μg.cm^{-2}.$hour^{-1}$. It can be concluded that no difference between the penetration rate between liposome cream and cream without liposome. It maybe happens because of the liposome should be reduced it particle size. Liposome particle size has an important role in penetration into the skin. Smaller size of liposome can penetrate easier into skin layer than bigger size liposome [37].

Cumulative 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 cannot be defined. Therefore, the sample taking should be in every minutes for an hour.

The penetration on 5% liposome cream shows instability of the cumulative amount of penetrated α-mangostin. It may be 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 the stratum corneum [32; 36]. Penetration speed is inversely proportional with viscosity. The thicker the

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

Drug partition coefficient also can influence the speed of drug penetration. α-mangostin is a drug that cannot 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 increase skin permeability by disrupt the composition of lipid bilayer stratum corneum, so that it can increase penetration α-mangostin. Other factor is the drug diffusion from the carrier into skin surface. Diffusion process of a drug is influenced by drug dissolubility of in carrier. If the drug substance 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 cumulative amount of α-mangostin penetrated into skin. Liposome cream with concentration 5%, 10%, and 15% that stored in low temperature (4 ± 2°C), room temperature (29 ± 2°C), and high temperature (40 ± 2°C) for 12 weeks can be stated as stable physically for its organoleptic, homogenity, viscosity, consistency, and globule diameter.

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 an 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.

Acknowledgment

This research was funded by Research Grant DIPA University of Indonesia year 2013.

References

[1] Wasitaatmadja, S.M. Penuntun Ilmu Kosmetik Medik. Jakarta: UI Press, 2007.

[2] Jusuf, N.K. Kulit Menua. Majalah Kedokteran Nusantara. Jakarta: FK – UI 2005; 184-188.

[3] Floyd, R.A., and Hensley K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases 2002, Neurobiol Aging 2002; Sep-Oct 23(5):795-807.

[4] Yaar, M & Gilchrest, BA. Photoaging: Mechanism, Prevention and Therapy. British Journal of Dermatology 2007; 157: 874-877.

[5] Graf, J. Anti-aging skin care ingredient technologies. In Cosmetic

[6] Rohdiana, D. Aktivitas Daya Tangkap Radikal Polifenol Dalam Daun Teh, Majalah Jurnal Indonesia 2001; 12(1): 53-58

[7] Sunarni, T., Aktivitas Antioksidan Penangkap Radikal Bebas Beberapa Kecambah Dari Biji Tanaman Familia Papilionaceae, Jurnal Farmasi Indonesia 2005; 2(2): 53-61

[8] Mardawati, E., F. Filianty, Herlina Marta. Kajian aktivitas antioksidan ekstrak skin manggis (*Garcinia mangostana* L.) dalam rangka pemanfaatan limbah skin manggis di kecamatan puspahiang kabupaten tasikmalaya. Jurusan Teknologi Industri Pangan. Fakultas Teknologi Industri Pertanian Universitas Padjadjaran, 2008.

[9] Martínez-Abundis, E., N. García, F. Correa, S. Hernández-Reséndiz, J. Pedraza-Chaverri, and C. Zazueta. Effects of alpha-mangostin on mitochondrial energetic metabolism. Mitochondrion 2010; 10 (2): 151-157.

[10] Liu, X., M. Zhao, J. Wang, B. Yang, and Y. Jiang. Antioxidant activity of methanolic extract of emblica fruit (Phyllanthus emblica L.) from six regions in China. Journal of Food Composition and Analysis 2008; 21: 219-228.

[11] Mukherjee, K. Pulok., M. Venkatesh, K. Maiti, K. Mukherjee and B.P. Saha. (2009). Value added herbal drug delivery systems-perspectives and developments. Indian Journal of Pharmaceutical Education and Research 2009; 43.

[12] Samad A., Sultana Y., Aqil M. Liposome drug delivery systems: an update review. Curr Drug Deliv 2007; Oct 4(4): 297-305.

[13] Lipowsky, R., and E. Sackmann. (1995). Handbook of biological physics Volume 1. Amsterdam: Elsevier Science B.V.

[14] Venkateswarlu, I., et al. (2011). A Review on Liposomes. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2: 739-751.

[15] Hyun-Ah Jung, B.-N. Su, W. J. Keller, R.G. Mehta, and A.D. Kinghorn. (2006). Antioxidant xanthones from the pericarp of Garcinia mangostana (mangosteen). J. Agric. Food Chem. 54: 2077-2082.

[16] Kondo, M., L. Zhang, H. Ji, Y. Kou and B. Ou. Bioavailability and antioxidant effects of a xanthone-rich mangosteen (Garcinia mangostana) Product in Humans. J. Agric. Food Chem., 2009; 57 (19): pp 8788–8792

[17] Zarena, A.S. and K.U. Sankar. Screening of xanthone from mangosteen (Garcinia mangostana L.) peels and their effect on cytochrome c reductase and phosphomolybdenum activity. Journal of Natural Products 2009; 2: 23-30.

[18] Krowczynski, L. Extended-Release Dosage Forms. USA, 1987; CRC Press.

[19] Crommelin, D.J.A., G.W. Bos and G. Storm. Liposome: Successful carrier systems for targeted delivery of drugs. Business Brifing: Pharmatech, 2003.

[20] Kosem, N., Y-H. Han, and P. Moongkarndi. Antioxidant and cytoprotective activities of methanolic extract from Garcinia mangostana Hulls. Science Asia 33, 2007; 283-292.

[21] Obolskiy D., Pischel I., Siriwatanametanon N. , and Heinrich M. (2009). Garcinia mangostana L.: A phytochemical and pharmacological review. Phytother Res. Aug 23(8): 1047-1065.

[22] Chairungsrilerd, N., K. Takeuchi, Y. Ohizumi, S. Nozoe, and T. Ohta. (1996). Mangostanol, a prenyl xanthone from Garcinia mangostana. Phytochemistry 43(5): 1099-1102.

[23] Pothitirat W, Gritsanapan W (2009). HPLC quantitative analysis method for the determination of alpha-mangostin in mangosteen fruit rind extract. Thai J. Agri. Sci, 2009; 42(1): 7-12.

[24] Moongkarndi, P., N. Kosema, S. Kaslungka, O. Luanratana, N. Pongpan, and N. Neungton. (2004). Antiproliferation, antioxidation and induction of apoptosis by Garcinia mangostana (mangosteen) on SKBR3 human breast cancer cell line. Journal of Ethnopharmacology 90: 161–166.

[25] Pedraza-Chaverri, J., N. Cárdenas-Rodríguez, M. Orozco-Ibarra, J.M. Pérez-Rojas. Medicinal properties of mangosteen (Garcinia mangostana). Food and Chemical Toxicology 46, 2008; 3227–3239

[26] Márquez-Valadez, B., R. Lugo-Huitrón, V. Valdivia-Cerda, L. R. Miranda-Ramírez, V. P.- D. la Cruz, O. González-Cuahutencos, I. Rivero-Cruz, R. Mata, A. Santamaría, and J. Pedraza-Chaverrí. The natural xanthone alpha-mangostin reduces oxidative damage in rat brain tissue. Nutritional Neuroscience 2009; 12(1): 35-42.

[27] Lionberger, D.R. and M.J Brennan. Topical nonsteroidal anti-inflammatory drugs for the treatment of pain due to soft tissue injury: diclofenac epolamine topical patch. J Pain Res. 2010; 3: 223–233.

[28] Panwar, A.S., N. Upadhyay, M. Bariagi, S. Gujar, G.N. Darwhekar, D.K. Jain. Emulgel: A review. Asian Journal of Pharmacy and Life Science 2011; 1(3): 333-343.

[29] Mitkari, B.V., S.A. Korde, K.R. Mahdik and C.R. Kokare. Formulation and Evaluation of Topical Liposome Gel for Fluconazole. Indian Journal of Pharmaceutical Education and Research, 2010; 44: 324-333.

[30] Kendall, K and M.R. Kosseva. Nanoparticle aggregation influenced by magnetic fields. Colloids and surfaces A: Physicochem. Eng. Aspects 2006; 286: 112-116.

[31] Tranggono, R.I dan F. Latifah. Buku pegangan ilmu pengetahuan kosmetik. J. Djajadisastra (Ed.) UI Press 2003; ix + 223.

[32] Martin, A., Swarbrick, J., and Cammarata, A. (1983). Farmasi Fisik. Jakarta: UI Press, 1143-1183.

[33] Zats, J. L. and Kushla, G. P. (1996).Gels. In H. A. Lieberman, M. M. Rieger and G.S. Banker (Ed.). Pharmaceutical dosage forms: Disperse systems (2nd Ed., Vol.2, pp. 507). New York: Marcel Dekker.

[34] Yi-Hung Tsai, Jia-You Fang, Pao-Chu Wu, and Yaw-Bin Huang. 1995. Percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from gel and ointment bases: In vitro formulation evaluations in pigs and in vivo bioengineering methods in humans. International Journal of Pharmaceutics 1996; 130(1): 121–135.

[35] Wester, R. C., & Maibach, H. I. (1990) In vitro testing of topical pharmaceutical formulations. Dalam: Topical drug delivery formulations. New York: Marcel Dekker Inc.

[36] Barry BW. Novel Mechanisms and Devices to Enable Successful Transdermal Drug Delivery. Eur. J. of Pharm. Sci. 2001; 14: 101-114.

[37] Verma, D.D., S. Verma, G. Blume, A. Fahr. Particle size of liposomes influences dermal delivery of substances into skin. International Journal of Pharmaceutics, 2003; 258: 141-151.

[38] Williams, AC. (2003). Transdermal and Topical Drug Delivery. London, Pharmaceutical Press.

[39] Dragicevic-Curic N, et al. Temoporfin-loaded liposome gels: Viscoelastic properties and in vitro skin penetrastion. Int. J. of Pharm 2009; 373: 77-84.

[40] Sarheed, O. Combination treatments of chemical enhancers with low frequency ultrasound for the transdermal delivery of hydrocortisone. Canadian Journal of Pure & Applied Sciences, 2013; 7(3): 2463-2473.

AUTHORS

First Author – Desy Muliana Wenas, Herbal Master Faculty of Farmacy Universitas of Indonesia
Second Author – Mahdi Jufri, Herbal Master Faculty of Farmacy Universitas of Indonesia
Third Author – Berna Elya, Herbal Master Faculty of Farmacy Universitas of Indonesia

Correspondence Author – D. M. Wenas
Email: desym3wjo@gmail.com