

Development of Ethosome Containing Bitter Melon (*Momordica charantia* Linn.) Fruit Fraction and *In Vitro* Skin Penetration

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ABSTRACT

Objective: Bitter melon fruit, which is containing charantin, has poor penetration through the skin. This problem can be solved with the lipid vesicle called ethosomes that offers better skin penetration. This study was aimed to develop ethosomes for improving skin penetration ability of charantin. **Methods:** Bitter melon was extracted with ethanol 80% and fractionated with dichloromethane. Ethosomes were formulated with a various concentration equal to 2% (F1), 3% (F2) and 4% (F3) of bitter melon fruits fraction (BMFF) and prepared using thin layer hydration method. The obtained ethosomes were characterized, then the penetration study was conducted using Franz diffusion cells. **Results:** The results showed that the BMFF was a dry, brown-greenish fraction and gave a positive test for a steroid. The entrapment efficiency of ethosomes F1, F2 and F3 was $91.50 \pm 0.40\%$, $92.62 \pm 0.26\%$ and $83.85 \pm 1.10\%$, respectively. Moreover, the particle size (Dv90) of ethosomes F1, F2 and F3 was 1083.33 ± 15.27 nm, 1736.67 ± 11.55 nm and 1976.67 ± 5.77 nm, respectively. Ethosomes F1, F2 and F3 resulted polydispersity index of 0.42 ± 0.02 , 0.35 ± 0.05 and 0.50 ± 0.11 , as well as zeta potential of -54.33 ± 0.75 , -57.50 ± 0.44 and -50.60 ± 0.98 , respectively. Besides, all ethosomes had a spherical shape. The research revealed that ethosome F2 was the optimal ethosome among another formulas. Cumulative percentage of penetrated stigmaterol glycoside for ethosome F2 was $18.25 \pm 0.08\%$, while the control solution did not penetrate within 20 h. **Conclusion:** This research demonstrated that the ethosome could increase the skin penetration of stigmaterol glycoside, which is charantin content, from the fraction of bitter melon fruit. **Key words:** Bitter melon fruits, Charantin, Ethosomes, Skin penetration.

INTRODUCTION

Bitter melon fruit (*Momordica charantia* Linn.) has extensive study as a hypoglycemic agent and it is known to treat diabetes because of its ability to lower blood sugar.¹⁻³ Polypeptide-p and charantin work as the main compounds on bitter melon fruit that has hypoglycemic activities. However, charantin is the most widely researched compound and has been proven that it is more effective than polypeptide-p and tolbutamide (oral hypoglycemic agents) as a hypoglycemic agent in bitter melon.⁴

Charantin is a steroidal glycoside consist of a mixture of 1:1 sitosterol glycoside and stigmaterol glycosides. When charantin is given orally, in acid circumstances such as in the stomach, charantin glycosides will be in the hydrolyzed form of aglycons (sterols) and sugar.² However, Harinantenaina and colleagues (2006) reported that aglycons from charantin do not have a significant effect on the blood glucose levels. It shows that the hypoglycemic effect occurs because of steroid glycosides of charantin.^{5,6} Thus, it is necessary to find other alternative routes to solve the problem.

A transdermal route has the advantage that can avoid drug degradation by acids in the digestive tract. Active substances in transdermal drug should be penetrated through the skin, so it can get into the blood stream.⁷ However, charantin is difficult to penetrate through the skin, due to its large

molecular weight (578.494 Da).² This penetration problem can be overcome with vesicle application called ethosome.

Ethosome is a lipid-based vesicle that is used to increase drug delivery. Ethosome is formulated from the phospholipids, alcohol with a high concentration (20-45%) and water. Ethosome can be used for large molecules like peptides and proteins. A high concentration of ethanol can disrupt the lipid bilayer. Ethanol gives the ability of vesicles to penetrate through the stratum corneum. Moreover, ethanol causes the lipid membrane density even more tenuous, thus increasing the ability of drug distribution.⁸ Therefore, this study was aimed to formulate the dichloromethane fraction of bitter melon (*Momordica charantia* Linn.) fruit into ethosome vesicle to overcome the penetration problem.

MATERIALS AND METHODS

Materials

Dried simplisia of bitter melon obtained from Research Institute for Spices and Medicinal Plants (BALITTRO) and determined by Conservation Center of Bogor Botanical Gardens as well as Indonesian Institute of Sciences (LIPI), stigmaterol glycoside (PT. Indogen Intertama, Indonesia), β -sitosterol glycoside (Chromadex, USA), phospholipon 90G (Lipoid, Germany), ethanol 96%

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(Merck, Germany), dichloromethane (Merck, Germany), propylene glycol, aqua dm (Brataco, Indonesia), n-hexane, methanol (Merck, Germany), sodium hydroxide (Merck, Germany), potassium hydrogen phosphate (Merck, Germany), female *Sprague Dawley* rats (Bogor Agricultural University, Indonesia). The ethics committee (No. 0360/UN.2F1/ETIK/2018) from Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia approved the use of animals in this study. All solvent used in this study was an analytical grade or higher.

Plant materials and extract preparation

Dried simplisia was crushed with a grinder to get the coarse powder. The fruit powder was stored in a tightly closed container protected from light until further use.⁹ The extraction method was adapted from Atmaputri (2016) with modifications. First, the bitter melon fruits powder was macerated using solvent n-hexane and ethanol 80% three times during 24 h with a comparison between the active substance and the solvent (1:10; 1:5; 1:5). The simplisia was extracted with n-hexane to eliminate fat and chlorophyll. N-hexane and ethanol extract was collected and evaporated with rotary vacuum evaporator temperature 50°C to obtain a viscous extract.

Bitter melon extract partition

Partition of ethanol extract of bitter melon was conducted using a separating funnel with solvents dichloromethane (DCM) and water. Ethanol extract of bitter melon was reconstituted with water. Then, dichloromethane solvent was added into a mixture of water and ethanol extracts. The ratio between the active substance and the solvent was 1 to 10. Subsequently, the mixture was incorporated into a separating funnel. DCM layer was collected and evaporated using a water bath to obtain dried dichloromethane fraction of bitter melon.⁹

Stigmasterol glycoside and β -Sitosterol glycoside identification in BMFF

Charantin is able to be identified based on the compounds in it, which are stigmasterol glycoside (STG) and β -sitosterol glycoside (BSG). Both were determined by HPLC method adapted by Desai (2014) with modification.¹ HPLC system (Shimadzu LC-20AD, Japan) was performed with reversed phase column (C18, 250 × 4.6 mm, 5 μ m diameter particles) and mobile phase consists of methanol and water (98:2, v/v). Samples were analyzed with an injection volume of 20 μ L, the flow rate of 1 mL/min and detection at 204 nm using a UV detector. Analysis was made by creating a mix of 5 μ g/ml STG and BSG standard solutions, as well as dichloromethane fraction of bitter melon solution as a sample. In methanol medium, the solution was injected as much as 20.0 μ L on HPLC column. Then retention time between the standard solution and sample solution were observed and compared for identification.

Determination of Stigmasterol glycoside content in BMFF

Calibration curves of STG were established by plotting the areas of peaks against seven concentrations of STG standards (12.22-102.34 μ g/mL). This calibration curve was used to determine the STG contents of the fraction. The concentration of sample solution was \pm 5000 μ g/mL in methanol medium. The sample solution was injected as much as 20.0 μ L on HPLC column. The area of STG in samples was plotted into a regression equation to determine STG content.¹

BMFF characterizations

The yield of bitter melon extract and fraction

BMFF yield was calculated against the amount of fraction that was obtained.

Organoleptic of BMFF

The process was done using the senses to specify the color and smell of BMFF.

Steroid identification of BMFF

The presence of steroid in the dichloromethane fraction of bitter melon fruits was tested using Lieberman Burchard's method. BMFF was dissolved in acetic anhydride and concentrated sulfuric acid (2:1). The presence of the red-green ring in solution showed that the fraction had steroid.¹⁰

Water content determination of BMFF

Determination of water content was carried out by the gravimetric method. The bitter melon fraction (1 gr) was inserted in the container. Then, the fraction was dried at a temperature of 105°C for 5 h and weighed. The drying process was continued with weighed approximately 1 h until the difference between two consecutive weighing not exceeding than 0.25%.⁹

Remaining Dichloromethane solvent test in BMFF

This test was performed using gas chromatography equipped with a flame ionization detector.⁹

Formulation of BMFF Ethosomes

Ethosome formulation was adapted from Barupal, Gupta and Ramteke (2010) with modifications and formulation is displayed in Table 1. The ethosome was prepared by thin layer hydration method using rotary evaporator vacuum. First of all, BMFF and phospholipon 90G were dissolved with dichloromethane. Then, the dissolved solution was poured into the round-bottom flask. Dichloromethane in the flask was evaporated at a temperature of 37°C and rotated at 50-150 rpm using a rotary vacuum evaporator to form a thin film on a round bottom flask. The thin layer film was flushed with nitrogen and stored in 7°C up to 24 h. The thin film then was hydrated with a mixture solution of phosphate buffer pH 7.4, ethanol and propylene glycol to produce an ethosomal suspension. Once the ethosomal suspension was formed, sonication was done for two minutes.^{11,12}

Ethosomes Characterizations

The Entrapment efficiency of BMFF Ethosomes

This method was adapted from Park and his colleagues (2013) with modifications. The amount of STG in BMFF entrapped-ethosomes was determined by HPLC analysis. The entrapment efficiency was measured by an indirect method. The ethosome suspension (2.5 mL) was dissolved in methanol ad 5 mL. Then, 2 ml of ethosome solution was diluted again with methanol ad 50 ml (25x dilution factor). This solution was analyzed as a total STG in ethosomes. The free STG in supernatant (1 mL) was analyzed after centrifuged the phytosome suspension at 13,000 rpm for 240 min. The supernatant contained free STG was diluted with methanol HPLC (1:1 v/v).¹³ Then, it was diluted

Table 1: Formulas of BMFF ethosomes.

Materials	F1 (% b/v)	F2 (% b/v)	F3 (% b/v)
Dichloromethane Fraction of Bitter Melon	2	3	4
Phospholipon 90G	3	3	3
Ethanol	30	30	30
Propylene glycol	5	5	5
Buffered Phosphate pH 7.4	Ad 100	Ad 100	Ad 100

again with methanol (25x dilution factor). The entrapment efficiency was calculated as the following formula:

$$\text{Entrapment Efficiency (\%)} = (\text{Total STG-Free STG}) / (\text{Total STG}) \times 100$$

Particle size distribution and zeta potential of BMFF Ethosomes

Particle size distribution and zeta potential of ethosome formulas were measured by dynamic light scattering (DLS) technique (Malvern Zetasizer, Malvern Instrument, UK). Samples were dispersed in distilled water (3:25) before measurement.¹⁴

Morphology of BMFF Ethosomes

Ethosome morphology was observed using transmission electron microscopy (TEM) (Microscope Tecnai 200 kV D2360, USA). A drop of the ethosome that had been dispersed by water was placed onto the carbon-coated copper grid and dried at room temperature, leaving a thin film. The film was colored using phosphotungstic acid solution and imaged.

Stability study of BMFF Ethosomes dispersion

Stability evaluation of ethosome suspension was performed by storing the suspension at high ($40^\circ \pm 2^\circ\text{C}$), room ($25^\circ \pm 2^\circ\text{C}$) and low ($7^\circ \pm 2^\circ\text{C}$) temperatures. During 12-weeks, organoleptic, pH, particle size distribution, polydispersity index, zeta potential and STG content in the BMFF ethosomes were evaluated properly.

In vitro skin penetration test

The penetration test was performed using Franz diffusion cells with a diffusion area of 1.76 cm^2 . The abdomen skin of female *Sprague-Dawley* rats aged 2-3 months was used as a membrane in the test. All of the methods for sacrificing the animals have been approved by Ethical Clearance Committee of Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia No. 0360/UN.2F1/ETIK/2018. The receptor compartment was filled with 10 mL mixture solution of phosphate buffer pH 7.4 and methanol (90:10) and stirred with a magnetic stirrer at a speed of 300 rpm ($37 \pm 2^\circ\text{C}$). The skin was mounted to the donor compartment with the dermis facing the receptor compartment. A sample of 5 ml was applied to the skin surface. At several time intervals, 2.0 mL of samples were taken from receptor compartment and equal amount was replaced after each sampling. Then 1 ml samples were diluted with 10 ml methanol. STG content in samples were analyzed by HPLC method. The cumulative amount permeated at each time interval was measured.¹⁴

RESULTS

Plant materials and extract preparation

Ethanol 80% and n-hexane extract of bitter melon fruits had a viscous and specific smell. However, ethanol 80% extract had brown extract while n-hexane had dark brown extract.

Bitter melon extract partition

Dichloromethane and water fraction of bitter melon fruits had a dry and specific smell. However dichloromethane had brown-greenish fraction while water fraction had a brown fraction.

Stigmasterol glycoside and β -Sitosterol glycoside identification in BMFF

The retention time of STG and BSG standard solution consecutively is 10.803 s and 12.002 s. The STG retention times on sample solution were 11.198 s; 11.235 s; and 11.218 s. The BSG retention times on sample solution were 12.002 s; 12.042 s; and 12.020 s.

Determination of Stigmasterol glycoside content in BMFF

The equation of linear regression from calibration curve was $y = 5478.7x + 9497.9$ with the value of the correlation coefficient (r) = 0.9995. Based on the analysis, the area obtained was then calculated based on the calibration curve, so the content of STG in the fraction was $1.38 \pm 0.01\%$.

BMFF characterizations

The yield of bitter melon extract and fraction

The yield of n-hexane and ethanol 80% extract consecutively was 1.92% and 21.23%. The yield of dichloromethane and water fraction consecutively was 10.12% and 86.32%.

Organoleptic

Dichloromethane fraction of bitter melon fruits had dry, brown-greenish fraction and specific smell.

Steroid identification

Identification result of steroids on the dichloromethane fraction was positive.

Water content determination of BMFF

Based on the calculation, the water content of the dichloromethane fraction was 2.23%.

Remaining Dichloromethane solvent test in fraction

Based on the result, dichloromethane concentration in the BMFF was 270 ppm.

Formulation of BMFF Ethosomes

The final form of the ethosome suspension was brown greenish with a specific smell of ethanol and charantin. Ethosome formula with the larger concentration of fraction produced darker/concentrated suspension.

Characterization of BMFF Ethosomes

The Entrapment efficiency of BMFF Ethosomes

Calibration curve for entrapment efficiency was created by HPLC analysis. The equation of linear regression was $y = 6839.2x + 25259$ with the value of the correlation coefficient (r) = 0.9992. The entrapment efficiency of BMFF ethosomes is shown in Table 2.

Particle size distribution, polydispersity index and zeta potential

The particle size distribution of BMFF ethosomes of F1, F2 and F3 is given in Figure 1. Table 3 shows the particle sizes of BMFF ethosomes, which are represented as volume distribution in the values of D_v10 , D_v50 , D_v90 and $D_{v-average}$. Polydispersity index (PDI) and zeta potential of each ethosome are shown in Table 2. The results showed that PDI value of all ethosomes was less than 0.6 and zeta potential were smaller than -30 mV.

Table 2: Entrapment efficiency, polydispersity index, and zeta potential of BMFF ethosomes.

Formula	Entrapment Efficiency (%)	Polydispersity Index	Zeta Potential (mV)
F1	91.5 ± 0.4	0.42 ± 0.02	-54.33 ± 0.75
F2	92.62 ± 0.26	0.35 ± 0.05	-57.5 ± 0.44
F3	83.85 ± 1.10	0.5 ± 0.11	-50.6 ± 0.98

Morphology of BMFF Ethosomes

Figure 2 shows TEM micrographs of BMFF ethosomes, that appeared a spherical shape.

Stability study of BMFF Ethosomes dispersion

Organoleptic stability

Based on organoleptic observation, all formulas had discoloration into a brighter brown at low, room temperature and high temperature.

pH stability

Based on the measurement using pH meter, every formula gave pH value that was similar with pH of buffer phosphate pH 7.4. However, the results showed that pH value had decreased during 12 weeks. The results showed that pH of BMFF ethosomes dispersion of F1, F2 and F3 was decreasing gradually as shown in Figure 3.

Particle size distribution and zeta potential stability

The stability of particle size, zeta potential and polydispersity index during 12 weeks are presented in Figures 4-6 respectively. During 12 weeks of storage, there was a significant alteration in particle size, zeta potential and polydispersity index of BMFF ethosomes. They showed the increasing of Dv_{90} and zeta potential.

Stigmasterol glycoside content in BMFF Ethosomes dispersion

The results showed significant decreasing of STG content in BMFF ethosome dispersion during 12 weeks of storage, that is shown in Figure 7.

Selection of Ethosome for penetration study

The best ethosome formulation was chosen to be evaluated for skin penetration. Selected formula was a formula, which was a spherical shape, the highest percentage of entrapped BMFF, PDI approached to 0 and zeta potential more than ± 30 mV. According to the evaluation of BMFF ethosomes, ethosome F2 was selected, since it was spheric, PDI of 0.35, zeta potential of -57.50 mV and the highest percentage of STG entrapped (92.62%).

In vitro skin penetration test

Penetration profile of STG from BMFF ethosome F2 is given in Figure 8. The result showed that the cumulative amount of penetrated-STG from BMFF ethosome F2 was $138.59 \pm 1.33 \mu\text{g}/\text{cm}^2$ (18.25%). However, the control solution did not penetrate at all within 20 h. Penetration of BMFF ethosome F2 showed a lag time of 1.5 h and the flux value of $12.14 \mu\text{g}/\text{cm}^2\cdot\text{h}$.

Table 3: Particle size measurement of BMFF ethosomes.

Formula	Dv_{10} (nm)	Dv_{50} (nm)	Dv_{90} (nm)	Dv_{average} (nm)
F1	696 ± 2.65	883.33 ± 6.35	1083.33 ± 15.27	905.1 ± 6.56
F2	727.67 ± 18.9	1240 ± 20	1736.67 ± 11.55	1233.37 ± 10.33
F3	1386.67 ± 15.27	1670 ± 10	1976.67 ± 5.77	1658.16 ± 9.9

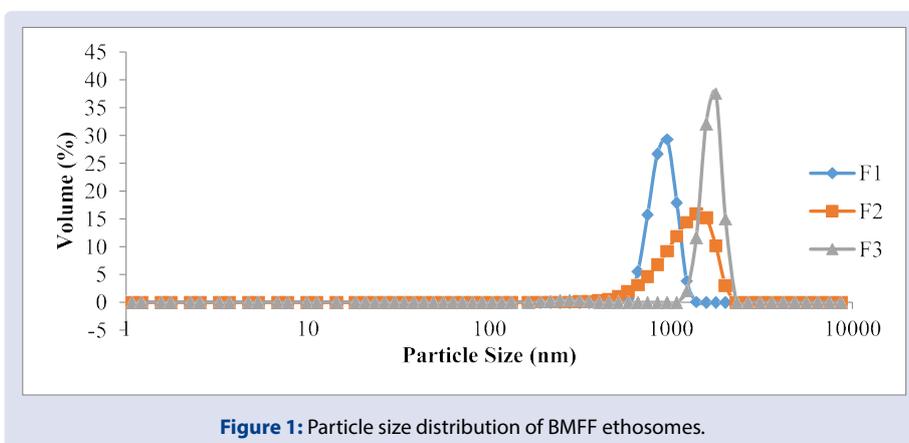


Figure 1: Particle size distribution of BMFF ethosomes.

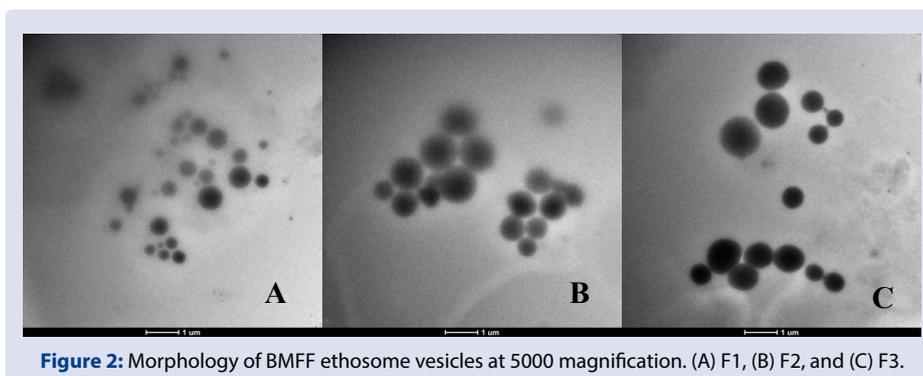
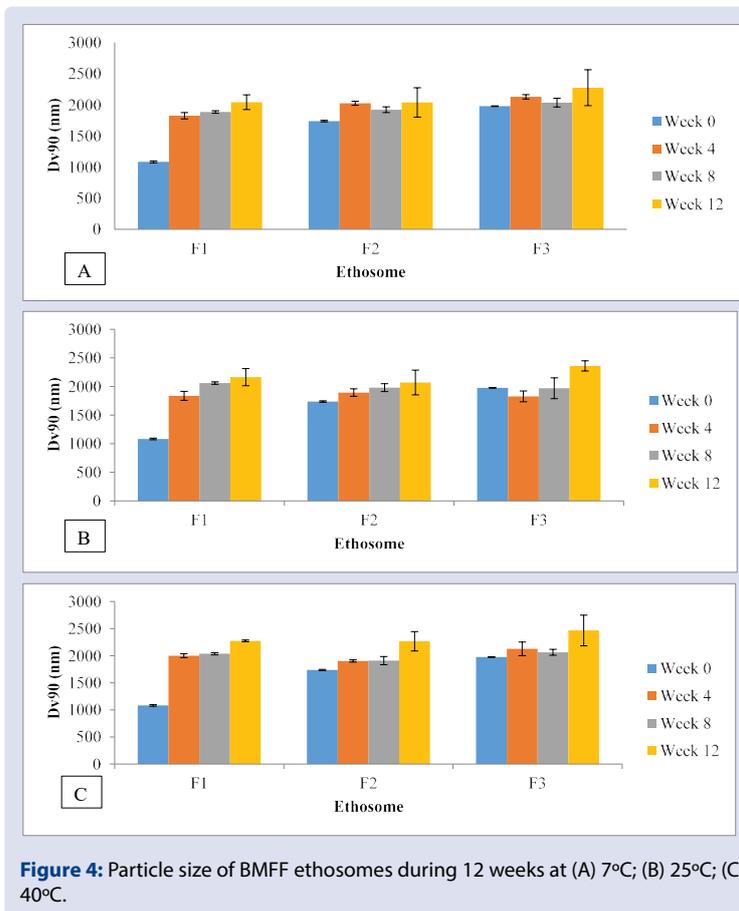
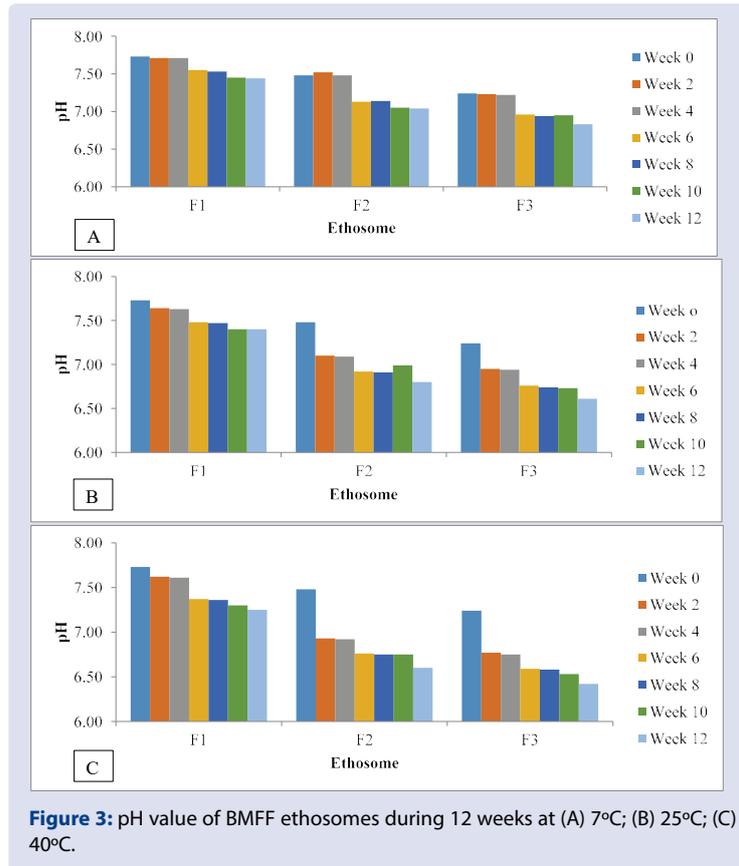


Figure 2: Morphology of BMFF ethosome vesicles at 5000 magnification. (A) F1, (B) F2, and (C) F3.



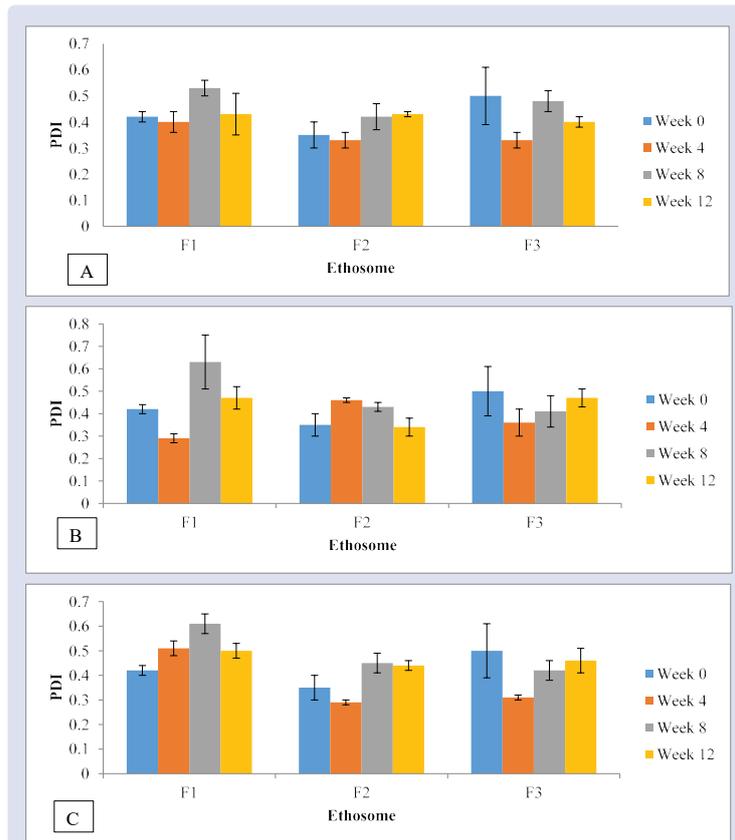


Figure 5: Polydispersity index of BMFF ethosomes during 12 weeks at (A) 7°C; (B) 25°C; (C) 40°C.

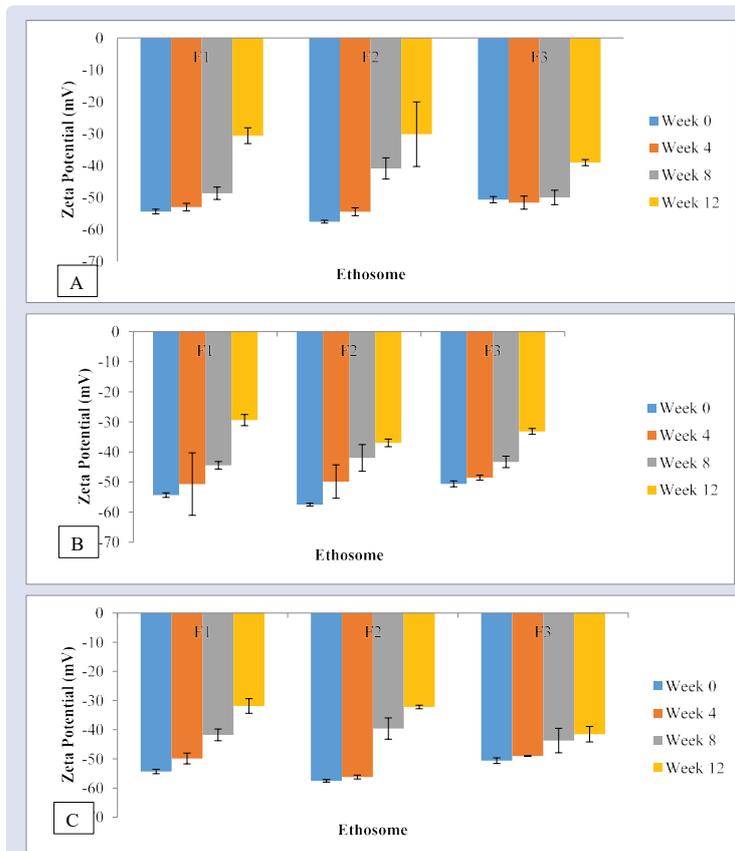


Figure 6: Zeta potential of BMFF ethosomes during 12 weeks at (A) 7°C; (B) 25°C; (C) 40°C.

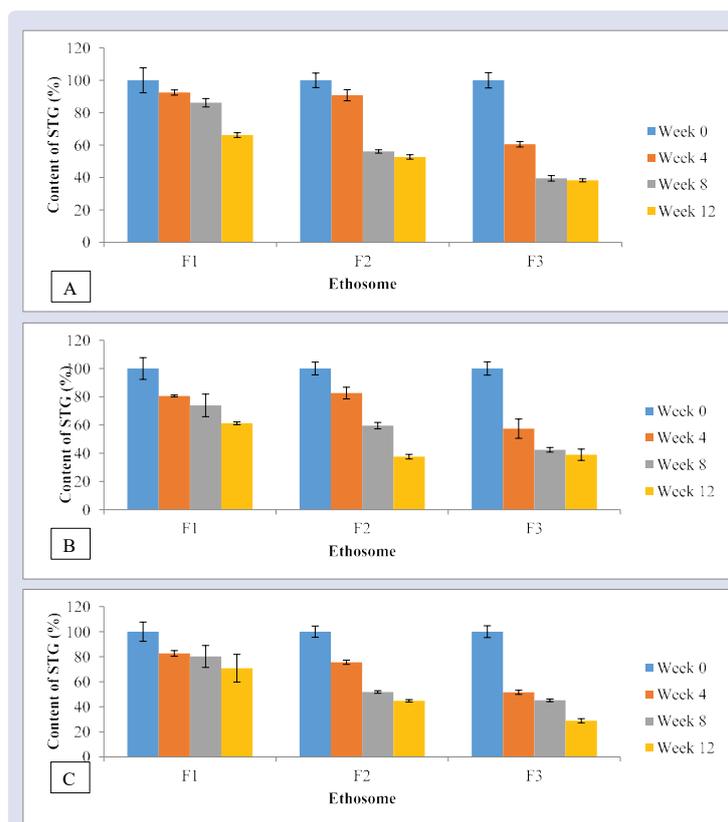


Figure 7: Stigmasterol glycoside content in BMFF ethosomes during 12 weeks at (A) 7°C; (B) 25°C; (C) 40°C.

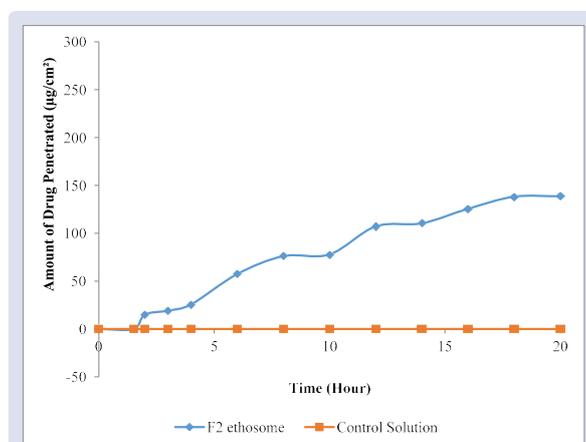


Figure 8: Cumulative amount of penetrated- STG from ethosome F2 and STG solution (control).

DISCUSSION

Maceration was selected as the extraction method for bitter melon. Maceration uses simple tools, easy to do and does not damage the compounds which are not heat resistant. However, the disadvantage is maceration requires a lot of solvents and needs a long time. Simplisia of bitter melon was macerated using two solvents namely n-hexane and ethanol 80%. N-hexane was used to attract nonpolar compounds like fat and chlorophyll. Ethanol 80% was chosen based on the research of Pitinapong, *et al.* (2007) which stated that the extraction efficiency of the sample increases directly proportional to increase the percentage of ethanol above 50% in the solvent.^{2,9}

Partition process of the bitter melon extract was done by liquid-liquid partition method. In this process, the dichloromethane fraction was expected to get the desired compound (charantin) more than the ethanol extracts. Charantin has a high solubility on semipolar solvents like dichloromethane and chloroform.^{2,9}

Stigmasterol glycoside and β -sitosterol glycoside identification in BMFF revealed that sample solution showed the presence of β -sitosterol glycoside (BSG) and stigmasterol glycoside (STG) in HPLC chromatogram after its retention time compared with a mixture of STG and BSG standard solutions. The result revealed that the retention time of sample and standard was compatible. The retention time of a

compound was typical but not specific because of column condition which could affect the little difference between standard and sample retention that were analyzed.

Calibration curve to determinate of stigmasterol glycoside content in BMFF was created with STG standard concentration plotted in X-axis and area in Y-axis. Based on the analysis, the area obtained was then calculated into the calibration curve. A minimal content of STG ($1.38 \pm 0.01\%$) might be due to solvents and extraction methods used were not optimal, so STG in bitter melon was not maximally extracted. Research about the best method for bitter melon fruit extraction should be done to give a maximal content of STG in the extract.

One of the bitter melon fruit extract and fraction characterizations was the yield of bitter melon fruit extract and fraction. The yield calculation of extract and fraction was taken to know the efficiency level of the extraction and partition. Identification result of steroids on the dichloromethane fraction with Lieberman Burchard's method showed that fraction contained a steroid. There was a red-green ring on the fraction solution.¹⁰ Water content measurement is required to know that water content in an extract or fraction does not pass the specified minimum limits. It met the requirement that the water content in an extract or fraction may not exceed 10%.⁹ According to the International Conference on Harmonization (ICH) (2011), dichloromethane concentration that is still allowed is 600 ppm.¹⁵ Based on the result, the BMFF had an eligible concentration of dichloromethane.

Ethosomes were formulated with a various concentration equal to 2% (F1), 3% (F2) and 4% (F3) of bitter melon fruits fraction (BMFF) and prepared using thin layer hydration method. The components of ethosomes were a phospholipid, ethanol, active substances, propylene glycol and buffered phosphate pH 7.4. A phospholipid has a similar structure to the lipid bilayer of the skin. Phospholipid and ethanol are expected to increase membrane permeability of the vesicle to the skin.¹¹ Propylene glycol is used as a penetration enhancer and necessary to hold the leakage of layers.¹⁶ A thin film was applied nitrogen gas to expel oxygen which oxidizes the phospholipids and stored for 24 h in the refrigerator to eliminate residual organic solvents. The residual organic solvents will trigger instability of ethosome and toxic if it is absorbed into the skin. The liquid suspension was sonicated to decrease the particles size. Sonication performed within two min to prevent the outbreak of the vesicles. The final form of the ethosome suspension was brown-greenish with a specific smell of ethanol and charantin. Ethosome formula with the larger concentration of fraction produced darker/concentrated suspension.

Ethosome characterizations are necessary for determining the quality of ethosomes. One of the characterizations is entrapment efficiency. The entrapment efficiency obtained for F1, F2 and F3 were $91.50 \pm 0.40\%$, $92.62 \pm 0.26\%$ and $83.85 \pm 1.10\%$, respectively. The result showed that the entrapment efficiency would increase with the increase of the fraction concentration.¹³ However, F3 had the lowest entrapment efficiency because phospholipids might only entrap the fraction maximally about 3%.

Particle size distribution must also be measured to characterize ethosomes. The results showed that the order of ethosomes from the smallest particle size was $F1 < F2 < F3$. They showed that the more active substance that was incorporated into the ethosome, the bigger particle size of the ethosome. The value of a polydispersity index (PDI) is the other physical properties of ethosome. PDI shows the degree of heterogeneity of particles. If PDI value is closer to zero, the degree of heterogeneity of particle size is smaller. PDI with good value is less than 0.6.¹⁷ It can be concluded that F1, F2 and F3 were homogenous. Zeta potential was also measured to characterize ethosome. The particle which shows the value of zeta potential is higher than +30 mV or smaller than -30 mV shows a high degree of stability. The more positive

or negative values of zeta potential, then more repulsive force between particles. It causes an increase of inter-particle distances. As a result, the particles tend not to flocculate/aggregate.¹⁸ F1, F2 and F3 showed zeta potential values which were smaller than -30 mV.

Stability evaluations of ethosome suspensions were done for 12 weeks. Based on organoleptic observation, F1, F2 and F3 ethosomes had discoloration. All formulas had discoloration into a brighter brown at low, room temperature and high temperature. The discoloration might be caused by the oxidation of fraction in ethosomes. Besides, photolysis might occur because of a bright storage area at low temperature.

Based on pH measurement, every formula gave pH value that was similar with pH of buffer phosphate pH 7.4 at week 0. The pH value increased because of the concentration of ethanol in formulas. However, the results showed that pH of ethosomes had decreased during 12 weeks of storage. It was caused by the oxidation of fraction that increased the acidity. This oxidation process occurs might due to the absence of additional antioxidants in all ethosomes.

Particle size distribution and zeta potential stability of ethosomes were also measured. During 12 weeks of storage, all formulas showed the increasing of zeta potential value. It caused ethosomes became less stable. However, zeta potential of ethosomes still showed values that were smaller than -30 mV except for F1 that had -29.4 ± 1.85 mV. During 12 weeks of storage, there was a significant alteration in polydispersity index of BMFF ethosomes. However, they still had good values of polydispersity index. Furthermore, F1, F2 and F3 showed increasing of Dv_{90} value. Particle size affects the stability of an ethosome suspension. The larger diameter or the size of the dispersed particles then deposition become more rapid and suspension become more unstable.¹⁸

STG content in ethosome suspension was also measured for stability study. The results showed significant decreasing of STG content in BMFF ethosome dispersion during 12 weeks of storage. It was caused by the oxidation of sterol on stigmasterol glycoside structure. The results were occurred because of the interactions between lipid in ethosome formulas and temperatures that made drastic effects on the total contents of the stigmasterol oxides formed and also on the reaction pathways of oxidation. During heating at high temperatures for prolonged periods, more stigmasterol was oxidized. The oxidation of stigmasterol was slower when the storage temperature was lower.¹⁹

In skin penetration study, ethosome penetration was represented by ethosome F2. Based on the results, ethosome F2 had a greater amount of STG penetrated and better flux value than control solution. The control solution did not penetrate within 20 h. The control solution could not be detected by the HPLC method because the area was too small. The flux demonstrated that the penetration rate of ethosome F2 was faster than the control solution. The increased penetration of ethosome F2 was caused by some mechanisms from ethosome. Phospholipid that has a similar coefficient partition with lipid in the stratum corneum can increase the penetration of STG. The effects of ethanol and propylene glycol as penetration enhancers will increase the fluidity of lipid bilayer and leads to an increase in permeability of the stratum corneum to the drug.

CONCLUSION

All BMFF ethosomes formulas had good criteria as ethosomes that were evidenced by particle size distribution, morphology, polydispersity index, zeta potential and entrapment efficiency. However, stability of BMFF ethosomes should be improved for long term application. In addition, BMFF ethosomes could significantly increase the STG (in BMFF) flux through the skin. It suggests that BMFF ethosomes is a promising dosage form for transdermal delivery.

ACKNOWLEDGEMENT

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CONFLICTS OF INTEREST

There is no conflicts of interest in this study.

ABBREVIATIONS

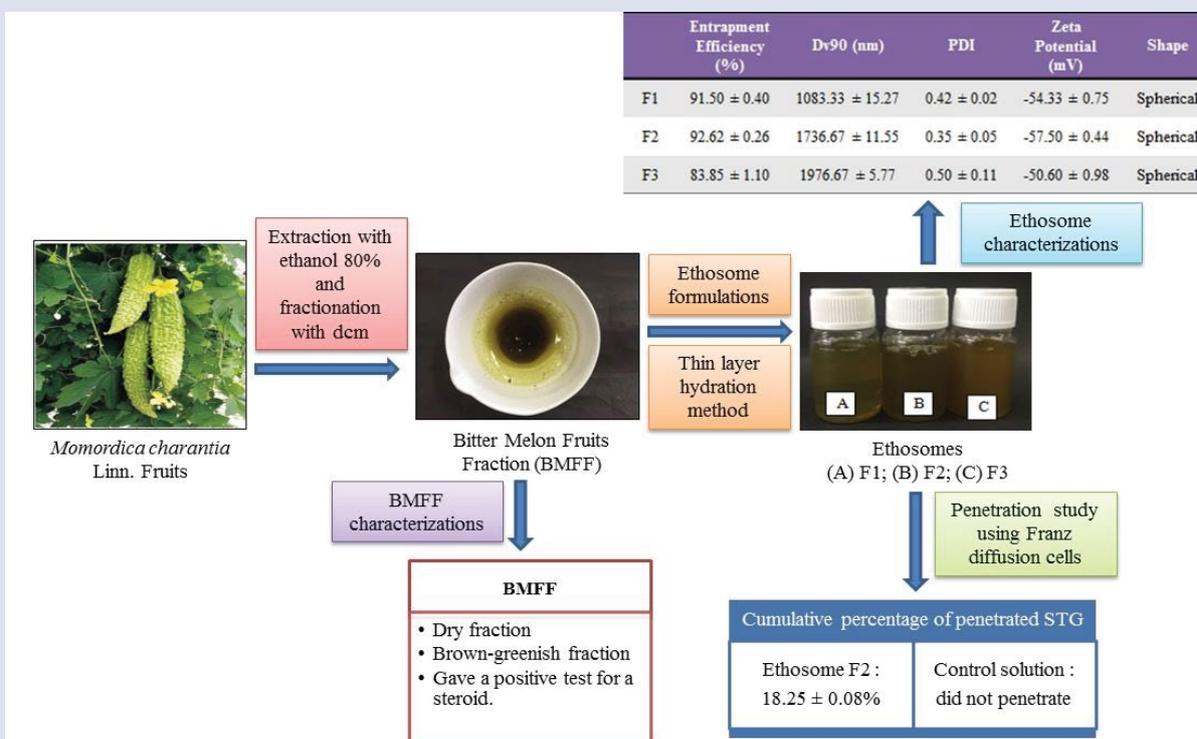
BMFF: Bitter melon fruits fraction; **DCM:** Dichloromethane; **STG:** Stigmasterol glycoside; **BSG:** β -Sitosterol glycoside; **HPLC:** High performance liquid chromatography; **UV:** Ultraviolet; **DLS:** Dinamic light scattering; **TEM:** Transmission electron microscopy; **PDI:** Polydispersity index.

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GRAPHICAL ABSTRACT



SUMMARY

Ethosome was used to overcome skin penetration problem for BMFF. Ethosomes were formulated with a various concentration equal to 2% (F1), 3% (F2) and 4% (F3) of bitter melon fruits fraction (BMFF) then the penetration study was conducted using Franz diffusion cells. BMFF was a dry, brown-greenish fraction and gave a positive test for a steroid. All formulas had a spherical shape. The entrapment efficiency of ethosomes F1, F2 and F3 was $91.50 \pm 0.40\%$, $92.62 \pm 0.26\%$ and $83.85 \pm 1.10\%$, respectively. Moreover, the particle size (Dv90) of ethosomes F1, F2 and F3 was 1083.33 ± 15.27 nm, 1736.67 ± 11.55 nm and 1976.67 ± 5.77 nm, respectively. Ethosomes F1, F2 and F3 resulted polydispersity index of 0.42 ± 0.02 , 0.35 ± 0.05 and 0.50 ± 0.11 , as well as zeta potential of -54.33 ± 0.75 , -57.50 ± 0.44 and -50.60 ± 0.98 , respectively. Ethosome F2 was chosen as a optimal ethosome among other formulas. Cumulative percentage of penetrated stigmaterol glycoside for ethosome F2 was $18.25 \pm 0.08\%$, while the control solution did not penetrate within 20 h. Therefore, BMFF ethosomes could significantly increase the STG (in BMFF) flux through the skin.

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