

DEVELOPMENT AND EVALUATION OF CREAM PREPARATION CONTAINING PHYTOSOME FROM AMLA FRUIT EXTRACT (*PHYLLANTHUS EMBLICA* L.)

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ABSTRACT

Objective: This study aims to develop and evaluate the phytosome of *Phyllanthus emblica* L. fruit extract.

Methods: The formation of phytosome was performed by using three methods, namely solvent evaporation, antisolvent, and thin layer formation method. The phytosome was evaluated for its entrapment efficiency, particle size, polydispersity index, Fourier Transform Infra-Red (FTIR), and Transmission Electron Microscope (TEM) methods. The phytosome was made into cream with a concentration of 1%. The cream stability was evaluated at a room temperature of 40 °C for 28 d. The permeation of the total phenolic content of cream-containing phytosome and cream-containing extract without phytosome technology was evaluated by diffusion studies.

Results: The best method to prepare phytosome was an antisolvent method with the efficiency of entrapment, particle size, and polydispersity index equal to 66.99±0.01%, 298.53 nm±12.04, and 0.323±0.01 respectively. Evaluation by using TEM and FTIR spectroscopy also confirmed the formation of spherical phytosome. There were no significant changes in the cream's organoleptic, pH, and viscosity during storage at room temperature. The permeation test through skin snake and Spangler's membrane showed that cream containing phytosome had a better diffusion percentage than cream containing extract without phytosome technology.

Conclusion: The results of this study showed that the antisolvent method was a potential method to develop phytosome of *Phyllanthus emblica* L. fruit extract and cream containing phytosome was effective in increasing the permeation.

Keywords: Phytosome, *Phyllanthus emblica* L. fruit extract, Cream, Permeation studies

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INTRODUCTION

Skin is the largest organ in the human body and the first defence against dangerous external materials [1]. UV irradiation and oxidative stress are the main extrinsic factors that have been identified can cause premature aging, skin damage, and skin cancer. The presence of excess reactive oxygen species triggers oxidative stress. Under normal conditions, the endogenous antioxidant system in the skin effectively counteracts reactive oxygen species. When the skin was exposed to the intrinsic or extrinsic factor which is cause oxidative stress, the effectiveness of the endogenous system of antioxidants decreases. It is important to support endogenous antioxidant systems by consuming food or applying compounds that have antioxidant activity to skin [2].

Phyllanthus emblica L. (synonym *Emblia officinalis* Gaertn.) grows in subtropical and tropical regions such as India, China, Malaysia, and Indonesia. A previous study showed that *Phyllanthus emblica* L. fruit water extract had a total phenolic content of 34.22% with a gallic acid content of 20.5% [3]. The water extract of *Phyllanthus emblica* L. has activities such as antibacterial, anticancer, antiaging, skin lightening, and skin cancer prevention [1, 4, 5]. Phenolic compounds are compounds in plants. Its chemical structures can capture free radicals, such as radical oxygen species. The majority of the presence of phenolic compounds is in glycoside form. It causes the use of phenolic compounds in the cosmetics field to be limited due to the low permeability of the epidermal barrier [6]. The technology of forming a complex between phospholipids and compounds in plants known as phytosome has been widely used in topical use to increase permeation and stability [7–10].

There are some phytosome preparation methods, namely solvent evaporation, antisolvent, and thin layer formation [11]. The study to compare the phytosome preparation methods of *Phyllanthus emblica* L. still needs to be completed. In contrast, the optimum method should be important information in the preparation of phytosome, particularly in phytosome for the pharmaceutical dosage form. Therefore, this study compares three phytosome preparation methods to determine the optimum method for phytosome

preparation of *Phyllanthus emblica* L. In addition, the study aimed to develop and evaluate cream preparations containing phytosome from *Phyllanthus emblica* L. fruit extract.

MATERIALS AND METHODS

Materials

Phyllanthus emblica L. powder used in this study was collected from Aceh in July 2016. It was authenticated in Herbarium Bandungense, School of Life Science and Engineering, Bandung Institute of Technology, with specimen number 2340/11. CO3.1/PP/2016. Lipoid S100 was purchased from Landson Pharmaceutical Industry. All other reagents and chemicals used in the study were analytical grade.

Methods

Crude extract preparation

Phyllanthus emblica L. water fruit extract was gained by reflux method with a ratio 1:10 between crude powders and water at temperature 110-120 °C for 3 x 2 h. The resulting extract was concentrated using a freeze dryer.

Total phenolic content

The Folin Ciocalteu reagent measured total phenolic content. The procedure was described in Indonesian Pharmacopoeia of Herbal. Total phenolic content was determined by reacting 1 ml of extract (100 µg/ml) with 5 ml of Folin Ciocalteu reagent 7.5%. After 1 hour of incubation, the absorbance was read with a spectrophotometer UV-Vis at a maximum wavelength of 730 nm against blank. Gallic acid was used as a standard. Total phenolic content was expressed as gram gallic acid Equivalent per 100gram extract weight (g GAE/100 g). Analysis was done in triplicate for each sample [12].

Antioxidant activity assessment by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity method

Each 2 ml of DPPH 50 µg/ml was added to 2 ml of extract 3-20 µg/ml. The reaction mixture was incubated in the dark for 30 min at

room temperature. Absorbance was read at a maximum wavelength of 517 nm against blank. Ascorbic acid was used as standard. The scavenging activity percentage was read as the calculation of the inhibition percentage using the following equation.

$$\text{Inhibition percentage (\%)} = \frac{\text{Control Abs.} - \text{Extract Abs.}}{\text{Control Abs.}} \times 100$$

Analysis was done in triplicate for each sample.

Phytosome preparation

Preparations of phytosome were prepared by three different methods: solvent evaporation, antisolvent, and film formation. As an initial step, the extract was solved in methanol, and phospholipids were solved in dichloromethane resulting in a clear mixture [9].

Solvent evaporation method

The clear mixture was refluxed at 50 °C for 3 h in a 500 ml round-bottom flask. The result was evaporated with a vacuum at 50 °C for 1 hour in a round-bottom flask. The dried residue was stored at room temperature in the desiccators overnight. The precipitate was added to water as a hydration process for 30 min in a round-bottom flask with a speed of 210 rpm [11].

Antisolvent method

The clear mixture was refluxed at 50 °C for 3 h in a 500 ml round-bottom flask. The result was evaporated with a vacuum for 30 min at 50 °C. 100 ml of n-hexane was added, and the precipitate resulted. The precipitate was dried under a vacuum at 50 °C. The dried residue was stored at room temperature in the desiccators overnight. The precipitate was added to water as a hydration process for 30 min in a round-bottom flask with a speed of 210 rpm [11].

Film formation method

The clear mixture was evaporated in a round-bottom flask with a vacuum at 50 °C for 1 hour. The thin layer formation was stored at room temperature in the desiccators overnight. The precipitate was added to water as a hydration process for 30 min in a round-bottom flask with a speed of 210 rpm [11].

Entrapment efficiency

Entrapment efficiency was determined using a spectrophotometer UV-Vis. A known amount of phytosome preparation of various methods was taken at 1 ml. Each phytosome preparation was added 5 ml of water. The proportion of encapsulated phenolic content was determined by centrifuging the mixture at 5000 rpm for 60 min. Total phenolic content from the supernatant was measured using a spectrophotometer UV Vis at a maximum wavelength of 733 nm. The measurement of total phenolic content was done in triplicate for each sample. The calculation of entrapment efficiency followed the equation:

$$\text{EE (\%)} = \frac{T-S}{T} \times 100$$

Where,

T Phenolic content is the total amount present in a quantity of phytosome taken

S Phenolic content amount in the supernatant

T-S Amount of phenolic content entrapped

Extract: phospholipid optimization

The phytosome was prepared with various ratios (4:1; 1:1; 1:2; 1:3) between extract and phospholipids using the optimum method of phytosome preparation.

Phytosome evaluation

Entrapment efficiency

Entrapment efficiency was measured using a spectrophotometer UV-Vis. A known amount of prepared phytosome of various methods was taken at 1 ml. For each prepared phytosome was added 5 ml of water. The proportion of encapsulated phenolic content was determined by centrifuging the mixture at 5000 rpm for 60 min.

Total phenolic content from the supernatant was measured using a spectrophotometer UV Vis at a maximum wavelength of 733 nm. The measurement of total phenolic content was done in triplicate for each sample. The calculation of entrapment efficiency followed the equation:

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T-S Amount of phenolic content entrapped

Particle size analysis and polydispersity index

Particle size and polydispersity index of phytosome were measured using *Particle Size Analyzer*.

Optimization particle size

Various sonication times (5 s, 10 s, 15 s, and 20 s) were performed using a probe sonicator.

Transmission electron microscopy (TEM)

Transmission electron microscopy was used to analyze the surface morphology. 10 µl of phytosome was dropped onto the specimen and then closed by a grid with mesh 400 for 1 minute. After that, 10 µl of uranyl acetate was dropped onto the grid for 30 min until the grid was dried and analyzed the samples using transmission electron microscopy.

Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy was used to study the interaction between extract and phospholipids. The spectrums of IR were obtained from extract, phospholipids, physical mixture of extract and phospholipids, and phytosome of extract-phospholipids by the potassium bromide (KBr) method. KBr pellets were made by adding a sample to KBr with a ratio of 1:100 until homogeneous. Each sample pellet was analyzed in the range between 4000 and 400 cm⁻¹.

Cream base optimization

Cream bases were optimized by the formula explained in table 1.

Cream base evaluation

The cream base evaluation was obtained from base stability for eight days. The evaluations were analyzed from organoleptic determination, pH, and viscosity measurements. Each test was done in triplicate.

Cream formulation of phytosome

The optimum cream base was formulated with the addition of phytosome 1%. All ingredients were weight and placed in separate beakers (oil and water phases). The cream preparation containing phytosome was made by mixing oil-and water-phase ingredients. Water phase ingredients (triethanolamine, propylene glycol, glycerin, propylparaben, disodium EDTA, and water) were heated at 70°-80 °C. Oil-phase ingredients (stearic acid, cetyl alcohol, and isopropyl myristate) were melted at temperatures 70°-80 °C. At the same range temperature of both phases, the oil-phase was added to the water-phase while stirring for 15 min until homogenous cream was formed. Phytosome was added to homogenous cream.

Diffusion studies

Diffusion tests were performed using diffusion flow through cells with various membranes (the snake's skin and Spangler membranes). A phosphate buffer of pH 7.4 ml was used as a receptor compartment. The temperature was maintained at a temperature of 37 °C. The cream preparation was weighed around 450 mg and placed on a membrane cell. The receptor solution flowed through the bottom of the membrane by a peristaltic pump. The receptor compartment container has a stirrer to homogenize the buffer

solution. 3 ml of receptor compartment solution was taken for total phenolic content determination using spectrophotometer UV-Vis

and replaced with 7.4 phosphate buffer with the same volume at 15, 30, 45, 60, 120, 180, 240, 300, 360, 420, and 480 min.

Table 1: Composition of cream base optimization

Ingredients	Percentage of amount (%)		
	F1	F2	F3
Stearic acid	5	10	15
Cetyl alcohol	1.5	1.5	1.5
Triethanolamine	0.67	0.67	0.67
Propilen glycol	1.5	1.5	1.5
Isopropyl myristate	5	5	5
Glycerin	1.90	1.90	1.90
Propylparaben	0.20	0.20	0.20
Disodium EDTA	0.40	0.40	0.40
Aquadest	add to 100	add to 100	add to 100

Statistic study

Each sample analysis was performed in triplicate. All results were presented the means (\pm SD) of at least three times experiments. Statistical analysis was using ANOVA (* $P \leq 0.05$).

RESULTS

Preparation of water extract

The fruit extract of *Phyllanthus emblica* L. was refluxed for 3 x 2 h. The yield obtained from this research was 46.44%.

Total phenolic content

The total phenolic content of *Phyllanthus emblica* L. water extract measured by the Folin-Ciocalteu method was shown in terms of gallic acid equivalent (the equation of standard curve: $y = 0.0098x + 0.0134$, $r^2 = 0.9949$). In our study, the total phenolic content of *Phyllanthus emblica* L. water extract was 197.46 ± 5.50 mgGAE/g.

Antioxidant activity assessment by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity method

This reducing reaction resulted in the color change from purple to yellow. IC_{50} of *Phyllanthus emblica* L. water extract is 14.85 ± 0.34 μ g/ml, whereas IC_{50} of vitamin C is 3.94 ± 0.39 μ g/ml.

Preparation and phytosome evaluation

This study resulted that the antisolvent method had higher entrapment efficiency than other methods. Because of the entrapment efficiency value, the antisolvent method was chosen for phytosome preparation. Various weight ratios were determined for the optimum ratio by entrapment efficiency measurement. The weight ratio 1:3 between extract and phospholipids had the optimum result by highest entrapment efficiency than others and smaller particle size and polydispersity index. This weight ratio was used for the next step.

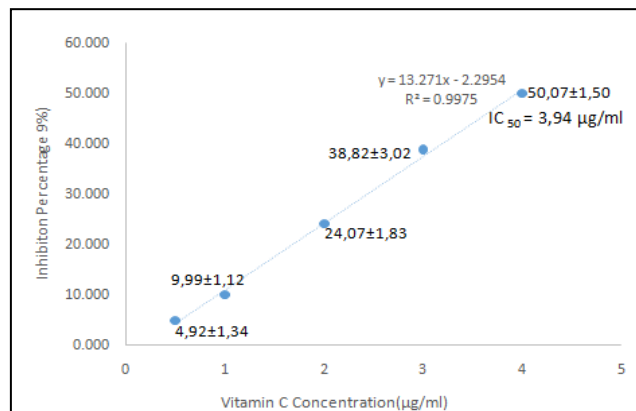


Fig. 1: Inhibition concentration of vitamin C

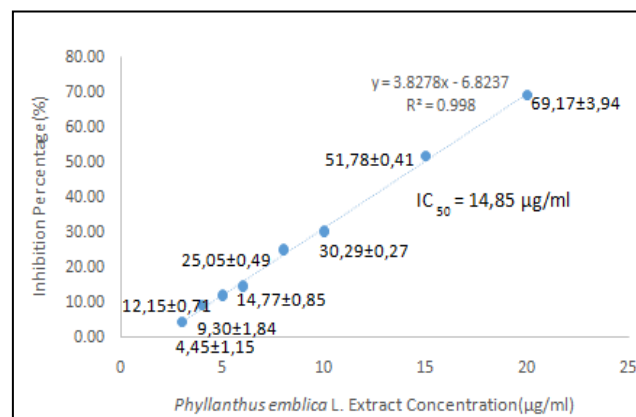


Fig. 2: Inhibition concentration of *Phyllanthus emblica* L. extract

Table 2: IC₅₀ value comparison*

Samples	IC ₅₀ (µg/ml)
Vitamin C	3.94±0.39
<i>Phyllanthus emblica</i> L. water extract	14.85±0.34

*Data were expressed as mean±SD of three experiments

Table 3: Entrapment efficiency of total phenolic content with various methods*

Method	Entrapment efficiency (%)
Solvent Evaporation	35.54±1.92
Antisolvent	38.44±0.63
Thin Layer Formation	14.27±1.23

*Data were expressed as mean±SD of three experiments

Table 4: Entrapment efficiency of total phenolic content by antisolvent method with various weight ratios between *Phyllanthus emblica* L. extract and phospholipids*

Weight ratio extract: phospholipid	Mean entrapment efficiency (%)	Particle size (µm)	Polydispersity index
4:1	52.66±5.56	3.78±1.26	0.963±0.01
1:1	58.96±0.80	1.09±0.95	0.451±0.04
1:2	62.89±0.01	2.39±1.05	0.783±0.48
1:3	66.99±0.01	1.15±0.20	0.475±0.08

*Data were expressed as mean±SD of three experiments

Table 5: Phytosome particle size and polydispersity index with various sonication times*

Sonication time (second)	Mean particle size (nm)	Polydispersity index
5	820.73±88.34	0.354±0.03
10	553.93±112.85	0.270±0.04
15	298.53±12.04	0.323±0.02
20	122.83±3.14	0.350±0.04

*Data were expressed as mean±SD of three experiments

Surface morphology

The surface morphology of the phytosome of *Phyllanthus emblica* L. extract-phospholipids was examined by TEM in fig. 5. It showed that the phytosome of *Phyllanthus emblica* L. extract-phospholipids has a spherical form and the particle size was less than 500 nm. Depending on the particle size analyzer, the particle size of the phytosome was 298.53±12.04 nm.

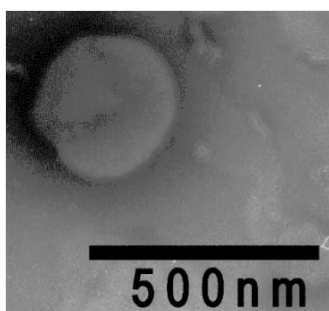


Fig. 3: Phytosome morphology surface determination using a transmission electron microscope

Infrared spectroscopy

The band reduction intensity between 3400 cm⁻¹ and 3500 cm⁻¹ (stretching of hydroxyl) and 2900 cm⁻¹ (stretching of CH from a fatty acid chain) in phytosome than a physical mixture. The band in the phytosome showed a disappear band at 1111.00-1178.51 cm⁻¹ compared with the band in extract and physical mixture.

Where,

— Phytosome between *Phyllanthus emblica* L. extract and phospholipids

— A physical mixture between *Phyllanthus emblica* L. extract and phospholipids

Optimization of cream base

The optimum cream base was evaluated by organoleptic, pH, and viscosity for eight days at room temperature and 40 °C.

Organoleptic

There were no organoleptic changes from all cream bases.

pH and viscosity measurement

Statistical analysis showed significant changes from the F3 cream base because the significant value was (*P<0.05, **P<0.004). In comparison, other cream bases had no significant changes in pH value. The pH stability of the cream base for eight days at room temperature and at the temperature of 40 °C was shown in fig. 7 and fig. 8.

Diffusion studies

In vitro diffusion tests were performed on cream-containing phytosome and cream-containing extract without a phytosome system. Snakeskin and Spangler's membranes were used to evaluate this diffusion test. The cream containing phytosome showed a better absorbance, read using spectrophotometer UV-Vis measurement, than cream containing extract without the phytosome system. Depending on the absorbance value from the spectrophotometer UV-Vis measurement, cream-containing extract without the phytosome system was difficult to measure because of the little absorbance.

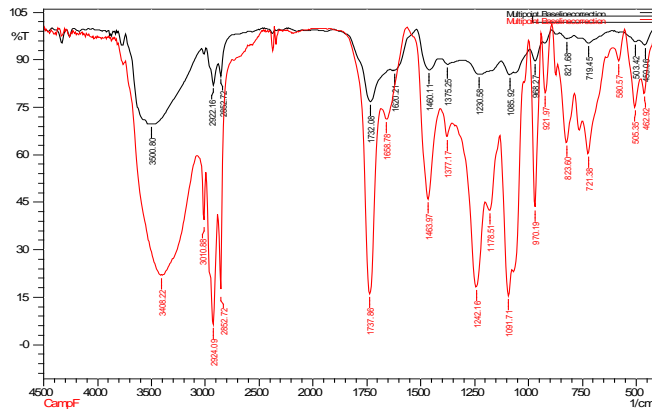


Fig. 4: Infrared spectrum comparison between phytosome of *Phyllanthus emblica* L. water extract-phospholipid and physical mixture of *Phyllanthus emblica* L. water extract and phospholipid

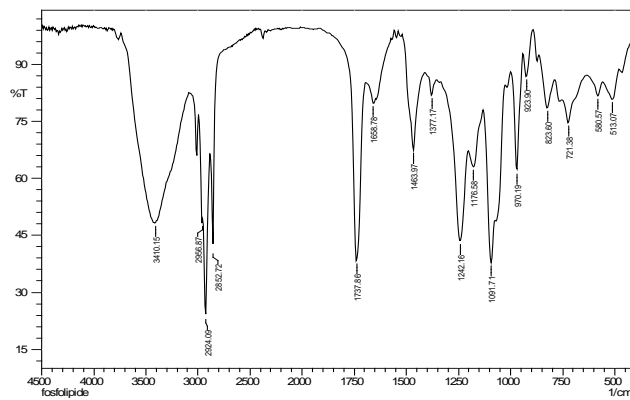


Fig. 5: Infrared spectrum of phospholipids

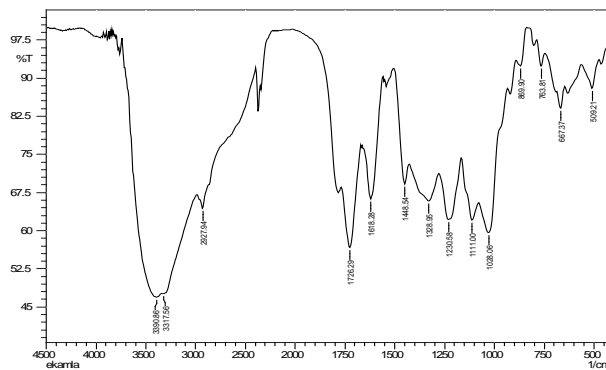


Fig. 6: Infrared spectrum of *Phyllanthus emblica* L. extract

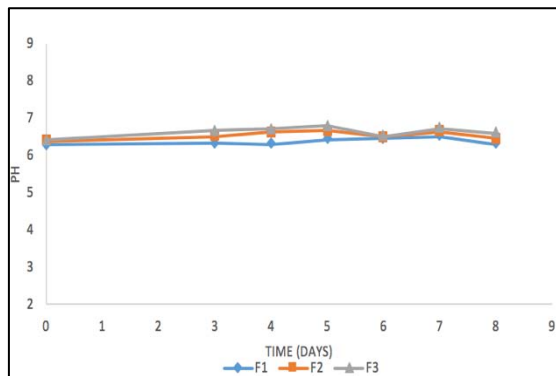


Fig. 7: pH stability of various cream bases during eight days at room temperature

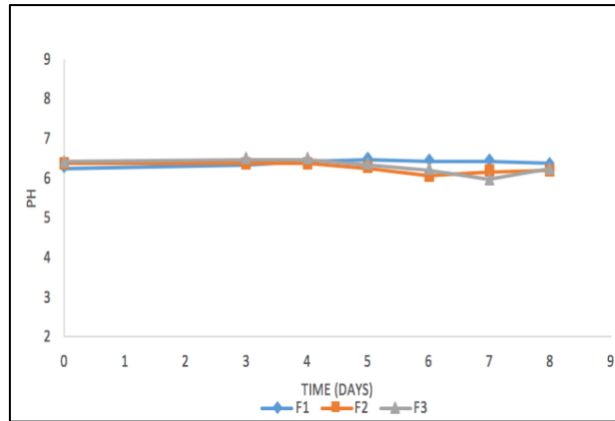


Fig. 8: pH stability of various cream bases during eight days at 40 °C

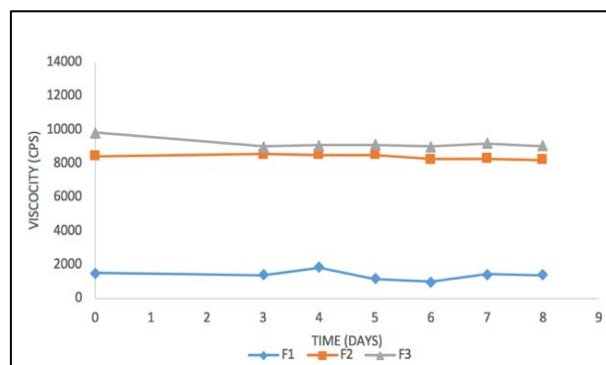


Fig. 9: Viscosity stability of various cream bases during eight days at room temperature

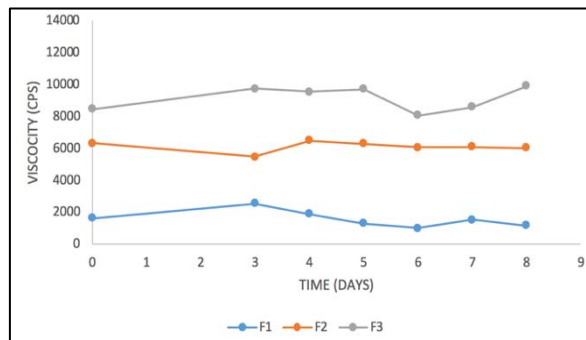


Fig. 10: Viscosity stability of various cream bases during eight days at 40 °C

Preparation and evaluation cream containing phytosome

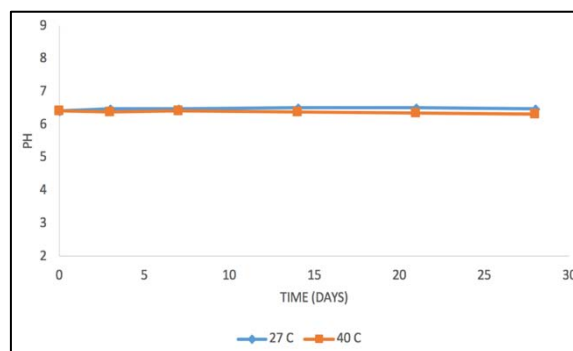


Fig. 11: pH stability of cream containing phytosome during 28 d at room temperature 27 °C and 40 °C

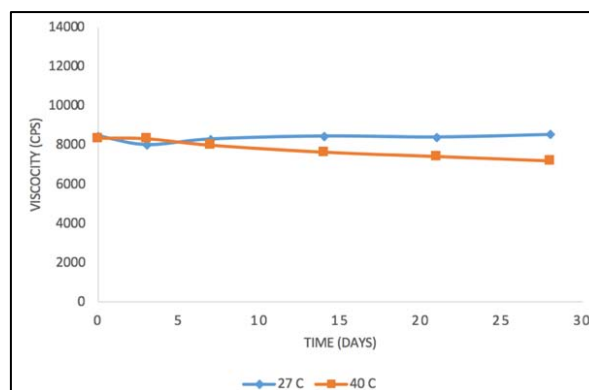


Fig. 12: Viscosity stability of cream containing phytosome during 28 d at room temperature 27 °C and 40 °C

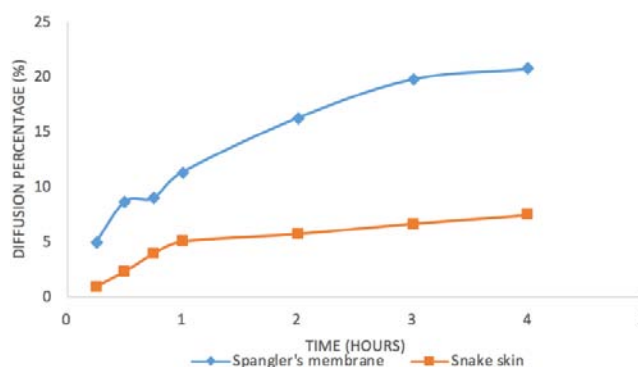


Fig. 13: Diffusion percentage of total phenolic content in cream containing phytosome using snake skin and spangler's membrane by spectrophotometry UV Vis method

DISCUSSION

Phytosome is the delivery system formed by the interaction between the phytochemicals and phosphatidylcholine in phospholipid, obtaining in the formation of hydrogen bonds between them [11].

Total phenolic content was measured by the Follin-Ciocalteu method. This method is based on forming the phosphotngstat-phosphomolibdat complex from phenolic hydroxyl compound oxidation. The complex had a blue color which could be detected by a spectrophotometer [12]. Previous studies have reported that the total phenolic content of *Phyllanthus emblica* L. extract was 188-237 mgGAE/g and 342.2±1.74 mgGAE/g [13]. In comparison, the total phenolic content of this study was 197.46±5.50 mgGAE/g.

DPPH scavenging capacity method depends on hydrogen atom scavenging by DPPH to antioxidant sample. DPPH is reduced to become diphenylpicrylhydrazine (DPPH-P). IC₅₀ was used to determine the antioxidant capacity of the sample compared to standard. The sample that had IC₅₀<50 ppm, was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with IC₅₀>150 ppm [14].

This study showed that *Phyllanthus emblica* L water extract was very strong antioxidant. Phenolic hydroxyl (-OH) could be an antioxidant substance because OH is an electron donor to free radicals or others reactive species [15].

There were some methods to produce the phytosome system, namely solvent evaporation method, antisolvent method, and thin layer formation method. This study showed the optimum method of phytosome of *Phyllanthus emblica* L. extract-phospholipids preparation was an antisolvent method with a weight ratio 1:3 between extract and phospholipids. It resulted higher entrapment efficiency than others method and others weight ratio. The entrapment efficiency determination was measured by indirect measurement to free phenolic content.

Time optimization of the sonication process was performed to determine the optimum time that resulted in the optimum particle size and polydispersity index. This study showed that higher sonication time resulted lower particle size. Energy (E) during sonication process not only depend on applied power but also depend on time (E=Pxt) [16]. Longer sonication time is linear with power that resulted to break down the particle size. The optimum sonication time is 15 seconds.

The morphology of phytosome of *Phyllanthus emblica* L. extract-phospholipids was determined using TEM. It showed the spherical shape with the particle size lower than 500 nm. Depending to particle size analyser, particle size of phytosome was 298.53±12.04 nm. Particle size below 300 nm has an ability to reach lower epidermis [17].

Infrared spectroscopy was used to determine the interaction between extract and phospholipids. The infrared spectroscopy spectra of extract, phospholipids, phytosome and physical mixture were gained by the potassium bromide (KBr) method. The spectrum confirmed the interaction between extract and phospholipid in phytosome. The spectrum was indicated that the phytosome of *Phyllanthus emblica* L. extract-phospholipids was formed [11].

Depending to the evaluation of cream base optimization, F2 is the optimize cream base. Due to, F3 had a significant changes of pH value and F1 at 40 °C had viscosity less than 2000 cps after 8 d. Good cream has a viscosity in a range 2000-50000 cps [18].

Depending to absorbance value from spectrophotometer UV-Vis measurement, cream-containing extract without phytosome system was difficult to measure because of the little absorbance. It can be caused by the small amount of total phenolic compound which penetrates or due to the poor stability of the total phenolic compound in the receptor compartment. It be evidence by dissolving gallic acid standard in phosphate buffer pH 7.4, and the colour changes from clear to greenish was occurred. Cream containing

Phytosome had a better diffusion percentage than cream containing extract without phytosome system. It can be caused the form of phytosomes affected the permeation and improved the stability of total phenolic compound in receptor compartment.

The diffusion percentage of total phenolic content in cream containing phytosome using Spangler membranes was higher than snake skin membranes. The permeation process is influenced by the physiological properties of the skin such as water and lipid content, and the thickness of the skin. Snake skin membranes was chosen because snake skin has a coefficient of stratum corneum thickness that does not differ significantly from human stratum corneum thickness, but the water content in stratum corneum of snake skin is significantly different from water content in human stratum corneum [19]. Spangler's membrane is a Whatman paper which is impregnated with a mixture of several lipids that represent semi polar lipids contained in the epidermal extracellular matrix [20]. So that, it can be more facilitate permeation of phytosome as vesicles system.

CONCLUSION

The optimum preparation of phytosome was an antisolvent method which has an efficiency of entrapment, particle size, and polydispersity index equal to $66.99 \pm 0.01\%$, $298.53 \text{ nm} \pm 12.04$, and 0.323 ± 0.01 respectively. Evaluation by using TEM and FTIR spectroscopy also confirmed the formation of spherical phytosome. There were no significant changes in organoleptic, pH and viscosity of the cream during storage at room temperature. But there was a significant change in viscosity of the cream at temperature of 40°C . The permeation test through skin snake and Spangler's membrane showed that cream containing phytosome had better diffusion percentage compared to cream containing extract without phytosome technology.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

All authors have none to declare

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