Formulation And Evaluation Of Liposome Moringa Oleifera Seed Oil (*Moringa Oleifera* L.) As Anti-Aging

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Abstract.
Moringa oil is a natural vegetable oil obtained from the juice of the ripe kernels of the Moringa tree (Moringa oleifera L). Moringa seeds have an oil content of up to 40%, and have antioxidant activity with vitamin E content reaching 51% but have low stability. The purpose of this study was to find the best formulation as an anti-aging on liposome delivery system with a ratio of phosphatidylcholine soy lechitin and cholesterol. The method used is thin layer hydration (Thin Film Hydration). The results of the observation on the best formula are the 6th formula on organoleptic examination which is milky yellow in color, has a distinctive smell of lecithin and cholesterol and is in the form of a thick suspension, with a pH of 6.0, particle size 83.98nm using a particle size analyzer and entrapment efficiency of 96.8 % using a UV-Vis spectrophotometer.

Keywords: *Moringa oleifera seed oil, liposome, anti-aging.*

I. INTRODUCTION

The skin is the largest organ in the body and covers the entire outer surface of the body. The skin consists of three layers namely the epidermis, dermis, and hypodermis. The skin serves as the body's primary protection against pathogens, UV rays and chemicals as well as mechanical injury. The process of skin destruction which is marked by the appearance of wrinkles, scales, dryness, and cracks, black spots appear caused by free radicals. Free radicals are the cause of premature aging of the skin, because free radical attacks on tissues can damage fatty acids and eliminate elasticity, so that the skin becomes dry and wrinkled. [1],[2].Aging or skin aging is a biological process generally involving intrinsic factors (genetic, cellular metabolism, hormones and metabolic processes) and extrinsic factors (long-term exposure to light, pollution, ionizing radiation, chemicals and toxins). Cumulative and progressive changes in the structure and physiology of the various layers of the skin, as well as changes in the appearance of the skin such as wrinkles and spots. These factors can cause skin oxidative stress, which can lead to skin cell and tissue damage. Anti-aging is a part of cosmetics that contains ingredients to reduce wrinkles and increase the moisture level of the skin. The main function of anti-aging preparations is to reduce wrinkles and age spots. Based on a report from global industry analysis, in 2021 to 2026, it is estimated that the demand for anti-aging products will reach $88.30 billion [3],[4].Moringa (Moringa oleifera) is a plant originating from the sub-Himalayan region of Northwest India and is also spread throughout Indonesia. Traditionally, Moringa seed oil is used as a natural skin moisturizer to remove fine lines on the face and treat scalp irritation. The antioxidants in Moringa seed oil have UV absorption activity which can increase protection against the negative effects of solar radiation on the skin.

Previous researchers have stated that Moringa seed oil contains high levels of vitamin E (tocopherol) reaching up to 51%, this means that Moringa seed oil has the potential to used for various cosmetic products. The highest antioxidant activity obtained the IC_{50} value of Moringa seed oil with a value of 62 g/ml. However, the content of vitamin E (tocopherol) in Moringa seeds has unstable stability to air and light [5],[6].One method of drug delivery system that is good and can be used topically is the liposome method. Previous studies have found that liposomes are one of the unique drug delivery systems, because their amphiphilic character allows solubilization or encapsulation of active substances, both hydrophobic and

hydrophilic, and the use of liposomes in cosmetic products has a good penetration rate and room temperature stability. Liposomes can protect the active substance from being degraded and liposomes increase the stability of the active substance which has low stability8-12. Liposomes are mainly composed of phospholipids of natural origin and cholesterol. Phospholipids have an important role with amphiphilic properties and self-assembly to encapsulate or active substances. Phospholipids play an important role in the composition of cell membranes so that they are very suitable as a constituent of liposomes22,23. Cholesterol has been widely used to improve the characteristics of the liposome bilayer membrane. The structure of cholesterol is composed of hydrocarbons in the form of steroid rings that can fill the space between the alkyl chains in the bilayer membrane and has an important function because of its ability to modulate the physico-chemical properties of cell membranes [7]-[10].

II. METHOD

a. Ingredients

Moringa oleifera were procured from VegIndian Exports, (Erode, Tamil Nadu, India), Phosphatidylcholine Soy lecithin from soybean oil (USA), Cholesterol from woll fat (Merck KGaA, Germany), Chloroform (Merck, Germany), Methanol (Merck, Germany) and Aquadest.

b. Preparation of Liposomes

Liposomes are formulated with a mixture of phosphatidylcholine soy lecithin, cholesterol and moringa seed oil (fat phase). Liposomes were made using the Thin Film Hydration method. The liposome formula made can be seen in Table 1. All ingredients were weighed according to the formulation then the fat phase was dissolved in an organic solvent, namely chloroform:methanol (2:1) and then put into a round flask. Then it was evaporated with a rotary evaporator at a temperature of 60°C with a rotation speed of 200 rpm and a pressure of 200 mBar for 1 hour to obtain a thin film of fat and allowed to stand for 1 night in a desiccator under vacuum until dry. After that, the fat layer formed in the form of a dry film was then hydrated using water at a temperature of 60°C with a magnetic stirrer, then sonicated using a sonicator for 1 hour and left at room temperature for 2 hours for the formation of intact vesicles [11]-[13].

Table 1. Moringa seed oil liposome formulation

Formulation	Moringa seed oil IC_{50} (µg/ml)	lecithin (%)	Cholesterol (%)	Aquadest
F1	186	8	0,5	Ad 100
F2		8	1	
F3		8	1,5	
F4		10	0,5	
F5		10	1	
F6		10	1,5	
F7		12	0,5	
F8		12	1	
F9		12	1,5	

c. Organoleptic Examination

The liposomes formed were visually observed with a dark/black background. If the turbidity has a bluish shadow, it indicates that the sample particles are in a homogeneous condition, while the turbidity with a gray color indicates that there is a dispersion of liposome particles in the dispersion [14],[15].

d. Characterization of pH

Determination of the pH of the preparation is carried out using a pH meter in a way that the instrument is first calibrated using a standard neutral pH buffer solution (pH 7.01) and an acidic pH buffer solution (pH 4.01) until the instrument shows the pH value. Then the electrodes were washed with distilled water, then dried with tissue paper. Then the electrode is immersed in the sample, until the instrument shows a constant pH value. The number shown by the pH meter is the pH value of the preparation [16].

e. Characterization of Particle Size

Determination of particle size using a particle size analyzer (FRITSCH Analysette 2.2 Nanotech). The working principle of the tool is to use Laser Diffraction (LAS), which is when the particles pass through the laser beam and the light scattered by the particles is collected beyond the range of angles that are directly

opposite each other. The distribution of these scattered intensities which will be analyzed by the computer as a particle size distribution [17].

f. Characterization entrapment efficiency (*EE%*)

Determination of the entrapment efficiency (EE%) in this study using a UV-Vis spectrophotometer. 1 ml lipoosm was dissolved in methanol in a ratio of 1:10 liposomes diluted and centrifuged at 6000 rpm for 60 minutes at 4˚C. The supernatant obtained was pipette 1 ml and put into a 10 ml volumetric flask, then the volume was adjusted with methanol to the limit line. The solution was then measured its absorption at a wavelength of 218 nm [18]. The amount of adsorption is then calculated using the following formula:

$$Count\ rate = \frac{\boldsymbol{A-intercept}}{\boldsymbol{slope}}\ \boldsymbol{x}\ (\boldsymbol{dilution\ factor})$$

$$\text{EE\%} = \frac{C\ total - count\ rate}{C\ total}\ x\ 100\%$$

III. RESULT

The manufacture of this Moringa seed oil liposome preparation uses vesicle-forming materials such as phosphatidylcholine, soy lecithin and cholesterol. Lecithin as the main constituent of biological membranes in liposomes is used as a bilayer component to absorb Moringa seed oil, while cholesterol is used to maintain the stability of vesicles from the formed bilayer [19] and Moringa seed oil is used as an ingredient that has anti-aging activity. The data from the evaluation can be seen in Table 1.Based on organoleptic observations, the 9 formulas have a milky yellow color, according to the color of the liposome vesicle-forming material and Moringa seed oil which has a yellow color, characteristic odor, and a thick suspension dosage form. The higher the concentration of phosphatidylcholine soy lecithin and cholesterol concentration, the thicker the liposome preparation of Moringa seed oil. The observed pH of the 9 liposome formulas was still in accordance with the skin pH, which was between 4.5 - 6.5, so it was safe to use and did not cause irritation to the skin. Measurement of pH aims to ensure the safety of Moringa seed oil liposomes when applied to the skin. If the pH of the preparation is too acidic it will cause skin irritation and if the pH of the preparation is too alkaline it will cause scaly skin [20] so the pH of the preparation must match the skin pH, which is 4.5-6.5 [21].

Determination of the particle size of Moringa seed oil liposomes was carried out using a Particle Size Analyzer. Particle size is an important factor because it can affect the amount of sorption of the active substance in a delivery system. The ability of liposomes to penetrate the stratum corneum which is able to increase the penetration of the active ingredients in the topical preparation. Particle size is one of the factors for increasing the permeability, thereby increasing the amount of penetration of the active substance in the preparation in the stratum corneum. This is due to the liposome component as a penetration factor that can increase the permeability of the stratum corneum, because liposome vesicles are fused with the lipid components of the stratum corneum in the skin, so that the vesicles are able to penetrate deep into the skin layer [22]. Based on the results of the particle size analysis of the 9 liposome formulas of Moringa seed oil, the formula that has the best particle size is seen in formula 6 with a value of 83.98nm. The particle size results in the formula are included in small unilamellar vesicles (SUV) vesicles that meet vesicle size requirements ranging from 20-100 nm [23],[24].

Tabel 2. Evaluation of moringa seed oil liposome formula

Formulation	pH	Particle Size (nm)	Entrapment Efficiency (EE%)	Organoleptic
F1	6,26 ± 0,05	45,07	38,53	Milky yellow suspension Smells typical of lecithin and cholesterol
F2	6,03 ± 0,05	46,34	42,53	Milky yellow suspension Smells typical of lecithin and cholesterol
F3	6,13 ± 0,1	49,28	53,9	Milky yellow suspension Smells typical of lecithin and cholesterol
F4	6,5 ± 0	51,6	73,26	Milky yellow suspension Smells typical of lecithin and cholesterol

F5	6,0 ± 0	51,73	88,54	Milky yellow suspension Smells typical of lecithin and cholesterol
F6	6,0 ± 0	83,98	96,8	Milky yellow suspension Smells typical of lecithin and cholesterol
F7	6,0 ± 0	107,92	55,76	Milky yellow suspension Smells typical of lecithin and cholesterol
F8	6,0 ± 0	117,51	66,06	Milky yellow suspension Smells typical of lecithin and cholesterol
F9	6,0 ± 0	154,55	83,61	Milky yellow suspension Smells typical of lecithin and cholesterol

Determination of the entrapment efficiency was carried out using a UV-Vis Spectrophotometer. The entrapment efficiency test was carried out to determine the amount of moringa seed oil trapped in liposome vesicles. In this study using the centrifugation method, with the principle of the centrifugation method, namely the separation of active substances that are not adsorbed on liposome preparations. Moringa seed oil liposome preparations were centrifuged for 1 hour at 6000 rpm. The supernatant from the centrifugation is Moringa seed oil which is not adsorbed whose absorbance will be measured using a UV-Vis Spectrophotometer [25]. Determination of entrapment efficiency was used to describe the amount of active substance that was successfully adsorbed into liposome vesicles. The greater the value of the entrapment efficiency, the faster the penetration phase of the active substance through the skin caused by the greater the concentration gradient that encourages passive diffusion in penetration. Based on the analysis of the entrapment efficiency of the 9 formulas, it can be seen that the 6th formula has an adsorption efficiency of almost 100% with the obtained entrapment efficiency value of 96.8%.

IV. CONCLUSION

The liposome formulation of Moringa seed oil as anti-aging in this study was obtained from 9 formulas, the best formula was obtained, namely formula 6 on organoleptic examination which was milky yellow in color, had a characteristic odor of lecithin and cholesterol and the form of a thick suspension, with a pH of 6.0, particle size 83.98nm and 96.8% entrapment efficiency.

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