Stability and antioxidant tests of ethanol extract liposome of moringa leaves (*Moringa oleifera*)

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ABSTRACT

Moringa leaf potentially has an antioxidant effect because it contains Quercetin having poorsolubility in water. Liposomes as carriers of drug compounds can increase the solubility of quercetin through an entrapment system in the lipid bilayer. This study aimed to determine the stability and antioxidant activity of ethanol extract liposome of moringa leaves. This research method used experimental research utilizing moringa leaf ethanol extract in different concentrations (0,125%, 0.25%, and 0.5%) which was formulated into phospholipids by extrusion method. Preparation of liposome utilized Lipoid S-75 and cholesterol (1:1) by ethanol injection method then was characterized and stability tested. Meanwhile, the antioxidant test of ethanol extract liposome of moringa leaves used DPPH assay. The characterization results showed that particle size of moringa leaf ethanol extract (0.125%, 0.25%, 0.5%) liposomes were 112, 145, and 175 nm, with polydispersity index (PDI) of 0.34, 0.28, 0.12 respectively. Moreover, the entrapment efficiency (%EE) of all formulations showed that 0.25% of moringa leaf ethanol extract liposome had the highest load into liposome (93%) compared to 0.125% (80%) and 0.5% (60%)only the best result (0.25%) of size, %EE and stability which continued for the antioxidant test. Six weeks of stability study described that size and retention profile of liposomes were stable at 25°C and 37°C (0.25%). The result of the antioxidant test was obtained by inhibitory concentration (IC_{50}) 124.41 ppm (only ethanol extract) and 61.78 ppm (loaded-liposome) which was included in the moderate and strong category respectively. It can be concluded that moringa leaf ethanol extract liposome has strong antioxidant activity as a promising nano nutraceutical formulation.

Keywords: *antioxidant, ethanol extract, liposome, moringa leaf*

1. INTRODUCTION

Indonesia is a country that has the largest population with a variety of diverse community activities. One of the main problems in developing countries particularly Indonesia is pollution resulting free radicals which can cause negative effects on health. For example alcohol, radiation, medications, chronic stress, and poor diet. Therefore, some researchers are developing natural products to reduce the negative impacts of free radicals. One of the natural antioxidants is moringa leaves which can give solution to reduce the negative effects of pollution [1].

The raw materials of moringa leaves are very abundant and easy to get in Indonesia. Unfortunately, moringa leaves are only used for vegetables and animal feed so Moringa leaves are useful to be developed into medicinal raw materials as natural antioxidants [2].

One of the most important components of the Moringaplantisantioxidants and thehighestcontent is found in Moringa leaves. Studies show that fresh Moringa leaves have 7 times antioxidant capacity higher than vitamin C [3] and one of the flavonoid derivatives, namely quercetin, has 4-5 times stronger antioxidant power than vitamins C and E [4].

Moringa leaf extract contains flavonoid compoundssuchas Quercetin which are used as an antioxidantbuthavepoorsolubility in water. There are some advantages of liposome preparation such as reducing toxicity, increasing efficacy, increasing drug

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solubility, delivering drugs to specific target organs, and avoiding general damage before reaching the target site [5,6]. Therefore, to improve the solubility and increase the bioavailability of Quercetin, it was formulated into liposomes as lipid carriers [7,8].

2. MATERIALS AND METHODS

2.1. Materials

Lipoid S-75 was purchased from Lipoid Gmbh, Germany. Cholesterol, potassium dihydrogen phosphate, disodium hydrogen phosphate, ethanol 96% were bought from Sigma Aldrich, Germany.

2.2. Extraction of moringa leaves using rotary evaporator

Moringa leaves that have been in the form of hassle are weighed as much as 100 grams and soaked in 1 L of 96% ethanol with a ratio of 1:10. The extraction process was carried out for 5 days and was accompanied by shaking every 24 hours. The sample was filtered using filter paper to separate the filtrate and residue then continued with evaporation using a rotary evaporator to produce a thick extract.

2.3. Liposomal preparation of moringa leaf ethanol extract (MLEE) using ethanol injection

Two beaker glasses were prepared to consist of one beaker for phosphate buffer pH 7.4 and others for ethanol 96% solution. Lipoid S-75 (0.55%), cholesterol (0.125%), and MLEE 0.125% as formula 1 (F1)were dissolved with ethanol 96% while stirring until homogenous solution was formed. After that, ethanol solution was injected quickly into phosphate buffer solution while stirring for 30 minutes, 250 RPM to get opaque suspension then was done extrusion and continued with purification with PD-10 column. The above procedure is repeated in the same way for formula 2 (F2) 0.25% and formula 3 (F3) 0.5%.

2.4. Characterization of MLEE-loaded liposomes

MLEE-loaded liposomes were diluted to a total lipid concentration (0.1 mM in 100 mM phosphate buffer) before measuring the mean diameter size and polydispersity index (PDI). All determinations were recorded at room temperature (25°C) using a particle size analyzer (SZ-100, Horiba).

Moreover, all formulas of MLEE-liposomes absorbances were measured to calculate the entrapment efficiency percentage (%EE) using Spectrophotometry UV-Vis.

2.5. Stability tests of MLEE loaded liposomes

Liposomal colloidal stability under storage conditions was studied by monitoring their mean size and size distribution with PSA every week for 6 weeks upon storage in phosphate buffer at 4°C. Besides that, the stability studies of all liposomal formulation were performed at 25° and 37°C to see MLEE retention into liposomes for 24 hours. MLEE-liposome (5 mL) was analyzed by dialysismethodusing float-A-lyzer 300 kDa and tween 80 as an outer medium were collected at 25° and 37°C at different intervals (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours). The concentrations of MLEE containing Quercetin and the lipids were measured in the collected liposomal samples fraction and compared with the initial concentration of Quercetin and lipids, as described above for the determination of %EE.

2.6. Determination of antioxidant activity (IC_{50}) of MLEE-Liposome and free MLEE

Moringa leaf extract was dissolved in 96% ethanol to get a concentration of 400 ppm. The solution put into a 100 mL volumetric flask until sufficient to mark the limit by making test solutions with series of 20, 40, 60, and 80 ppm. This solutions were taken 2 mL from each the test solution and put into the vial then added 2 mL of 0.1 mM DPPH solution, shaken using a vortex until homogeneous and incubated for 30 minutes in thedarkroom. After that, the absorbance of the test solution was measured using a UV-Vis spectrophotometry at a DPPH wavelength of 516 nm. The above protocol was repeated in the same way as measuring samples of MLEE loaded liposomes

3. RESULTS AND DISCUSSION

3.1. Moringa leaf ethanol extract yield

The table 1 shows the yield of ethanol extract of moringa leaf 14.7% which is in accordance with the requirements of the yield between 10-15% which provides information that the extraction process has taken place perfectly.

Table 1. The yield of MLEE

Sample weight (gram)	Solvent volume (mL)	Extract weight (gram)	% yield
100	1000	14.7	14.7%

3.2. Characterization of MLEE loaded liposomes

The characterization results written in table 2 show that the three formulas have homogeneous particle size distributions with particle sizes between 100 - 200 nm.

This fits with literature which states that the liposome particle size is in the range of 100 - 200 nm with a PDI smaller of 0.7 [9,10]. Meanwhile, the results of entrapment efficiency show that formula 2 (0.25%) is the highest compared to formula 1 (0.125%) and 3 (0.5%). This means that flavonoid compounds such as quercetin are highly entrapped in the lipid bilayer of liposomes.

Tabel 2. The results of MLEE loaded liposomes characterization

Formula	Size (nm)	PDI	%EE
F1 0.125%	112	0.34	80%
F2 0.25%	145	0.28	93%
F3 0.5%	175	0.12	60%

3.3. Stability tests of MLEE-loaded liposomes and free MLEE

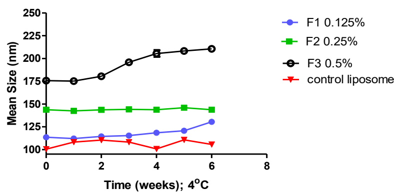

Figure 1. The stability of all liposomal formulations and free MLEE based on mean size change at 4°C

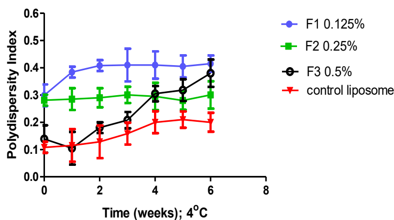

Figure 2. The stability of all liposomal formulations and free MLEE based on PDI change at 4°C

The stability tests, both graphs showed storage stability for 6 weeks. This study wants to see size and PDI of MLEE-liposomes change. In the sixth week, both graphs showed no change in size and PDI for formula 2 (F2) which is the same as the control liposome (Figure 1 dan 2). It means that F2 0.25% is stable compared to F1 and F3 which tend to change. This is related to cholesterol content which formula 2 also contains 0.25% cholesterol with the right ratio of 1:1 because cholesterol can make stable liposomes due to the rigid chain in the lipid bilayer [11].

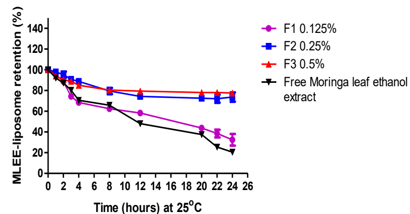

Figure 3. The percentage of MLEE-liposomes retention at 25°C

Meanwhile, the study also sees to the retention of liposomes in each formulation at 25° and 37°C. Both graphs showed that F2 and F3 kept stable on both temperature for 24 hours using a float lyzer and tween 80 as outer medium to keep sink condition (Figure 3 dan 4).

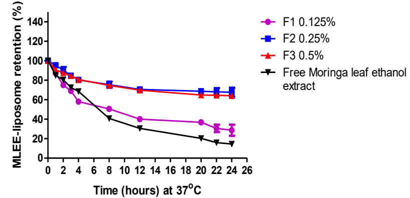

Figure 4. The percentage of MLEE-liposomes retention at 37°C

Otherwise, F1 and free moringa leaf ethanol extract tend to decrease liposome retention. It means that F1 and free drugs are not stable. This is also related to the cholesterol content ratio.

3.4. Antioxidant activity of MLEE-loaded liposome and free MLEE

Based on the results of the antioxidant test, the graph showed that 0.25% moringa leaf extract loaded liposome (F2) had an inhibition concentration (IC_{50}) of 61.78 ppm which was included in the strong category while free moringa leaf extract of 124.41 ppm which was included in the medium category (Figure 5). This means that the Quercetin compound that is trapped into liposome as a targeted delivery system is more effective in inhibiting free radicals [12].

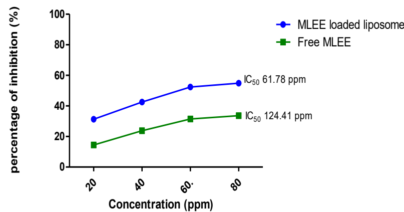

Figure 5. The inhibition percentage (IC_{50}) of MLEE loaded liposomes and free MLEE

4. CONCLUSION

Moringa leaf ethanol extract loaded liposome 0.25% (F2) is a stableformulationbased on the characterization and evaluation. Moreover, the antioxidant test gives IC_{50} of moringa leaf extract loaded-liposomeis more stronger than free moringa leaf extract. Therefore, moringa leaf extracts are very suitable to be formulated into liposomes as a candidate fordegenerative diseases such as cancer, diabetes, and so on.

AUTHORS' CONTRIBUTIONS

All authors take an equal part in this research.

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