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Preparation and Characterization of Moringa Leaf Extract in Ethosomal Vesicles with Variations in Phosphatidylcholine and Ethanol Concentrations

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ABSTRAK

Kelor merupakan salah satu tanaman obat yang memiliki khasiat sebagai antioksidan. Senyawa yang diduga memiliki aktivitas antioksidan yaitu kuersetin. Kuersetin termasuk senyawa hidrofobik yang tergolong dalam Biopharmaceutical Classification System (BCS) II, yang berarti memiliki permeabilitasnya yang tinggi dan kelarutan yang rendah. Untuk mengatasi kelemahan dan meningkatkan bioavailabilitas zat aktif, maka diatasi dengan enkapsulasi zat aktif ke dalam suatu sistem pembawa yang berukuran nano. Etosom merupakan sistem pembawa yang cocok digunakan untuk obat yang bersifat lipofilik, hidrofilik atau amfifilik. Penelitian ini bertujuan untuk mengetahui preparasi dan karakterisasi etosom yang mengandung ekstrak daun Kelor dengan variasi konsentrasi fosfatidilkolin dan etanol. Etosom dibuat dalam 3 formula dengan variasi konsentrasi fosfatidilkolin dan etanol yaitu F_1 1% dan 20%, F_2 2% dan 30% dan F_3 3% dan 40%. Hasil karakterisasi etosom menggunakan PSA (Particle Size Analyzer) didapatkan hasil ukuran partikel untuk F_1, F_2 dan F_3 secara berturut-turut sebesar 202,3 nm, 185 nm dan 146,3 nm dengan nilai rata-rata PDI (polydispersity index) sebesar 0,346, 0,293 dan 0,196. Selanjutnya pengujian persen efisiensi penjerapan dimana didapatkan hasil untuk F_1, F_2 dan F_3 secara berturut-turut sebesar 36%, 35% dan 41%. Etosom ekstrak daun Kelor F_3 memiliki karakteristik yang paling baik dengan ukuran partikel paling kecil dan persen efisiensi penjerapan paling tinggi.

Kata Kunci: *Ekstrak ; Daun Kelor; Etosom; Foafatidilkolin; Etanol*

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ABSTRACT

Moringa is a medicinal plant with antioxidant properties, and quercetin is one of the compounds considered to contribute to this activity. Quercetin is a hydrophobic compound classified as Biopharmaceutics Classification System (BCS) Class II, characterized by high permeability and low solubility. To overcome this limitation and improve the bioavailability of the active compound, the active compound can be encapsulated in a nanosized carrier

system. Ethosomes are vesicular carrier systems suitable for lipophilic, hydrophilic, and amphiphilic compounds. This study aimed to prepare and characterize ethosomes containing Moringa leaf extract using different concentrations of phosphatidylcholine and ethanol. Three formulations were prepared: F1 containing 1% phosphatidylcholine and 20% ethanol, F2 containing 2% phosphatidylcholine and 30% ethanol, and F3 containing 3% phosphatidylcholine and 40% ethanol. Characterization using a Particle Size Analyzer (PSA) showed particle sizes of 202.3 nm, 185 nm, and 146.3 nm for F1, F2, and F3, respectively, with mean polydispersity index (PDI) values of 0.346, 0.293, and 0.196. The corresponding entrapment efficiencies were 36%, 35%, and 41%, respectively. F3 exhibited the most favorable characteristics, with the smallest particle size and the highest entrapment efficiency.

Keywords: *Extract ; Moringa leaf; Ethosomes; Phosphatidylcholine; Ethanol*

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1. Introduction

Indonesia possesses abundant natural resources. Approximately 80% of the world's medicinal plant species can be found in Indonesia, making plant-derived materials used for treatment readily accessible. One plant widely recognized by the public is Moringa [1]. Moringa, also known as *The Miracle Tree*, is considered exceptional compared with many other plants because of its nutritional value and medicinal properties [2]

Moringa leaves are the most widely utilized part of the plant. They contain various phytochemical constituents, including alkaloids, flavonoids, triterpenoids, steroids, and anthraquinones [3]. The flavonoids present in Moringa leaves may contribute to biological and pharmacological activities in the body. Quercetin is a major flavonoid found in Moringa [4]. Structurally, quercetin is a polar polyphenolic compound; however, based on its solubility profile, it is poorly soluble in water. To overcome this limitation and improve the bioavailability of the active compound, it can be encapsulated in a nanoscale carrier system [5].

Nanomedicine technology offers advantages in controlling the release of active compounds and enhancing their bioavailability [6]. One nanoscale carrier system is the ethosome [7]. Ethosomes are lipid vesicular carriers modified from liposomes. These lipid vesicles consist of phospholipids, relatively high concentrations of alcohol, and water. Advantages of ethosomes include the ability to increase drug concentration in the skin, accommodate large molecules, support broad applications in cosmetics and herbal drug technology, and entrap hydrophilic, lipophilic, or amphiphilic drug molecules [8].

The principal components of ethosomal formulations are phospholipids and an alcohol, typically ethanol. Phospholipids function as vesicle-forming agents and form a lipid bilayer surrounding the aqueous compartment of the ethosome [9]. Phosphatidylcholine is commonly used in ethosomes because it is neutral and relatively less expensive than other phospholipids. Ethanol can be used at concentrations of 20-45%, where it contributes to vesicle flexibility and acts as an efficient skin penetration enhancer. In addition, ethanol can improve the solubility of drugs formulated in ethosomes [10]. The high ethanol content of ethosomes can enhance the encapsulation of hydrophilic or lipophilic active compounds, which is

particularly suitable for natural extracts containing chemically diverse groups of compounds [11].

Given the poor solubility and low bioavailability of the active compound, this study explored an ethosomal vesicular carrier system as an approach to improve bioavailability. Variations in phosphatidylcholine and ethanol concentrations were investigated to obtain a Moringa leaf extract ethosomal formulation with the most favorable particle size and entrapment efficiency. Accordingly, this study aimed to prepare and characterize Moringa leaf extract-loaded ethosomes using different concentrations of phosphatidylcholine and ethanol.

2. Methods

Materials and Equipment

The equipment used in this study included a stirring rod, porcelain dish, funnel (Pyrex), cuvettes, disposable syringes, beakers (Pyrex), graduated cylinders (Pyrex), a homogenizer (Thinky Centrifugal Homogenizer ARM-310), a hot-plate magnetic stirrer (Heidolph), volumetric flasks (Pyrex), a Particle Size Analyzer (Horiba SZ-100, Japan), micropipettes, Pasteur pipettes, horn spoons, a centrifuge (Puspirin 6), spatulas, a UV-Vis spectrophotometer (Shimadzu), a stopwatch, an analytical balance (Chyo), Eppendorf tubes, and vials.

The materials used in this study were distilled water, ethanolic Moringa leaf extract (*Moringa oleifera*), cholesterol (Sigma-Aldrich, USA), ethanol, and Lipoid S-75 (Lipoid, Germany).

Preparation of Moringa Leaf Samples

Moringa leaves (*Moringa oleifera*) were collected from Tuladengi Village, Dungingi District, Gorontalo City. Fresh, undamaged leaves from the third to fifth stalks were selected. The leaves were washed under clean running water and then air-dried until completely dry. The dried leaves were ground using a blender to obtain simplicia powder. A 100 g portion of the powdered leaves was macerated with 1 L of 96% ethanol at a ratio of 1:10 for 3 days, with stirring every 24 h. The sample was then filtered through filter paper to separate the filtrate from the residue. The Moringa leaf macerate was subsequently evaporated using an evaporator to obtain a viscous extract free of alcohol. Evaporation was continued until a thick mass (viscous Moringa leaf extract) was formed. The viscous extract was weighed, and the percentage yield was calculated.

Quercetin Analysis Using UV-Vis Spectrophotometry

The analysis was performed by preparing a 1000 ppm stock solution by dissolving 0.01 g of pure quercetin in 10 mL of 96% ethanol. Subsequently, 1 mL of the stock solution was diluted in 10 mL of 96% ethanol to prepare a working stock solution. Standard solutions at concentrations of 5, 10, 15, 20, and 25 ppm were then prepared by taking 5 mL of the stock solution and diluting with 96% ethanol to the mark in a 10 mL volumetric flask. The solutions were analyzed using UV-Vis spectrophotometry over a wavelength range of 550-300 nm. This wavelength range was used to optimize the calibration curve and obtain a linear regression equation using $y = bx + a$.

Preparation of Moringa Leaf Extract Ethosomes

Moringa leaf extract ethosomes were prepared using the ethanol injection method. Lipoid S-75, cholesterol, and Moringa leaf extract were dissolved in 96% ethanol using a magnetic stirrer at 300 rpm for 20 min at 30°C (mixture 1). Water was

heated separately to 30°C, after which mixture 1 was injected into the aqueous phase while stirring with a magnetic stirrer for 30 min until ethosomes were formed. The Moringa leaf extract ethosomes were then sonicated for 5 min over three cycles and stored under refrigeration.

Table 1. Formulation of Moringa Leaf Extract Ethosomes

Ingredient	Concentration (%)		
	F_1	F_2	F_3
Moringa Leaf Extract	0.1%	0.1%	0.1%
Lipoid S-75	1%	2%	3%
Cholesterol	0.2%	0.2%	0.2%
96% Ethanol	20%	30%	40%
Water	ad 100%	ad 100%	ad 100%

Characterization of Ethosomes

Particle Size Measurement

Particle size was measured using a Particle Size Analyzer (PSA). The Polydispersity Index (PDI) was also determined using the PSA to assess the homogeneity of the particle-size distribution [12].

Entrapment Efficiency Measurement (%)

Using UV-Vis spectrophotometry, the percentage of Moringa leaf extract entrapped within the ethosomes was calculated using the following equation:

$$\%EE = \frac{\text{Entrapped Sample Concentration}}{\text{Initial Sample Concentration}} \times 100\%$$

3. Results and Discussion

In this study, Moringa leaf samples were extracted using the maceration method to obtain a viscous Moringa leaf extract. The results are presented in the following table.

Moringa Leaf Extraction Results

Table 2. Yield of 96% Ethanolic Moringa Leaf Extract

Sample Weight (g)	**Extract Yield (%)**
100 g	13.5%

A total of 13.5 g of viscous Moringa leaf extract was obtained, corresponding to an extract yield of 13.5%. This result met the specified requirement for viscous Moringa leaf extract, which is not less than 9.2% [13].

Analysis of Pure Quercetin Using UV-Vis Spectrophotometry

Table 3. Absorbance Values of Pure Quercetin

Concentration (ppm)	Absorbance (λ = 374.4 nm)	Linear Regression Equation
5	0.2613	$R^2 = 0.997$
10	0.5633	a = 0.030
15	0.7603	b = 0.049
20	1.0330	y = 0.049x + 0.030
25	1.2730	

UV-Vis spectrophotometric measurement produced the quercetin regression equation y = 0.049x + 0.030. Linearity was indicated by a coefficient of determination (R^2) of 0.997. A value close to 1 indicates that the regression relationship is highly linear and that absorbance and concentration are strongly correlated. The pure quercetin calibration curve was subsequently used to determine the content of quercetin-derived flavonoids present in the Moringa leaf extract ethosomes.

Preparation of Moringa Leaf Extract Ethosomes

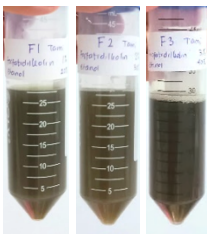

Figure 1. Prepared Moringa Leaf Extract Ethosome Formulations

Figure 1 shows that the prepared Moringa leaf extract ethosomes consisted of Moringa leaf extract, Lipoid S-75, cholesterol, ethanol, and water. The formulations were prepared by varying the concentrations of Lipoid S-75 and ethanol across three formulations. The Lipoid S-75-to-ethanol ratios were F1 (1:20), F2 (2:30), and F3 (3:40). All three formulations produced a dark green ethosomal suspension with no visible phase separation.

Characterization of Ethosomes

Particle Size Measurement

Table 4. Particle Size and PDI Measurements

Formula	Size (nm)	Mean Size (nm)	PDI	Mean PDI
F1	200 202	202.3	0.35 0.33	0.346

	205		0.36	
	185		0.28	
F2	186	185	0.29	0.293
	184		0.31	
	148		0.20	
F3	145	146.3	0.21	0.196
	146		0.18	

The results in Table 4 show that the three Moringa leaf extract ethosomal formulations had particle sizes ranging from 146.3 to 202.3 nm. According to [14], an ethosomal vesicle meets the nanoscale criterion when its particle size or vesicle diameter is within the range of 10-1000 nm. F3 had a smaller particle size than F1 and F2, which was associated with the increased ethanol concentration. According to [15], ethanol alters the surface characteristics of ethosomal vesicles. At high concentrations, ethanol promotes interpenetration of hydrocarbon chains, thereby reducing vesicle membrane thickness and consequently vesicle size [16]. In the study by [17], ethosome particle size decreased as the concentrations of phosphatidylcholine and ethanol increased.

Table 4 also presents the polydispersity index (PDI) values of the Moringa leaf extract ethosomes. The PDI is an indicator of molecular mass distribution within a given sample. The mean PDI values were 0.346 for F1, 0.293 for F2, and 0.196 for F3. According to [18], a PDI value of ≤ 0.5 meets the criterion for a homogeneous particle-size distribution. Formulations are more stable at lower PDI values, whereas higher PDI values indicate less uniform particles and may lead to faster flocculation. All three formulations had PDI values within the range of 0.08-0.7, which represents the upper range within which the distribution algorithm operates optimally.

Entrapment Efficiency Measurement (%)

Table 5. Entrapment Efficiency of Moringa Leaf Extract Ethosomes

Formula	Absorbance	Entrapment Efficiency (%)
1	0.2072	36%
2	0.2026	35%
3	0.2344	41%

Table 5 shows that the entrapment efficiencies of F1, F2, and F3 were 36%, 35%, and 41%, respectively. According to [18], there is no specific threshold for determining whether vesicles meet a particular entrapment-efficiency requirement; however, a higher percentage indicates that a greater amount of compound is entrapped within the vesicles. The values obtained for the three formulations were considered acceptable because a previous study by [19] reported an entrapment efficiency of 15.026% for kaffir lime leaf extract ethosomes. The use of an extract rather than pure quercetin may account for the observed entrapment efficiency.

F3 showed the highest entrapment efficiency, while F1 and F2 had lower values. This was attributed to the increased amount of phosphatidylcholine added to the ethosomal formulation. Increasing the phosphatidylcholine concentration can increase the entrapment efficiency of a vesicular carrier. The choline moiety of phosphatidylcholine contributes to binding the extract; therefore, a greater amount of

phosphatidylcholine provides greater loading capacity, resulting in more extract being entrapped within the vesicles [20].

4. Conclusion

Based on the results, Moringa leaf extract was successfully formulated into nanosized ethosomes using the ethanol injection method in three formulations. All three formulations exhibited characteristics that met the specified criteria. F3 showed the most favorable characteristics, with the smallest particle size of 146.3 nm and the highest entrapment efficiency of 41%.

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