ORIGINAL ARTICLE

Effect of Surfactant Concentration on the Entrapment Efficiency Niosomes Aqueous Extract of Cassava Leaves (*Manihot esculenta* Crantz)

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Abstract

Context: Aqueous extract of cassava leaves (*Manihot esculenta* Crantz) has a high rutin content. Rutin has benefits as an antioxidant, anti-inflammatory, analgesic, and antidiabetic. The plant extract has low bioavailability and has a large molecular weight. Therefore, a carrier system like niosomes is required to allow aqueous leaves extract of cassava through to absorb in the body. **Aims:** The purpose of this study was to determine the effect of variation concentration of Span 40 that can trap aqueous extract of cassava leaves in niosomes optimally. **Materials and Methods:** Niosomes were done by thin-layer hydration method with concentration of Span 40 which was varied into three formulas: Formula A (100 µmol), Formula B (150 µmol), and Formula C (200 µmol). **Statistical Analysis Used:** The data analysis using SPSS analyzed by one-way ANOVA. **Results:** The rutin contained in cassava leaves of this research is 42.52%. Entrapment efficiency test used dialysis membrane method and the result showed that the increased concentration of Span 40 is used; the entrapment efficiency is also increasing. The result of SPSS test using one-way ANOVA of three formulas did not show significant difference ($P > 0.05$). **Conclusions:** Optimum concentration of Span 40 in trapping aqueous extract of cassava leaves is Formula A (100 µmol) which was 95.9%.

Key words: Cassava leaves, entrapment efficiency, niosomes, rutin, Span 40

INTRODUCTION

Cassava leaves (*Manihot esculenta* Crantz) have been reported and contain flavonoids, tannins, saponins, phenolics, and terpenoids.[1] Plant extracts are difficult to absorb into the body either in oral or topical dosage form because their multiple ring large size which cannot absorb by passive diffusion, or due to their poor lipid solubility; severely limiting their ability to pass across the lipid-rich biological membranes, resulting poor bioavailability.[2,3] Therefore, a carrier system like niosome is required to allow aqueous extract of cassava leaves through to absorbed in the body.

Niosomes are vesicular systems that can entrap lipophilic, hydrophilic, and amphiphilic drug in aqueous layer and vesicular membrane, respectively. Two basic components of niosomes are non-ionic surfactant and cholesterol.[4,5] Niosomes can improve the oral bioavailability of poorly soluble drugs and also enhance the skin permeability of drugs when applied topically.[6] Niosomes are characterized for different attributes, one of them is entrapment efficiency. These characteristics can be affected by the type and amount of surfactant used.[7]

As the surfactant/lipid level increases, the amount of drug to be encapsulated into niosomes system also increases.[8] Research that has been done before has suggested that the increased amount of surfactants used in niosome formulations does not always show a positive effect on the amount of entrapment efficiency. That is because the type and amount of surfactant can affect the permeability of the niosome membrane so as to have a capability to trap a number of active substances.[5]

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One of the non-ionic surfactants that can be used is Span 40. Span 40 has better entrapment efficiency compared to other non-ionic surfactants.[9] Research on niosomes formula of aqueous extract of cassava leaves using Span 40 has never been done. This study aims to determine the effect of variation concentration of Span 40 that can trap aqueous extract of cassava leaves in niosomes optimally.

MATERIALS AND METHODS

The used materials were cassava leaves from Sungai Raya Dalam, St. Pontianak, Indonesia, sorbitan monopalmitate (Sigma-Aldrich), cholesterol (Sigma-Aldrich), chloroform, methanol, rutin (Sigma-Aldrich), dialysis tubing cellulose membrane cutoff between 12,000 and 14,000, aquadest, phosphate buffer, and plate silica gel GF254.

Extract preparation

Cassava leaves was extracted by decoction method. One kilogram of fresh leaves of *M. esculenta* Crantz was boiled in ±4 L of aquadest for 30 min (starting at 96°C). The extract was filtered and deposited at temperature (Tc) of 2–8°C. The precipitate formed then was filtered and the residue in the oven at 80°C for ±1 h or until dry. After that, it was stored in a desiccator.

Thin-layer chromatography profile

TLC aims to identify of flavonoid rutin compounds. Extract and standard compounds were eluted using mobile phase butanol: acetic acid: water (BAA) with a ratio of 4:1:5 and a stationary phase is a plate of silica gel GF_{254}. The TLC plate is viewed under ultraviolet (UV) 254 nm and UV 366 nm. Flavonoid compounds are detected by $AlCl_3$ reagent.

Determination of total flavonoids

Determination of total flavonoid begins with the determination of maximum rutin wavelength (λ) in ethanol: water (1:1) solvent, which is scanned using UV-visible spectrophotometer in the range of 450–500 nm. λ maximum obtained is 511.5 nm. The total flavonoid is determined using the Zhou method. Two milliliter sample solution was put into 5 mL measuring flask and added 0.15 mL of $NaNO_2$ 5% and leaves for 6 min then added 0.15 mL AlCl3 10% and also left for 6 min, then added 2 mL of NaOH 1 M and immediately added aquadest to the final volume of 5 mL, then shaken and left for 15 min. Absorbance is measured at a wavelength of 511.5 nm.

Solubility determination of extract

About 0.1% extract was dissolved in 10 mL each of chloroform, methanol, aquadest, and phosphate buffer (pH 7, 7.4, 7.6, and 8). The mixtures were then stirrer at 700 rpm for ±45 min.

The production of niosomes

Niosomes were prepared using the thin-layer hydration method with modifications,[10,11] span and cholesterol in various comparisons according to Table 1. Cholesterol used was 15% of Span 40 concentration. Span 40 and cholesterol were dissolved in 10 mL of chloroform mixture in a round-bottom flask. The flask was allowed to rotate at 400 rpm for 5 min in a rotary evaporator at 48 ± 2°C under reduced pressure (400 mbar) to obtain a thin-layer niosomes. Then, the round-bottom flask is removed and put into the desiccator and left for 24 h. Thin-layer niosomes hydrated with 10 mL of phosphate buffer pH 8 containing 0.3% aqueous extract of cassava leaves and were allowed to rotate at 48 ± 2°C at 400 rpm until thin layer to form niosomes suspension and particle size reduction was done by magnetic stirrer for 1 h with speed 1000 rpm.

Determination of entrapment efficiency

Niosomes entrapment aqueous extract of cassava leaves was measured the entrapment efficiency by separation method using dialysis membrane with modifications.[10,11] Niosomes suspension was inserted 2 mL into dialysis membrane. In medium receivers, 25 mL of ethanol: water (1:1) was used. The entrapment efficiency testing was conducted for 2 h. Measured the rutin from extract level which was not caught on the receiving medium at a wavelength of 511.5 nm using UV spectrophotometer. The replication was done 3 times for each formula. The entrapment efficiency could be calculated as follows:

$$\%\ \text{Entrapment efficiency} = \frac{A - B}{A} \times 100\%$$

Description: A = Rutin from extract level which was added in the formula; B = Rutin from extract level in the receiver medium (unentrapped).

Vesicles morphological observation

Morphological observation was done using a light microscope Zeiss Discovery V.12. The niosomes suspension was checked for pH using pH meters and suspension was analyzed particle size, particle size distribution, and polydispersity index with particle size analyzer Horiba Scientific.

The data analysis

The percentage entrapment efficiency analysis was done using SPSS analyzed by one-way ANOVA.

Table 1: Span 40 comparison

Material	FA	FB	FC
Span 40 (μmol)	100	150	200
Cholesterol (μmol)	15	22.5	30
Chloroform (mL)	10	10	10

RESULTS AND DISCUSSION

Result of extracting

Cassava leaves are used 1 kg and produce dry extract of 11.4084 g with rendemen value of 1.14% (w/w). The extract results obtained in the form of fine powder, yellowish-green, distinctive smell, and taste a bit bitter [Figure 1].

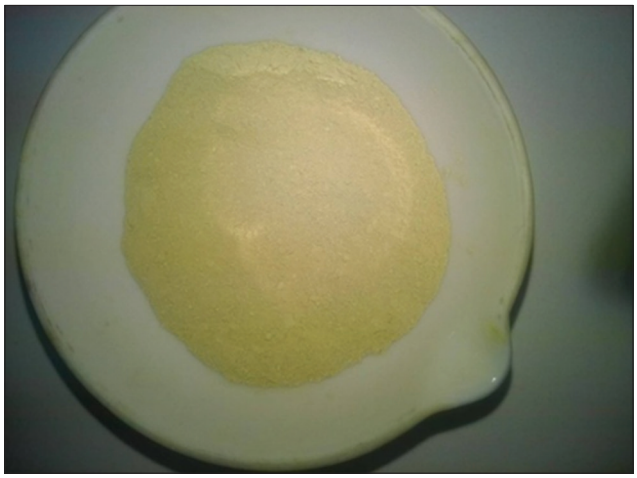

Figure 1: Aqueous extract of cassava leaves

Thin-layer chromatography

Based on the research that has been done before, cassava leaves have high flavonoid content compared to other compounds.[12] One of the flavonoid compounds that are in the cassava leaves is rutin.[13] Determination of TLC profile of flavonoids, spot of extract compared with the standard rutin. This is to prove if there are the same spot and the same Rf value with the standard rutin, then it is that in extract there is a compound flavonoid rutin. However, the result of TLC pictures only a qualitative parameter needs quantitative chromatographic fingerprinting required for rutin content as several phytoconstituents have same Rf value. To detect the presence of flavonoid compound, the plate is sprayed with $AlCl_3$ 5% reagent. If the spot produces yellow and yellow glow stains on UV 366 nm, then indicates the presence of flavonoid groups.[14]

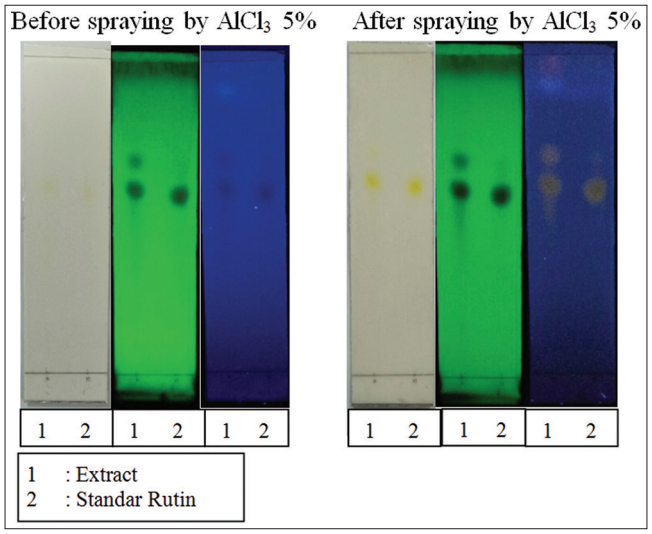

Figure 2: Thin-layer chromatography profile

Based on the results obtained [Figure 2], it can be concluded that aqueous extract of cassava leaves contains a rutin compound. Other results mentioned that rutin can be isolated using Amberlite XAD4 resin yield 0.75% of local Kaliki varieties; 0.15% of Manteiga varieties, and 0.24% of the Valencia variety.[15] Using the Kromatografi Lapis Tipis-Densitometry method of Kasetsart-50, cassava leaves yield 12.3% of rutin isolation.[16] Other studies using the same method resulted in rutin 0.66% of old cassava leaves, 0.32% of young cassava leaves, and 0.15% of yellow cassava leaves while using gravimetric method yield 0.53% and 0.30%, respectively, on old and young cassava.[17] In addition, the rutin from rubber yam leaves can be isolated by maceration method and extracted successively with n-hexane and chloroform produces crystals of rutin 0.105%.[18] It can be concluded that rutin can be used as marker from plant of cassava leaves.

Result of total flavonoid

Determination of total flavonoid using Zhou method.[19] The purpose of determining the total flavonoid to calculate the rutin level in the extract which will be used to determine the entrapment efficiency of aqueous extract of cassava leaves niosome. The principle of determination of flavonoid level is a reaction between flavonoids with $AlCl_3$ 10% will form yellow complex and with the addition of NaOH 4% will form a burgundy color complex that measured its absorbance at wavelength of 511.5 nm.[20] Absorbance obtained is calculated using standard curve rutin that is $y = 0.005x - 0.0151$ with correlation coefficient value $r = 0.9998$. The result of the determination of total flavonoid content from aqueous extract of cassava leaves was 425.2074 mg RE/g extract.

Solubility of extract

Solubility optimization was conducted to determine the amount of aqueous extract of cassava leaves to be made in the niosomes system. Chloroform and methanol are selected because they are volatile organic solvents and can be used in the preparation of thin-layer niosome. About 0.1% concentration of extract is slightly soluble in 10 mL of chloroform, methanol, or in chloroform: methanol (3:7, 5:5, 7:3, and 9:1) ratio and yields of particle deposits. Aquadest is chosen because the medium of hydration of thin layer is the water phase. Aqueous leaves extract of cassava is slightly soluble in aquadest because an extract with 0.1% concentration cannot soluble and produce many large particles. The variation of pH performed on phosphate buffer is pH 7, 7.4, 7.6, and

8. Used the pH range because the rutin is more stable in a low concentration of alkaline and rutin will decomposed in acidic solution, with an increase of pH from 7 to 11 rutin will decomposed under these conditions.[21,22] Based on the results of the solubility test, 0.1% aqueous extract of cassava leaves is soluble in phosphate buffer pH 8 due to phosphate buffer pH 8 the solution more clear than phosphate buffer pH 7, 7.4, and 7.6 and also chloroform, methanol, and aquadest. After that, the increase of amount extract until their solubility through saturation is marked by increasingly murky solution. Extracts with a concentration of 0.3% have shown saturation, in which the concentration composition is increased to 0.4% containing particulate deposits. The concentration used in hydration a thin layer is 0.3% extract in phosphate buffer pH 8.

The production of niosomes

The thin-layer hydration method has two stages of preparation, which is to make a thin layer around the round-bottom flask by dissolving Span 40 and cholesterol with chloroform and hydrate a thin layer with a hydration medium containing the extract. Chloroform was chosen because it can dissolve Span 40 and cholesterol and is highly volatile. Span 40 is selected because Span 40 has a small hydrophilic group and a long lipophilic that will produce a thick and non-fragile niosomes.[23] Cholesterol is used as a stabilizing agent, in which cholesterol maintains vesicle rigidity and prevents leakage. Cholesterol used is 15% of Span 40 concentration. Addition of too much cholesterol will cause niosome vesicles become stiff and break easily, so the maximum cholesterol that can be used is 20%. The maximum cholesterol that can be used is 20%.[24]

A thin layer formed white on the wall of a round-bottom flask. Rotation speed will affect the condition of the thin layer formed. If it is too fast, it will cause foam/froth that will make the thin-layer hollow and if too long, then the thin layer will accumulate on the bottom of the round-bottom flask. The resulting thin layer is stored in the desiccator aiming to refine the chloroform removal process that remains in the round-bottom flask.

The thin layer is hydrated at the phase transition Tc of Span 40 which is 48 ± 2°C. This hydration process aims to develop vesicles to form vesicles that can absorb aqueous extract of cassava leaves optimally. The process of vesicle formation occurs spontaneously; the water phase will cause the double layer to bend itself and form a closed bubble, where the aqueous extract of cassava leaves is trapped in the middle. After forming the niosomal suspension, it is necessary to reduce the particle size by stirrer for 1 h. The working principle is that high-speed rotation generated by stirrer will break the bond between particles so that the particle size is smaller. Niosomes aqueous extract of cassava leaves on each formula replicated 3 times. The result of niosomes looked like greenish-yellow suspension with odorless and has pH 7.75 on the three formulas.

Entrapment efficiency

The percentage of entrapment efficiency calculated from the receiving medium is assumed to be unentrapped. Calculation of unentrapped rate using the linear regression equation of standard rutin curve is y = 0.005x−0.0151 with UV-visible spectrophotometer. The results obtained [Table 2] were analyzed by one-way ANOVA, indicating that there was no significant difference ($P > 0.05$) between the increases of Span 40 concentration to increase the entrapment efficiency in all three formulas. Hence, it can be concluded that the increase Span 40 concentration can increase the percentage of entrapment efficiency, but the percentage increase is not significantly different between formulas A, B, and C.

The effect of the variation of surfactant concentration on the entrapment efficiency is similar with the previous research. Based on the results of the previous studies, an increase in Span concentration of 40 will increase the entrapment efficiency of niosomes sodium ascorbyl phosphate,[23] as well as the niosome of *Gymnema sylvestre* extract.[25] This might be due that increasing concentration of surfactant will make the niosomes membrane less permeable and hence promotes entrapment efficiency.[5] However, after a certain point, the increase in surfactant concentration cannot increase significantly. This might be due to the magnitude of the concentration of the adsorbed active substance depending on the ability of the drug to be disposed on the polar and non-polar part of the lipid molecule that forms the vesicle and its ability to diffuse into the vesicles during hydration.[4]

Morphology of niosomes

The result of light microscopy [Figure 3] showed round vesicles.

Morphology of niosomes was done using a light microscopy, but the results are less specific. Scanning or transmission electron microscopy result needs to be produced for exact shape and size of vesicles. The particle size data and the polydispersity index of each niosomal formula are shown in Table 3. The particle size data show the difference of each formula and are relatively large. The results can be due to aqueous extract of cassava leaves used and are not perfect soluble so it can affect the particle size. Based on literature, length of the alkyl chain of the surfactant affecting

Table 2: Entrapment efficiency of niosomes aqueous extract of cassava leaves

Formula	Span 40 (μmol): cholesterol (μmol)	Entrapment efficiency (%)±standard deviation
A	100:15.0	95.90±0.13
B	150:22.5	96.08±0.15
C	200:30.0	96.09±0.16

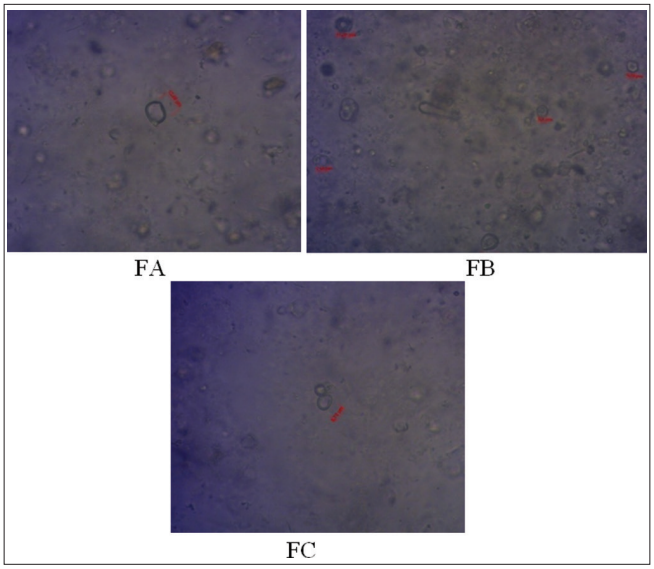

Figure 3: Morphology of niosomes aqueous extract of cassava leaves

Table 3: Characterization parameter of noisome aqueous leaves extract of cassava

Formula	Particle size (nm)	Index polydispersity (PI)
A	3446.7	0.240
B	5763.5	0.207
C	2327.1	0.596

the diameter of the vesicle, the longer the alkyl chain then generally gives a relatively large vesicle size.[5]

The polydisperse index (PI) describes the range of particle size distribution and determines whether there is aggregation. Homogeneously dispersed particles have a tendency to physically stable and there is no aggregation of particles. The particle size distribution is expressed as a monodispersion if the PI is in the range of 0.01–0.7.[26] The polydisperse index of each formula shows that the niosomes of aqueous extract of cassava leaves are monodisperse.

CONCLUSIONS

From the present study, it could be concluded that the increase of Span 40 concentration in niosomes formulation of aqueous extract of cassava leaves will increase the entrapment efficiency but is not significantly different which optimum concentration of Span 40 in trapping aqueous extract of cassava leaves is Formula A (100 µmol) which was 95.9%.

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