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Span 60 as Surfactant of Topical Microemulsion of Purple Sweet Potato (*Ipomoea batatas* L.) Ethanol Extract and Antioxidant Activity Test using Dpph Method

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Abstract: Objective: To determine the antioxidant activity of microemulsion of purple sweet potato ethanol extract that is formulated using a various concentration of Span 60.
Methods: The purple sweet potato was extracted with ethanol using maceration method. Then IC_{50} value of ethanol extract of purple sweet potato was determined, and formulated in microemulsion with various concentration of Span 60. Each formula has a concentration ratio of Span 60 as follows, Formula A (FA) 0.75%, Formula B (FB) 1%, and Formula C (FC) 1.25%, and use cosurfactant PEG 400 in the ratio of surfactant: cosurfactant 1: 1 for each formula. Measurement of antioxidant activity was conducted using DPPH method. The physicochemical properties and stability of microemulsion is tested for 28 days, covering organoleptic, pH, specific gravity and centrifugation tests. The most stable microemulsion was tested for its antioxidant activity by the value of percent inhibition, then the size of microemulsion globules was observed using PSA (Particle Size Analysis).
Results: IC_{50} of ethanol extract of purple sweet potato obtained at 38.246 ppm. Organoleptic and centrifugation test showed the occurrence of creaming at FC. pH was in the range of pH that is safe for the skin (5.8 to 5.9) with the density of microemulsion that's relative stable. FA is the most stable formula. FA showed antioxidant activity with percent inhibition of 81.37254% with globule size from 111.1 to 152.4 nm.
Conclusion: Based on research that has been done, it can be concluded that microemulsion formula A with a concentration of 0.75% Span 60 generates the most stable microemulsion and has antioxidant activity with percent inhibition of 81.37254%.
Keywords : purple sweet potato, antioxidant, DPPH, microemulsion, Span 60.

Introduction

Premature aging is the process of skin aging faster than it should. The signs of premature aging of the skin are dry, scaly, rough skin, accompanied by the appearance of wrinkles and dark stains or spots. Premature aging can be prevented by avoiding the factors that accelerate the process, one of which free radicals. Free radicals can be overcome or neutralized by using antioxidant[1].

Purple sweet potato (*Ipomoea batatas* L.) is a plant that has been shown to have antioxidant activity. Various studies have shown that some classes of flavonoids contained in purple sweet potato has antioxidant properties, one of which is anthocyanins[2,3]. These compounds are hydrophilic, easily oxidized and stable at low pH (pH 1-2). Heating and exposure to light for long periods can lead to a decrease in the number of components and damage anthocyanin[4]. One of drug delivery systems that can be used to solve the above problems is a microemulsion.

Microemulsion is oil and water dispersion system which is stabilized by the interface layer of surfactan[5]. Microemulsion advantages when compared with emulsion is thermodynamically stable, clear and transparent[6].

Microemulsion that was made is the Water / Oil (W / O) type, in which the outer phase is oil, to protect anthocyanin from exposure to light. To form a W/O microemulsion system, it takes surfactant having an HLB range (Hydrophyle Lypophyle Balance) of 3-6. The surfactant used was Span 60 (Sorbitan monostearate) which was a solid wax and having HLB of 4.7. So then, the formulation of microemulsion of purple sweet potato ethanol extract is made by using span 60 surfactant. The extract is varied in order to obtain the optimum concentration which can provide antioxidant activity. Besides, the microemulsion was also subjected to several tests including globule size and physical stability tests. Measurement of antioxidant activity using DPPH method was conducted on the most stable microemulsion.

Materials and Methods

Materials used are purple sweet potato, ethanol 96%, concentrated HCl, powdered zinc or magnesium, glacial acetic acid (Merck), FeCl3, AlCl3, HCl 2 M, chloroform, H2SO4, NaOH 2 M, distilled water, methanol (Merck), HCL 1%, DPPH (Sigma-Aldrich), sorbitan monostearate (Span 60), DMDM-Hydantoin, BHT, PEG 400, olive oil (Bertolli).

Phytochemical Screening

Phytochemical screening was conducted on the screening of flavonoids, phenolics, anthocyanin, alkaloids, saponins, triterpenoids and tannins.

Antioxidant Activity Test on Purple Sweet Potato Ethanol Extract using DPPH Method

Antioxidant activity test was conducted using DPPH by ratio of 1:1 using UV-Vis spectrophotometry[7]. The sample solution is made in 5 concentrations which are 5, 50, 100, 120 and 200 ppm. The concentration of DPPH solution used was 30 ppm. The absorbance was measured at a wavelength of 516.5 nm. Percent inhibition (%inhibition) is calculated from the obtained data of absorbance.

$$\%\text{inhibition} = \frac{Ablank - Asample}{Ablank} \text{x} 100\%.$$

IC_{50} is calculated from a linear regression curve at various concentrations of standard versus % antioxidant activity.

Formulations and Preparations of Topical Microemulsion of Purple Sweet Potato Ethanol Extract

Microemulsion was made into 4 formulas (Table 1). The oil phase is made by mixing span 60, PEG 400, BHT and olive oil with a stirring speed of 700 rpm ±. The aqueous phase is made by dissolving the ethanol extract of purple sweet potato and DMDM-Hydantoin into distilled water. Then the water phase is dispersed into the oil phase, and stirred at 1000 rpm for 1.5 hours using temperature of 40◦C to form a clear microemulsion. Furthermore, the clear microemulsion was sonicated to reduce the size of the globules by using sonication type of bath for 24 minutes.

Evaluation of microemulsion

The evaluation was conducted for 28 days includes:

Organoleptic test

Organoleptic test was done by looking for changes in color, odor, and clarity, and by observing whether or not sediment's formed.

Table 1. Variation of Purple Sweet Potato Microemulsion Formula

Materials	Composition (% w/w)			
	A	B	C	D
Purple sweet potato ethanol extract	38,246	38,246	38,246	-
Distilled water	20	20	20	20
Span 60	0,75	1	1,25	0,75
PEG 400	0,75	1	1,25	0,75
DMDM-Hydantoin	0,5	0,5	0,5	0,5
BHT	0,1	0,1	0,1	0,1
Olive oil	Ad 100	Ad 100	Ad 100	Ad 100

pH Test

pH test was conducted using a pH meter at a room temperature[9].

Determination of Density

The density was measured using a pycnometer at a temperature of 29º C. Pycnometer was weighed (A), and then filled with water and weighed again (A1). After that, water was removed until the pycnometer was dry. Microemulsion was loaded into the pycnometer and weighed (A2). Density of the microemulsion was calculated as follows[9].

$$\frac{A2 - A}{A1 - A} \text{ x density of water (at 29°C)}$$

Globule size determination of microemulsion

The globule size distribution was measured on the most stable microemulsion by using Particle Size Analysis (PSA) type of Beckman Coulter.

Sentrifugation Test

Microemulsion was put into a centrifuge tube and then centrifuged at 3000 rpm for 30 minute[10].

Antioxidant Activity Test on Microemulsion of Purple Sweet Potato Ethanol Extract using DPPH Method

Antioxidant activity test performed on the formula of the most stable microemulsion and on negative control (Formula D) by DPPH method using UV-Vis spectrophotometry.

Results and Discussion

Samples of purple sweet potato was made into simplisia and extracted with 96% ethanol: glacial acetic acid: water (25: 1: 5) using maceration method. Results of phytochemical screening of the ethanol extract of purple sweet potato shows that extracts containing flavonoids, phenolic, anthocyanins, alkaloids, tannins, saponins, and triterpenoids (Table 2).

IC_{50} value of the ethanol extract of purple sweet potato was determined by DPPH method using UV-Vis spectrophotometer, and obtained at 38.24 ppm (Table 3). This value is gained by inserting the absorbance obtained in the linear regression equation with the equation y = 0,1072x + 45.9. IC_{50} values obtained here were incorporated into the formulation of microemulsion of purple sweet potato ethanol extract.

Table 2. Phytochemistry Screening Results of Purple Sweet Potato Ethanol Extract

Test	Reagents	Colours	Results
Flavonoids	Mg + Concentrated HCL	Red	+
Phenolics	$FeCl_3$	Blackish blue	+
Anthocyanines	HCL 2M NaOH 2M	Red Green-Blue	+ +
Alkaloids	Mayer Dragendroff Wagner	Brown precipitate White precipitate Brown precipitate	+ + +
Saponins	+ distilled water, shaken vigorously	Frothes	+
Triterpenoids/ Steroids	*Lieberman-Burchard*	Triterpenoids (Red) Steroids (Blue)	+ -
Tannins	Heated + Gelatin + NaCl	White precipitate	+

Description: (+) : contain tested compounds
(-) : do not contain tested compounds

Table 3. IC_{50} Determination Results of Purple Sweet Potato Ethanol Extract

Concentration (ppm)	Average Absorbance	% inhibition	Equations (y = bx +a)	IC_{50} (ppm)
Blank	0,78817±0,03	0	y = 0,1072x + 45,9	38,246
5	0,44983±0,09	42,92729		
50	0,35891±0,09	54,46287		
100	0,32794±0,02	58,39223		
120	0,32195±0,06	59,15221		
200	0,27171±0,01	65,52647		

Microemulsion of purple sweet potato ethanol extract was produced by varying the concentration of Span 60 as follows: 0.75% in formula A, 1% in formula B, and 1.25% in formula C. The results of the evaluation of organoleptic observations show that the microemulsion has good physical properties except in formula C. In the formula C, instability of microemulsion was occured in form of creaming on day 28, but it can be made homogenous again if it's shaken. At pH measurement on microemulsion, it was obtained that the pH range is safe for the skin with pH of 5.8 to 5.9, so that it can be said that the microemulsion was in good pH (Figure 1). The aim of this pH measurement was to determine the suitability of the microemulsion with skin pH which ranges from 4.5-6.5. The determination of density aims to look at the stability of the microemulsion stored for 28 days. The densities of formula A, B, and C respectively were 0.9106 to 0.9128 g / mL (Figure 2), 0.9106 to 0.9126 g / mL (Figure 3) and 0.9106 to 0,9135 g / mL (Figure 4). These results indicate that there is no great difference in density in each formula, with RSD of each formula less than 2%, in other words, the densities of microemulsion formula A, B and C were good. Centrifugation test performed to illustrate the physical stability of the microemulsion for 1 year. The results of centrifugation test on formulas A and B for 28 days showed that phase separation does not occur, there was no precipitation, and that the microemulsions remain clear. In formula C, phase separation occurs in day 21st and 28th . So, it can be said that formula C was not stable in a long time storage.

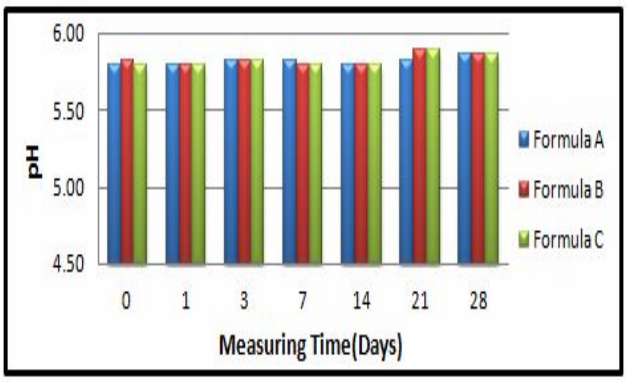

Figure 1. pH Measurement Results

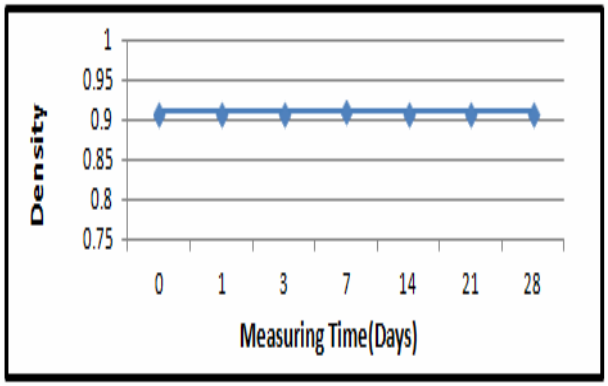

Figure 2. FA Density Test Graphic Results

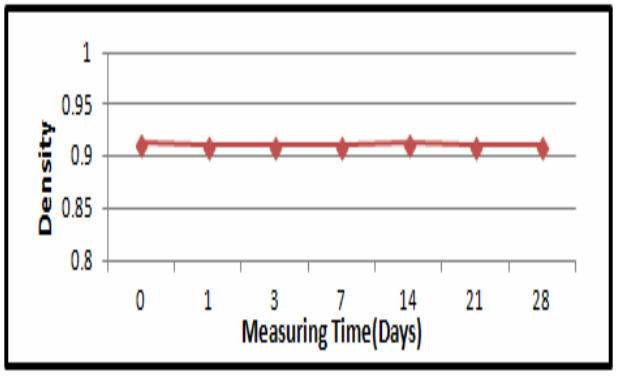

Figure 3. FB Density Test Graphic Results

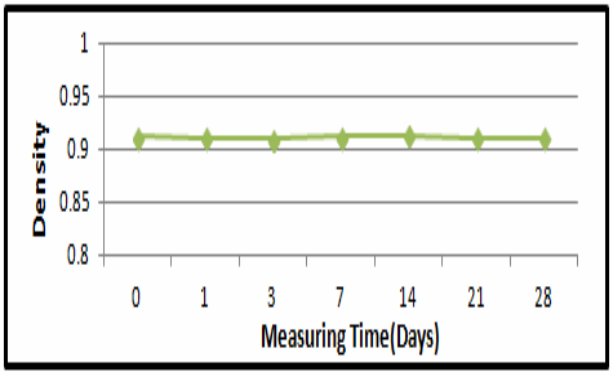

Figure 4. FC Density Test Graphic Results

After the test of stability for 28 days, it was found that the most stable formula is formula A with 0.75% concentration of Span 60. So, the measurement of globules size and antioxidant activity test were conducted on formula A. The measurement results showed that the size of 90.45% globules of microemulsion formula A was in range of 111.1 to 152.4 nm.

Antioxidant activity test was conducted on formula A and D. Formula D is negative control which's microemulsion formula without added extract of purple sweet potato. Results showed that formula A provides a fairly high attenuation against free radicals when compared with formula D, so it can be seen that the extract of purple sweet potato on formula A played a role in providing antioxidant activity. Formula A also provide percent inhibition higher than percent inhibition of purple sweet potato ethanol extract, so it can be said that formula A was able to help protect anthocyanins in purple sweet potato ethanol extract (Table 4).

Table 4. Antioxidant Activity Test Results of Topical Microemulsion

Sample Names	% Inhibition
Negative control	52,74076
Formula A	81,37254
Purple sweet potato ethanol extract 38,246 ppm	50,000

Statistical analysis between formula A and ethanol extract of purple sweet potato showed significant difference between the two formulas, with a significance value of 0.03 ($p < 0.05$), therefore, it can be concluded that formula A is able to increase antioxidant activity of purple sweet potato ethanol extract.

Conclusion

Based on research that has been done, it can be concluded that ethanol extract of purple sweet potato (Ipomoea batatas L.) has IC_{50} value of 38.246 ppm, and categorized as very powerful antioxidant. The most stable formula of microemulsion is formula A with 0.75% concentration of Span 60. Percent inhibition of microemulsion formula A is 81.37254%.

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