International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.3, pp 234-239, 2016

Topical Microemulsion's Formulation of Purple Sweet Potato *(Ipomoea batatas* L.) Ethanol Extract as Antioxidant by using Various Concentration of Span 80

Rise Desnita[1]*, Maria Veronika[1], Sri Wahdaningsih[1]

[1]Pharmacy, Faculty of Medicine, Tanjongpura University, Pontianak, Indonesia

Abstract: Purposes: Determine IC_{50} value of the ethanol extract of purple sweet potato that has good antioxidant activity. Make clear and stable microemulsion's formulation containing purple sweet potato ethanol extract by using various concentration of span 80, i.e 20%, 25% and 30% (w/w) and determine the percent inhibition of formula microemulsion are the most stable.
Methods: Determination antioxidant activity of purple sweet potato ethanol extract by using DPPH method. Then do the manufacture of microemulsion containing purple sweet potato ethanol extract as much as third formula with a variation span 80 levels, they are Formula A (20% Span 80), Formula B (25% Span 80) and Formula C (30% Span 80). Physical stability of microemulsion was evaluated for 28 days to define which concentration span 80 could make clear and stable microemulsion form. The most stable formula then determined its percent inhibition.
Results: Purple sweet potato ethanol extract has a IC_{50} value of 38.25 ppm. Span 80 with concentrations of 20%, 25% and 30% could make clear and stable microemulsion form. The third formula produces a stable microemulsion perfomed so determining the percent inhibition of the formula is determined based on a formula that contains a concentration of the smallest span 80 because in the smallest concentration microemulsion can already be formed that formula A. Percent inhibition of formula A was 80.78092%.
Conclusion: Formula micoremulsion ethanol extract of purple sweet potato that is made has antioxidant activity and good stability.
Keywords: Purple sweet potato ethanol extract, Antioxidant activity, microemulsion, Span 80, physical stability.

Introduction

The free radicals produced in the body under normal circumstances would be neutralized by antioxidants in the body. However, if the level is too high then the ability of inadequate endogen antioxidant to neutralize free radicals resulting in an unbalanced state between free radicals with antioxidants[1]. High levels of free radicals in the body can trigger a variety of diseases. Therefore, our bodies require an exogen antioxidants that may help protect the body from free radicals. One of the plants of Indonesia that has been shown to have antioxidant activity is purple sweet potato *(Ipomoea batatas L.)*. Based on the research that has been done previously stated that the antioxidant activity in the purple sweet potato is directly proportional to the levels of anthocyanins contained in the purple sweet potato[2].

Skin is one of the organs that are susceptible to exposure of free radicals, so we need a topical preparation of natural antioxidants to protect the skin. Anthocyanins have low stability and a hydrophilic

compound that have problems in its penetration past the *stratum corneum* which lipophilic. One way to increase its penetration and stabilititation on the skin was formulated in topical microemulsion dosage form.

Microemulsion's formulation in this study using a type of water-in-oil microemulsion (W/O). The selected surfactant is a nonionic surfactant lipophilic Span 80 with HLB value of 4.3 as it can affect the properties of the skin barrier and the partition coefficient carrier - *stratum corneum,* So, it can increasing the penetration rate of active substances[3].

Based on the things that have been presented in this study will be made of a topical antioxidant preparations microemulsion type W/O uses a variation span 80 levels in order to obtain the microemulsion clear, transparent and effective as drug delivery systems.

Materials and Methods

Material

Purple sweet potato, 96% ethanol, aquadest, BHT, DMDM-hydantoin, olive oil, span 80, DPPH (Sigma-Aldrich), magnesium powder, $FeCl_3$, $AlCl_3$, 2M HCl, 2M NaOH, methanol p.a (Merck), chloroform, 10% NaCl, gelatin, glacial acetic acid (Merck), H_2SO_4.

Method

Intake and Extraction Sample

The sample used is purple sweet potato *(Ipomoea batatas* L.) were taken from Hamlet VI Rasau Jaya I RT / RW 02/03 Rasau Jaya District of Kubu Raya West Kalimantan which is farmed. Purple sweet potato taken 3 months old, leather and dark purple tuber flesh. The samples were then made into botanicals and macerated using ethanol, acetic acid and water (25: 1: 5).

Phytochemical screening

Phytochemical screening of the purple sweet potato ethanol extract is done with tube test includes examining alkaloids, phenolics, flavonoids, anthocyanins, tannins, saponins, terpenoids and steroids.

Antioxidant Activity Test of Purple Sweet Potato Ethanol Extract by Using DPPH Method

DPPH test solution was added a solution of 30 ppm in the ratio 1: 1. Vortex further mix for 2 minutes then test solutions incubated at 37ºC for 30 minutes. The same treatment is performed on the blank DPPH solution of 30 ppm. Then the absorbance of test solution was measured by UV-Vis spectrophotometer at the wavelength of maximum[4].

Making the microemulsion

Microemulsion prepared by mixing the water phase into the oil phase. The aqueous phase consisted of ethanol extract of purple sweet potato and DMDM-Hydantoin while the oil phase consists of Span 80, BHT and olive oil. The mixture is stirred with a *magnetic stirrer* at a speed of 500 rpm until dissolved and homogenized for 1 hour at 40 ° C. Furthermore, microemulsion that has formed sonicated to reduce the size of the globules by using sonication type of bath for 24 minutes (3 cycles).

Table 1. Composition of Formula microemulsion Varying concentrations of Span 80

Material	Composition (% w / w)			
	A	B	C	D
ethanol extracts of *Ipomoea batatas*	0.0038	0.0038	0.0038	-
Aquades	20	20	20	20
Span 80	20	25	30	20
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

Description: D = negative control

Evaluation microemulsion preparations

Observation of the microemulsion was conducted over 28 days. Evaluation was conducted on the organoleptic to see any change in color, odor, clarity, phase separation and sediment, test the pH by using a pH meter, the determination of specific gravity was measured using a pycnometer at a temperature of 29º C, the test centrifugation at 3000 rpm for 30 minutes [7] and globule size determination using the tool *Particle Size Analysis* (PSA) type of Beckman *Coulter.*

Antioxidant Activity Test Preparations microemulsion with DPPH method

DPPH test solution was added a solution of 30 ppm in the ratio 1: 1. Divortex further mix for 2 minutes and then allowed to stand at 37 ° C for 30 minutes. The same treatment is performed on the blank DPPH solution of 30 ppm. Then the absorbance of test solution was measured by UV-Vis spectrophotometer at the wavelength of maximum [4].

Data analysis

Analysis of the test data from the antioxidant activity microemulsion was performed using *SPSS software* version 18 based test with independent sample t-test.

Results and Discussion

Selection method of extraction by maceration anthocyanin compounds based on sensitivity to high temperatures, where the method of extraction is done without heating and performed at room temperature. Ethanol extract of purple sweet potato obtained after maceration process is 200 grams or value yield of 9.79%. Based on phytochemical screening performed on the ethanol extract of purple sweet potato showed a positive extract contains secondary metabolites, alkaloids, phenolics, flavonoids, anthocyanins, tannins, saponins and triterpenoid (Table 2). Extract obtained is then tested antioxidant activity using DPPH. The presence of the antioxidant activity of the ethanol extract of purple sweet potato can be seen from the decline in the value of the absorbance of DPPH which shows that there has been a radical scavenging DPPH by the sample resulting diazo bond on DPPH reduced [8.] The antioxidant activity of the extract was determined by IC_{50} values which describe the concentration of test compound that can capture radicals by 50%. The smaller the IC_{50} value means the higher the antioxidant activity [9.] IC_{50} value of the ethanol extract of purple sweet potato by the equation y = 0.1072 x + 45.90 is 38.25 ppm which indicates that the extract has a very strong antioxidant activity (less than 50 pg / ml). IC_{50} values are then used to calculate the concentration of the extract in the microemulsion is equal to 0.0038%. Linear regression curve can be seen in Figure 1.

Table 2. Results of phytochemical screening Purple Sweet Potato Ethanol Extract

No.	Compound	Testing Methods	Positive results (Theory)	Result
1.	Alkaloids	Reagent Meyer Reagent Dargendrof Reagent Wagner	White precipitate Brown precipitate Brown precipitate	- + +
2.	Phenolic	FeCl3 + 1%	green, red, purple, blue, or black strong	+
3.	Flavonoids	Test Willstater Sianidin	red, orange and green	+
4.	Anthocyanin	Heating + HCl 2 M + NaOH 2 M	red and green color blue that fades slowly	+
5.	Tanin	+ NaCl 10% and 1% gelatin	White precipitate	+
6.	Saponin	Test Forth	Stable foam for not less than 10 minutes	+
7.	Terpenoids Steroids	Test Liebermen- Burchard	Red / red ring Blue / purple	+ -

Description: (+): contains compounds that are tested;
(-): Does not contain compounds that are tested

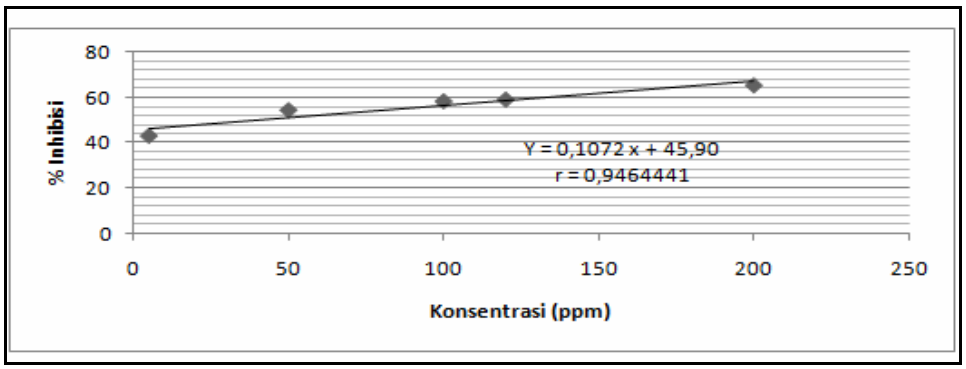

Figure 1. Linear regression curve test the antioxidant activity of ethanol extracts of *Ipomoea batatas* using DPPH

The best conditions are obtained to produce a microemulsion is clear and stable, the stirring speed of 500 rpm, stirring time of 1 hour and a temperature of 40ºC. In this study created three formulas microemulsion with 80 different span ratio as in Table 1. Preparation of the microemulsion is done by stirring technique. Stirring should not be too fast or too slow. If too fast, droplets in the microemulsion more easily collide so that the particle size becomes larger and can lead to foam more, while if it is too slow ingredients are difficult homogeneous. If the temperature is too low to use the process of merging or emulsification difficult. The increase in temperature will increase the kinetic energy of the droplets so as to facilitate incorporation [9.] Of the three formulas that made it was found that the concentration span of 80 by 20, 25 and 30% used can produce a microemulsion is clear and stable. This is because the concentration span of 80 used has reached or exceeded the critical micelle concentration.

Microemulsion formula A, B, and C. The resulting yellow, distinctive smell of olive oil, clear, and do not look any phase separation during 28 days of storage. The third formula has a pH and specific gravity stable. pH is obtained which is between 5.73 to 5.83 (Figure 2). This indicates that preparations are made in accordance with the skin physiological pH (4.5-6.5) so it can be acceptable for use on skin. Gravity of formula C produced greater than formula A and B as Span 80 are used as surfactants in the formula microemulsion also have a specific gravity and concentration span 80 were added to each formula varies so that it can be concluded that the increase of specific gravity between the formula comparable to the increasing concentration of span 80 were used (Figure 3,4,5). The third formula centrifugation test results showed no visible separation of phases, remained stable and clear. This indicates that the microemulsion was made to have good stability. Globule size determination made to the formula of the most stable microemulsion physically after storage for 28 days. However, based on the results of the evaluation showed that the three formulas stable during storage. Therefore, the determination of the size of the globules made to the formula at a concentration of 80 is the smallest span that formula A containing a span of 80 at 20% for the smallest concentration of the microemulsion can already be formed. The average diameter of the globules obtained microemulsion formula is 120.5 nm with a standard deviation (SD) of 11.6 is composed of 90.4% which globulnya size from 111.1 to 152.4 nm and 9.5% the size globulnya 36561.4 - 94392.3 nm.

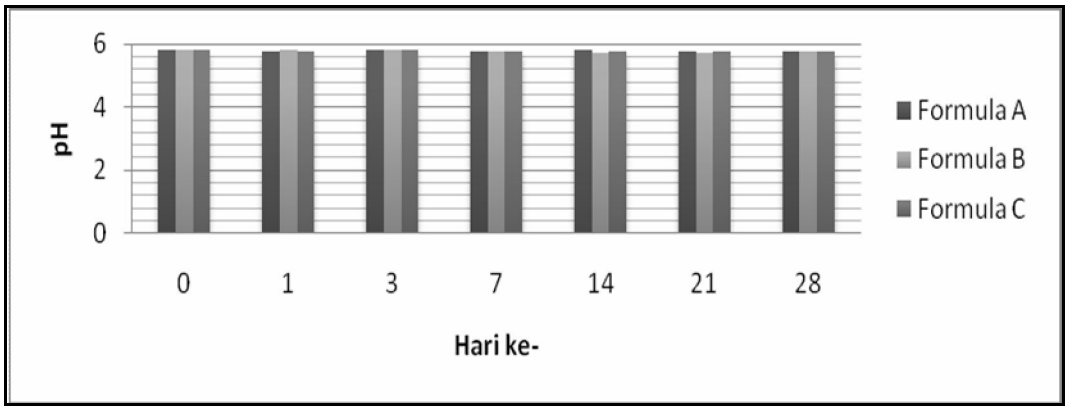

Figure 2. Graph microemulsion pH Test preparations

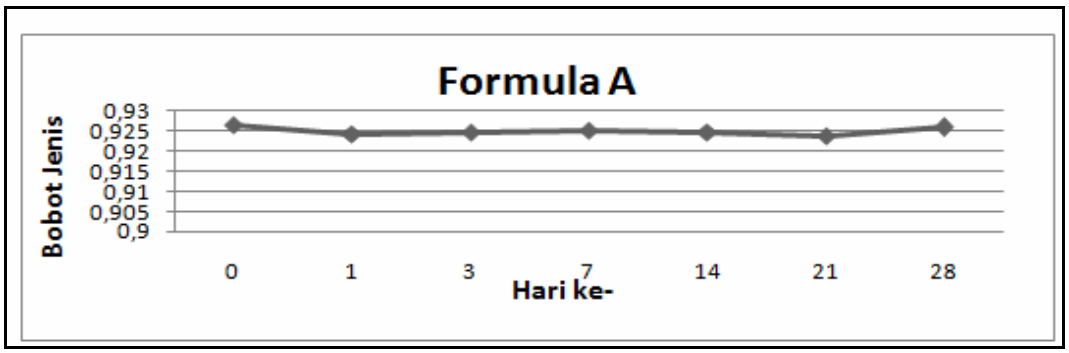

Figure 3. Graph Thickness Measurement Formula A

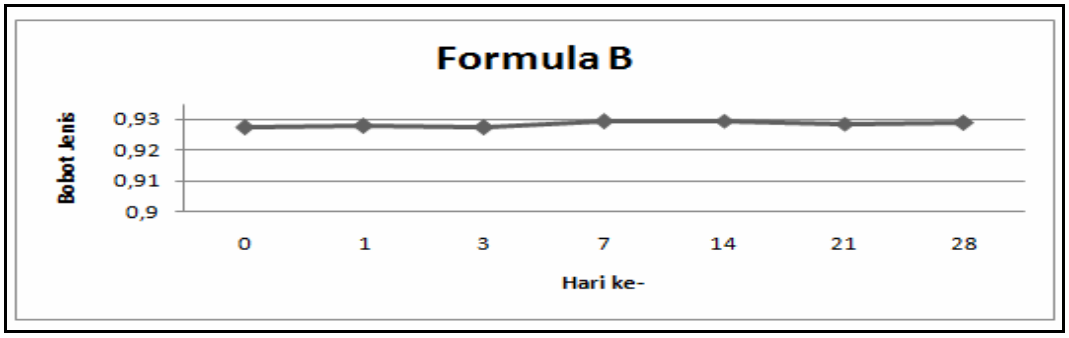

Figure 4. Graph Thickness Measurement Formula B

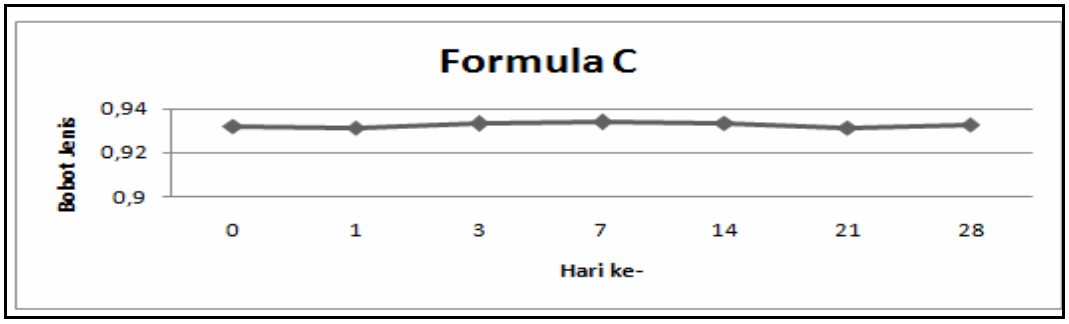

Figure 5. Graph Thickness Measurement Formula C

Activity test antioxidants microemulsion with DPPH performed on a formula that compared to the negative control is a microemulsion formula without the ethanol extract of purple sweet potato. Antioxidant activity assay results obtained based on the percent inhibition of formula A is then compared with the percent inhibition of the ethanol extract of purple sweet potato were not formulated into dosage forms microemulsion (Table 3). Percent inhibition of formula A has increased compared with the ethanol extract of purple sweet potato. This may be due to the microemulsion additives such as BHT and olive oil which can also provide antioxidant activity. Based on statistical analysis showed no significant difference between the percent inhibition of formula A and the ethanol extract of purple sweet potato with a p-value of 0.000 ($p < 0.05$) so that it can be concluded that the percent inhibition of formula A is higher than the ethanol extract of purple sweet potato. This shows, microemulsion are made to maintain the antioxidant activity of the ethanol extract of purple sweet potato.

Table 3. Results of Measurement of Antioxidant Activity microemulsion preparations with UV-Vis Spectrophotometer (X ± SD)

Samples	% Inhibition
Formula A	80.78 ± 0.01
Formula D	60.71 ± 0.20
Ethanol extracts of *Ipomoea batatas*	50 ± 0

Conclusion

Based on the results of the study it can be concluded that the ethanol extract of purple sweet potatoes have antioxidant activity with IC_{50} values of 38.25 ppm. Results of the evaluation for 28 days from the microemulsion ethanol extract of purple sweet potato made indicate that span 80 can form a microemulsion which is stable at a concentration of 20%, 25% and 30%. Formula A with a concentration of 20% span 80 have antioxidant activity with the percent inhibition of 80.78092%.

References

1. Harjanto. The recovery of oxidative stress in the exercise olahraga. Journal of Medicine YARSI. 2004, 12 (3), 81-87.
2. Husna EL, Melly N, Syarifah R. The content of anthocyanin and antioxidant activity of fresh purple sweet potato and other dairy products. AGRITECH. 2013, 33 (3); 296-302.
3. Arrellano A, Santoyo S, Martin C and Ygartua P. Surfactant effects on the in vitro perutaneous absorption of diclofenac sodium. European Journal of Drug Metabolism and Pharmacokinetics.1998, 23; 301-312.
4. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for Estimating antioxidant acitivity. Song klanakarin J.Sci. Technol. 2004 26 (2); 211-219.
5. Ministry of Health of the Republic of Indonesia. I ndonesia pharmacopoeia. Edition IV. Jakarta: Ministry of Health; 1995.. 72, 413, 1190.
6. Ministry of Health of the Republic of Indonesia. Farmako pe Indonesia. Edition IV. Jakarta: Ministry of Health; 1995.. 72, 413, 1190.
7. Ansel HC. Introduction of pharmaceutical dosage forms. Edition IV. Jakarta: Indonesia University Press; 2005.. 145-146, 376-381, 389.
8. Zuhra CF, Juliati BT, Herlince S. The antioxidant activity of flavonoids from leaves katuk *(Sauropus androgunus* (L) Merr.). Biological Journal of Sumatra. 2008; 3 (1): 7-10.
9. Yuhernita and Juniarti. Analysis of secondary metabolites of the methanol extract of leaves surian potential as an antioxidant. Makara, Science. 2011; 15 (1): 48-52.
10. Lachman L, Lieberman, Herbert A, Kanig, Joseph L. Theory and practice of industrial pharmacy II. Translation of The Theory and Practice of Industrial Pharmacy, by Suyatmi, Siti. Jakarta: UI-Press; 1994.. 1029-10901.

*****