J. Islamic Pharm. 2019. 4(1): 9-18

FORMULATION AND CHARACTERIZATION OF SUNSCREEN MICROEMULSION OF PINEAPPLE EXTRACT (A*nanas comosus* (L.)) WITH SYNERGISTIC EFFICACY ON SUN PROTECTION FACTOR (SPF)

Roihatul Mutiah[1], Yolanda C Sukma[1], Dewi Sinta Megawati[1], Rahmi Annisa[*1]

[1]*Department of Pharmacy, Faculty of Medical and Health Sciences, Universitas Islam Negeri Maulana Malik Ibrahim, Malang, Indonesia*

*Correspondent author: rahmiannisa@farmasi.uin-malang.ac.id

ABSTRACT

Pineapple (Ananas comosus (L.)) is plants that are often found in Indonesia. People often use pineapple as a food requirement. People only use the fruit, and the skin is only wasted into unused waste. Part of pineapple skin contains flavonoids and tannins which have the ability as photoprotection of UV light. To improve the ability of the preparation as a sunscreen, a microemulsion system is used as a system for delivering active substances. This study aims to determine the effect of variations in the concentration of pineapple peel extract in microemulsion preparations that can provide sunscreen effects through in vitro SPF values. This formulation uses pineapple peel extract with various concentrations of 5%, 10%, 15%, and 20%. The parameters observed in this study include organoleptic, pH, freeze-thaw, type of microemulsion, particle size, viscosity, and SPF value of the preparation in vitro. The results showed that the microemulsion of pineapple peel extract gave results following the specifications and variations in the concentration of pineapple peel extract did not affect the SPF values of microemulsion preparations with F1 results of 2.0294, F2 of 2.1391, F3 of 2.9322 and F4 of 3.7759. All formulas fall into the minimum type of protection.

Keywords: Pineapple skin, Photo protector, Microemulsion, Sun Protection Factor (SPF)

INTRODUCTION

Sun exposure has positive effects and adverse effects on the skin. These effects depend on the length and frequency of exposure, the intensity of sunlight, and the sensitivity of exposed individuals. Based on the wavelength and physiological effects, UV light is divided into three groups, namely UV-A, UV-B, and UV-C. UV-A has a wavelength of 320-400 nm, which causes the brown color on the skin without inflammation so that it is called the pigmentation area. UV-B has a wavelength of 290-320 nm, which can irritate the skin. UV-C has a wavelength of 200-290 nm and cannot reach the earth's surface because most of it has been absorbed by the ozone layer [1].

J. Islamic Pharm. 2019. 4(1): 9-18

The risk of being exposed to excessive UV rays can be overcome by using sunscreen, and some sunscreens are synthetic or natural. Natural sunscreen can be taken from plants; natural sunscreens also have the advantage of having lower side effects compared to synthetic sunscreens, one of the main ingredients of natural sunscreens comes from plants. Pineapple is one of the herbs that have medicinal properties containing vitamins A and C, calcium, phosphorus, magnesium, sodium, potassium, and bromelain enzymes. Part of the exhausted pineapple like the skin of a that has an uneven and small spiny texture on the outer surface also contains nutritious substances. Pineapple skin contains flavonoids, alkaloids, tannins, and steroids [2]. Other studies that have been carried out also prove that the skin of pineapple contains flavonoids and tannins, which can work as sunscreen active ingredients [3].

The microemulsion is a system consisting of water, oil, and amphiphilic compounds (surfactant and cosurfactant). Microemulsions are homogeneous, thermodynamically stable, isotropic, and low viscosity. The average particle size of microemulsions is in the range 0.1-1.0μm [4]. Previous research has reported on the formulation of sunscreen creams from pineapple peel extract with extract concentration levels of 2%, 4%, and 8% indicating the presence of a minimum level of sunscreen [3]. Therefore, further research is needed regarding the development of sunscreen preparations from pineapple peel extract in a more stable form of microemulsion gel, with high levels of solubilization, to increase bioavailability.

MATERIAL AND METHOD

Materials

Rotary evaporator (IKA RV Basic 10), oven (Memmert UN 55), Particle Size Analyzer (PARTICA), magnetic stirrer (Pyrex), pH meter (TOA DKK Model HM30R), viscometer Brookfield, ultrasonic bath (ELMER) and UV-Vis spectrophotometer (CAMAG).

Pineapple peels, 96% ethanol, *virgin coconut oil* (VCO), tween 80, span 80, PEG 400, water.

Formula

Formulation of Microemulsion of Pineapple Extract (*Ananas comosus* (L.)) used in this study supplemented as Table 1.

J. Islamic Pharm. 2019. 4(1): 9-18

Pineapple Extraction

Ultrasonic extraction method was performed in this study. The main purpose of this method is increasing cell wall's permeability based on waves propagation. When the waves propagate, the medium that passes through will experience vibration. The vibration will provide intensive stirring in the extraction process. Stirring will increase the osmosis between the material and the solvent so that it will improve the extraction process [5].

Extraction of pineapple peel is done using the ultrasonic extraction method. Ultrasonic extraction was carried out by weighing 50 grams of simplicia, then adding 96% ethanol as much as 200 mL and then for 2 minutes, the process was repeated three times and then filtered. After that, the simplicia was added with 96% ethanol as much as 150 mL to be sonicated for 2 minutes; the process was repeated three times and carried out the screening process. Then, add 96% ethanol as much as 150 mL again and do the same process as before. After the sonication process and filtration are complete, the filtrate results are concentrated using a rotary evaporator at 55^{o} C. The resulting extract is a dark brown thick extract after which the extract is placed inside. Then the thick extract was put into the oven to evaporate the water in the thick extract of pineapple peel at a temperature of 55^{o}C for one week and then weighed the extract obtained [6]. In this study, from 50 grams of simplicia obtained the extract weight of 13.2 grams with a yield of 26.4%.

Water Content Determination

We used HC 103 Moisture Analyzer (Mettler Toledo®) to determine water content of simplicia. It took 3-5 minutes to analyze 1 gram of sample. This procedure was replicated five times. In this study, the water content was 4, 202% ± 1.02.

Preparation of Microemulsion Pineapple Extract

Water phase of microemulsion consist of Tween 80 and water, while oil phase contains Span 80, PEG 400 and VCO. Water phase prepared by mixed 50°C pre-heated Tween 80 and water homogenously using magnetic stirrer. Oil phase prepared by mixed Span 80, PEG 400 and VCO into beaker glass using magnetic stirrer, then pineapple peel extract added into the mixture homogeneously. Water phase solution added into the mixture followed by heating at 50°C and 1000 rpm magnetic stirring for ±30 minutes.

J. Islamic Pharm. 2019. 4(1): 9-18

After that, the microemulsions formed clear and transparent were stable and allowed to stand for 24 hours.

Evaluation of Preparation

Organoleptic observation was performed to know the occurrence of significant changes in the final preparation. The dosage form that meets the requirements is a transparent or clear microemulsion, distinctive smell, and consistency like a solution [8][9].

pH

The test aims to determine the suitability of the pH value of the preparation with skin pH. The pH meter will show a stable number then recorded. PH requirements that can be tolerated not to irritate the skin are 5-9 [10].

Freeze-thaw-thaw

Test The test *freeze* aims to determine the stability of the preparation during storage from time to time and ensure that the preparation will not change during storage within 1 year. First, microemulsion preparations of ± 5 grams were tested for stability by storing alternately at cold temperatures (4 ± 2^{o}C) and high temperature (40 ± 2^{o}C) for 24 hours. This process is calculated as 1 cycle. If there is no phase separation, the preparation is declared physically stable [11].

Micoemulsion Type Test

Type examination of microemulsion was carried out using methylene blue over the surface of sample on an object glass. After the treatment, sample was observed using microscope. If the microemulsion is a type of water in oil, the particles of methylene blue dye will cluster on the surface, if the preparation is a type of oil in water then the methylene blue dye will dissolve in it and diffuse evenly into all parts of the water.[12]

Viscosity Test

Measurements were carried out with BrookfieldViscometer Cone and Plate. First turn on the appliance, after that installed cone is appropriate. Then the tested microemulsion was put into a container (plate). After that, the plate is raised to the position below the cone; the motor will drive the cone according to the selected speed, the microemulsion sample will be squeezed between the cone and plate. After that, the button is pressed start and waited for several minutes until the dosage viscosity value appears [13].

Particle Size Test Particle

J. Islamic Pharm. 2019. 4(1): 9-18

Size measurements were carried out on physically stable formula microemulsions. The aim is to determine the particle size in microemulsion preparations. The particle size was measured by a *particle size analyzer* (PARTICA®). Microemulsion samples are inserted in the content contained in the particle size test equipment until the monitor is *ready to* indicate that the sample is ready, then closed the tool after that on the monitor click "run" after which the particle size data of the preparation will appear. The particle size that met the requirements for microemulsion preparations was 0.1-1.0 µm [4].

Test of SPF Value of Pineapple Extract

Determination of the effectiveness of sunscreen was carried out by determining the SPF value in vitro with UV-Vis spectrophotometry. The concentration of extract used was 5000 ppm, 10000 ppm, 15000 ppm, and 20000 ppm. Pineapple peel extract was taken as much as 0.05 grams, 0.1 gram, 0.15 grams, and 0.2 grams. Then diluted using ethanol 96% to 10 mL. After that, 96 ml of ethanol was added as much as 1 ml into the cuvette then the cuvette was included in a UV-Vis spectrophotometer (library).

Test the Value of SPFPineapple Skin Extract

MicroemulsionMicroemulsion preparations were weighed as much as 0.05 grams, 0.1 gram, 0.15 grams, and 0.2 grams. Each microemulsion was put into a glass jar and then diluted with 96% ethanol and then put in a 10 mL volumetric flask. The concentration of preparations obtained was 5000 ppm, 10000 ppm, 15000 ppm, and 20000 ppm, respectively. Then, the absorbance value was measured using a UV-Vis spectrophotometer. The absorbance spectrum of the sample in the form of solution is obtained in the range 290-320 nm, every interval of 5 nm[9].

RESULT AND DISCUSSION

Preparation of Pineapple Extract (*Ananas comosus* (L.)) Microemulsion Sunscreen

The organoleptic evaluation was carried out to determine the physical characteristics of microemulsion preparations, including visual observations of color, aroma, and clarity. The organoleptic test results of microemulsion preparations shown in Table 2.

The pH test was carried out in order to determine the pH value of the preparation of pineapple bark extract microemulsions. Microemulsion preparations should have a pH that can

J. Islamic Pharm. 2019. 4(1): 9-18

be tolerated not to irritate the skin which is 5-9, because if the preparation is too acidic it can cause skin irritation, and if the pH is too alkaline it can cause scaly skin12]. Test for normality *Kolmogorov-Smirnov test* indicates normality test results have significance 0.875> 0.05, which means that data have a normal distribution of data, then the next stage of homogeneity test using a test *Levene* that shows the significance 0.531> 0.05 means that data has data distribution homogeneous. Based on the test, the *One Way* ANOVAdata has a significance of 0,000 <0,05, which means there are significant differences in each formula.

Freeze-thaw Test of Sunscreen Microemulsion

Freeze-thaw tests include organoleptic test and pH test after Freeze-thaw. Microemulsion preparations for each cycle were carried out by organoleptic testing. The results showed that the microemulsion of pineapple peel extract was stable for 6 cycles seen from the absence of changes in color, odor, and clarity.

During the 6 storage cycles of pineapple skin extract microemulsion and pH value evaluation after the freezing stability test *thaw*, pH generally decreased in each formula. However, the decrease in pH that occurs is still within the pH range which does not irritate the skin, and the changes that occur are not significant, and the organoleptic microemulsion preparations also do not change, and it can be said that the preparation is stable. After that, a statistical analysis was performed by comparing the pH values of the preparations before and after the stability testing of freeze-*thaw*. The statistical analysis chosen was *paired t-test,* based on the test, the five formulas did not have a significant difference because the analysis results had a significance of 0.423> 0.05.

Evaluation of type microemulsion, viscosity and particle size

Based on dissolved methylene blue in the microemulsion, it was found that the preparation was oil-in-water (*o/w*) type microemulsion.The viscosity test is carried out to determine the thickness of the preparation. Viscosity is the resistance of a separate preparation to flow or spread. The viscosity values in microemulsion preparations are presented in Table 3. The particle size of microemulsions is an essential characteristic in a microemulsion preparation, so particle size is needed to determine that the microemulsion preparations made are following the specifications. The results of the measurement of particle size of

J. Islamic Pharm. 2019. 4(1): 9-18

microemulsion preparations were obtained in the range of standard sizes of microemulsion particles of 0.1-1.0 µm. The results of particle size measurements are presented in Table 3.

Evaluation of SPF Value

SPF value for pineapple peel extract with a concentration of 5000 ppm was 1.1431, then for a concentration of 10000 ppm the results were 2.5179, the concentration of 15000 ppm obtained an SPF value of 2.9483 and for a concentration of 20000 ppm at 3.6172. Based on Kolmogorov-Smirnov normality test, data obtained in this study were normally distributed with the significance level of 0.303 (>0.05). Data homogeneity were tested using Levene Test and showed that all data were homogeneously distributed with significance level of 0.280 (>0.05). Unfortunately there is no significant differences between SPF values of each formula, this result based on significance value of One Way ANOVA test (0.301>0.05).

SPF value of formula 0 (0%) is 1.8694, which classified as a minimum protection. While formula 1 (5%) showed SPF value of 2.0294, according to FDA this formula can only provide minimal protection against sunlight. Formula 2 (10%) obtained an SPF value of 2.1391, in this formula it can provide minimal protection against sunlight, it is also the same as formula 3 (15%) obtained SPF value of 2.9322 which means it can provide minimal protection for skin from sun exposure, while for formula 4 (20%) obtained a value of 3.7759 the value is classified as a minimum protection according to the FDA. The results of testing the SPF values of microemulsion preparations were analyzed using the *Kolmogorov Smirnov test normality test* and showed significant results of 0.216 > 0.05, which means that the data has a normal distribution of data, then the next stage is a homogeneity test using the test *Levene* which shows a significance of 0.180> 0.05, meaning that the data has a homogeneous data distribution. Based on the test *One Way* ANOVA the SPF value data of microemulsion preparations showed a significance of 0.060> 0.05 meaning that there was no significant difference in SPF values in each formula, it can be said that on each formula with different concentrations (0%, 5 %, 10%, 155, 20%) did not have a significant effect on the SPF value.

CONCLUSION

Preparation of pineapple bark extract microemulsion as a sunscreen showed a good result, evaluated in organoleptic, pH test, particle size, viscosity, type of microemulsion and freeze

test. The concentration of extract did not affect the SPF value of the preparation. SPF value of formula 0 (0%), formula 1 (5%), formula 2 (10%), formula 3 (15%), and formula 4 (20%) respectively is 1.8624, 2.0294, 2.1391, 2, 9322, and 3.7759.

REFERENCES

[1] Hassan I., Konchok D., Abdul S., Parvaiz A. 2013. Sunscreens and Antioxidant Photo-Protective Measures: an Update. Post Graduate Department of Dermatology, STD & Leprosy, Govt. Medical College, Srinagar, University of Kasmir, J & K, India

[2] Kalaiselvi M, Kalaivani K. 2012. Phytochemcial analysis and anti-lipid peroxidative effect of Jasminum sambac (L.) Ait oleaceae. Pharmacologyonline; 1: 38-43.

[3] Damogalad, Viondy, Hosea Jaya Edy, Hamidah Sri Supriati. 2013. Formulasi Krim Tabir Surya Ekstrak Kulit Nanas *(Ananas comosus L Merr)* Dan Uji In Vitro Nilai *Sun Protecting Factor* (SPF). Pharmacon Jurnal Ilmiah Farmasi – Unsrat ;2(02): 39-44.

[4] Nikumbh, VK, Sevankar, SG, and Patil, MP 2013. Formulation Development, In Vitro Evaluation of Microemulsion-based gel Loaded With Ketoprofen. *Informa Healthcare, USA.*

[5] Departemen Kesehatan Republik Indonesia. 2000. Parameter Standar Umum Ekstrak Tumbuhan Obat. Edisi I. Direktorat Jenderal Pengawasan Obat dan Makanan. Direktorat Pengawasan Obat Tradisional. Jakarta.

[6] Direktorat Jendral Pengawasan Obat dan Makanan. 2000. Metode Analisis PPOM. Departemen Kesehatan RI. Jakarta.

[7] Winarno, FG. 2002. Kimia Pangan dan Gizi. Gramedia. Jakarta.

[8] Hendradi, E., Purwamti, T., Suryanto, AA 2013. Characterization of Preparations and Diclofenac Sodium Release Test with Microemulsion System in HPMC Gel Base. PharmaScientia. Vol 1, No. 2.

[9] Yulianti, Erlina., Adeltrudis Adelsa., And Alicia Putri. Determination of Value SPF *(Sun Protection Factor)* of 70% Ethanol Extract Temu Mangga *(Curcuma mango)* and 70% Ethanol Extract Cream Temu Mangga *(Curcuma mango)* Vitro *In* Method Using spectrophotometry. Essay. Faculty of Medicine and Health Sciences. Brawijaya University. Poor.

J. Islamic Pharm. 2019. 4(1): 9-18

[10] Murahata, RI and Aronson, PM 1994. The Relationship Between Solution pH and Clinical Irritancy for Carboxylic Acid-based Personal Washing Products. J. Soc. Cosmet. Chem. 45: 239-246.

[11] Fauzy, A. Effect of Fish Oil Concentration on Curcumin Penetration in Microemulsion Preparations. 2012. Thesis. Faculty of Mathematics and Natural Sciences, University of Indonesia. Depok

[12] Utami, Suci Syafitri. 2012. In Vitro Formulation and Penetration Test of Nanoemulsion Preparation, Nanoemulsion Gel, and Curcumin Gel. Essay. Faculty of Mathematics and Natural Sciences, University of Indonesia. Depok

[13] Martin, A., Swarbrick, J., Cammarata, A., 1993. Physical Pharmacy. UI-Press. Jakarta. Thing 1077-1090.

J. Islamic Pharm. 2019. 4(1): 9-18

Table 1. Formulation of Microemulsion of Pineapple Extract (*Ananas comosus.*L)

Materials (%)	Function of	Concentration% (*w/w*)				
		F0	**F1**	**F2**	**F3**	**F4**
EKBN	Active Material	-	5	10	15	20
Tween 80	Surfactant	29	29	29	29	29
Span 80	Surfactant	13,2	13,2	13, 2	13.2	13.2
PEG 400	Co-Surfactant	12	12	12	12	12
VCO	Oil Phase	4	4	4	4	4
Aquades	Solvent	ad 100				

Table 2. Organoleptic Test Results and PH Preparation

Formula	Color	Aroma	Description	pH
F0	Yellow	KS 80	Clear	5.93
F1	Yellow	KS 80	Clear	6.60
F2	KK	KS 80	Clear	6.57
F3	KK	KS 80	Jernih	6.37
F4	Coklat	KS 80	Clear	6.63

KK : Brownish Yellow
KS 80 : Typical Span 80

Table 3. Viscosity, particle size and SPF value of microemulsion preparations Pineapple Extract

Formula	Particle size (µm)	Viscosity (cPs)	SPF value	Protection type
F0 (0%)	0,537	102,3	1,8624	Minimal
F1 (5%)	0,1867	127,3	2,0294	Minimal
F2 (10%)	0,2198	498,9	2,1391	Minimal
F3 (15%)	0,0944	511,7	2,9322	Minimal
F4 (20%)	0,1674	601,2	3,7759	Minimal

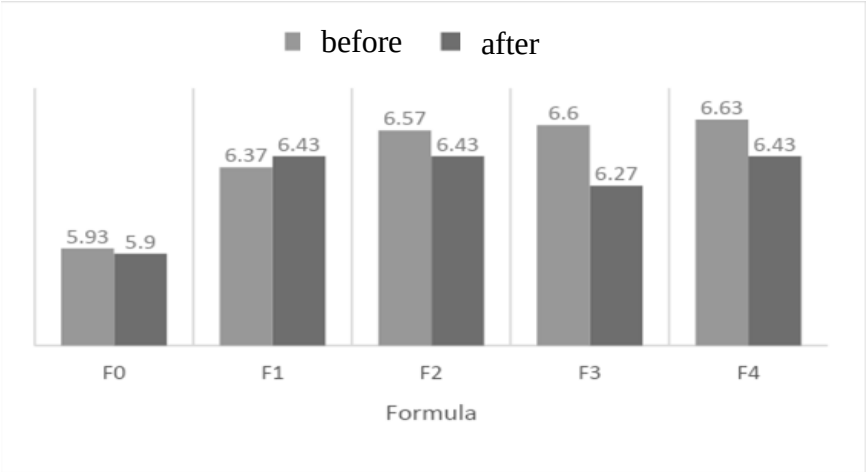

Figure 1. pH Testing Graph After Freezeaw Microemulsion *from of* Sunscreen Preparation Pineapple Extract *(Ananas comosus.L)Microemulsion*