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Determination Of SPF (Sun Protection Factor) Values And Evaluation Of Sunscreen Cream With ZnO and TIO_2 Nanoparticles Combination In Vitro As Active Ingredients.

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ABSTRACT

Cream preparation was made by mixing oil phase and water phase, then active ingredients namely the combination of TiO_2 and ZnO nanoparticles were added using capping agent of Hibiscus rosa sinensis leaf extract (F3). This cream was then used as the main cream. Comparative creams were also made using formulas of F0, F1, F2, F4 and F5. The effectiveness test for cream preparations was done in vitro by testing the absorbance using UV-Vis spectrophotometer. Determination of SPF values was calculated by the Mansur equation. After 28-day storage, the results showed that the pH values and cream viscosity were within the range of values corresponding to the Indonesian National Standard for sunscreen product. The results of UV detection showed that F3 cream made from combination of TiO_2 and ZnO nanoparticles as active ingredients using capping agent of Hibiscus rosa sinensis leaf extract has the highest SPF value of 6,927. This value has met the minimum value of the FDA (Food Drug Association).

Keywords: ZnO Nanoparticle, Sunscreen cream, TiO_2, SPF

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INTRODUCTION

Indonesia as a tropical country, naturally gets increasing exposure to sunlight intensity due to global warming. Sunlight containing ultraviolet (UV) can prevent or treat bone disorders by activating provitamin D_3 (7-dehydrocolrolrol) found in skin epidermis in to vitamin D3 [1]. Sunscreen compounds are compounds that can protect skin against erythema caused by UV light [2]. Compared with chemical sunscreens, physical sunscreen is preferably for its low toxicity properties. In addition, physical sunscreen agents are likely to be stable to light, does not exhibit phototoxic reactions and also effectively protects both UVA and UVB [3].

The effectiveness of sunscreen is usually indicated by Sun Protection Factor (SPF). Sunscreen products should contain SPF15 or higher [4]. ZnO is one of the physical sunscreens that does not undergo chemical decomposition when exposed to UV radiation. ZnO in nanometer size also has transparent appearance when applied on skin [5]. In addition to ZnO, TiO_2 in sunscreen has material characteristics that are safe, effective, and broad spectrum. TiO_2 works specifically, i.e by reflecting UV light. This material also has high photo stability and low toxicity levels [6]. But in smaller particle size, this material can contribute to the mechanism of UV light absorbing [7]. In this study, ZnO synthesis using sonochemistry method was performed. Sonochemistry method has the advantage of breaking aggregates of large particles into small to nano-sized particles [8]. One of capping agents that can decrease ZnO particle size is hibiscus (*Hibiscus rosa sinensis*) leaf extract. Selection of capping agents derived from natural materials more widely done because it is easy to obtain, economical, and environmentally friendly [9].

MATERIALS AND METHODS

Tools and Materials

The tools used in this study are as follows: X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), Transmission Electron Micrographs (TEM), UV-VIS Spectrophotometer, 35 kHz ultrasonic, oven, magnetic stirrer (Stuart), pH meter (Hanna) thermometer, mortar and pestle, analytical scales (Sartorius), spatulas, nonalcoholic wipes, aluminum foil, vapor plate, small knife, and scissors. It also includes glassware which includes erlenmeyer, beaker, measuring cup, stirring rod, dropper, measuring flask, and funnel.

The materials used in this study are as follows: $Zn(CH_3COO)_2.4H_2O$ (Merck), NaOH (Merck), aqua DM (Brataco), methanol (Merck), Whatman filter paper no.1 and no. 42. *Hibiscus rosa sinensis* is also used freshly, TiO_2 (Merck), VCO (Virgin coconut oil) (Fico Bagoes), stearic acid, cetyl alcohol, methyl paraben, TEA (triethanolamine), propyl paraben, glycerine and isopropanol (Merck).

Procedures

In the synthesis of ZnO nanoparticles, 50 mL of $Zn(CH_3COO)_2.4H_2O$ 0.1 M were mixed with 100 mL of NaOH 0.1 M in the Erlenmeyer flask, then inserted into ultrasonic vessels at a temperature of 35 kHz for 2 hours. The mixture was then cooled and filtered using Whatman filter paper no. 42 and washed 3 times using aqua DM and the last wash using methanol. The obtained solids were then oven-dried for 8 hours at 95 °C. The resulting material was then calcined at 600 °C. for 1 hour.

The making of sunscreen cream started by weighing materials that will be used. Water-soluble (water-phase) materials such as triethanolamine, glycerin, methyl paraben were mixed into aquadest and heated to 70 °C. While materials belong to oil phase such as VCO, stearic acid, cetyl alcohol and propyl paraben were mixed and heated at the same temperature of 70 °C. The water phase was then added gradually into the oil phase and stirring process was performed to obtain homogenous cream preparation. Then the active ingredients (TiO_2 and ZnO nanoparticles) were added to the cream and re-stirred. The formed creams were removed in a container and cooled to room temperature until thickened cream preparation (F2) was obtained. The same procedure is performed for creams preparation of F0, F1, F3, F4, and F5, according to the formula contained in Table 1.

Table 1. Cream Formulas

Material	Total (%) (b/b)					
	F0	F1	F2	F3	F4	F5
VCO	5	5	5	5	5	5
Stearic Acid	20	20	20	20	20	20
Cetyl Alcohol	2	2	2	2	2	2
Glyserin	5	5	5	5	5	5
Propyl Paraben	0,05	0,05	0,05	0,05	0,05	0,05
ZnO (without capping agent*)*	-	2,5	2,5	-	-	-
TiO_2	-	-	2,5	2,5	-	2,5
ZnO (with capping agent)	-	-	-	2,5	2,5	-
Triethanolamine	2	2	2	2	2	2
Metyl Paraben	0,1	0,1	0,1	0,1	0,1	0,1
Aquades	Add 100%	Add 100%	Add 100%	Add 100%	Add 100%	Add 100%

Description: F0 (cream base), F1 (cream made from nanoparticles as active ingredients without capping agent), F2 (cream made from ZnO nanoparticles as active ingredients without capping agent and TiO_2), F3 (active ZnO nanoparticle cream with capping agent of Hibiscus rosa sinensis leaf extract and TiO_2), F4 (cream made from ZnO nanoparticles as active ingredients with capping agent of Hibiscus rosa sinensis leaf extract), F5 (cream made from TiO2 as active ingredients)

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RESULTS AND DISCUSSION

Synthesis Results of ZnO Nanoparticles

In the synthesis process of ZnO nanoparticles, sonochemistry method utilizing 35 kHz ultrasonic waves was used, the results of the sonication process produced yellowish sediment. Filtering was performed using Whatman paper no. 42 and washed 3 times using aquadest to remove the impurities present in the solution. 8 hours heating at 95^oC was perfomed and produced greenish powder, but the powder has undergone color change into white color after calcination process was performed on temperature of 600^oC for 1 hour. The calcination process was performed to decompose the volatile substances existing in the materials and minimize nanoparticle sizes [10].

Characterization ZnO Nanoparticles

Characterization using X-Ray Difraction (XRD)

The resulting nanoparticle powder was characterized using X-Ray Diffraction (XRD) to determine the size and to see its crystallinity. When compared to the three difractograms presented in Figure 1, the diffraction of ZnO nanoparticles with capping agent of calcined Hibiscus rosa sinensis has the highest crystallinity properties.

There was a widening peak in difractogram of ZnO nanoparticles synthesized by capping agent of *Hibiscus rosa sinensis* leaf extract. The magnitude of peak width indicated that ZnO formed still containing an amorphous phase. The amorphous phase containing in ZnO nanoparticles was due to the presence of hydroxyl group residues derived from capping agent which may affect the crystallinity of ZnO nanoparticles material formed [11]. In addition, the content of organic compounds suspected to be present in ZnO nanoparticles affected the crystallinity of ZnO nanoparticles. In ZnO nanoparticle difractogram with *Hibiscus rosasinensis* leaf extract, ZnO specific peak appeared at 2θ with angles range of 20^o-40^o.

XRD results were also used to find the average size of ZnO particles by using the Scherrer equation. The results show that ZnO nanoparticles synthesized with capping agent *Hibiscus rosa sinensis* leaf extract and then calcined had size of 33 nm, whereas ZnO nanoparticles without capping agent had size of 44.95 nm. There was difference in size indicating that the addition of *Hibiscus rosa sinensis* leaf extract can limit the growth of nanoparticles so as not to form aggregates.

The calcination process can also affect the properties of nanoparticle materials, one of which can reduce the size of ZnO nanoparticle crystal. The calcination temperature in ZnO nanocrystalline formation process greatly affects the size of ZnO crystals formed.

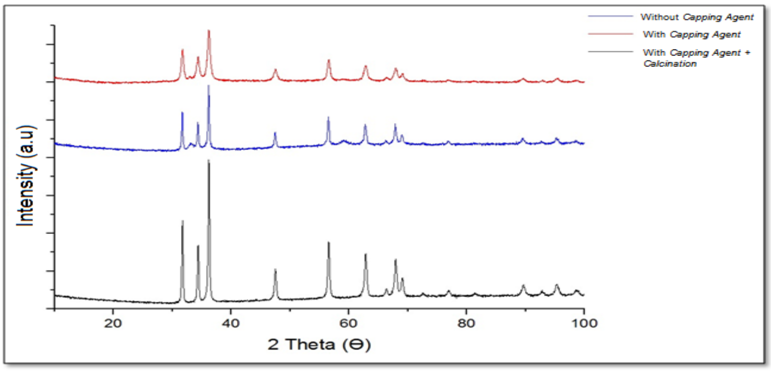
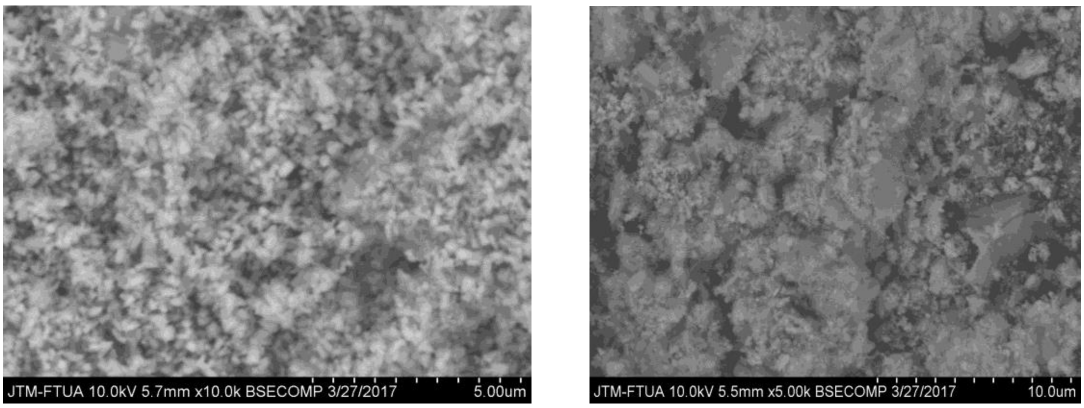


Fig 1. comparison of ZnO nanoparticles difractogram

Characterization using Scanning Microscope Electron (SEM)



(a) **(b)**

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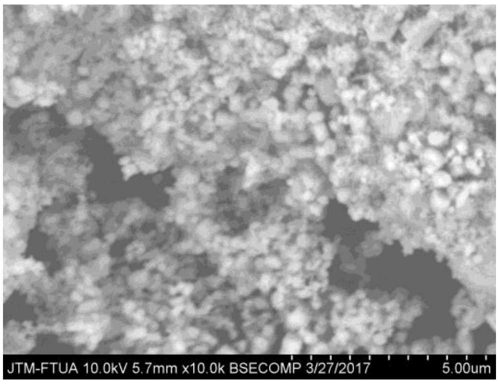


(c)

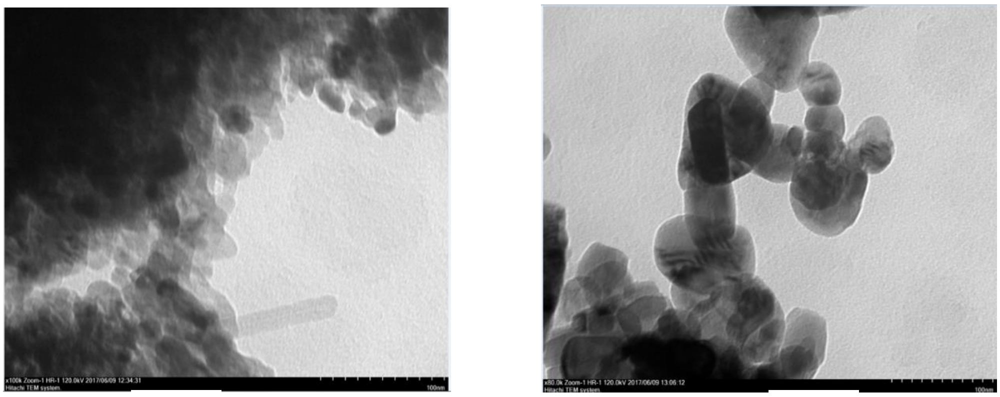
Fig 2. SEM analysis of ZnO nanoparticles (a) without the capping agent (b) with the capping agent of *Hibiscus rosa sinensis* leaf extract (c) with the capping agent of calcined *Hibiscus rosa sinensis* leaf extract

The SEM analysis of ZnO nanoparticles without capping agent can be seen in Figure 2a. The ZnO nanoparticle material produced in the form of aggregate. While the result of SEM characterization of ZnO nanoparticles synthesized with the capping agent of *Hibiscus rosa sinensis* leaf extract (Figure 2b) shows the morphology of ZnO nanoparticles which have aggregates-like shape with uneven size.

In ZnO nanoparticles synthesized with the addition of the calcined *Hibiscus rosa sinensis* leaf extract (Fig. 2c), it is clearly visible that granular nanoparticles are formed and the difference with synthesized nanoparticles without the addition of the extract is also visible. This is because *Hibiscus rosa sinensis* leaf extract used as the capping agent can inhibit the agglomeration of ZnO particles by delaying rapid direct contact between particles so that the size is evenly distributed.

Characterization using Transmission Electron Micrographs (TEM)

The transmission electron micrographs (TEM) of the bare and ZnO nanoparticles are shown in Fig. 3. Fig. 3a shows that most of the bare ZnO nanoparticles are spherical and their diameter is about 33 nm. This result is in accordance with the value calculated from the X-ray diffraction. Due to large specific surface area and high surface energy, some nanoparticles aggregate. The aggregation occurred probably during the process of drying. Fig. 3b shows the TEM of the surface modified nanoparticles with *capping agent*, illustrating that aggregation was alleviated and dispersion was improved.



(a) **(b)**

Fig 3. TEM photographs of: (a) ZnO nanoparticles without capping agent;
(b) ZnO nanoparticles.with capping agent

Cream

The cream was made by melting method. Melting at 70°C to facilitate the emulsification process due to higher temperatures will increase energy in emulsification process. The oil phase used in the cream base included VCO, stearic acid, and cetyl alcohol. The use of VCO in the preparation was performed because it provides soft and smooth texture on skin. In addition, short and medium chain fatty acids such as lauric acid and oleic acid are easily absorbed by skin so it can increase the penetration rate of active ingredients from VCO based cream preparation [12]. States that VCO contains lauric acid (53%) and tocopherol (0.5 mg/100g of coconut oil) which can serve as antioxidant in order to increase the effectiveness of sunscreen cream preparation [13].

The water phase used in this formula included triethanolamine (TEA) and aquadest. The neutralization reaction of TEA stearate occurs when stearic acid and TEA are mixed together at a temperature above the stearic acid melting point. The formed product turns into TEA stearate salt, otherwise known as TEA soap [14]. Glycerin was used as a humectant that works to moisturize the skin. The preservatives used in the dosage base were methyl paraben and propyl parabens. Methyl paraben has more activity in fungal group whereas propyl paraben has more for the bacterial group, so the combination of both provides good protection against both groups of microorganisms. Microbial contamination in pharmaceutical preparations can degrade the quality of cream preparation with the occurrence of discoloration, change of odor, and pH [15].

UV light protection test was performed in vitro. SPF values are used to measure the capacity of sunscreens in preventing UV radiation. The SPF values obtained from absorbances at 290-320 nm wavelengths with 5 nm intervals can be seen in Fig. 4.

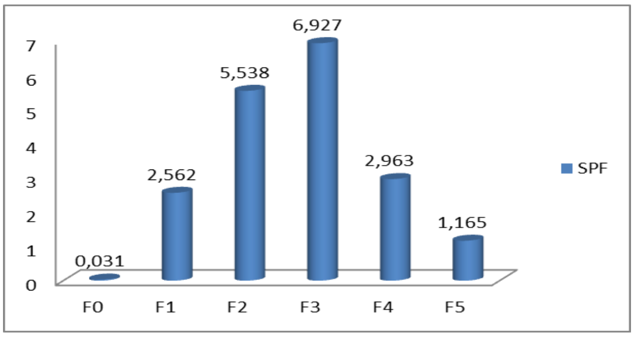


Fig 4. Chart of SPF Value

ZnO and TiO_2 are inorganic compounds that can be used as physical sunscreen which work by reflecting the UV radiation. But with their smaller particle size, these materials can play role in the mechanism of absorbing UV light. The combination of TiO_2 and ZnO nanoparticles with capping agent of *Hibiscus rosa sinensis* leaf extract can increase SPF cream value. It has been proven by greater SPF value of 6.927. The SPF value is greater than cream containing ZnO nanoparticles without the capping agent of *Hibiscus rosa sinensis* leaf extract and TiO_2, with the value of 5,538.

Cream Evaluation

Cream Homogeneity

The examination for homogeneity of cream preparations was carried out for 8 week storage. On day-0, all formulations showed that the cream preparations were homogeneous, but on the 7th day, the F2 and F3 creams showed that they were non-homogeneous due to the small particles of the solids were separating. While F0, F1 and F5 creams showed that they were still homogeneous up to 28 days storage.

Cream pH level

The pH test aims to determine the safety of cream preparations when used so as not to irritate skin. The pH evaluation of cream preparations was performed on a weekly basis for 8-week storage. Test results of pH values at the beginning of cream making are more likely to decrease insignificantly until day 28. According to the decrease in preparation pH level can be caused by hydrolysis of acidic compounds that can be triggered by temperature rise during storage. However, it still meets the requirements of safe pH values for skin which ranges from 5 to 8 [16].

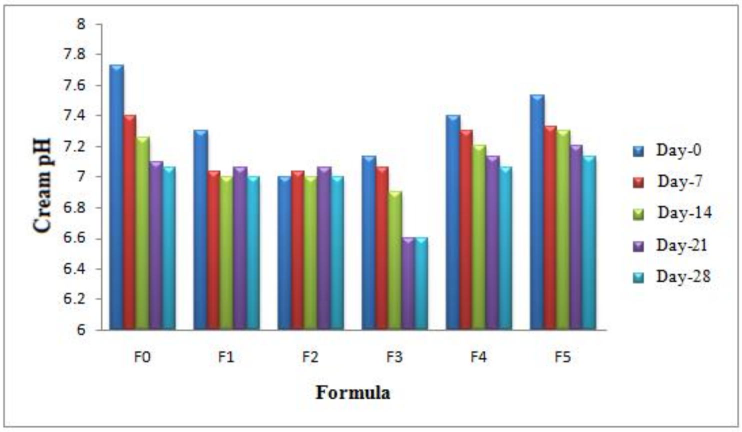


Fig 5. Measurement Results of Cream pH level

Cream Viscosity

Viscosity evaluation was performed to know the consistency of cream and stability of dosage to certain shelf-life. In this study, the viscosity decreases along with the length of storage. This is due to the decrease in viscosity is associated with phase separation. The complete separation of the emulsion occurs due to the hydrolysis of compounds used as emulsifiers [17]. Hydrolysis that occurs in the cream can change the TEA stearate to TEA and stearic acid. However, the resulting viscosity values are still in accordance with the standard, which are within the range of 2,000-50,000 centipoise.

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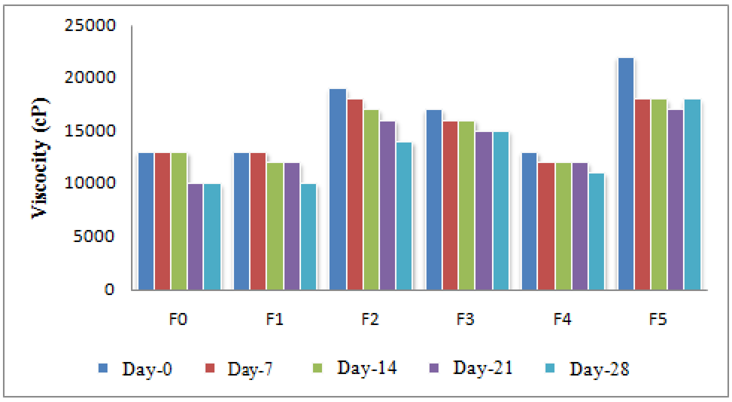

Fig 6. The Chart of Viscosity of Cream on Day 0 to Day 28

CONCLUSIONS

The cream preparations with active ingredients of ZnO nanoparticles combination synthesized by the capping agents of *Hibiscus rosa sinensis* leaf extract and TiO_2 have been successfully made. Result of UV protection effectiveness test showed that F3 cream made from active ingredients of TiO_2 and ZnO nanoparticles combination using the capping agent of *Hibiscus rosa sinensis* leaf extract has highest SPF value that is 6,927. This value has met the minimum value of FDA (Food Drug Association). In addition, the evaluation of sunscreen cream for 28-day storage showed that pH values and cream viscosity were within the range of values corresponding to the National Standar for sunscreen product.

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