Pharmaciana
Vol. l0, No.1,March 2020, Page. 61-68
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v10i1.14051

UV protection test of the ethanol fraction of papaya cream *(Carica papaya L.)* added with titanium dioxide

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Submitted: 06-09-2019 ***Reviewed: 28-09-2019*** ***Accepted: 08-03-2020***

ABSTRACT

Cream containing papaya fruit (10% of the 70% ethanol fraction) has been reported to effectively protect skins from ultraviolet (UV) light, though with a minimum protection ability. This study was aimed to determine the effects of adding titanium dioxide to sunscreen composed of 70% ethanol fraction of papaya flesh on the cream effectiveness, as measured by SPF values and physical properties. The ethanol fraction was obtained by fractionating the 70% ethanol extract of papaya fruit using n-hexane and ethyl acetate. Then, it was added with three different concentrations of titanium dioxide, namely, 1% (FI), 3% (FII), and 5% (FIII), to form cream preparations. These sunscreens were tested for their effectiveness in UV protection by in vitro spectrophotometry and based on the resultant SPFs. Based on the results of the study, the cream prepared with no titanium dioxide had SPF= 1.1283, while the SPFs of cream preparations added with 1%, 3%, and 5% titanium dioxide were 2.0572, 2.5708, and 2.8832, respectively. At these three concentrations, titanium dioxide increased the SPFs by 45%, 56%, and 61%, respectively. The cream preparations were found to have excellent physical properties. Based on the results of the statistical Kruskal-Wallis test, there are significant differences ($p<0.05$) between FI, FII, and FIII.

Keywords: SPF, cream, papaya, titanium dioxide

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INTRODUCTION

All living organisms require sunlight for survival. Although human needs it as a source of energy and for healthy skin and bones, its radiations also pose a negative impact on the skin, especially, UVA, UVB, and UVC. When the epidermal tissue of the skin is overexposed to these UV lights, it can develop into erythema, sunburn, degeneration of the skin, and skin cancer (Ismail *et al*., 2014).

Sunscreen potentially reduces the effects of exposure to UV rays because it can protect the skin from UV radiation. Although sunscreens that contain chemicals are widely developed, natural materials that are considered safer and more affordable are preferred. Papaya is an example of natural ingredients reportedly effective in UV protection (Islamiyati, 2018).

According to Islamiyati (2018), cream containing 10% of the 70% ethanol fraction of papaya flesh has a protective activity similar to that of sunscreen, though less optimal (SPF= 2.2570). With a higher SPF, sunscreens can provide better skin protection. In response to this, various substances have been added to increase SPFs, including the addition of titanium dioxide to papaya cream. Based on the mechanism of action, there are two categories of sunscreens: chemical sunscreen (absorbing radiation) and physical sunscreen (reflecting radiation) (Gustiani *et al*., 2015).

Titanium dioxide is one of the physical sunscreens that can protect the skin against UV rays (Zulkarnain *et al*., 2013). Its inorganic compounds have a better effect compared to their organic counterparts, which can only absorb UV radiation (Smijs and Pavel, 2011). Titanium dioxide is a very efficient sunscreen with low unfavorable effects like irritation and sensitization (Antoniou *et al*., 2008). With the addition of titanium dioxide to cream containing 70% ethanol fraction of papaya flesh, the effectiveness of these sunscreens is expected to increase.

MATERIALS AND METHODS

Materials

The materials used in this research were papaya fruits collected from Karang Kemiri Village, pharmaceutical-degree titanium dioxide, 70% Ethanol, N-hexane, Ethyl acetate, and the ingredients of the cream with pharmaceutical degrees (i.e., stearic acid, Cera alba, TEA, Vaseline alba, propylene glycol, nipagin, and aquadest).

Methods

Simplisia preparation

The papaya fruits were sorted out from impurities and washed with running water until clean. Afterward, they were mashed, placed on a black cloth, and dried in the sun. The resultant simplisia was ground using a blender until powdered simplisia was produced. It was then stored in a tightly closed dry container in a room protected from sunlight (Rahayu *et al*., 2009).

Preparation of papaya fraction

A total of 3.5 kg of the powdered simplisia was extracted by maceration using 70% ethanol solvent. After filtration, the filtrate was concentrated in a water bath at 40^{o}C until a dense extract was obtained. The dense extract was fractionated twice with 100 mL of n-hexane using a separating funnel. Then, the 70% ethanol fraction was collected and fractionated with 50 mL of ethyl acetate using a separating funnel, and this procedure was repeated four (4) times to obtain an optimal 70% ethanol fraction (Eka, 2014).

Cream formulation

The sunscreen cream was prepared from the 70% ethanol fraction of papaya fruit by mixing oil-phase with water-phase ingredients that had been prepared separately in a warm state. The oil phase (Cera alba, Vaseline alba, stearic acid, and titanium dioxide) was formed over a water bath at

70^oC until it melted, while the water phase was made by dissolving the ingredients (nipagin, propylene glycol, and TEA) with warm water. Both phases were mixed until homogeneous, added with the ethanol fraction of papaya fruit, and stirred until a homogeneous state and room temperature were achieved (Anief, 2006). The composition of the papaya sunscreen cream is presented in Table I.

Table I. The formulation of titanium dioxide-papaya sunscreen

Ingredients	F I (%)	F II (%)	F III (%)	F IV (%)
The ethanol fraction of papaya fruit	10	10	10	10
Titanium dioxide	1	3	5	-
Stearic acid	3	3	3	3
Cera alba	2.5	2.5	2.5	2.5
TEA	4	4	4	4
Vaseline alba	8	8	8	8
Propylene glycol	5	5	5	5
Nipagin	0.15	0.15	0.15	0.15
Aquadest until	100	100	100	100

Notes:
FI = formulated with 1% titanium dioxide
F II = formulated with 3% titanium dioxide
F III = formulated with 5% titanium dioxide
F IV = formulated without titanium dioxide

Testing of the physical characteristics of the cream

Organoleptic observation

The organoleptic test of the papaya cream included observations of color, texture, and odor (Faradiba, 2013).

Homogeneity test

The papaya cream was examined for its homogeneity by applying it on a glass object and observing the absence or presence of coarse grains (Suhery *et al*., 2016).

pH measurement

A homogeneous solution made by diluting the papaya cream in a water solvent was measured for its pH with a pH meter. The readings of the pH meter were then recorded (Puspitasari *et al*., 2017).

Spreadability test

Five hundred mg of the papaya cream was applied on a round glass scale and pressed with another round glass with a known weight for 5 minutes. Afterward, weights of 50 and 100 g were placed consecutively on top of the round glass for 1 minute. This procedure was replicated five (5) times (Miranti, 2009), and the diameter of the cream spread was measured.

Adhesion test

Five hundred mg of the papaya cream was placed on a glass object with a known area, covered by another glass object, and pressed with a weight of 1 kg for 5 minutes. The glass object was mounted on a test instrument and released with an 80-gram load, then the time needed to separate the two glass objects was recorded. This procedure was replicated five (5) times and reproduced for all cream formulas (Susilowati *et al*., 2014).

Determination of the SPFs of the cream

The effectiveness of the papaya sunscreen cream was determined from its SPF, which was measured in vitro by spectrophotometry.

Sample preparation

One gram of the papaya cream was weighed, transferred to a 100 mL volumetric flask, and then diluted with 70% ethanol. After that, the cream was cultivated for 5 minutes then filtered with a filter paper. Ten mL of the first filtrate was removed. A total of 5 mL of the solution was pipetted out into a 25 mL volumetric flask and then diluted with ethanol (Setiawan, 2010).

SPF calculation

The absorption spectrum of the test solution in cuvettes was obtained using a UV-Vis spectrophotometer at wavelengths of 290-320 nm with 70% ethanol as blank. The uptake of the test solution was measured at 290-320 nm, with an interval of 5 nm. Finally, the SPF of the cream preparations was analyzed by Mansur's method.

The mathematical equation for in vitro SPF calculation in a spectrophotometer is as follows:

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda) \quad \text{...........................(1)}$$

where:
EE : Erythemal effect spectrum
I : Solar intensity spectrum (*EE x I* produces a constant number, as presented in Table II)
Abs : Absorbance of the sunscreen sample
CF : Correction factor (10) (Mansur *et al*., 1986).

Table II. The values of *EE x I* at varying wavelengths between 290 and 320 nm (Mansur *et al*., 1986)

Wavelengths (λ nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

RESULTS AND DISCUSSION

Physical characteristics of the cream

The sample determination revealed that the papaya fruit used in this study was *Carica papaya L*., a species of the Caricaceae family. From 3.5 kg of papaya fruit, 280 grams of powdered simplisia were obtained. The maceration of this simplisia (using 70% ethanol) produced 60 grams of papaya extract, with a yield of 21.43%. Afterward, this ethanol extract was fractionated with n-hexane and ethyl acetate, producing 30 grams of papaya fraction with a 50% yield.

The four creams prepared previously (FI, FII, FIII, and FIV) were each subjected to organoleptic observation and homogeneity, pH, spreadability, and adhesion tests. The organoleptic test results showed that the four formulas produced cream preparations with a cream color, a distinctive smell of the papaya fraction, and a smooth texture. Meanwhile, the cream added with titanium dioxide had a white color. Based on the homogeneity test results, all of the four formulas created homogeneous creams.

Table III. The Physical characteristics of the papaya cream

Samples	Mean±SD		
	pH	**Diameters of the Spread (cm)**	**Adhesive strength (seconds)**
FI	7.49±0.044	4.45±0.586	4.60±1.140
FII	7.59±0.046	3.98±0.400	10.2±1.643
FIII	7.71±0.036	3.81±0.451	32.2±6.723
FIV	7.76±0.042	5.42±0.680	1.40±0.548

Notes:
FI = formulated with 1% titanium dioxide
FII = formulated with 3% titanium dioxide
FIII = formulated with 5% titanium dioxide
FIV = formulated without titanium dioxide

The pH measured proved that the four formulas (FI, FII, FIII, and FIV) fulfilled the requirements for a safe-to-use sunscreen, namely, 4.5-8.0 (SNI 16-4399-1996). The higher the concentration of titanium dioxide added to the cream, the higher the pH value (Anggraini *et al*., 2013). The Kruskal-Wallis test revealed a significant difference ($p<0.05$) between FI, FII, FIII, and FIV, meaning that differences in the concentration of titanium dioxide affect the pH of the cream. It is possible because titanium dioxide has a pH of 7.5 or tends to be alkaline (National Library of Medicine, 2019).

The spreadability test determines the ability of a dosage form to spread over a particular area from the diameter of the spread. Based on the results of this test (Table III), FI and FIV created a categorically good spread because FI had the least amount of titanium dioxide, and FIV did not contain titanium dioxide. Titanium dioxide has a high specific gravity (3.9-4.3 g/cm^3), which in this study was a solid powder that could reduce the spread of the cream (National Library of Medicine, 2019). The independent samples t-test further confirmed the significant difference ($p<0.05$) between FI and FIV.

For topical preparations, the excellent adhesion is when the debonding time is more than 4 seconds (Ulaen *et al*., 2012). The test results showed that the adhesive strength of FI, FII, and FIII met the requirements of good adhesion (i.e., more than 4 seconds), but that of FIV did not (i.e., less than 4 seconds). In conclusion, a higher concentration of titanium dioxide can prolong the debonding of two glass objects adhered to each other using the papaya cream or, in other words, strengthen the adhesion. Titanium dioxide has a high specific gravity (3.9-4.3 g/cm^3), which can increase the adhesion of the cream (National Library of Medicine, 2019). Based on the Kruskal-Wallis test, there is a statistically significant difference ($p<0.05$) between FI, FII, and FIII, which is believed due to differences in the concentration of the added titanium dioxide that potentially affect the stickiness of the cream. The results of the adhesion test can be seen in Table III.

Sun Protection Factor (SPF) of the Cream

The Sun Protection Factor (SPF) of the papaya cream added with titanium dioxide was measured in vitro by spectrophotometry (using a UV-Visible spectrophotometer). The tests were reproduced five times at wavelengths of 290-320 nm, with an interval of 5 nm. The SPF was determined by preparing the cream samples (FI, FII, FIII, FIV, and positive control) and measuring their absorbances.

Table IV. The SPFs of the Papaya Sunscreen

Samples	Sun Protection Factor (SPF)					Mean
	I	II	III	IV	V	
FI	2.0542	2.1122	2.0865	2.0178	2.0153	2.0572
FII	2.6509	2.5922	2.5475	2.5308	2.5325	2.5708
FIII	2.8999	2.9557	2.8628	2.8328	2.8648	2.8832
FIV	1.1336	1.1206	1.1374	1.1308	1.1189	1.1283
C (+)	37.7535	37.6922	36.0588	38.6594	37.4430	37.5214

Notes:
FI = formulated with 1% titanium dioxide
FII = formulated with 3% titanium dioxide
FIII = formulated with 5% titanium dioxide
FIV = formulated without titanium dioxide
C (+) = positive control = market products with scientifically proven SPFs (i.e., Garnier)

When added with titanium dioxide, cream containing 10% of the 70% ethanol fraction of papaya flesh can increase the SPF of the cream preparations. It is higher than FIV (SPF= 1.1283) but lower than the cream in the market. The results showed that the higher the concentration of the added titanium dioxide, the higher the SPF of the cream produced. Titanium dioxide is an active substance known to function as a sunscreen, whose physical protection mechanism involves reflecting UV lights (Anggraini *et al*., 2013). It is composed of inorganic particles that spread microparticles in the upper skin layer, increasing the protection factor against sunlight (Latha, 2013).

As presented in Table V, the effectiveness of the cream can be categorized based on its SPF. The Kruskal-Wallis test of the SPFs of FI, FII, FIII, FIV, and positive control resulted in $p<0.05$, indicating a statistically significant difference between the five cream preparations.

Table V. The Effectiveness of the Sunscreen Cream Preparations

Samples	SPF	Category
Formula I	2.0572	Minimal activity
Formula II	2.5708	Minimal activity
Formula III	2.8832	Minimal activity
Formula IV	1.1283	No activity
Control (+)	37.5214	Ultra activity

CONCLUSION

The addition of titanium dioxide to cream prepared with 70% ethanol fraction of papaya (*Carica papaya* L.) flesh has been proven to increase the effectiveness, pH value, and adhesive strength of the sunscreen creams, although with SPFs lower than the ones available in the market.

ACKNOWLEDGMENT

The authors would like to thank the LPPM of Stikes Serulingmas Cilacap for funding this research.

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