International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 1, 2022

Original Article

PHYSICAL STABILITY, PHOTOPROTECTIVE EFFECT, AND PRIMER IRRITATION TEST OF CREAM O/W LIME PEEL EXTRACT (*CITRUS AURANTIFOLIA*) AS A SUNSCREEN

WARDIYAH[1], ULYA SAFRINA[1*], HARPOLIA CARTIKA[1], NANDA PUSPITA[1]

[1]Department of Pharmacy, Poltekkes Kemenkes Jakarta II, DKI Jakarta, 10560, Indonesia
*Email: ulya.syafrina@poltekkesjkt2.ac.id

Received: 10 Nov 2021, Revised and Accepted: 15 Dec 2021

ABSTRACT

Objective: This research aimed to discover the physical stability, photoprotective effect, and primer irritation of cream oil in water (O/W) lime peel extract as sunscreen.

Methods: The cream formulations were prepared using different concentrations of lime peel extract (5%, 10%, and 15%). Physical stability test performed was cycling test, storage in high temperature (40±2 °C), room temperature (25±2 °C), and low temperature (4±2 °C). Then tested for SPF value using a UV/Vis spectrophotometer and primer irritation test using rabbits.

Results: All cream formulations have homogeneity, pH, viscosity, and dispersion values that meet the requirements. The SPF value of extracts and cream preparations gave Sun Protection Factor (SPF) values above 15, indicating that the extracts and the three formulas had sunscreen protection activity in the ultra-category. The SPF value and total phenol decreased in cold and hot storage but were relatively stable at room temperature storage. The results of the irritation test on rabbits showed moderate irritation in the base group and slight irritation in the extract group.

Conclusion: Lime peel extract sunscreen cream has good physical stability, a photoprotective effect in the ultra-category, and mild to moderate level of irritation.

Keywords: Sunscreen, Lime peel extract, SPF

 DOI: https://dx.doi.org/10.22159/ijap.2022.v14s1.04 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Sunlight emits ultraviolet (UV) radiation consisting of UVA (wavelength 315–400 nm) and UVB (wavelength 280–315 nm). Both of which can cause damage to the skin and eyes. Exposure to UV rays on the skin can cause several adverse effects such as wrinkles, skin pigmentation, premature aging, and inflammation. In addition, the skin has a vital role as protection against exposure to pathogenic substances and pollutants [1]. Therefore, the skin needs protection to prevent skin damage. Sunscreen is a preparation with an active substance that can actively absorb, scatter, or reflect sunlight energy that penetrates the skin. Sunscreen is closely related to the value of SPF (Sun Protecting Factor). The used SPF value is to determine the amount of ability of UV sunscreen products. The higher the SPF value of the sunscreen substance, the more effective it is to protect the skin from the adverse effects of UV rays. According to the FDA (Food Drugs Administration), an effective sunscreen product has an SPF value above 15 and medium protection. Furthermore, the maximum SPF value is above 50 [2].

Lime (*Citrus aurantifolia*) is one of the plants from the Rutaceae family that grows in subtropical and tropical areas, a group of thorny green plants with a height of 3-5 meters [3, 4]. Lime is widely used for medicine, cosmetics, cooking spices, and beverage ingredients. While the fruit skin would only be thrown away, this waste would pollute the environment. Several research reports state that lime peel has a fairly good effect on health, including antibacterial and antioxidant [5]. Lime is rich in flavonoids. The research using fruit peels and leaves of lime in three regions in Calabria, Italy, showed methanol extracts containing rutin, apigenin, quercetin, kaempferol, and nobiletin [6]. Lime's secondary metabolites are flavonoids, alkaloids, coumarins, limonoids, carotenoids, phenolic compounds, and essential oils. These compounds exhibit important biological activities for human health, such as antioxidant, anticancer, anti-inflammatory, cardiovascular protection, and neuroprotective effects [7]. This study uses lime peel extract because the peels contains a lot of total phenol and flavonoid compound, which play an important role as sunscreen compared to other plant parts such as fruit and leaves [8].

Ethanol extract of lime peel has antioxidant activity with an IC_{50} of 54.458 g/ml, a strong antioxidant. The chemical content in the lime peel can capture free radicals in body tissues, such as skin, to prevent aging and skin tissue damage [9, 10]. The study results on lime leaves showed antioxidant activity of IC_{50} 93.41µg/ml [11]. The smaller the IC_{50} value, the higher the free radical scavenging activity [12]. Thus, antioxidants overcome the damage effects to human skin caused by free radicals, the main factors in the aging process, and skin tissue damage. Thin Layer Chromatography (TLC) results showed that the compounds in the ethanolic extract of lime peel are flavonoids and vitamin C [9]. From the previous research, the lime extract has an SPF value of 40.15 at a 300 g/ml concentration and belongs to sunscreen ultra-protection. The value of SPF is directly proportional to the total phenol content in lime peel [13]. The value of total phenol content is determined using the Folin-Ciocalteu method [14]. The essential oil of lime is rich in terpenoids compounds and potential to be developed as anticholinesterase drugs. Using the ABTS (2,2 azinobis (3-etilbenzotiazolin)-6-asam sulfonat) method, the antioxidant testing of lime essential oil gave an IC_{50} value of 19.6 g/ml TEAC (Trolox Equivalent Antioxidant Capacity) [15].

The mechanism of sunscreen preparation is divided into two groups. Firstly, the physical blocker group works physically by reflecting or deflecting UV radiation. Secondly, the chemical sunscreen group works to absorb UV rays. Sunscreen is available in various forms, such as creams, gels, and lotions. Creams are more popular with the public than other forms because creams can maintain skin moisture and softness, which are requirements of sunscreen products, penetrate the skin easily, and spread evenly. Meanwhile, gel and lotion preparations contain more water than cream. Therefore, both preparations are very volatile and are lost when sweating [16, 17]. Creams have two types, which are W/O and O/W. The penetration of the O/W type cream is much stronger than the O/W type because the oil lasts longer on the skin surface and can penetrate the skin layer further. While the type of cream is W/O with a vanishing cream base, it is easy to wash and does not remain (sticky) [16]. This study uses O/W cream type because its shows better effectiveness

on sunscreen products [18]. To guarantee the product's safety, an irritation test for topical had been done using test animals. Rabbits are usually used as an irritation test because rabbit skin is more sensitive than human skin. The irritation test used a dose of 0.5 grams of sample on 1 x 1 inch of skin [19].

As a tropical country with year-round sun exposure, Indonesia makes the use of sunscreen important to avoid the harmful effects of UV rays. The potential of lime peel as an antioxidant and sunscreen is quite significant, but its utilization is not optimal. Therefore, in this study, the stability of lime extract cream preparation and its activity as a sunscreen would be tested. The research was conducted by making formulations of cream preparation type O/W to determine its activity and irritation using rabbits as test animals.

MATERIALS AND METHODS

Materials

The material used in this research was lime peel (*Citrus aurantifolia*) obtained from Balai Penelitian Tanaman Rempah dan Obat/BALITRO Bogor West Java, Indonesia. The sampel was determined at LIPI (Indonesian Institute of Sciences). The plant sample certificate number is 834/IPH.1.01/If.07/III/2018; the lime peel obtained was then extracted using the maceration method with 70% ethanol to obtain a thick extract. The cream used stearic acid, glycerin, triethanolamine, sodium tetraborate, nipagin, and aqua dest for the basic ingredients. Cream material obtained from TandT Chemical with pharmaceutical grade quality. For total phenol and SPF testing, it used ethanol p. a 96% (Merck), aquadest, Folin-Ciocalteu reagent (Merck), Na_2CO_3 (Sigma Aldrich), gallic acid (Sigma Aldrich), stearic acid (Sigma Aldrich), glycerin (Merck), sodium biborate (Merck), triethanolamine (Merck), and methylparaben (Merck).

Instruments

The tools used for the formulation and testing of creams are steam dish, measuring cup, beaker glass (Pyrex), UV-Vis's spectrophotometer (Shimadzu), stirring rod, homogenizer (Daihan Scientific), scales (OHAUS), watch glass, plastic pot, dropper, spatula, water bath (Memmert), slide, microscope (Boeco), Brookfield viscometer (Brookfield), pH meter (Mettler Toledo), thermometer, refrigerator, measuring flask (Pyrex), pipette filler, and oven (Memmert).

Raw material inspection

Lime peel (*Citrus aurantifolia*) was obtained from BALITTRO (Center for Medicinal and Aromatic Plant Research) and determined at LIPI (Indonesian Institute of Sciences). It was to determine the type of plant purchased following the desired plant by looking at the results of the analysis certificate. For cream bases, such as stearic acid, glycerin, triethanolamine, sodium tetraborate, aqua distillate, and methylparaben (nipagin), the examination followed the requirements of Indonesian Pharmacopoeia.

Extract production

Lime peel extract was made by maceration using 70% ethanol. The lime peel powder was weighed 200 grams 5 times, put into a maceration vessel, added 75 parts of 70% ethanol solvent (1500 ml), stirred, and let stand for 5 d. Next, the macerate was filtered using a flannel cloth and put into a brown bottle. The simplicia dregs were added with 25 parts of the filtered liquid, 500 ml, stirred, and let stand for 2 d. Then, the macerate was filtered. The obtained macerate evaporated in an evaporator at a temperature of 40-50 °C and then concentrated over a water bath at a temperature of±50 °C to obtain a thick extract [20].

Extract identification

Lime peel extract was identified qualitatively by using color reagents to detect the secondary metabolite compounds contained in the extract. The compounds tested were phenols, flavonoids, and tannins because the three compounds were suspected of having acted as sunscreen [21].

Sunscreen cream formulation

The production of lime peel extract used three formula (table 1), namely doses of 5%, 10%, and 15% and used an O/W type cream base. All necessary materials were weighed according to the usage requirements.

Table 1: Lime peel extract cream formulation type O/AW

Ingredients	Formula (%)		
	A	B	C
Extract	5	10	15
Stearic acid	14.1	14.1	14.1
Glycerin	10	10	10
Na Tetraborate	0.25	0.25	0.25
Triethanolamine	1	1	1
Nipagin	0.12	0.12	0.12
Aqua ad	50 g	50 g	50 g

Sunscreen cream stability test

Testing the physical stability of the preparation was done for 28 d of storage at 3 different temperatures, which were low temperature (4±2 °C), room temperature (25±2 °C) and high temperature (40±2 °C) (18). During the storage, observations were made in preparation evaluation, including organoleptic observations, pH, homogeneity, dispersion, and viscosity on day 0 and after every 7 d.

Determination of total phenolic content

Determination of total phenolic content was carried out using the Follin-Ciocalteu method. The standard used was gallic acid. The measurement results are expressed in % w/w equivalent gallic acid. The total phenolic content of the sample was read by weighing 0.2 grams of lime peel extract and then diluted to 100 ml with 96% ethanol: aqua dest (1:1) so that the concentration was 2000 ppm. Then 25 ml pipetted and dissolved with 96% ethanol: aqua dest (1:1) until the volume was 50 ml so that the concentration of 1000 ppm was obtained. From a concentration of 1000 ppm, 0.2 ml of sample solution was pipetted, and 15.8 ml of distilled water was added 1 ml of Folin-Ciocalteau reagent, shaken. Let stand for 8 min, then add 3 ml of 20% Na_2CO_3 into the mixture, let stand the solution for 1 h at room temperature. The absorption was measured using a UV-Vis spectrophotometer at the maximum absorption wavelength, which would give a blue complex. Perform three repetitions so that the total phenolic content obtained was as mg GAE/g sample [14].

Determination of SPF value

Determination of the SPF value *in vitro* using UV/Vis spectrophotometry. The measured absorbance value was at a wavelength of 290-320 nm and intervals of 5 nm with a thickness of A = 1 cm [22]. The solvent used was ethanol p. a 96%. The following formula determines the SPF value in Eq. 1:

Where CF = correction factor (10), EE = Erithermal Efficiency, I = Simulation of solar radiation [22].

Primary irritation test

The test animal was a female white rabbit (17 w old, with a bodyweight of±3.0 kg) with an experimental time of 72 h. Three female white rabbits were used. Each rabbit with its back shaved

was applied homogeneously to 0.5 grams of lime extract cream formula. The positive control used 3% benzophenone cream and negative control, namely base, then covered with gauze. Observations of erythema and edema that occurred were carried out at 24 and 72 h after exposure [19]. Each test preparation calculated the sum of the erythema index and edema index. Then, the irritation index was calculated in the following Eq. 2:

$$\text{Primary Irritation Index} = \frac{\text{Total erythema 24–48–72 hours} + \text{Total edema 24–48–72 hours}}{\text{Total of Rabbits}} \quad \text{(Eq.2)}$$

The obtained degree of irritation was by comparing the irritation index obtained with the following scores (table 2):

Table 2: Irritation degree score

Evaluation	Score
Not irritating	0.0
Very little irritation	0.1-0.4
Slight irritation	0.41-1.9
Moderate irritation	2.0-4.9
Severe irritation	5.0-8.0

RESULTS AND DISCUSSION

Physical and chemical examination of simplicia and lime (*Citrus aurantifolia*) peel extract

The physical and chemical characteristic of simplicia and lime (*Citrus aurantifolia*) peel extract was presented in table 3.

Phytochemical screening of the extract

The result of phytochemical screening of lime (*Citrus aurantifolia*) peel extract was presented in table 4 It was shown positive results in the identification test of secondary metabolites such as phenols, flavonoids, and tannins.

Table 3: Organoleptic examination of simplicia orange peel

Examination	Observation
Smell	Special lime
Color	Yellow
Form	Coarse powder
Weight of dry simplicia	950 g
Thick extract weight	180.6 g
Extract yield	19.01%

Table 4: Extract identification test

Substance	Reaction	Observation	Result
Phenol	Substance+FeCl3	Dark green, red, purple, blue, or strong black	+
Flavonoids	Substance+Mg+Concentrated HCl	Red	+
Tannins	Substance+FeCl3	Dark green/black/blue	+

The data follows the theory that lime peel has phenolic compounds such as phenols, flavonoids, and tannins. It is known that flavonoids act as natural antioxidants because they contain hydroxyl groups capable of scavenging free radicals. Conjugated aromatic benzene can absorb UV-A or UV-B rays, which can cause adverse effects on the skin to have the potential as a sunscreen.

Phenol and flavonoid compounds are secondary metabolites that are very potential as antioxidants and sunscreens. The mechanism of the antioxidant effect of phenolic compounds is reducing free radicals and chelating metals. Phytophenols are one of the effective compounds to prevent aging. Meanwhile, the flavonoid compounds in the *Citrus sp*., such as glycosides (hesperidin, naringin) and the O-methylated glycones of flavones (nobiletin and tangeretin), were reported to be more abundant in the skin than other parts of the fruit. Flavonoid compounds in the citrus sp family are very potent as an antioxidant and anti-aging [23, 24].

Physical stability test results

The lime peel extract cream was tested for stability for 28 d and at three different temperatures. Evaluation of the stability of the cream preparation was carried out on days 0, 7, 14, 21, and 28. The parameters evaluated were organoleptic (odor, color, and consistency), homogeneity, pH, viscosity, and spreadability.

The results of organoleptic observations of cream preparations for each formula had distinctive organoleptic properties. For 28 d, the cream was stored at storage temperatures (4±2 °C), (25±2 °C), and (40±2 °C). At 28 d of storage at low temperature, room temperature, and hot temperature, there was a change at hot temperatures (40±2 °C), namely on storage days 7, 14, 21, and 28, the consistency of the cream melts. Still, for the color and odor, no change occurs. At low-temperature storage (4±2 °C), the consistency of the cream hardens, and at room temperature storage, the cream consistency was stable.

The homogeneity test of the cream was carried out to observe the changes in the homogeneity that occurred during the 28-day storage period. Based on visual observations using a microscope, the results were homogeneous. The visualization of the cream appliance to the slide only and seen with the help of light, the cream was homogeneous for each particle. In the parameters of color, the result of cream mixing was also very good. Furthermore, testing using showed that the cream was smeared between the object-glass and the cover glass. It appeared in table 5, that the cream was placed at low temperature (4±2 °C), room temperature (25±2 °C) and high temperature (40±2 °C) was homogeneous.

Table 5: Homogeneity of cream on storage days 7, 14, 21, and 28

Temperature	Formula	Storage time day-				
		0	7	14	21	28
Temperature (4±2 °C)	A	NA	H	H	H	H
	B	NA	H	H	H	H
	C	NA	H	H	H	H
Temperature (25±2 °C)	A	H	H	H	H	H
	B	H	H	H	H	H
	C	H	H	H	H	H
Temperature (40±2 °C)	A	NA	H	H	H	H
	B	NA	H	H	H	H
	C	NA	H	H	H	H

Note: NA= Not applicable; H=Homogeneous, The pH examination (table 6) was carried out to observe the acidity value of the cream made based on the requirements. The standard in determining pH is 4.5-6.5.

Table 6: pH of cream on storage days 7, 14, 21, and 28

Temperature	Formula	The pH value cream day-				
		0	7	14	21	28
Temperature (4±2 °C)	A	NA	4.82	4.77	4.77	4.76
	B	NA	4.65	4.66	4.64	4.65
	C	NA	4.68	4.70	4.69	4.70
Temperature (25±2 °C)	A	4.79	4.80	4.82	4.82	4.81
	B	4.61	4.62	4.63	4.62	4.63
	C	4.62	4.64	4.65	4.66	4.66
Temperature (40±2 °C)	A	NA	4.83	4.82	4.82	4.80
	B	NA	4.66	4.65	4.64	4.64
	C	NA	4.63	4.62	4.63	4.64

Viscosity is a measure of the resistance of a liquid to flow. Viscosity testing was using a Brookfield Viscometer with spindle no. 64. The more significant the viscosity, the harder the liquid to flow. Table 7 shows the viscosity of lime peel extract cream for 28 d.

Table 7: The viscosity of cream on storage days 7, 14, 21, and 28

Temperature	Formula	Viscocity value day-(Cp)				
		0	7	14	21	28
Temperature (4±2 °C)	A	NA	21344	21462	22378	23814
	B	NA	20473	21982	22729	24210
	C	NA	22749	22731	23091	23552
Temperature (25±2 °C)	A	19860	20164	20261	20571	20923
	B	19050	19553	19739	19842	19996
	C	20730	20936	21146	21384	21412
Temperature (40±2 °C)	A	NA	18720	17846	17329	16739
	B	NA	17937	17562	16963	16241
	C	NA	19349	18743	17529	16272

The spreadability test was presented in table 8, It was carried out to measure the ability to spread the cream on the skin. The wider the spread area produced by a cream, the better it can spread when applied to the skin.

Table 8: Spreadability of cream on storage days 7, 14, 21, and 28

Temperature	Formula	Spreadability day-(cm)				
		0	7	14	21	28
Temperature (4±2 °C)	A	NA	4.9	4.9	4.8	4.8
	B	NA	5.0	4.9	4.8	4.8
	C	NA	5.3	5.2	5.0	4.9
Temperature (25±2 °C)	A	5,0	5.0	5.0	5.2	5.2
	B	5,0	5.0	5.1	5.1	5.1
	C	5,4	5.4	5.3	5.2	5.2
Temperature (40±2 °C)	A	NA	5.1	5.2	5.3	5.4
	B	NA	5.1	5.2	5.4	5.5
	C	NA	5.5	5.5	5,6	5.6

From the evaluation of the stability test parameters in Tables 5, 6, 7, and 8, there are no significant differences in all parameters at room temperature, cold temperature, and hot temperature after 28 d of observation. Thus, changes in the consistency of cream preparations occur at hot and cold temperatures but do not affect the ability to spread cream preparations. A good formulation must have a consistency that is not too dense. Therefore, it requires little pressure to apply the preparation on the skin surface [25, 26].

Total phenolic content test results

Testing of total phenol content was carried out using the Folin-Ciocalteu method, where gallic acid was used as a standard. Using spectrophotometry was used to obtain a calibration curve equation to plot the absorption of extracts and cream preparations. The total phenolic content test began with determining the maximum wavelength by a standard solution of gallic acid at a concentration of 500 ppm, which resulted in an absorption peak at a wavelength of 754 nm. The used maximum wavelength is subsequently to determine the total content of phenolic compounds.

The results of measuring the absorbance of a standard amount of gallic acid with a concentration series of 300-700 ppm at 754 nm obtained the regression equation $y = 0.0015x+0.116$; $R_2 = 0.994$. The total levels of phenolic compounds in lime peel extract, suncream base, and lime peel extract cream (Formula A, Formula B, and Formula C) were determined by plotting the sample absorbance on the linear regression equation of the gallic acid standard calibration curve. The total phenolic value in lime peel extract showed the number 137.57 mg GAE/gram sample. The suncream base did not have a total phenolic value because the value was-0.15 mgGAE/gram sample. The value of the phenolic compound content produced from the three formulas was also linear with the active substance concentration. The higher the active substance concentration, the higher the value of the phenolic compound content obtained.

Table 9: Total phenolic content of extract and base of lime peel sunscreen cream

Sample	Total phenolic mgGAE/g sample
Extract	137.57±1.73
Base	-0.15±0.05
Formula A	23.45±1.50
Formula B	31.07±1.18
Formula C	37.48±1.22

Data presented in mean+SD, n= 3

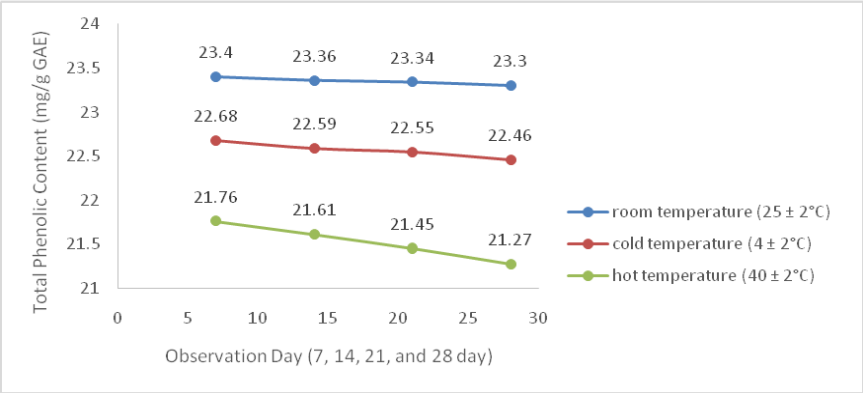

Fig. 1: Phenolic content of formula A in various temperatures and storage times

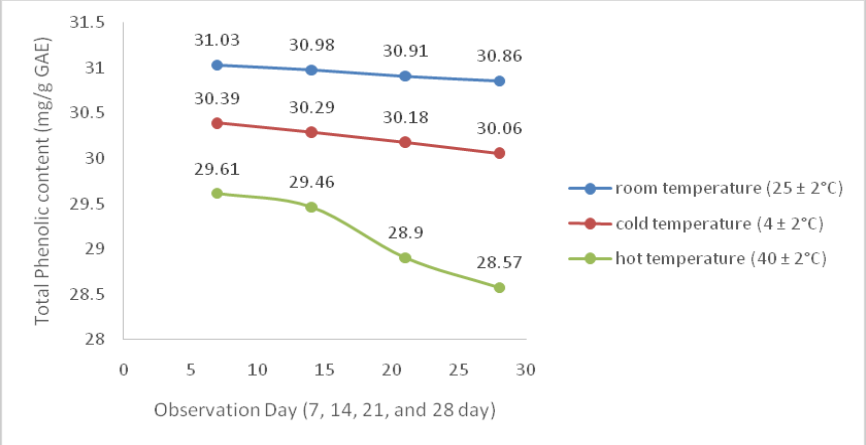

Fig. 2: Phenolic content of formula B in various temperatures and storage times

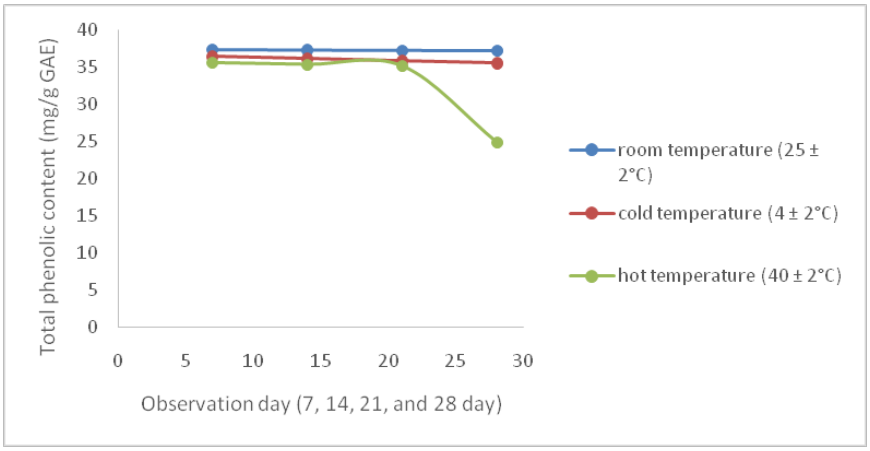

Fig. 3: Phenolic content of formula C in various temperatures and storage times

Total phenol testing was also carried out on samples at various treatment temperatures for 28 d. Fig. 1-3 show a decrease in the total phenolic content at cold and hot temperatures on each formula's 7th to 28th day. This is in line with previous studies, which showed that the total phenolic content was relatively stable at storage temperatures of 4 °C and 25 °C for 15 d of observation [27]. Another study has shown that polyphenolic compounds can be significantly degraded at temperatures above 50 °C. Total phenolic content could decrease significantly at 55 °C and 75 °C due to an increase in the oxidation reaction of phenol compounds. The oxidation reaction rate of polyphenolic compounds at a temperature of 55 °C was eight times higher. Meanwhile, at a temperature of 75 °C, it was 16 times higher than at a cold temperature (2 °C) [28]. Differences in total phenol levels were also influenced by variety, sampling time, sampling location, and plant age [13]. The extraction method and solvent also affect the total phenolic content. The Sokhlet method with methanol as the solvent can produce higher total phenol levels than other methods because, at hot temperatures, phenol compounds attracted more solvent during extraction [6].

SPF test results

The results of the SPF values were shown in Tables 10 and 11. It shown the SPF of the extract, cream base, and formulas A, B, and C.

Table 10: SPF value of ethanolic lime extract

SPF value	200 ppm	300 ppm	400 ppm	500 ppm
SPF Value	14.39±0.33	17.59±0,79	21.86±0,60	24.41±0,97

Data presented in mean+SD, n=3

Table 11: Results of the SPF value of lime peel cream

SPF value			
Formula	**2000 ppm**	**4000 ppm**	**6000 ppm**
Base	0.43±0,20	0.75±0,07	0.95±0,08
A	6.64±0,69	13.44±0,43	19.88±0,30
B	11.17±0,75	22.69±0,60	29.94±0,66
C	18.57±0,45	29.20±1,21	36.08±0,82

Data presented in mean+SD, n=3

The tests were carried out at levels of 2000 ppm, 4000 ppm, and 6000 ppm. The level calculation is based on the weight of the cream tested. Therefore, at levels of 2000 ppm, it is equivalent to 100 ppm in Formula A, 200 ppm in formula B, and 300 ppm in Formula C. Levels of 4000 ppm are equal to 200 ppm in Formula A, 400 ppm in formula B, and 600 ppm for formula C. Meanwhile, levels of 6000 ppm are equivalent to 300 ppm for formula A, 600 ppm for formula B, and 900 ppm for formula C. Based on the test results, levels of 300 ppm for extracts and cream preparations had SPF values above 15. Therefore, the SPF value indicates that the extract and the three formulas have sunscreen protection in the medium category. This is in line with the total phenolic content, where Formula C has the highest total phenolic content and shows the highest SPF value compared to other formulas [26]. This difference in SPF value could be caused by various factors, one of which is the variation in the concentration of the active substance in the formula [29, 30].

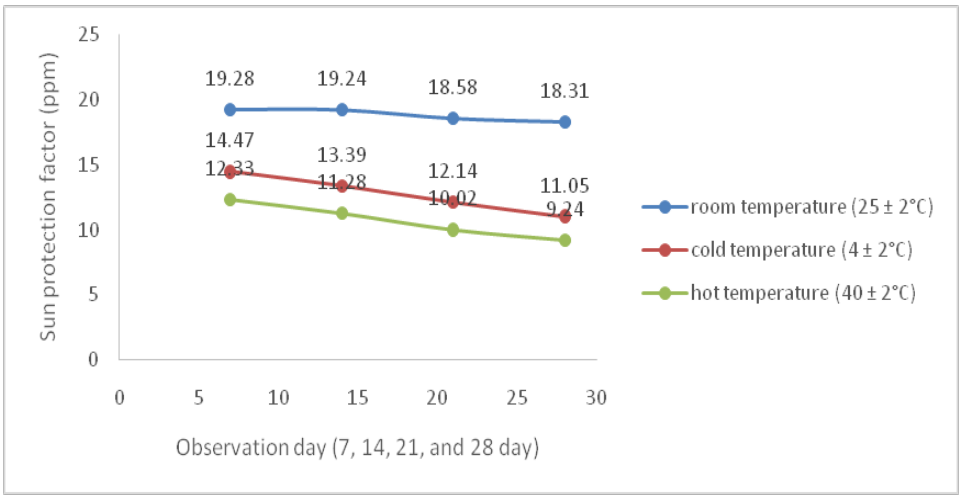

Fig. 4: The SPF value of formula a content of 6000 ppm

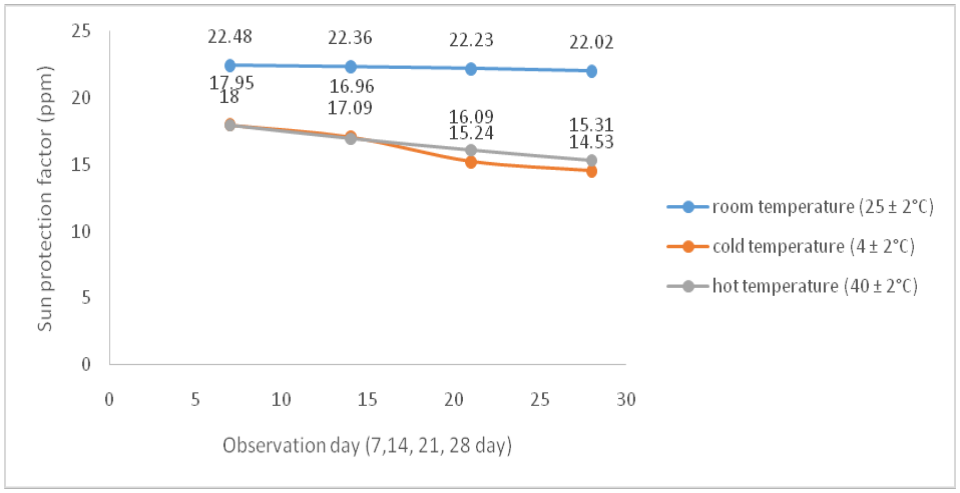

Fig. 5: The SPF value of formula B content of 4000 ppm

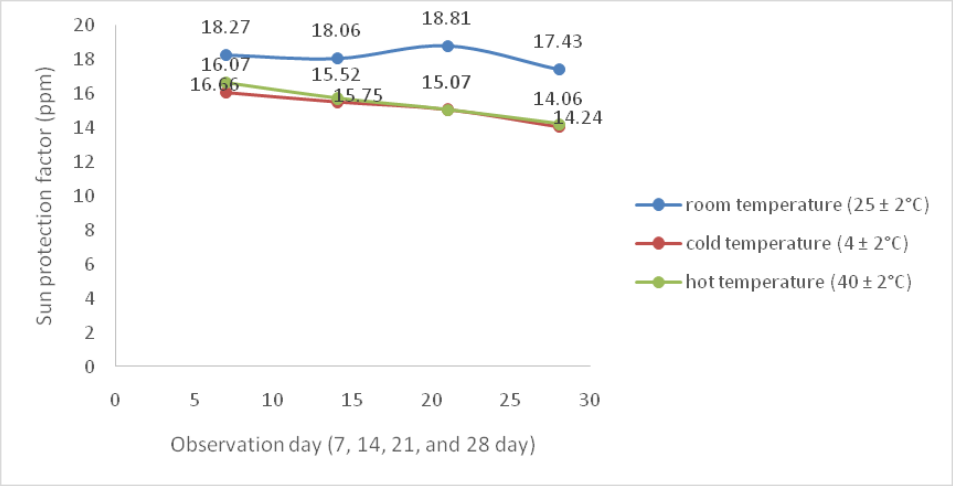

Fig. 6: The SPF value of formula C content of 2000 ppm

Based on the test results in fig. 4-6, storage for 28 d at room temperature showed that the SPF value of the cream was relatively stable. Meanwhile, at cold temperatures and hot temperatures, there was a decrease in the SPF value. Thus, the storing time of the cream affects the SPF value at cold and hot temperatures because the longer the storage, the lower the SPF value.

Primary irritation test results

Irritation is an inflammatory symptom that occurs on the skin or mucous membranes immediately after prolonged or repeated treatment using chemicals or other substances. Irritation test is carried out on cosmetic preparations before being sold to the public to prevent side effects on the skin. The irritation test was carried out *in vivo* on guinea pigs. Observations for irritation tests were performed at 0, 24, 48, and 72 h after the test preparation by observing the skin reactions that occurred with two observation parameters: the level of erythema (redness reaction) and the level of edema (swelling reaction) that occurred. Then, the results of these observations are given a score of 0 to 4 according to the severity. The calculated irritation index in table 12 was from the erythema and edema index with a score of 0-8 degrees of irritation.

Table 12: Irritation test results (n = 4)

Group	24 h		48 h		72 h	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
Base 1	0	0	3	3	3	3
Normal 1	0	0	0	0	0	0
Extract 1	0	0	0	0	0	0
Base 2	0	0	0	0	0	0
Normal 2	0	0	0	0	0	0
Extract 2	0	0	0	0	0	0
Base 3	0	0	2	2	2	2
Normal 3	0	0	0	0	0	0
Extract 3	0	0	2	2	2	2
Base 4	0	0	0	0	0	0
Normal 4	0	0	0	0	0	0
Extract 4	0	0	0	0	0	0

In this study, there was an incidence of erythema in the base and test extract groups with an erythema index of 3 (moderate to severe erythema) and 2 (clearly circumscribed erythema). In the base group, 50% of the incidence of edema also appeared, which are edema with a score of 3 (moderate edema (upper edge±1 mm)) and edema with a score of 2 (well-defined edge edema) at 48 h to 72 h. There was a 25% incidence of edema in the test extract group with a score of 2 (demarcated peripheral edema) at 48 h to 72 h. The presence of edema in the test extract group was possible because the base used could induce edema. Thus, the degree of irritation for the base group was 2 (moderate irritation), and for the extract, the group was 1 (slight irritation). In another study, irritation tests for Citrus sp., lemon oil, orange oil, and mandarin peel oil caused irritation reactions in test animals. However, after being tested on human subjects, no irritation appeared after the administration of lemon oil (concentration up to 20%) or mandarin peel oil (8%) [31]. There is no other research data that shows the level of irritation of the skin of lime fruit in test animals and human subjects.

CONCLUSION

The sunscreen cream of lime peel extract formula A (5%), formula B (10%), formula C (15%) has physical instability that occurs at high temperature and cold temperature storage on the consistency parameter of the preparation. However, the physical evaluation of the preparation on all parameters still meets the requirements. The testing results of the lime peel extract antioxidant activity with an IC_{50} value of 45.87 ppm were included in the category of very strong antioxidants. The most significant total phenol value of sunscreen cream with lime peel extract was formula C at 37.48 mg GAE/g sample, formula B at 31.07 mg GAE/g sample, and formula A at formula A 23.45 mg GAE/g sample. The SPF values of extracts and cream preparations had SPF values above 15, indicating that the extracts and the three formulas had sunscreen protection activity in the ultra-category. However, the total phenol and SPF values tended to decrease during storage at cold and hot temperatures, although not significantly. The results of the irritation test on rabbits showed

moderate irritation in the base group and slight irritation in the extract group.

ACKNOWLEDGMENT

The authors are very grateful to the Politeknik Kesehatan Kemenkes Jakarta II, Indonesia, for funding this research. We also thank all the participants who contributed to this study.

FUNDING

This work was funded from DIPA Politeknik Kesehatan Kemenkes Jakarta II, Indonesia.

AUTHORS CONTRIBUTIONS

Wardiyah, W. contributed to concept and study design; acquisition, analysis, and interpretation; drafted manuscript; critically revised manuscript and gave final approval. Safrina, U. contributed to analysis and interpretation and critically revised manuscript. Cartika, H. contributed to the concept and study design; acquisition, analysis, and interpretation; drafted manuscript; and critically revised manuscript. All authors gave final approval and agreed to be accountable for all aspects of work, ensuring integrity and accuracy.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Lin TY, Wu PY, Hou CW, Chien TY, Chang QX, Wen KC, Lin CY, Chiang HM. Protective effects of sesamin against UVB-induced skin inflammation and photodamage *in vitro* and *in vivo*. Biomolecules. 2019;9:(479):1-23. doi: 10.3390/biom9090479, PMID 31547364.
2. Schalka S, Reis VM. Sun protection factor: meaning and controversies. Anais Brasileiros de Dermatologia. 2011;86(3):507-15. doi: 10.1590/s0365-05962011000300013, PMID 21738968.
3. Apraj V, Thakur N, Bhagwat A, Mallya R, Sawant LP, Pandita N. Pharmacognostic and phytochemical evaluation of citrus aurantifolia (Christm) swingle PEEL. Pharmacognosy Journal. 2011;3(26):70-6. doi: 10.5530/pj.2011.26.12;.
4. Al-Aamri MS, Al-Abousi NM, Al-Jabri SS, Alam T, Khan SA. Chemical composition and in-vitro antioxidant and antimicrobial activity of the essential oil of citrus aurantifolia L. leaves grown in Eastern Oman. Journal of Taibah University Medical Sciences. 2018 Feb 1;13(2):108-12. doi: 10.1016/j.jtumed.2017.12.002.
5. Singh B, Singh JP, Kaur A, Singh N. Phenolic composition, antioxidant potential and health benefits of citrus peel. Food Res Int. 2020 Jun;132:109114:109114. doi: 10.1016/ j.foodres.2020.109114.
6. Loizzo MR, Tundis R, Bonesi M, Menichini F, De Luca D, Colica C, Menichini F. Evaluation of citrus aurantifolia peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. J Sci Food Agric. 2012;92(15):2960-7. doi: 10.1002/jsfa.5708;, 922960PMID 22589172–7.
7. Lv X, Zhao S, Ning Z, Zeng H, Shu Y, Tao O, Xiao C, Lu C, Liu Y. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chem Cent J. 2015;9:68. doi: 10.1186/s13065-015-0145-9, PMID 26705419.
8. Pandey BP, Rupak T, Anil U. Total phenolic content, flavonoids content, antioxidant and antimicrobial activities of the leaves, peels and fruits of locally available citrus plants collected from Kavre District of Nepal. Int J Pharmacog Chinese Med. 2019;3:1-6.
9. Barros HR de M, Ferreira TAP de C, Genovese MI, Barros HR, Ferreira TA, Genovese MI. Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. Food Chemistry. 2012;134(4):1892-8. doi: 10.1016/j.foodchem.2012.03.090, PMID 23442635.
10. Azman NFIN, Azlan A, Khoo HE, Razman MR. Antioxidant properties of fresh and frozen peels of citrus species. Curr Res Nutr Food Sci J. 2019;257(72):331-9. doi: 10.12944/CRNFSJ.7.2.03.
11. Fajarwati Nilam. Uji aktivitas antioksidan pada ekstrak daun jeruk nipis (Citrus aurantifolia) dengan menggunakan metode DPPH (1,1-diphenyl-2-picrylhydrazyl). Univ Islam Negeri Syarif Hidayatullah; 2013.
12. Gomis Tena J, Brown BM, Cano J, Trenor B, Yang PC, Saiz J, Clancy CE, Romero L. When Ddoes the IC50 accurately assess the blocking potency of a drug? J Chem Inf Model. 2020;2360(603):1779-90. doi: 10.1021/acs.jcim.9b01085, PMID 32105478.
13. Inan O, Ozcan MM, Aljuhaimi F. Effect of location and citrus species on total phenolic, antioxidant, and radical scavenging activities of some citrus seed and oils. J Food Process Preserv. 2018;42(3):e13555. doi: 10.1111/jfpp.13555.
14. A Agbor G, Vinson JA, Donnelly PE. Folin-ciocalteau reagent for polyphenolic assay. IJFS 2014:147-56. doi: 10.19070/2326-3350-1400028.
15. Tundis R, Loizzo MR, Bonesi M, Menichini F, Mastellone V, Colica C, Menichini F. Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of citrus aurantifolia swingle, C. aurantium L., and C. Bergamia Risso and Poit. peel essential oils. J Food Sci. 2012;77(1):40-6H40-6. doi: 10.1111/j.1750-3841.2011.02511.x.
16. Joseph A. Pharmaceutlcal dosage forms and drug delivery systems. J Pharm Sci. 1990;1(7):1044.
17. Aulton ME. Pharmaceutics: the science of dosage form design. 2nd ed. Edinburgh: Churchill Livingstone; 2002.
18. Ngoc LTN, Tran VV, Moon JY, Chae M, Park D, Lee YC. Recent trends of sunscreen cosmetic: an update review. Cosmetics. 2019;16(6):64. doi: 10.3390/cosmetics6040064.
19. Lee BM, Kacew S, Hyung Sik Kim HS. Lu's basic toxicology: fundamentals, target organs, and Rrisk Aassessment. 7th ed. CRC Press; 2017.
20. Sivanandham V. Phytochemical techniques-a review. World J Sci Res. 2015;1:80-91.
21. Farida Y, Azela W, Lestar ME, Pratami DK. The quality parameters, total flavonoids determination and antioxidant activity compound of andaliman fruit andaliman fruit (Zanthoxylum acanthopodium DC.) extract. Int J App Pharm. 2021;10:34-40. doi: 10.22159/ijap.2021.v13s2.07.
22. Dutra EA, Oliveira DAG da CDAGdC, Kedor-Hackmann ERM, Santoro MIRM. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Rev Bras Cienc Farm. 2004;40(3):381-5. doi: 10.1590/S1516-93322004000300014.
23. Li S, Wang H, Guo L, Zhao H, Ho CT. Chemistry and bioactivity of nobiletin and its metabolites. J Funct Foods. 2014;6:2-10. doi: 10.1016/j.jff.2013.12.011.
24. Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, Ahmad Nayik G. Citrus peel as a source of functional ingredient: a review. Journal of the Saudi Society of Agricultural Sciences. 2018;17(4):351-8. doi: 10.1016/j.jssas.2016.07.006.
25. Estanqueiro M, Conceicao J, Amaral MH, Santos D, Silva JB, Lobo JMS. Characterization and stability studies of emulsion systems containing pumice. Braz J Pharm Sci. 2014;50(2):361-9. doi: 10.1590/S1984-82502014000200016.
26. Ahmady A, Amini MH, Zhakfar AM, Babak G, Sediqi MN. Sun protective potential and physical stability of herbal sunscreen developed from afghan medicinal plants. TJPSTurk J Pharm Sci. 2020;17(3):285-92. doi: 10.4274/tjps.galenos.2019.15428, PMID 32636706.
27. Chang MY, Lin YY, Chang YC, Huang WY, Lin WS, Chen CY, Huang SL, Lin YS. Effects of infusion and storage on antioxidant activity and total phenolic content of black tea. Applied Sciences. 2020;10(8):2685. doi: 10.3390/app10082685.
28. Moldovan B, Popa A, David L. Effects of storage temperature on the total phenolic content of cornelian cherry (Cornus mas L.) fruits extracts. J Appl Bot Food Qual. 2016;89. PMID 208211.
29. Khang DT, Tien LTT, Men TT, Thuy NP. Potential of fermented fruit peel liquid in cosmetics as a skincare agent. Cosmetics. 2021;8(2):33. doi: 10.3390/cosmetics8020033.
30. Panchal C, Sapkal E, Paradkar L, Patil A, Padhiar J, Parekh P, Patel A, Singh S. Comparison and determination of sun protecting factor (SPF) of some citrus fruit juices. Int J Res Pharm Sci. 2015;6:31-4.
31. Burnett CL, Fiume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG, Shank RC, Slaga TJ, Snyder

PW, Gill LJ, Heldreth B. Safety assessment of citrus-derived peel oils as used in cosmetics. Int J Toxicol. 2019;38(2_Suppl):33-5933S-59S. doi: 10.1177/1091581819862504, PMID 31522650.