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RESEARCHF ARTICLE

The effect of additional bitter gourd seed oil on the effectiveness of zinc oxide sunscreen cream

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Abstract

Background: Sunscreen can protect the skin from the harmful effects of ultraviolet (UV) exposure. This study formulated sunscreen cream using a combination of zinc oxide (ZnO) and bitter gourd seed oil. **Objective:** This study was to determine the effect of adding bitter gourd seed oil in various concentrations on the characteristics of the cream, the effect of irritation on the skin, and the effectiveness of sunscreen in vitro. **Method:** The ZnO cream combined with bitter gourd seed oil was prepared in various concentrations of F0 (0%), F1 (6%), F2 (9%), and F3 (12%). After that, the physical characteristics and in vitro sunscreen activity were evaluated. **Result:** The results showed that all formulations met the requirements of organoleptic and homogeneity tests. Cream indicates the type of oil in water (O/W) with a pH of 4.5-8. Spreadability tests are in the range of 5-8 cm, while the irritation test on the skin of the rat's back showed no signs of erythema or oedema. The SPF (Sun protection factors) were 5.64, 10.3, 16.1, and 22.8, with %TE (Erythema transmission) values of 0.983, 0.764, 0.182, and 0.006, while the %TP (Pigmentation Transmission) were 0.981, 0.885, 0.211, and 0.006. LSD results in each test showed that the four formulations had significant differences (p <0.05). **Conclusion:** The analysis concluded that adding bitter gourd seed oil could increase the in vitro effectiveness of ZnO sunscreen cream preparations and did not cause irritation to the skin.

Introduction

Sunscreen is a cosmetic preparation that can inhibit ultraviolet (UV) rays from penetrating the skin. The mechanism of action of sunscreen can be divided into two when exposed to the skin, there are absorbing UV rays (chemical sunscreens) and reflecting or scattering UV rays (physical sunscreens) (Gadri *et al*., 2012).

Zinc oxide (ZnO) is a compound safe to use in physical sunscreens with a maximum concentration of 10%. Excessive use of this maximum concentration can irritate and leave a white coating on the skin. Its use in low concentrations also provides a less-than-optimal UV blocker. Zinc oxide needs to combine with other materials that can maintain the activity of zinc oxide as a UV blocker if it is used in low concentrations (Ermawati *et al.,* 2020).

Vegetable oil has been shown to have acted as anti-UV radiation and can reduce skin irritation. Bitter gourd seed oil is a vegetable oil that contains flavonoids, tannins, polyphenols, and phytosterols, which have the potential as antioxidants (Montenegro & Santagati, 2019). Bitter gourd seed oil has the same composition as the oil on the skin's surface. Based on its fatty acid profile, it also contains alpha-oleo stearic acid with more than one conjugated bond in its structure so that it can produce a reasonably high SPF value and has the potential to be used as anti-UV radiation. Good UV anti-radiation effectiveness will result in a high sun protection factor (SPF) value. In contrast, the erythema transmission percentage (%TE) and pigmentation transmission percentage (%TP) values are low (Ranjithkumar *et al.,* 2016).

The cream base is chosen because it relates to zinc oxide, which is insoluble in water. An oil phase in the cream formulation can help dissolve the zinc oxide so it can be mixed homogeneously in the cream preparation (Juwita *et al*., 2013). Based on this background, research will determine the potential of bitter gourd seed oil combined with zinc oxide as a physical sunscreen in cream-based preparations on the effectiveness of in vitro sunscreen.

Methods

Materials

Bitter gourd seed oil was obtained from Ethereal Ingredients Private Limited. Zinc oxide is Pharma Grade; stearic acid cetyl alcohol, glyceryl monostearate, petrolatum, triethanolamine, glycerine, and BHT (butylated hydroxytoluene) are Cosmetic Grade. Methyl paraben and propyl paraben from UENO Fine Chemicals Industry Ltd Japan. The female Wistar rats aged 2-3 months, 200-250 grams from the Biomedical laboratory of the Faculty of Pharmacy, Jember University.

Procedure

Formulation

The research formula used four sunscreen cream formulations with varying concentrations of bitter gourd seed oil there are F0 (without bitter gourd seed oil), F1 (3%), F2 (6%), and F3 (9%). Each cream formula contains the active ingredient of zinc oxide 5%.

Sunscreen preparation

The oil phase consisting of bitter gourd seed oil, zinc oxide, stearic acid, cetyl alcohol, glyceryl monostearate, petrolatum, BHT, and propylparaben melts in a water bath at 70°C – 80°C. The water phase consists of glycerine, triethanolamine, methylparaben, and distilled water heated in a water bath at the same temperature. The melted and mixed oil phase mixes into the preheated mortar. The water phase was gradually added while stirring for 3 minutes until forming a creamy mass. The zinc oxide used previously crushes and sieves using a mesh sieve no. 100 (Chandra, 2015).

Evaluation

Organoleptic test

The test was carried out by visually observing the cream preparation's texture, aroma, and colour.

Homogeneity test

This test is carried out by applying 0.1 grams of the cream preparation on a glass object and then covering it with another. A good cream preparation should show a homogeneous composition and no visible coarse grains.

Spreadability test

This test was carried out by weighing 0.5 grams of cream, then placing it in the middle of the spreadability tester plate, covering it with another plate, adding a load, and leaving it for 1 minute. The load is added every 1 minute at 5-gram intervals, and the distribution is recorded until a constant diameter. A good cream preparation has a spreadability value ranging from 5–8 cm (Meliala *et al.*, 2020).

pH test

The pH test is carried out by measuring the pH of the cream using a digital pH meter (Meliala *et al.,* 2020).

Cream type test

This test was carried out by dripping the cream with methylene blue and covering it with another object glass, after which it was observed under a microscope (Putri *et al*., 2019).

Irritation test

The test was conducted according to the ethical clearance (EC) No.1266/UN25.8/KEPK/DL/2021. This test used the Draize skin test method on four female wistar rats. The shaved backs of rats were 2x2 cm in size, and then 0.2 grams of cream was applied to each rat and left for one day. The duration of the observations was 24 and 48 hours after testing. (Savitri *et al.,* 2019).

Draize (1944) categorises skin irritation index responses characterised by oedema and erythema reactions starting from a value of 0 (no reaction), 1 (minor reaction), 2 (clearly demarcated reaction), 3 (moderate to severe reaction), 4 (severe reaction). The primary skin irritation index value is calculated to determine the irritation index response, which consists of 4 categories, namely non-irritating (0.0-0.4), mild irritation (0.5-1.9), moderate irritation (2.0-4.9), and severe irritation (5.0-8.0).

In vitro test

SPF test

The absorption spectrum of the sample was measured using a UV spectrophotometer at a wavelength of 290-400 nm with isopropyl alcohol as a blank. Absorbance values were recorded every 5nm interval. The area under the curve (AUC) is calculated at a wavelength of 290-400 nm (Fonseca & Rafaela, 2013). The AUC value at each wavelength is added up to obtain the total AUC value and proceed with calculating the SPF value using subtraction (Petro, 1981):

$$\%TP = \frac{\Sigma(T.Fp)}{\Sigma Fp}$$

Where AUC: the area under the absorption curve; Ap: absorbance at λ p nm; Ap-a: absorbance at λp-a n; λp-λp-a: difference λ of p dan p-a.

Erythema transmission value test (%TE)

A cream solution was prepared from 0.5 grams of cream dissolved in 25 ml of isopropyl alcohol, then 5ml of the solution was pipetted and added with 10ml of isopropyl alcohol. Wavelengths were observed in 292.5-337.5 nm with an observation range of 5nm. TE percent can be calculated by the equation (Abdassah *et al.,* 2015; Cumpelik, 1972):

$$\%TE = \frac{\Sigma(T.Fe)}{\Sigma Fe}$$

Where T: %TE; Fe: erythema flux setting; ΣFe: the total amount of solar erythema flux; ΣT. Fe: the amount of erythema flux.

Pigmentation transmission test (%TP)

A cream solution was prepared from 0.5 grams of cream dissolved in 25 ml of isopropyl alcohol, then 5 ml of the solution was pipetted and added with 10 ml of isopropyl alcohol. Wavelengths were observed in the 332.5nm – 372.5nm range with an observation range of 5 nm. TP percent value can be calculated by the equation (Abdassah *et al.,* 2015; Cumpelik, 1972):

$$\%TP = \frac{\Sigma(T.Fp)}{\Sigma Fp}$$

Where T: %TP; Fp: pigmentation flux setting; ΣFp: the total amount of sunlight pigmentation flux; ΣT. Fp: the amount of pigmentation flux.

The %TE and %TP for sunscreen cream preparations are grouped into four categories, as shown in Table I.

Table I: %TE and %TP value categories

%TE	%TP	Categories
<1	<1 – 2	Total block
1 – 6	3 – 40	Ultra protection
6 – 12	45 – 86	Suntan
12 – 18		Fast tanning

Data analysis

The statistical analysis used in this study was a statistical test way system analysis (One Way ANOVA) with a 95% confidence level. The results of the ANOVA test, if a sig.<0.05, can be continued with the LSD test.

Results

The cream formulation

Sunscreen cream containing bitter gourd seed oil makes in four formulations (Table II).

Table II: Sunscreen cream preparation formula

Ingredients	Concentration (%)			
	F0	F1	F2	F3
Bitter gourd seed oil	-	**6**	**9**	**12**
Zinc oxide	5	5	5	5
Stearic acid	10	10	10	10
Cetyl alcohol	1	1	1	1
Gliceryl monostearate	4	4	4	4
Petrolatum	5	5	5	5
Propylparaben	0.04	0.04	0.04	0.04
Glycerin	10	10	10	10
TEA	2	2	2	2
BHT	1	1	1	1
Metylparaben	0.36	0.36	0.36	0.36

The process for making this sunscreen cream was divided into blank sunscreens (without the addition of bitter melon seed oil) and sunscreens with varying levels of bitter gourd seed oil to compare both physical and in vitro effectiveness as a sunscreen.

Evaluation of cream

Organoleptic test

The F0, F1, F2, and F3 creams are white cream and soft textured. There was a slight colour difference between the formulation, although there were differences in the composition of the bitter gourd seed oil concentration. The F1, F2, and F3 had a distinctive aroma of bitter gourd seed oil, while the smell of cream F0 has a distinctive odour of zinc oxide because this formulation does not contain bitter gourd seed oil.

Homogeneity test

The cream formula contained insoluble zinc oxide, so the texture was not rough and homogeneous; There was a need for the fornulation to be sieved with mesh number 100 before being mixed. All formulas (F0, F1, F2, and F3) showed no coarse grains and phase separation, so the cream preparations were homogeneous.

Spreadability test

The results of the spreadability test for each formula were 5.7 ± 0.15, 6.4 ± 0.20, 7.1 ± 0.25, and 7.8 ± 0.20 cm. The results of the spreadability test for each formulation met the expected diameter. The spreadability value is increased by adding bitter gourd seed oil concentration.

pH test

The pH test results for each formula were 7.73 ± 0.04; 7.67 ± 0.01; 7.52 ± 0.02; 7.42 ± 0.02. The pH test results showed that the addition of bitter gourd seed oil concentration affected the pH value of the resulting zinc oxide cream. The results of the LSD analysis showed that each formula was significantly different, as indicated by the value of $p<0.05$.

Cream type test

Based on the addition of methylene blue, the cream has the type of oil in water (O/W).

Irritation test

The formulation did not cause any erythema and oedema reactions on the skin of the test animals. The skin irritation index value is 0, which indicates that the cream does not irritate the skin.

In vitro test

SPF test

The bitter gourd seed oil in this study was combined with zinc oxide to produce a synergistic effect as a sunscreen and to reduce the disadvantages of zinc oxide if used alone. The SPF value as shown in Table III. The addition of bitter gourd seed oils significantly increased the SPF value of the zinc oxide cream due to the presence of alpha-oleo stearic acid in bitter gourd seed oil, which has acted as anti-UV radiation (Arifin *et al*., 2020).

Erythema transmission value test

All formulas can provide total erythema sunblock protection from UV rays (%TE <1%). The F3 formula with the highest concentration of bitter gourd seed oil had the lowest percentage of TE value, therefore its effectiveness in protecting the skin from erythema was the best.

Pigmentation transmission test

The percentage of pigmentation transmission is shown in Table III. All formulas are included in the total block category (%TP <40%).

Table III: In-vitro effectiveness of zinc oxide sunscreen cream combined with bitter gourd seed oil

Formula	SPF value	Spf category	% TE	% TP	Category TE and TP
F0	5.64 ± 0.14	Extra	0.983 ± 0.009	0.981 ± 0.157	Total block
F1	10.37 ± 0.64	Maximal	0.764 ± 0.008	0.885 ± 0.087	Total block
F2	16.10 ± 1.66	Ultra	0.182 ± 0.014	0.211 ± 0.120	Total block
F3	22.80 ± 0.86	Ultra	0.006 ± 0.0007	0.006 ± 0.0007	Total block

Formula F3, with the highest concentration of bitter gourd seed oil, has the lowest percent TP value and is the most effective sunscreen, so adding bitter gourd seed oil significantly gives complete protection against skin darkening.

Discussion

Bitter gourd seed oil gives a distinctive odour in the Zinc oxide cream due to increased concentration. A Cream is a dispersion system consisting of oil and aqueous phases. Phase separation often occurs when there is a difference in density between the ingredients of the cream (Jumaa et al., 2002). The cream formula contains insoluble zinc oxide, but the texture is not rough, it is homogeneous due to sieved with mesh number 100 before being mixed. All formulas (F0, F1, F2, and F3) showed no coarse grains and phase separation, so the cream preparations were homogeneous. Bitter gourd seed oil significantly increases the zinc oxide cream's spreadability value. It reduces the cream's viscosity due to the oil consistency, making it thicker as the oil concentration increases. The addition of bitter gourd seed oil significantly lowers the pH of the resulting zinc oxide cream because the pH of the bitter gourd seed oil tends to be acidic (pH=4.0), so along with the addition of the concentration of bitter gourd seed oil causes the pH to decrease. Bitter gourd seed oil is a vegetable oil with high nutrition that keeps the skin moist and prevents skin irritation. These results indicate that all cream formulations are safe because they do not irritate. The addition of bitter gourd seed oil could increase the SPF value and reduce the percentage of erythema and pigmentation; this is due to Alpha-oleo stearic with more than one conjugated bond, which could act as a chemical absorber by absorbing the emitted UV radiation (Arifin et al., 2020).

The ideal sunscreen agent is safe, non-irritating, non-toxic, photostable, and capable of providing complete protection to the skin against sun damage. It also needs to be formulated to be a cosmetically acceptable preparation that effectively blocks UVB and UVA rays, remains on the top layers of the skin even after sweating and swimming (Giacomoni et al., 2010), and has an SPF of 30 or more (Medeiros & Lim, 2010).

The cream formulation has the highest SPF value below 30; this SPF value can still be increased by increasing the concentration of bitter gourd seed oil. However, if the concentration of bitter gourd seed oil increased beyond the levels used in this study, it would cause the cream to break because the oil could not blend homogeneously. Therefore, further exploration needed to produce a stable cream formula using levels above the highest levels used in this study to optimize the SPF value.

Conclusion

Adding bitter gourd seed oil in various concentrations to the zinc oxide cream preparation increased the spreadability but decreased the preparation's pH ($p < 0.05$). The formulas did not cause an irritating reaction on animals and could produce sunscreen cream that met the requirements. The addition of bitter gourd seed oil in the zinc oxide cream can increase the effectiveness of sunscreen cream, which is indicated by the higher SPF and lower %TP and %TE values.

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Conflict of interest

The authors certify that there are no actual or potential conflicts of interest to declare regarding the subject matter discussed in this manuscript.

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