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IN VITRO AND IN VIVO FORMULATION AND EVALUATION OF ANTI-AGING CREAM WITH PROPOLIS EXTRACT

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Propolis, Anti_aging Cream.

Abstract

Propolis contains flavonoids with antioxidant activity that can prevent the impact of UV exposure. Propolis has antioxidant activity, so its components can be used as an anti-aging (AA) cream cosmetic formulation. This development can be used as innovations in propolis products. AA cream is usually made of fatty acids and their derivatives. This study aims to formulate an AA cream preparation with propolis extract and its concentration variations to obtain a good physico-chemical formulation. The AA cream preparations with propolis extract use variations in concentration with a difference of 5%, 10%, and 15%. The physical and chemical evaluations included organoleptic tests, homogeneity, spreadability, adhesion, emulsion type, viscosity, and pH tests. The antioxidant value used the DPPH method. The statistical test used SPSS One-Way ANOVA, followed by a dependent test with a confidence level of 95%. As a result, the selected formula was Formula-1 with a 5% propolis concentration. The potential IC_{50} of the three formulas based on the antioxidant test before the cycling test were: F1 = 5.16 ppm, F2 = 71.47 ppm, F3 = 159.93 ppm; while after the cycling test were: F1 = 29.55 ppm, F2 = 117.9 ppm, and F3 = 228.61 ppm.

Introduction

The sun is an important source of energy in human life. Sunlight can nourish the skin and bones. However, excessive exposure to sunlight will have a negative impact, such as causing the skin to become rough and scaly (Artiningrum and Harvianto, 2019). Aging results in human physical changes in posture, energy, skin, and others. The anatomy of the human body is different from one another. Aging can occur in humans, where the effects can be seen in the skin structure that is inelastic and wrinkled, and the skin becomes looser and tends to dry out. Antioxidants can be used to prevent aging caused by free radicals. A cosmetic formula created to prevent or treat aging in optimal comfort. Cosmetic preparations include gel, cream, powder, or ointment (Maya and Mutakin, 2018). Anti-aging creams are products usually made with fatty acids and their derivatives. The anti-aging activity in the cream can be increased by adding other active ingredients. In this study, the active ingredient used was propolis. Propolis is a substance produced by bees, consisting of a mixture of bee saliva and plant exudates collected by bees. Propolis is believed to be one of the relatively safe natural ingredients with many benefits. The benefits of propolis depend on its chemical content, which consists of flavonoids, terpenoids, and polyphenols (Amanda et al., 2019). Propolis has antioxidant activity, so its components can be used as anti-aging cosmetic formulations. These developments can be used as innovations in propolis products (Sahlan et al., 2017). Based on the background of the potential and benefits of propolis as a cosmetic product, a study was conducted to determine its effect on the physical and chemical properties of a cosmetic preparation, especially anti-aging cream (AA cream). This study formulated propolis into AA cream preparations with three different concentrations: 5%, 10%, and 15%.

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Materials and methods

Instruments

Analytical balance (Fujitsu), refrigerator (SHARP), Memmert Beschikking-Loading-Model 100-800 D-91107 (Schwabach) oven, ATC pH meter, Olympus-CX43 (Japan) microscope, pH meter (Laqua), viscosimeter (Brookfield), H-C-8 centrifuge health (Lemirza), UV-Vis spectrophotometry, and hotplate (Ika Type Hs-7).

Materials

Propolis extract, Lexemul cs-20, Olive oil, Triethanolamine (TEA), Stearic acid, Glycerin, Propilenglicol, Methylparaben, Propyl paraben and Aquadest

Reseach Procedure

AA cream formula was made with three formulations with variations in Propolis concentration of 5%, 10% and 15%.

Table 1. BB Cream formula design

Nama Bahan	Komposisi (% b/v)		
	F1	F2	F3
Ekstrak propolis	5	10	15
Lexemul cs-20	15	16	15
Olive oil	0,5	0,5	0,5
Triethanolamine	3	3	3
Stearic acid	3	3	3
Glycerin	5	5	5
Propilenglikol	10	10	10
Methyl paraben	0,1	0,1	0,1
Propil paraben	0,05	0,05	0,05
Aquadest	68,35	73,35	78,35

Making of AA Cream : The oil phase (stearic acid, Cs-20 lexemul, and olive oil) was melted in a water bath at 70ºC while stirring until homogeneously mixed. The aqueous phase (propylene glycol, glycerin, triethanolamine, nipagin, nipasol, and aquadest) was heated in a water bath at 70ºC while stirring until homogeneous. The oil phase was transferred into a hot mortar after melting. The water phase was added little by little while stirring regularly until homogeneous and formed a creamy mass.

In-Vitro Antioxidant Activity Test of AA Cream with Propolis Extract

Preparation of DPPH Solution

0.004 gram of DPPH was weighed carefully, then dissolved with pro-analysis methanol up to 50 ml. Later, it was added with enough solvent to mark the limit, shaken until homogeneous, then placed in a dark bottle.

Preparation of Blank Solution and Optimization of DPPH Wavelength

2 ml of DPPH solution was pipetted into a test tube, then added 2 ml of methanol (1:1) and homogenized with a vortex. Then, the mouth of the tube was covered with aluminum foil. Then, determined the absorption spectrum using a UV-Vis spectrophotometer at 400-800 nm and determined the maximum wavelength.

Preparation of Cream Test Solution

50 mg of cream was weighed, then dissolved in 50 ml of pro-analysis methanol; this was the main solution. After that, several concentrations were determined (20, 40, 60, and 80 ppm). Some concentrations were pipetted as much as 2 mL into a test tube. DPPH solution (0.4 mM) was added in a 1:1 ratio in each test tube, then measured using a UV-Vis spectrophotometer.

Absorption Measurement

Base control, test solution, and positive control with several concentrations were incubated at room temperature for 30 minutes. Then the absorption was measured at a maximum wavelength of 516 nm using a UV-Vis spectrophotometer. Vitamin C was used as a positive control.

Inhibition Percent Determination, IC_{50} Value

The inhibition percentage shows the activity of the radical. The inhibition percentage of DPPH radicals of each concentration of sample solution can be calculated using the formula:

$$\% \text{ Inhibition} = \frac{DPPH\ Blank\ Absorbance - Sample\ Absorbance}{DPPH\ Blank\ Absorbance} \mathbf{x\ 100\%}$$

After obtaining the percentage of inhibition from each concentration, the sample concentration and the percentage of inhibition obtained were plotted at temperatures x and y, respectively, in the linear regression equation y = a ± bx. This equation is used to determine the IC_{50} value of each sample.

Physical and Chemical Stability Test for Propolis AA Cream

The AA propolis cream underwent a cycling test using the freeze-thaw cycle method. The cycling test was carried out, and then the preparations were stored at 4 ± 20°C for 24 hours, then transferred to storage for 24 hours at 40 ± 20°C. During storage, these two temperatures are considered one cycle. A cycling test was carried out for 6 cycles or 12 days (Luthfiyana et al., 2016). The stability test of the physical and chemical properties of AA cream was carried out before and after the cycling test, with the following parameters:

Organoleptic Test

The organoleptic test was carried out by observing the preparation visually, including the smell, color, and texture of the cream.

Homogeneity Test

The homogeneity test was carried out by weighing 0.1 grams of the preparation and smeared on the object glass to form a thin layer.

Adhesion Test

The adhesion test was performed by placing 0.5 grams of AA cream preparation. Another slide was placed on top of the cream, then given a load of 1 kg for 5 minutes, then another load of 80 grams. The time was recorded until the glass object was separated (Azkiya et al., 2017). A good cream requirement has a stickiness of more than 4 seconds.

Spreadability Test

The spreadability test used a petri dish. The AA cream was weighed as much as 0.5 grams, placed in a petri dish, covered with another petri dish, and allowed to stand for 1 minute. Next, measure the diameter of the cream that was formed. Repeat the test with loads of 50, 100, and 150 grams. A good spreadability requirement for cream preparations is 5-7 cm.

pH Test

The pH test was carried out by weighing the preparation as much as 1 gram, diluted with 10 ml of distilled water until the solution became homogeneous. Then the pH of the solution was measured using a pH meter and read on the monitor to show a constant pH meter (Indonesian Ministry of Health, 1985). The pH of the face that meets the requirements is in the range of 4.5 – 8.

Viscosity Test

The viscosity test was carried out with a viscometer. The cream preparation was put in a wide-mouthed container, and then spindle number 1 was installed until it was immersed according to the stated limits. The rotor was turned on until

the needle showed a stable number, then read the viscosity of the cream. The viscosity requirement itself is in the range of 2,000 - 50,000 cps. (Saryanti et al., 2019).

Cream Type Test

The cream type test was carried out by the dilution method using aqua dest then stirring. If the cream preparation is homogeneous, it is an M/- type emulsion; if the preparation is insoluble, it is an A/M-type emulsion.

In-Vivo Effectiveness Test of AA Cream

The effectiveness test was carried out using a moisture analyzer to determine the ability of the preparation to reduce water evaporation on the skin. This research selected 12 volunteers between 18 to 24 years old who were willing to use the cream once a day at night, applied it to the inner arm along ±2 cm, and agreed not to use other products. This research was conducted for ten days (Pujihastuti, 2009).

Statistical Approach Data Analysis

The data obtained from the test results were then analyzed using the IBM SPSS Statistics 21.0 Windows method. Normality and homogeneity of the data were tested using the Shapiro-Wilk test method and continued with the One-Way ANOVA test. The next test is the paired sample T-test to determine the physical and chemical stability of AA cream before and after the cycling test.

Results and discussion

Flavonoid Test of Propolis Extract

The flavonoid test aimed to determine the flavonoid content in propolis extract. This test has the principle, namely the occurrence of color changes due to the addition of a color reagent, then matched the color changes that occurred with the color standard. The flavonoid test was carried out using propolis extract added with methanol, then heated. Methanol was used to dissolve the extract, and heating was carried out to speed up the reaction. Next, a small amount of concentrated Mg and HCl were added. Concentrated HCl aims to hydrolyze flavonoids into their aglycones, while Mg metal produces complex compounds. Then, the reduction of concentrated Mg and HCl metals will form orange or red-colored complex compounds. The results of the flavonoid test can be seen in table 2:

Table 2. Propolis Extract Flavanoid Test Results.

Treatment	Result
Propolis extract + methanol	Yellow liquid
Propolis extract and methanol, heated	clear brown liquid
Plus Mg powder	clear brown liquid
Plus concentrated HCI	orange red liquid

The results of the flavonoid test showed a red-orange color, so it contained flavonoid compounds.

Organoleptic Test

An organoleptic test is an observation made visually to determine the color, shape, and odor of the preparation without the aid of tools. The results of observing the propolis AA cream preparations before and after the cycling test can be seen in table 3.

Table 3. Organoleptic Test of AA Cream Propolis

Formula	Organoleptic Test of AA Cream Propolis		
	Parameter	Before *cycling test.*	After *cycling test.*
F1	Consistency	Rather thick	Thick
	Smell	No smell	No smell
	Color	White	Yellowish white

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F2	Consistency	Rather thick	Thick
	Smell	Smell of propolis	Smell of propolis
	Color	Beige	Light brown
F3	Consistency	Rather thick	Thick
	Smell	Smell of propolis	Smell of propolis
	Color	Light brown	Light brown

From the observations, it can be seen that the three formulas have good color concentrations, where the preparation before the cycling test was white to light brown. Meanwhile, the preparation after the cycling test was yellowish-white to light brown, as seen in Figure 1.

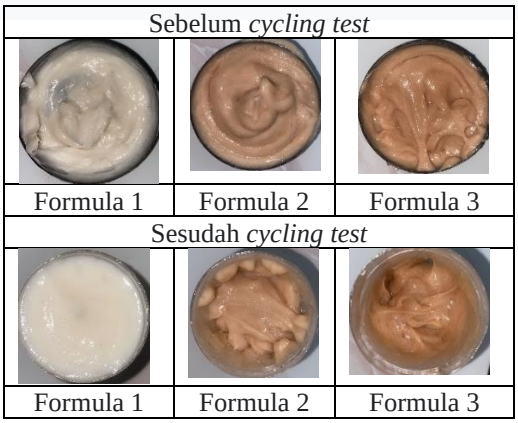

Figure 1. Observation of the color stability test of propolis anti-aging cream preparations after and before the cycling test.

The preparation changed after the cycling test was carried out. It happened because it had a different propolis concentration in each preparation amount.

Homogeneity Test

The homogeneity test aims to determine the mixture of ingredients used in making cream preparations, such as the active substance, the water phase, the oil phase that was physically observed, and the smoothness of the cream (Suciati et al., 2020). When being observed using a microscope, the cream has a texture that looks flat and does not clot. The preparation can be considered homogeneous if no coarse grains occur and no phase breakdown occurs (Azkiya et al., 2017). The application of the preparation is carried out evenly to minimize irritation to the skin. The homogeneity test results using propolis extract preparations of AA cream based on the observation results before and after the cycling test obtained homogeneous results of the AA cream preparations for all formulas. The AA cream is said to be homogeneous when the observation results meet the physical homogeneity requirements, in which the cream texture is flat and does not clot. There was no phase breakage in the preparations, and the preparation did not show any coarse granules (Azkiya et al., 2017). The results obtained showed that the variation in the propolis concentration did not affect the homogeneity of the AA cream preparation. The homogeneous preparation of AA cream indicated that the ingredients used were mixed perfectly.

Emulsion Type Test

Based on the test results, the AA cream preparation for all formulas was the M/A emulsion type because, when diluted using water, the AA cream preparation met the requirements for the M/A emulsion type, which was well dissolved

and homogeneous. Thus, it can be concluded that the variation of the propolis concentration in the AA cream preparation did not affect the type of emulsion preparation and had good emulsion type stability. It was evidenced by the production of a stable type of emulsion without any observed changes before and after the cycling test.

Adhesion Test

The data obtained from the adhesion stability test showed that the adhesion strength of the AA cream preparation increased with the propolis concentration increase, and the AA cream preparation adhesion decreased after the cycling test. Table 4 shows that all three formulas can adhere to the skin.

Table 4. Cream adhesion test results

Formula	Stickiness Test (seconds)	
	Before cycling test	After cycling test
F1	8,10 ± 1,60	7,23 ± 1,31
F2	10,15 ± 0,55	9,11 ± 0,87
F3	14,10 ± 0,42	11,65 ± 0,17

Based on the statistical analysis, the results before and after the cycling test showed a significance value of 0.010, where the value was less than 0.05 ($p<0.05$). It shows that the increase in the propolis concentration also increased the adhesion of the AA cream preparation. The AA cream adhesion increases because the greater the concentration of propolis, the thicker the consistency due to the reduced amount of distilled water added, so the viscosity increases. The adhesion value is directly proportional to the viscosity of the resulting preparation. The higher the viscosity, the longer the time the cream will stick to the skin (Azkiya et al., 2017). After the cycling test, there was a decrease in adhesion because, during the storage process, it was stored in a place with high humidity, resulting in the preparation containing a lot of water which affected the concentration of the cream.

Spreadability Test

The spreadability test aimed to determine the cream's ability to spread when applied to the skin. A good standard of spreadability is 5 to 7 cm. Spreadability can be related to viscosity; the smaller the viscosity value obtained, the greater the spreadability. The smaller the viscosity value of the cream, the easier it is to spread on the skin (Elcistia and Zulkarnain, 2018). Preparations that are easily applied to the skin will expand the area of skin that is in contact with the preparation, so that the possibility of the active substance being absorbed will be even greater.

Table 5. Cream adhesion test results

Formula	Spreadability test (cm)	
	Before cycling test	After cycling test
F1	5,00 ± 0,56	5,40 ± 0,34
F2	5,10 ± 0,58	5,90 ± 0,52
F3	5,90 ± 0,59	6,50 ± 0,74

The data obtained from the spreadability stability test showed that the AA cream preparation spreadability increased with the increase in the propolis concentration, and the AA cream preparation spreadability increased after the cycling test. It shows that the propolis affected the AA cream preparation spreadability, and this happened because the cream viscosity decreased during storage, which reduced the liquid's resistance to flow. The statistical analysis results, before and after the cycling test, showed a significance value of less than 0.05 ($p<0.05$). It shows that the increase in the propolis concentration affected the increase of the AA cream preparations' spreadability.

Viscosity Test

The viscosity stability test of cream preparations has a good viscosity value parameter, which is in the range of 2,000-50,000 cps. The higher the viscosity of the preparation, the more stable it will be; particle movement tends to be difficult due to the thicker the preparation. Preparations with too high a viscosity will be difficult to apply to the skin and pour into the container. Meanwhile, preparations with too low viscosity produce runny preparations and easily drip when applied so that they are not fully attached to the skin surface (Elcistia and Zulkarnain, 2018). Viscosity is influenced by temperature, pH, solution concentration, dissolved molecular weight, and pressure.

Table 6. Cream viscosity test results

Formula	Viscosity test (dPas)	
	Before cycling test	After cycling test
F1	49,33 ± 5,77	37,33 ± 5,77
F2	37,33 ± 5,77	35,10 ± 0,00
F3	32,12 ± 0,00	21,33 ± 5,77

Based on the statistical analysis of the results before and after the cycling test, the significance value was 0.00, less than 0.05 (p>0.05). It shows that the increase in the concentration of propolis increases the viscosity of AA cream preparations.

Viscosity increases with the increasing concentration of propolis extract because propolis extract is a thick extract, which can increase the thickness of the cream. Changes in viscosity are affected by storage, such as humidity and temperature. In addition, the viscosity can also be affected by the hygroscopic base component (Propylenglikol), which can cause the cream to absorb moisture from the outside air and increase the volume of water in the cream. The more the volume of water, the thinner the cream and the lower the viscosity.

During the storage process, the viscosity of the cream decreased. Temperature is the main factor in decreasing the viscosity value. High temperatures will increase the distance between particles, reducing the forces between particles.

pH Test

The pH test aimed to determine the acidity of the cream preparations that had been made, which were expected to conform to the pH standards used for topical preparations (Elmitra, 2017). The pH test was carried out to determine the safety level of the cream when used on the skin so as not to cause irritation. If the pH is too alkaline, it can cause the skin to become scaly, while if it is too acidic, it irritates the skin. The pH stability test for AA cream propolis preparations has a pH value parameter for topical preparations that are safe to use on the skin, namely 4.5-8.

Table 7. Cream pH test results

Formula	pH test	
	Before cycling test	After cycling test
F1	7,77 ± 0,03	7,08 ± 0,15
F2	6,83 ± 0,02	6,71 ± 0,12
F3	6,79 ±0,24	6,48 ± 0,53

Based on the test results, the variation in propolis concentration affected the pH of the preparation of AA cream with propolis extract. The data obtained from the pH stability test showed that the pH of the AA cream preparation

decreased along with the increased concentration of propolis, and the pH of the AA cream preparation decreased after the cycling test. Because propolis extract is acidic, the more propolis extract is used, the more acidic the pH of the preparation. It can be seen in the table that there was a decrease in pH after the cycling test was carried out due to the hydrolysis of acidic compounds during the storage period, resulting in a decrease in the pH of the cream.

The statistical analysis of the results before and after the cycling test showed a significance value of 0.000, where the value was less than 0.05 ($p<0.05$). It shows that the increase in the concentration of propolis affects the decrease in the pH of AA cream preparations.

Antioxidant Activity Test of Propolis Extract AA Cream

An antioxidant activity test was carried out on AA cream with propolis extract. The antioxidant activity test was carried out using the DPPH method. The DPPH method was chosen because it is simple, fast, and does not require a lot of reagents (Bahera, 2012). Antioxidant testing of AA cream with propolis extract was carried out to examine the antioxidant activity, which used vitamin C as a positive control. Based on the results of testing the activity of antioxidant cream against DPPH for each formula F1, F2, F3, negative control, and positive control, the most effective formula as an antioxidant cream with propolis extract is FI which contains 5% cream with IC_{50} value of 5.16 ppm before cycling test and 29.55 ppm after cycling test, which is a very strong antioxidant activity but not more than vitamin C. It was proven after doing the same research on DPPH using the active ingredient vitamin C as a positive control with an IC_{50} value of 4.48 ppm. Thus, it can be seen that one of the causes of the high effectiveness of the formula of antioxidant cream with propolis extract is the content of flavonoid compounds.

Table 8. IC50 Calculation Results

Formula	Before cycling test	After cycling test
Formula 1	5,16 ppm	29,55 ppm
Formula 2	71,47 ppm	117,9 ppm
Formula 3	159,93 ppm	228,61 ppm

The statistical results before and after the cycling test were based on statistical analysis showing the sig value of > 0.05. The IC_{50} test results were not significantly different before and after the cycling test.

In Vitro Effectiveness Test of AA Cream

The effectiveness test aims to determine the ability of the preparation to reduce the evaporation of water on the skin. The formulation tested was Formula 1, with the highest antioxidant effectiveness.

Table 9. *Humidity Test Results on Respondents*

Hari	Respondents (% Water)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	13,5	13,0	12,7	10,8	10,8	10,8	11,4	12,8	12,2	11,3	12,9	11,4
10	29,5	42,1	14,3	15,8	14,3	14,8	17,1	17,8	15,5	15,8	17,1	15,8

Based on the following table, the statistical test of the use of cream on the respondents, where the result is sig. >0.05. It can be seen that the use of AA cream is significantly different from the first day until the tenth day of using the AA Cream, so the anti-aging cream with propolis extract is effective in hydrating the skin.

Conclusion

Differences in the concentration of propolis extract can affect the physical and chemical properties of anti-aging cream preparations of propolis extract, either before or after the cycling test, where the higher the concentration of propolis used, the dispersion and adhesion of AA cream increases, and the viscosity and pH decrease, and F1 with a concentration of 5% has the best antioxidant activity in hydrating the skin.

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