Journal of Science and Technology Research for Pharmacy
Volume 3 Issue 2 Year 2023 Pages 28-37

E-ISSN 2776-0685
https://journal.unnes.ac.id/journals/JSTRP/index

Sunscreen Activity Test on Iler Leaf Ethanol Extract Cream (Coleus Scutellarioides (L) Benth) In Vivo

Zalzabila Adelya Putri*, Endah Widhihastuti [1]

[1]Department of Pharmacy, Universitas Negeri Semarang, Semarang, 50229, Indonesia

*Correspondence to: *zalzabila@students.unnes.ac.id*

Abstract: Indonesia is one of the countries known as a tropical climate country with a high intensity of sun exposure. The sun's UV rays have health benefits. However, UV rays also have negative effects on skin health. Therefore, additional protection against solar UV rays is needed, namely by using sunscreen preparations derived from Iler Leaves (*Coleus scutellarioides (L) Benth*) which contain flavonoid and phenolic compounds. The purpose of this study was to determine the most optimal ethanol extract cream formula of iler leaf (*Coleus scutellarioides (L) Benth*) after preparation evaluation and determine the sunscreen activity of iler leaf ethanol extract cream (*Coleus scutellarioides (L) Benth*) in vivo. This research is a real pure experiment or also called True Experimental Research and uses the posttest control group design method. The results obtained from the evaluation of the ethanol extract of iler leaf cream preparation formula are with a cream formula consisting of 1.5% iler leaf extract, 18% stearic acid, 2% TEA, 2% cetyl alcohol, 8% glycerin, 0.2% metal paraben, 0.02% propyl paraben and aquadest. And after in vivo testing on white rats, it was found that the ethanol extract cream preparation of iler leaves had sunscreen activity as evidenced by the erythema effect that appeared after exposure under sunlight. So it can be concluded that the most optimum cream formula contains 18% stearic acid and 2% TEA. And the iler leaf ethanol extract cream has sunscreen activity after in vivo testing.

Keywords: Sunscreen, in vivo, Iler leaf, Coleus scutellarioides (L) Benth

INTRODUCTION

Skin is the outermost organ that functions as a protector and has aesthetic value. It is also an important organ that serves as a barrier to the external environment. Therefore, the skin has a natural defense mechanism against the side effects of sun exposure. It is also known that Indonesia is one of the countries known as a tropical climate country with a high intensity of sun exposure or solar radiation (Theresia, 2014).

According to Jusmiati et al (2019), sunlight contains infrared and ultraviolet (UV) rays that have chemical effects. UV sunlight also has health benefits in helping the formation of vitamin D needed by bones. However, the sun's ultraviolet (UV) rays also have a negative impact on skin health, as UV rays can cause skin damage (Byren et al., 2017). Another negative impact of solar UV light is that it can cause erythema, DNA damage, premature aging, carcinogenesis and produce very high Reactive Oxygen Species (ROS). Excessive Reactive Oxygen Species (ROS) can cause DNA damage (Byren et al., 2017). Therefore, additional protection against sunlight is needed by using sunscreen preparations.

Sunscreen is a protective cosmetic that plays an important role in maintaining skin health, because most of the activities carried out daily take place outdoors and tend to be exposed to sunlight (Theresia, 2014). Available beauty products usually contain chemical sunscreen ingredients that cause side effects, such as sunburn, stinging or sensitivity (Byren et al., 2017). In contrast, sunscreen products made using natural ingredients are safer than sunscreen products made using synthetic ingredients, as sunscreen containing natural ingredients are safer and reduce the risk of skin damage than organic or chemical compounds that can irritate the skin and cause allergies. Therefore, alternative sunscreens made from natural ingredients are needed to be safer from side effects (Cefali et al., 2016).

One plant that contains phenolics and flavonoids and has the potential as a sunscreen ingredient is iler leaves or can be called miana leaves. Miana leaves or commonly referred to as iler leaves have the scientific name Coleus scutellarioides (L) Benth.

Research on sunscreen activity tests on iler leaf extract (Coleus scutellarioides (L) Benth) has previously been carried out by Amrillah et al (2015), but this research is still limited to in vitro research and the results show that the preparation of ethanol extract of iler leaves (Coleus scutellarioides (L) Benth) has good sunscreen activity.

According to Moelyono (2016) The chemical content of Iler leaves (Coleus scutellarioides L. Benth) is in the form of flavonoids, saponins, polyphenols, alkaloids, minerals and essential oil components. In the research of Amrillah et al (2015) it also explained that among the various kinds of phenolic compounds, flavonoids are probably the most important group of compounds. Besides fighting radicals induced by UV light, flavonoids may provide a protective effect against UV radiation by acting as a strong UV light absorber.

According to research by Almira et al (2019), the mechanism of flavonoids in protecting the skin from UV exposure is by absorbing UV rays that penetrate the skin. Flavonoids have a structure in the form of conjugated double bonds so that almost all flavonoids can act as chromophores. Flavonoids will absorb UV light and cause electron excitation from the ground state to higher energy orbitals.

METHODS

The tools used in this study are gloves, scissors, analytical scales, test tubes, measuring cups, beakers, Buchner funnels, Erlenmeyer, ovens, rotary evaporators, razor blades, gold razors, drop pipettes, vials, spatulas, watch glasses, glass stirrers, petri dishes, calipers, jar bottles, filter paper, aluminum foil, UV-Vis spectrophotometry, flat glass, pH paper and spreadability test kits,

The materials used in this study are ether, iler leaves, white rats (Rattus norvegicus), 50% methanol, Mg powder, concentrated HCl, ethanol, $AlCl_3$ 10%, potassium acetate, $FeCl_3$ 3%, 50% folin-ciocalteu reagent, Na_2CO_3 15%, stearic acid, TEA, cetyl alcohol, glycerin, methyl paraben, propyl paraben, distilled water, 5% acetic acid, and gallic acid.

Extraction Preparation

Preparation of Tiler Leaf Powder

Iler leaf samples are washed with running water, then sorted by observing the integrity of the shape of the leaf samples to be processed into simplicial. Then the sample is dried by drying in the oven at a temperature of 50°C until the sample is completely dry and physically when squeezed the leaf sample becomes crushed. The dried samples were then put into a blender to be pulverized.

Preparation of extracts from iler leaf powder

Extraction was carried out by maceration method using 96% ethanol solvent and 500 grams of iler leaves. The maceration process was carried out for 1x24 hours with 96% ethanol solvent totaling 5000 mL. After the maceration process was carried out for 1 day, the filtrate was separated from the residue by filtering using filter paper. The extraction process is continued by remaceration for approximately 2-3 days or repeated until the extract is colorless. The filtrate obtained was then concentrated using a Rotary Evaporator at 40°C to separate the solvent from the extract so that a thick extract was obtained. After that, the % yield was calculated.

Determination of Flavonoid Content

Flavonoid screening test

Phytochemical screening of flavonoid compounds was carried out by the amyl alcohol method, namely by means of a number of samples put into a test tube then added 0.1 mg magnesium powder and 0.4 mL of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol with the same volume) and added 4-5 drops of HCl, then the mixture was shaken. A positive reaction is indicated by the formation of an orange, yellow or red amyl alcohol layer in the amyl alcohol layer (Sugiarna et al., 2019).

Flavonoid content test

Preparation of 1000 ppm quercetin standard solution. Weighed as much as 100 mg of quercetin standard and dissolved with 96% ethanol up to 100 mL. Preparation of 100 ppm quercetin working standard solution. Standard solution was pipetted as much as 1 mL and the volume was sufficient to 10 mL with 96% ethanol to obtain a concentration of 100 ppm. Preparation of blank solution. Pipette 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid, add 96% ethanol up to 10 mL. Determination of operating time. Determination of operating time based on Suharyanto & Hayati (2021), which is carried out for 30 minutes.

Determination of maximum wavelength of quercetin

Quercetin 100 ppm working standard solution was taken as much as 1 mL added with 1ml AlCl3 10% and 8 mL 5% acetic acid. Take readings with UV-Vis spectrophotometry at a wavelength of 370-450 nm. The result of the maximum wavelength is used to measure the absorbance of the 96% ethanol extract sample of iler leaves (*Coleus scutellarioides (L) Benth*).

Preparation of quercetin standard curve

Quercetin standard concentrations of 20, 40, 60, 80, and 100 ppm were made in a 10 ml volumetric flask plus 3 ml of methanol and added 0.2 ml, then added 10% AlCl3 and 0.2 ml of 1M sodium acetate. After that added ad aquadest

until the limit mark. Then measured the absorbance based on the maximum wavelength that has been obtained and operating time.

Determination of total flavonoid content of Iler Leaves Extract (Coleus scutellarioides (L) Benth)

Weighed 100 mg of ethanol extract of iler leaves (Coleus scutellarioides (L) Benth) dissolved with 96% ethanol until the volume is 100 mL. Then pipetted 1 ml of sample and put in a 10.0 ml volumetric flask, then added 3 ml of methanol and added 0.2 ml of AlCl3 10%. After that, 0.2 ml of 1M Na Acetate was added and ad aquadest was added until the limit mark. Then the absorbance was measured using UV-vis spectrophotometric method at the maximum wavelength obtained.

Determination of Phenolic Content

Phenolic screening test. Qualitative analysis of phenolic compounds is carried out by means of a number of samples put in a test tube. Then added FeCl3 reagent solution as much as 3-5 drops, then shaken and waited for a while. Then observed the color change. Positive results are indicated by the presence of green, red, purple, blue or black colors (Sugiarna et al., 2019).

Test of phenolic content

Preparation of gallic acid standard solution. A total of 10.0 mg of gallic acid was dissolved in distilled water to a volume of 100.0 ml.

Determination of Wavelength and Operating Time

A total of 0.3 ml of gallic acid solution of 3ppm concentration plus 1 ml of Folin-Ciocalteau reagent (1:10) shake and let stand for 1 minute. In the solution added 2 ml of 15% Na2CO3 solution shake homogeneous, and then the absorbance was measured at a wavelength of 700-900nm. Determination of operating time based on Tahir et al (2017) is done for 120 minutes.

Preparation of Gallic Acid Standard Curve Series

A total of 0.3 ml of gallic acid solution with concentrations of 4, 5, 6, 7, 8, and 9 ppm was put in a 10 ml measuring flask, then added 1.5 ml of Folin (1:10) and allowed to stand for 3 minutes. Then added 1.2 ml of 7.5% Na2CO3 and added ad aquadest until the limit mark. Then measured the absorbance using UV-vis spectrophotometry at the maximum wavelength obtained and operating time.

Measurement of phenolic content

The sample solution was pipetted 0.3 ml and added 1 ml of Folin Ciocalteau reagent (1:10) then shaked. Let stand for 5 minutes added with 2 ml of 15% Na2CO3 solution and then let stand again at room temperature operating time range. The absorbance of the solution was measured with a UV-Vis spectrophotometer at the wavelength of maximum absorbance; 2-3 repetitions were made.

Cream Preparation

Optimization of Stearic Acid as Surfactant

Table 1. Formula of Iler Leaf Ethanol Extract Cream with Stearic Acid Base Combination

Ingredients	Function	F1	F2	F3
Iler Leaves Extract	Active substance	1,5	1,5	1,5
Stearic Acid	**Surfactant**	**18**	**12**	**6**
TEA	Emulgator	2	2	2
Cetyl alcohol	Emulgator, Stabilizer	2	2	2
Glycerin	Thickening agent	8	8	8
Methyl Paraben	Antimicrobial	0,2	0,2	0,2
Propyl Paraben	Antimicrobial	0,02	0,02	0,02
Aquadest	Solvent	ad 100	ad 100	ad 100

The preparation of this iler leaf extract cream is done by weighing the ingredients first. Then divide the ingredients separately with oil phase base (stearic acid, cetyl alcohol, and propyl paraben) and water phase base (triethanolamine, glycerin, methyl paraben). Both phases were heated at 70°C on a waterbath separately. After the two phases have melted and mixed completely, the water phase is gradually introduced into the still warm oil phase and stirred until the formation of a creamy mass. After that, the iler leaf extract was added and stirred again. Then added with aquadest and stir until evenly distributed and perfectly mixed (Endriyatno & Aida, 2023).

Optimization of TEA as Emulgators

Table 2. Formulas of Iler Leaf Ethanol Extract Cream with TEA Base Combination

Ingredients	Function	F1	F2	F3
Iler Leaves Extract	Active substance	1,5	1,5	1,5
Stearic Acid	**Surfactant**	**18**	**12**	**6**
TEA	Emulgator		Optimization Results	
Cetyl alcohol	Emulgator, Stabilizer	2	2	2
Glycerin	Thickening agent	8	8	8
Methyl Paraben	Antimicrobial	0,2	0,2	0,2
Propyl Paraben	Antimicrobial	0,02	0,02	0,02
Aquadest	Solvent	ad 100	ad 100	ad 100

The preparation of this iler leaf extract cream is done by weighing the ingredients first. Divide the ingredients separately with oil phase base (stearic acid, cetyl alcohol, and propyl paraben) and water phase base (triethanolamine, glycerin, methyl paraben). Both phases were heated at 70°C on a waterbath separately. After the two phases have melted and mixed completely, the water phase is gradually introduced into the still warm oil phase and stirred until the formation of a creamy mass. After that, the iler leaf extract was added and stirred again. Then added with aquadest and stir until evenly distributed and perfectly mixed (Endriyatno & Aida, 2023).

Cream Dosage Evaluation

Organoleptic. Observations made include the color and odor of the preparation that has been made. **Homogeneity.** The cream is taken sufficiently and applied to a flat layer of glass and then seen whether there are still coarse grains or not. **pH**. The universal indicator is dipped and allowed to stand for a while then adjust the color change of the universal indicator to the universal pH standard. **Spreadability.** Cream as much as 0.5 g was weighed and placed between 2 layers of glass objects that were given a load of 100 g. Measurement of the diameter of the cream that spread was carried out after approximately 1 minute. **Adhesion.** The cream was placed between two glass objects and then given a load of 1 kg for 5 minutes. The two objects were separated by pulling the glass object on top with a weight of 80 g, while the glass object below is held down with the other weight. The length of time it takes to separate the two objects is recorded as the adhesion time. **Stability.** The test was carried out for six cycles by storing the cream preparation at 4±2°C for 24 hours and then moving the cream preparation at 40±2°C for 24 hours (one cycle). After that, observations were made whether there was a phase separation of the cream preparation. **Emulsion Type.**
The emulsion type test uses the method that the cream that has been made is put into a glass and then diluted with water. If the cream can be diluted, then the emulsion type is type M/A otherwise if it cannot be diluted then the emulsion type is A/M.

In Vivo Testing

Treatment of test animals.

The test animals used were white rats (Rattus norvegicus) weighing approximately 100-150 grams and aged 2-3 months totaling 16 animals. Where divided into 4 groups, namely:

Group 1: Positive group with octyl methoxycinnamate administration
Group 2: Negative group without treatment
Group 3: Treatment group using ethanol extract cream of iler leaves

Group 4: Treatment group using ethanol extract of iler leaves

Before being treated, rats were adapted for ± 5 days by giving standard feed. Then all rats had their backs shaved with a length of ± 3-4 cm and a width of ± 2-3 cm. After that, the test animals were given the test compound in the shaved area as much as ± 1 g. Animals were allowed to contact the test compound for 1 hour to ensure the preparation could penetrate well into the animal's skin and ensure no irritation after applying the test compound. Then the test animals were exposed under direct sunlight for ± 6 hours.

Animal testing

After the test animals were treated, then the test animals were exposed under direct sunlight for ±6 hours.

Observation

Observations of erythema arising by macroscopic means with the aim of comparing the effects of erythema that occurs between the four groups and measuring the diameter of erythema that occurs using a caliper. Analysis was carried out on the anti-inflammatory properties of the compound measured by a score of 0-4 for the skin area that gave an erythema response. The erythema scores used were:

Score 0: no erythema
Score 1: very little erythema (barely visible) that is pink in color, with a diameter of ≤25 mm
Score 2: clearly visible erythema which is bright red in color, with a diameter of 25.10- 30.00 mm
Score 3: moderate to strong erythema which is dark red in color, with a diameter between 30.10 - 35.00 mm
Score 4: severe erythema forming crusts or wounds and brownish red, with a diameter ≥ 35.10

Data Analysis Technique

The data analysis techniques used in this study include normality test and homogeneity test, then continued with the One Way Anova test which serves to determine whether there is a difference in the effect of the treatment given.

RESULT AND DISCUSSION

Ethanol extract of iler leaf (Coleus scutellarioides (L) Benth)

Maceration is carried out using 96% ethanol as a solvent. The maceration process is carried out 3x24 hours with two remaceration or changes of new solvents which aim to extract the compounds contained in the sample thoroughly. Furthermore, the filtrate that has been produced is then evaporated using a rotary evaporator, so that a thick blackish green extract of 29 grams is obtained and a yield of 5.8% is also obtained. The yield is the percentage of the weight of the extract obtained with the weight of dry simplicial.

Phytochemical Screening

Phytochemical screening aims to determine the content of secondary metabolites contained in Iler leaf extract, so that compounds that have potential as natural sunscreens can be identified. According to research conducted by Aji et al (2023) iler leaves contain secondary metabolite compounds such as flavonoids, alkaloids, saponins, tannins, polyphenols and essential oils.

Flavonoids are one of the largest natural phenolic antioxidant compounds found in all plants. The presence of flavonoid compounds in iler leaves can make iler leaves have good antioxidant activity, which can ward off free radicals and can absorb UV rays (Ubaedilah & Supriyatna, 2023). The test results of flavonoid compounds on iler leaf samples showed positive results, with the addition of Mg powder and amyl alcohol and HCl resulting in the formation of a thick red amyl layer.

Phenolic compounds are compounds that have hydroxyl groups and are most abundant in plants. Phenolic compounds conjugate with benzene nuclei, causing resonance with electron transfer when exposed to ultraviolet (UV) light. Chemical compounds and compounds from phenolics have conjugation system similarities called photoprotective or can absorb UV light, both UV A and UV B (Hidayah et al., 2023). The test results of phenolic compounds on iler leaf samples in figure show positive results, with the addition of FeCl3 causing the formation of a blackish red solution.

Total Flavonoid and Total Phenolic

Total Flavonoid Content

Determination of the maximum wavelength has the aim of determining the measurement wavelength at which the complex compound between quercetin and $AlCl_3$ can provide optimum absorbance. In chemical analysis with spectrophotometric methods, determining the maximum wavelength is also an important component. In this

analysis, the wavelength used is the maximum wavelength, the maximum wavelength obtained is 436.5 nm which has an absorbance of 0.711.

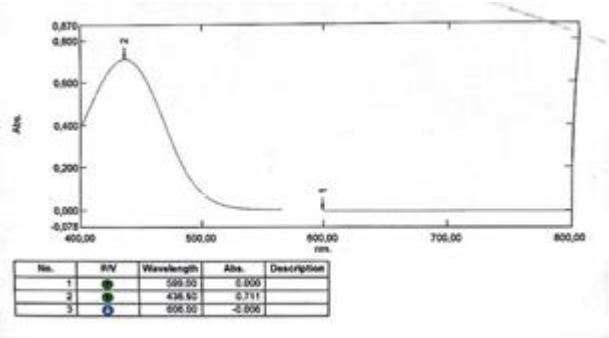

Figure 1. Wavelength Analysis of Quercetin

The determination of the standard curve aims to determine the relationship between the concentration of the solution and its absorbance value so that the sample concentration can be known. So that the determination of the standard curve obtained the equation y = 0.0072x + 0.0038. This equation is to calculate the flavonoid content in the iler leaf sample, with (y) as the absorbance value and (x) states the flavonoid content in the sample. And obtained a correlation coefficient (r) value of 0.9998. A good r value indicates that the curve between concentration and absorbance will be linear when the value is close to 1.

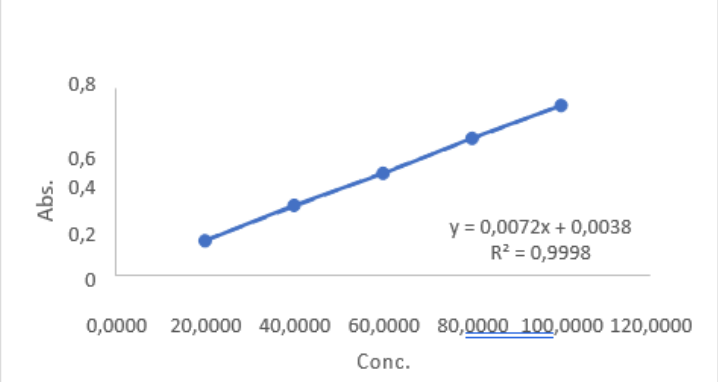

Figure 2. Standard Curve of Quercetin

The total flavonoid content in iler leaves is expressed as mg QE/100 g which is the equivalent of Quercetin in every 100 grams of sample. The average level of total flavonoids contained in iler leaves is 1.0398 mg QE/g. In previous research conducted by (Yanuarty et al., 2023) also conducted research on the determination of total flavonoid compounds from ethanol extracts of iler leaves, which obtained a value of 0.2673 mg QE/100 g. This means that the flavonoid content obtained is 0.0398 mg QE/g. This means that the flavonoid content obtained in the study is slightly different from the research conducted by (Yanuarty et al., 2023). This is caused by extrinsic factors, namely environmental factors. Environmental factors that influence are such as altitude, soil pH, light intensity, humidity, rainfall, soil texture and temperature.

Total Phenolic Content

The maximum wavelength is the wavelength that can have maximum absorbance during measurement. Based on the results of scanning the maximum wavelength of gallic acid obtained a maximum wavelength of 775 nm with an absorbance of 0.303. The calibration curve equation is the relationship between the x-axis and the y-axis. The x-axis shows the concentration obtained, while the y-axis shows the absorbance or absorbance obtained from the measurement results. Therefore, the linear regression equation of the calibration curve obtained is y = 0.0846x + 0.0646, with a correlation coefficient of r^2 =0.997. A good r value indicates that the curve between concentration and absorbance will be linear when the value is close to 1.

In the measurement of total phenolic compounds, three replications were made for data accuracy purposes. Based on the results of this study, the total phenolic content of ethanol extract of iler leaves was 0.5659 mgGAE /

gram extract, meaning that in every gram of ethanol extract of iler leaves there is phenolic equivalent to 0.5659 mg gallic acid.

Determination of total phenolic content was carried out using Folin Ciocalteu reagent. Folin Ciocalteu reagent is used because phenolic compounds can react with Folin to form a colored solution that can be measured for absorbance (Sitorus et al., 2020).

Formulation of Preparations

Stearic Acid Optimization

Stearic acid is one of the components in making cream preparations which acts as an emulsifying agent. In the cream there is a water phase and an oil phase so that a good emulsifying agent is needed to mix the two phases (Sepriliani & Devi, 2022). Therefore, this stearic acid functions as a cream base and emulgator in the oil phase.

The organoleptical test aims to examine and determine the physical quality of a preparation which includes color, shape (texture) and odor. The observation of the test Organoleptical evaluation is done visually using the five senses. From the results of organoleptical evaluation of the three formulas of iler leaf ethanol extract cream preparation, the results obtained are F1 and F2 are greenish brown, and F3 is gray brown. The color difference in the cream preparation is due to the different amounts of base content contained in each formula. For the overall shape has the same shape (texture) which is semi-solid. And the three formulas also have the same odor, namely the aromatic odor of the extract.

The homogeneity test was carried out with the aim of observing the mixing of the ingredients used. Homogeneity checks on all cream preparations, both F1, F2 and F3, show homogeneous results, characterized by all particles that are evenly dispersed on the glass object and there is no clumping in each preparation (Tungadi et al., 2023). So that stearic acid does not affect the homogeneity of the cream preparation.

The pH test is carried out to determine whether the resulting cream is acidic or alkaline by looking at the pH value obtained. The pH range in topical preparations is between 4.5-6.5 (Saryanti et al., 2019). In topical preparations, pH is related to the taste when applied, if the pH of the preparation is too acidic it will cause skin irritation, and if the pH of the preparation is too basic it will cause dry skin, so it is necessary to match the cream preparation with the pH of the skin. Skin is a layer that covers the surface of the body and plays an important role as a protector from various kinds of disturbances and external stimuli (Saryanti et al., 2019). The pH test results that have been carried out are that F1 has Ph 8, F2 has Ph 7, and F3 has Ph 6.

So that the pH of the three formulas that meet the requirements is in F3. Of the three formulas have different pH because the pH value of the preparation is also influenced by the amount of emulgator used. The more stearic acid, the pH will be low because of the many acid groups contained in stearic acid (Siti Hadija Beda, 2019).

The spreadability test is conducted to determine the speed of spread of the cream on the skin when applied to the skin. Good spreadability causes contact between the drug and the skin to be extensive, so that absorption into the skin takes place quickly. The results of the spreadability test of the three cream preparation formulas are F1 has a spreadability of 9.4 cm, F2 has a spreadability of 8.4 cm, and F3 has a spreadability of 7.6 cm. The diameter of the spreadability that is comfortable in its use for semisolid preparations is 5-7 cm. So that the results of the spreadability test show that F1, F2 and F3 do not meet the standards of good spreadability. The variation in stearic acid concentration affects the spreadability of the resulting cream preparation. The more stearic acid used will have a high viscosity, the smaller the spreadability, on the other hand, the less stearic acid used, the lower the viscosity so that the greater the spreadability (Saryanti *et al*., 2019).

The adhesiveness test was conducted to determine the adhesiveness of the cream on the skin by measuring the length of time the cream adhered to the adhesiveness test kit. This will relate to the length of time the cream contacts the skin until the desired therapeutic effect is achieved (Saryanti *et al*., 2019). The adhesion test that has been carried out obtained results, namely F1 has an adhesion of 0.64 seconds, F2 has an adhesion of 0.82 seconds, and F3 has an adhesion of 1.03 seconds. According to Murdiana *et al* (2022), the adhesion requirement for cream is more than 1 second. This means that of the three formulas that meet the requirements of good cream adhesion is F3 cream preparation. And F1 and F2 have not met the criteria for good cream adhesion requirements, although in F1 and F2 the adhesion is also almost close to 1 second. However, if the adhesion test results do not meet the specified range of requirements, it can affect the release of active substances when applied to the skin. These results are also influenced by the amount of stearic acid, the greater the concentration of stearic acid will produce greater adhesion because the cream preparation will be thicker so that its adhesion increases (Tari & Indriani, 2023).

Emulsion type test is conducted to determine the type of A/M or M/A in a cream preparation. The results of the cream emulsion test showed that F1, F2 and F3 had an M/A emulsion type. This is because the volume of the dispersed phase (oil phase) used in the cream is smaller than the dispersing phase (water phase), so that the oil globules will disperse into the water phase and form an M/A type emulsion (Pratasik et al., 2019). If stearic acid is mixed with TEA, it will form an anionic soap and form a stable and smooth M/A type emulsion (Elcistia et al., 2018). In

research conducted by Endriyatno & Aida (2023) where the cream formula tested in this study refers to research conducted by Endriyatno & Aida (2023) also produced cream preparation with type M/A.

The stability test is carried out with the aim of observing and knowing the stability of preparation and to determine the possibility of the preparation experiencing crystallization or evaporation (Yanuarti et al., 2021). The observation results showed that in the homogeneity test both before the cycling test (cycle 0) and during the cycling test (cycles 1-3) the cream structure showed a homogeneous arrangement. The observation shows that the emulsifying agent is able to unite the oil phase and water phase well so that the two ingredients are homogeneously mixed and stable. In addition, organoleptic testing conducted before the freeze thaw cycle (cycle 0) and during the freeze thaw cycle (cycles 1-3) showed that the preparation did not undergo changes in color, odor and shape, so this cream met the stability test standards.

So it can be concluded that the results of stearic acid optimization of the most optimal formula and the one that best meets the standards of cream preparation is F3 with a stearic acid content of 18%.

TEA Optimization

TEA is one of the components in making cream preparations which acts as an emulsifying agent. Emulsifying agent is one of the most important factors in the formulation of cream preparations, because in the cream there are two phases, namely the water phase and
the oil phase so that a good emulsifying agent is needed to combine the two phases (Sepriliani
& Devi, 2022). Therefore, TEA here functions as a cream base and emulgator in the water phase.

The organoleptical test aims to evaluate and determine the physical quality of a preparation which includes color, shape (texture) and odor. The observation of the organoleptical test is done visually using the five senses. From the results of organoleptical evaluation of the three formulas of iler leaf ethanol extract cream preparation, the results obtained are F1 and F2 are gray brown, and F3 is greenish brown. For the overall shape has the same shape (texture) which is semi-solid. And the three formulas also have the same odor, which is the typical aromatic odor of the extract. So that TEA has no effect on the organoleptic of the cream preparation.

The homogeneity test was carried out with the aim of observing the mixing of the ingredients used. Homogeneity checks on all cream preparations show that the results are homogeneous, characterized by all particles that are evenly dispersed on the glass object and there is no clumping in each preparation (Tungadi et al., 2023). So that TEA does not affect the homogeneity of the cream preparation.

The stability test is carried out with the aim of observing and knowing the stability of a preparation and to determine the possibility of the preparation experiencing crystallization or evaporation. The cream is declared stable if during the test there is no separation of the water phase and oil phase. The observation results showed that in the homogeneity test both before the cycling test (cycle 0) and during the cycling test (cycles 1-3) the cream structure showed a homogeneous arrangement. The observations showed that the emulsifying agent was able to It unites the oil phase and also the water phase well so that the two ingredients are homogeneously mixed and stable. In addition, organoleptic testing before the Freeze thaw cycle (cycle 0) and during the Freeze thaw cycle (cycles 1-3) did not experience changes in color, odor and shape of the preparation, so this cream met the stability test standards.

So it can be concluded that the results of the TEA optmation of the most optimal formula and the one that best meets the standards of cream preparation is F1 with a TEA content of 2% and with a stearic acid content of 18%.

In Vivo Testing

The purpose of this study was to prove the existence of sunscreen protection activity carried out on white rat test animals with iler leaf ethanol extract cream preparation. There were 4 treatments, namely negative control, positive control with octyl methoxycinnamate, treatment group with ethanol extract of iler leaves cream and treatment group with ethanol extract of iler leaves.

The erythema color on the skin of rats after sunscreen application also indicates the skin's response to UV exposure that is not sufficiently protected by the tested sunscreen preparation. If the sunscreen is ineffective, the skin may be overexposed to UV rays, which can cause damage such as erythema. The color of erythema on the skin can be visually described as redness seen after UV exposure. The color of erythema caused after being exposed under direct sunlight for approximately 6 hours causes different colors of erythema in several groups. So that the average color score in the negative control group falls into score category 1, namely very little erythema (almost invisible), which is pink. For the positive control group, the average also falls into the score 1 category, but there is 1 test animal that falls into the score 2 categories, which is clearly visible erythema, which is bright red in color. For the ethanol extract of iler leaf cream treatment group and the ethanol extract of iler leaf treatment group fall into the score 1 category, namely very little erythema (almost invisible), which is pink in color. It means that it can be concluded that the preparations tested on rats, namely octyl methoxycinnamate, ethanol extract cream of iler leaves and ethanol

extract of iler leaves have sunscreen protection activity compared to the negative control test group which was not given any treatment.

Table 3. In Vivo Testing Results

Groups	Mean erythema diameter
No treatment	0
Negative control	30,5 mm ± 2,61 *
Positive control	3,04 mm ± 0,97 *
Treatment	2,56 mm ± 0,33 *
Treatment	3,14 mm ± 1,20 *

**P value* > 0,05

The results of the analysis between the negative control group, positive control, treatment group (cream) and treatment group (extract) after being exposed to direct sunlight for 6 hours showed a difference. The results of ANOVA analysis between the negative control group and the treatment group (extract) showed results with P value = 0.000 (P < 0.05), between the positive control group and the treatment group (extract) showed results with P value = 1.000 (P > 0.05), between the treatment group (cream) with the treatment group (extract) showed results with P value = 0.973 (P > 0.05). So that the difference in the average diameter of erythema descriptively between the negative control group and the treatment group (extract) is significantly different. However, the difference in erythema diameter between the positive control group and the treatment group (extract), and between the treatment group (cream) and the treatment group (extract) is the same. So that the average difference in erythema diameter descriptively between the positive control group and the treatment group (cream) and between the treatment group (cream) and the treatment group (extract) is not significantly different.

CONCLUSION

Based on the results of the research and discussion described in the previous chapter, it can be concluded that the most optimum ethanol extract cream formula of iler leaves (Coleus scutellarioides (L) Benth) after preparation evaluation consists of 1.5% iler leaf extract, 18% stearic acid, 2% TEA, 2% cetyl alcohol, 8% glycerin, 0.2% metal paraben, 0.02% propyl paraben and aquadest. And the ethanol extract cream of iler leaves (Coleus scutellarioides (L) Benth) after in vivo testing has sunscreen activity as evidenced by the erythema effect that appears after exposure under sunlight.

ACKNOWLEDGMENTS

-

CONFLICT OF INTEREST

We declare that we don't have any conflict of interest.

REFERENCES

Aji, N. P., Noviyanty, Y., Fahlevi, R., S1, P., Klinis, F., Komunitas, D., Farmasi, F., Al-Fatah Bengkulu, S., & Penulis, K. (2023). Skrining Fitokimia dan profil KLT metabolit sekunder dari Ekstrak Etanol Daun Miana (Coleus scutellarioides Benth) Phytochemical screening and TLC profile of secondary Metabolites from the Ethanol Extract of Miana (Coleus scutellarioides Benth). *Agustus*, *6*(2), 149–157.

Byren D, Dufort M, Zahr A, Franco R. Zinc Oxide Technology and Superior UVA Protection.

American Academy of Dermatology. 2017;76(6):4931.

Cefali LC, Ataide JA, Moriel P, Foglio MA, Mazzola PG. Plant-based active photoprotectants for sunscreens. *International Journal of Cosmetic Science*. 2016;38(4):1–8.

Endriyatno, N. C., & Aida, F. (2023). FORMULASI KRIM EKSTRAK DAUN KEMANGI (Ocimum basilicum L.) DENGAN VARIASI KONSENTRASI TRIETANOLAMIN DAN ASAM STEARAT. *Forte Journal*, *3*(1), 43–49. https://doi.org/10.51771/fj.v3i1.420

Jusmiati A, Rolan Rusli, and Laode Rijai. 2019. "Aktivitas Antioksidan Kulit Buah Kakao Masak Dan Kulit Buah Kakao Muda." Joumal of Chemical Information and Modeling 53(9):1689- 99.

Moelyono MW, Uswatun A, Rochjana H, et al. Aktivitas Antioksidan Daun Iler Plectranthus scutellarioides (L.) R. Br. *J Farm Indones* 2016; 8: 271–276.

Saryanti, D., Setiawan, I., & Safitri, R. A. (2019). Optimasi Asam Stearat Dan Tea Pada Formula Sediaan Krim Ekstrak Kulit Pisang Kepok (*Musa paradisiaca L.*). *Jurnal Riset Kefarmasian Indonesia*, *1*(3), 225–237. https://doi.org/10.33759/jrki.v1i3.44

Sepriliani, I. W., & Devi, S. (2022). Pengaruh Konsentrasi Asam Stearat Sebagai Emulsifying Agent Terhadap Karakteristik Fisik Sediaan Krim Ekstrak Daun Pepaya (Carica Papaya L.). *Indonesian Journal on Medical Science*, 9(2). https://doi.org/10.55181/ijms.v9i2.369

Shofia Amrillah, M., Rusli, R., Fadraersada Laboratorium Penelitian dan Pengembangan Farmaka Tropis, J., Farmasi, F., Mulawarman, U., & Timur, K. (N.D.). Aktivitas Tabir Surya Daun Miana (Coleus Atropurpureus L. Benth) Secara In Vitro. In *Jurnal Sains dan Kesehatan. 2015* (Vol. 1, Issue 4).

Siti Hadija Beda, T. D. K. (2019). Perbandingan Konsentrasi Asam Stearat Terhadap Mutu Fisik Sediaan Krim Ekstrak Daun Kersen (Muntingia calabura Linn). Edible Medicinal And Non Medicinal Plants, 486–492.

Sitorus, F. C. E., Dwi Wulansari, E., & Sulistyarini, I. (2020). Uji Kandungan Fenolik Total Dan Aktivitas Antibakteri Ekstrak Kulit Buah Asam Paya (Eleiodoxa Conferta (Griff.) Burret) Terhadap Staphylococcus Aureus. Vol 15. Media Farmasi Indonesia, 15(2), 1617–1624.

Sugiarna, R., Farhan, N., Rusdi, M., Ikhlas Arsul, M., Farmasi Fakultas Kedokteran dan Ilmu Kesehatan Universitas Islam Negeri Alauddin Makassar, J., Yasin Limpo No, J. H., Sombaopu Kabupaten Gowa, K., Selatan, S., & Prodi Farmasi Fakultas Kedokteran dan Ilmu Kesehatan Universitas Islam Negeri Alauddin Makassar, M. (2019). Kadar Fenolik dan Flavonoid Total Ekstrak Etanol Daun Anggur (Vitis vinifera L) Total Phenolic and Flavonoid Content of Grapevine (Vitis vinifera L) Leaves Ethanol Extract. Ad-Dawaa Journal of Pharmaceutical Sciences, 2(2), 95–102.

Suharyanto, S., & Hayati, T. N. (2021). Penetapan Kadar Flavonoid Total Ekstrak Buah Gambas (Luffa acutangula(L.) Roxb.) dengan Metode Spektrofotometri UV-Vis. Pharmacon: Jurnal Farmasi Indonesia, 18(1), 82–88. https://doi.org/10.23917/pharmacon.v18i01.10916

Tahir, M., Muflihunna, A., & Syafrianti, S. (2017). PENENTUAN KADAR FENOLIK TOTAL EKSTRAK ETANOL DAUN NILAM (Pogostemon cablin Benth.) DENGAN METODE SPEKTROFOTOMETRI UV-VIS. Jurnal Fitofarmaka Indonesia, 4(1), 215–218. https://doi.org/10.33096/jffi.v4i1.231

Tari, M., & Indriani, O. (2023). Formulasi Dan Uji Stabilitas Fisik Sediaan Krim Ekstrak Sembung Rambat (Mikania micrantha Kunth). Jurnal Ilmiah Multi Science Kesehatan, 15(1), 192–211.

Theresia L. 2014. Molecular and Cellular Effect of UV Radiation. National Simposium Skin Photodamage Up Date. Jakarta

Tungadi, R., Sy. Pakaya, M., & D.as'ali, P. W. (2023). Formulasi dan Evaluasi Stabilitas Fisik Sediaan Krim Senyawa Astaxanthin. Indonesian Journal of Pharmaceutical Education, 3(1), 117–124. https://doi.org/10.37311/ijpe.v3i1.14612

Ubaedilah, N. A., & Supriyatna, A. (2023). Analisis dan Penerapan Manfaat Kandungan Senyawa Daun Miana (Coleus scutellarioides (L.) Benth.) di Kiaracondong, Kota Bandung. Hippocampus: Jurnal Pengabdian Kepada Masyarakat, 2(1), 75–82. https://doi.org/10.47767/hippocampus.v2i1.547

Yanuarty, R., Tobondo, N. A., Tandi, J., & Budiawan, E. (2023). Penetapan Metabolit Sekunder Dan Uji Aktivitas Antioksidan Ekstrak Etanol Daun Miana Secara Spektrofotometer Uv-Vis. Farmakologika Jurnal Farmasi, XX