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FORMULASI DAN PENETAPAN KADAR FLAVONOID TOTAL SEDIAAN GEL EKSTRAK DAUN AKALIFA (Acalypha wilkesiana Muell.Arg)

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ABSTRACT

Skin damage is mainly caused by ultraviolet radiation, which necessitates the use of sunscreen. Sunscreen is a skincare product that protects the skin from sun exposure. Plants that can be used as sunscreen include the leaves of akalifa (Acalypha wilkesiana Muell. Arg). Alkaloids, flavonoids, phenols, tannins, and saponins are all found in akalifa leaves. Phenolic compounds, particularly flavonoids, have potential as sunscreen. To formulate a gel preparation and conduct testing of the total flavonoid content of the gel formulation from acalypha leaf extract (Acalypha wilkesiana Muell. Arg.). This study uses a laboratory experimental research design by extracting akalifa leaves using the UAE (Ultrasonic Assisted Extraction) method with 96% ethanol as the solvent. The gel preparation of the akalifa leaf extract is formulated at concentrations of 6%, 8%, and 10%. The formula involves determining the total flavonoid content of the gel preparation using UV-Vis spectrophotometry at a wavelength of 430 nm. The determination of total flavonoid content in formulation 1 is 1,888 mg QE/gram, in formulation 2 is 2 mg QE/gram, and in formulation 3 is 2,102 mg QE/gram. In the three formulations of concentrations 6%, 8%, and 10% formulation 3 has the highest flavonoid content.

Keywords: akalifa leaves; gel; total flavonoid; UV-Vis spectroscopy; Acalypha wilkesiana Muell. Arg.

INTRODUCTION

As a tropical country, Indonesia receives sunlight throughout the year. The sun emits various types of rays, including ultraviolet (UV) rays consisting of UV-A, UV-B, and UV-C. UV rays have a significant impact on human health, both positive and negative (Wijayadi et al., 2024). Skin damage is mostly caused by ultraviolet radiation, particularly UV-B and UV-A (Tan Sukmawati Tansil et al., 2024).

One skin care product that can protect the skin from sun exposure is sunscreen. Sunscreen can be derived from natural ingredients or chemical synthesis. The use of synthetic substances such as oxybenzone, avobenzone, PABA derivatives (p-

aminobenzoic acid), TiO2, and ZnO often results in allergic effects, hypersensitivity, impaired vitamin D synthesis, and the accumulation of these substances may also pose the risk of melanoma cancer. Therefore, there is a need for sunscreen derived from plants as a substitute for chemical substances (Endriyatno et al., 2024).

One of the formulations commonly found for sunscreen use is gel. Gel is a semi-solid system where the movement of its dispersing medium is restricted by a three-dimensional network of particles or macromolecules dissolved in its dispersing phase (Fuji Nurfadilah et al., 2021). The following are the advantages of gel formulations, which include good dispersion, good drug release, and stability during storage with a cooling effect (Acnes et al., 2024).

Acalypha leaves can be developed into sunscreen because they contain flavonoid compounds. Many plants and foods include flavonoids, which are secondary metabolites of polyphenols. They have a number of bioactive properties, such as antiviral, anti-inflammatory, cardioprotective, anti-diabetic, anticancer, and anti-aging properties, as well as antioxidant qualities (Arifin & Ibrahim, 2018) and they counteract free radicals (Amini et al., 2020). Ultraviolet exposure that can be absorbed or blocked by chromosome groups includes UV-A and UV-B (Dalimunthe et al., 2024). By absorbing UV-A and UV-B photons that reach the skin, flavonoids help shield the skin from UV radiation exposure. Due to their conjugated double bond structure, nearly all flavonoids function as chromophores. (single conjugated bonds). When UV-B light strikes the skin, the flavonoids will absorb the UV-B rays that affect the skin, and when the electrons return to their original state, the absorbed UV-B light will then be emitted but with much lower energy. Most of the UV-B energy will be converted by the flavonoids into harmless heat energy for the skin. This mechanism will inhibit or minimize the occurrence of erythema due to UV exposure (Amini et al., 2020).

Therefore, research is needed to formulate the gel and establish the total flavonoid content in the developed gel preparation to determine the amount of compounds and their ability to block UV rays.

MATERIAL AND METHODS

Tools

The instruments utilized in this research include an analytical equilibrium (Ohaus), mortar, stamper, beaker glass (Iwaki), measuring cup (Iwaki), porcelain dish (Iwaki), Erlenmeyer flask (Iwaki), dropper pipette, blender object glass, waterbath (Memert), hot plate, stirring rod, test tubes, pH meter (Hanna), spreading apparatus, concentrating apparatus, viscometer (Rion VT-06), rotary evaporator (IKA RV 10 basic), Ultrasonic Assisted Extraction (Biobase), UV-Vis spectrophotometer (Shimadzu), filter paper, mesh 60 sieve, and UV shield gel container.

Materials

In this study, The resources utilized include acalypha leaves (Acalypha wilkesiana Muell. Arg), 96% ethanol, carbopol 940, ethanol p.a, methyl paraben, propyl paraben, propylene glycol, TEA, glycerin, aquades, magnesium, hydrochloric acid, and quercetin.

Plant Determination

The determination of the plant (Acalypha wilkesiana Muell. Arg) was carried out at the Plant Laboratory of Jember State Polytechnic, ensuring that the plants used are leaves of the Acalypha species with the Latin name (Acalypha wilkesiana Muell. Arg).

Extraction Process of Acalifa Leaves

The simplicia powder is put in a flask by Erlenmeyer and dissolved with 96% ethanol at a ratio of 1:5 (w/v), and extracted at a temperature of 40°C for 30 minutes using ultrasonic assistance at a frequency of 50Hz. After extraction, filtration is performed by passing the solution through Whatman paper. The resulting filtrate is subsequently gathered and focused on using a rotary evaporator until a dense extract is obtained. Concentration of the extract is performed at 40°C in temperature, operating at 60 rpm (Syamsul et al., 2020).

Gel Preparation

Formulation Assemble the equipment and supplies needed to make the gel. Then weigh 1 gram of carbopol 940, add hot aquades, and stir until it swells. Mix glycerin and propylene glycol and stir until homogeneous, then add methyl paraben and propyl paraben, stirring until homogeneous. Next, gradually add aquades until homogeneous. Finally, add the extract of acalypha leaves and TEA, and stir until homogeneous.

Preparation of Quercetin Stock Solution 1000 Ppm

To make a 1000 ppm stock solution, weigh 25 mg of quercetin and then dissolve it using ethanol p.a in a 25 ml volumetric flask. Then, pipette 1 ml of the 1000 ppm stock solution and dilute it with ethanol p.a into a 10 ml volumetric flask to obtain a 100 ppm solution ((Bachtiar et al., 2023).

Preparation of Quercetin Standard Curve

Standard quercetin solutions with concentrations of 2, 4, 6, 8, and 10 ppm are made from the main stock solution with a concentration of 100 ppm. The 100 ppm working stock solution is taken in various volumes: 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml, then placed into a 50 ml volumetric flask. Then add 3 ml of ethanol p.a, 0.2 ml of 10% AlCl3, and 0.2 ml of potassium acetate, and then dilute with aquadest to reach a total volume of 50 ml (Bachtiar et al., 2023)

The preparation of the blank solution

3 ml of ethanol p.a solvent is added to a 10 ml volumetric flask, followed by the addition of 0.2 ml of 10% AlCl3 solution and 0.2 ml of 1 M potassium acetate, then diluted with aquades to 10 ml (Riska et al., 2024).

Determination of Flavonoid Content

A 1000 ppm sample solution is prepared by weighing 25 mg of the akalifa leaf extract gel, then dissolving it in ethanol p.a using a 25 ml volumetric flask. Then, 0.5 ml is pipetted and transferred to a 10 ml volumetric flask. Next, 3 ml of ethanol p.a, 0.2 ml of 10% AlCl3, and 0.2 ml of 1 M potassium acetate are added. After that, aquades are

added to dilute the mixture to the appropriate level. The solution is let to stand for half an hour after being well mixed to ensure homogeneity. Following the duration, the sample's absorbance was measured at the greatest wavelength yield. By using the acquired absorbance value as variable Y in the linear regression equation developed from the quercetin standard curve, the concentration of flavonoids in the working sample solution (given in parts per million) is determined. To get the average absorbance value for each assay, the samples were produced in three replications (Bachtiar et al., 2023).

$$\mathrm{TCF} = \frac{\mathrm{c.v.fp}}{\mathrm{g}}$$

Description:
TFC: Total Flavonoid Content (mg QE/gram)
C: Flavonoid Concentration (value x)
V: Volume of Extract Used (ml)
Fp: Dilution FactorG: Weight of Sample Used (g) (Purnama Sari et al., 2024)

RESULT AND DISCUSSION

After reviewing the samples sent to the Plant Laboratory of Jember State Polytechnic on January 20, 2025, it was concluded that the sample used in this study is Acalypha leaves with Kingdom: Plantae, Division: Spermatophyta, Subdivision: Magnoliophyta, Class: Magnoliopsida, Order: Euphorbiales, Family: Euphorbiaceae, Genus: Acalypha, Species: Acalypha wilkesiana Muell. Arq. The identification letter number is 22/PL17.8/PG/2025.

Acalypha leaves are made into simplicia before undergoing the extraction process. The simplicia of Acalypha leaves is done by wet sorting, washing with running water to remove foreign materials, shredding, and drying the simplicia using an oven. After drying, it is made into powder using a blender until 1500 grams of simplicia powder is obtained (Murniyati et al., 2021). The UAE technique is used for the extraction of Acalypha leaves. UAE is an extraction method assisted by ultrasonic waves that spread through the solvent, creating a cavitation effect that causes heating and the formation of extract compounds (Fauziyah et al., 2022). Ultrasonics is a non-thermal extraction method that can increase mass transfer rates and break down cell walls with numerous microcavities, thereby shortening processing time and optimizing solvent use (Handaratri & Yuniati, 2019).

The preparation of the akalifa leaf extract gel was done with 3 formulas containing akalifa leaf extract at 6%, 8%, and 10%, respectively. In the preparation of the akalifa leaf extract gel formula, there are 2 main components, namely carbopol as the gelling agent and propylene glycol as the humectant. The use of Carbopol 940 is due to the fact that in small concentrations of 0.5%-2%, it can already produce gels with good characteristics. Carbopol can bind moisture from the air, which can cause the preparation to become thicker and have a lower spreading ability.

As a humectant, propylene glycol absorbs moisture from the surrounding air and lowers water evaporation from the preparation, preserving the gel's stability. Propylene glycol can prevent the skin from drying out immediately in addition to preserving the preparation's stability.

The addition of Triethanolamine (TEA) is intended as a pH regulator for carbopol because carbopol 940 is active as a gelling agent in a basic environment, specifically at a pH of 7-10. The reaction upon the addition of TEA is the occurrence of coagulation, marked by the thickening of the preparation. The mechanism involves the COO- groups from carbopol binding with TEA, thereby making the preparation thicker. This is due to the shift in equilibrium of carbopol from the acidic form after swelling in water to the salt form when exposed to TEA, which is basic (Irianto, 2021).

Results of the Total Flavonoid Content Test of Gel Preparations of Acalypha Leaf Extract

The determination of total flavonoid compounds aims to ascertain the amount of total flavonoid components in the gel preparation of the extract from the leaves of Acalypha wilesiana Muell.Arg. The determination of flavonoids is performed using UV-Vis Spectrophotometry. The flavonoid analysis process involves the reaction of flavonoid compounds with a color-forming reagent, in this case AlCl3, to produce absorbance at a visible wavelength. The creation of a compound between aluminum chloride and the keto group at C-4 and the hydroxyl groups at C-3 or C-5 that are near to flavon and flavonol groups is the basis for the colorimetric method's premise of measuring flavonoid concentration (Suharyanto & Hayati, 2021).

At this stage, the addition of aluminum chloride induces the formation of stable acid complexes with the ortho-hydroxyl groups present in rings A or B of flavonoid compounds. The addition of acetic acid is used to identify the 7-hydroxyl group to maintain absorption in the visible range (Oktaria & Marpaung, 2023). The initial stage in flavonoid testing involves measuring the maximum wavelength in the range of 400-800 nm from a quercetin solution that has an absorbance of 0.272% and a wavelength of 430.40 nm at a concentration of 8 ppm in p.a. ethanol. To determine absorbance values, a standard quercetin stock solution was then made at concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm (Mulyaningsih et al., 2022). A standard curve connecting the quercetin solution's absorbance and concentration can be made using the absorbance data from the concentration. This yields a linear regression equation of y=0.0088x + 0.2016 with a correlation coefficient (r) = 0.9988. A linear calibration curve and a correlation between the absorbance value and the quercetin solution content are shown by a r value approaching 1. The total flavonoid content in the gel extract sample of Akalifa leaves may be ascertained by comparing it to the quercetin calibration curve equation (Panca *et al*., 2022).

For flavonoid content determination, quercetin was used as the standard compound. The choice of quercetin as the standard solution is based on its widespread distribution in various plant species. About 60-75% of the flavonoids in plants consist of quercetin and its glycosides. Quercetin is also classified as a flavonoid that forms complexes with AlCl3. The absorbance of samples prepared in three replicates is used to calculate the total flavonoid content in the gel preparation of the akalifa leaf extract. The linear regression equation y = bx + a, which is derived from the quercetin standard curve, is then applied to this absorbance result (Maqfirah *et al.*, 2023).

According to the Lambert-Beer law, the range of total flavonoid content based on its absorbance values is between 0.2-0.8. And the absorbance values produced from the

nine samples all fall within this range. The results obtained from the gel formulation of akalifa leaf extract contain flavonoid content (Mulyaningsih *et al*., 2022). From the data above, it is obtained that the highest total flavonoid content in the gel formulation of akalifa leaf extract is in formula 3 with an average value of 2.102 mg QE/gram, where formulation 3 has the highest concentration of akalifa leaf extract This suggests that an extract's potential to function as a sunscreen is enhanced by its concentration, which also increases its overall flavonoid content.

The largest class of phenols found in nature are flavonoid compounds. These substances are red, purple, blue, and yellow plant pigments (Asmoro Bangun, 2021). The flavonoid compounds contained in akalifa leaves have many benefits in the health sector, including aromatherapy, antibacterial, antioxidant, preventing oxidative diseases such as cardiovascular diseases and certain types of cancer, heart disease, stroke, and other conditions that weaken the immune system, absorbing and neutralizing free radicals, anti-inflammatory and antidiabetic. Flavonoid compounds' conjugated double bonds enable electronic changes in molecules, which in turn allow them to absorb UV light (Endriyatno *et al*., 2024). Flavonoid compounds as natural antioxidants provide a good alternative for the body to combat the formation of Reactive Oxygen Species (ROS). ROS in the skin network causes premature aging (Riska et al., 2024). This compound has 15 carbon atoms with a chemical structure of C6-C3-C6, of two benzene rings connected by an oxygen-containing heterocyclic ring (Sanjaya *et al*., 2023).

CONCLUSION

In all three formulations the gel extract of akalifa leaves with the highest total flavonoid content is formulation 3 with a concentration of 10% akalifa leaf extract.

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TABLE

Table 1. Formulation Gel

Ingredient	Formulate			Fungtion
	F1 (%)	F2 (%)	F3 (%)	
Acalypha leaf extract	6	8	10	active ingredient
Carbopol 940	2	2	2	*Gelling agent*
Metyl paraben	0,2	0,2	0,2	preservative
Propyl paraben	0,03	0,03	0,03	preservative
Propylene glycol	5	5	5	humectant
TEA	1	1	1	*Alkalizing agent*
Glycerin	25	25	25	humectant
Aquadest	ad 100	ad 100	ad 100	solvent

Table 2. % Yield

Simplicity Weight	Extract Weight	Yield(%)	Requirements
1500 gram	273 gram	18,2%	not less than 10%

Table 3. Result of Flavonoid Level Determination

Sample	Total Flavonoid Content Test Results ± SD (mg QE/gram)
F1 (6%)	1,888 ± 0,018
F2 (8%)	2 ± 0,018
F3 (10%)	2,102 ± 0,018