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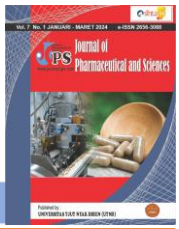

Formulation and Antioxidant Activity Test of a Moisturizing Gel Preparation from Celery Leaf Extract (*Apium graveolens* L.)

Formulasi dan Uji Aktivitas Antioksidan Sediaan *Moisturizer* Gel dari Ekstrak Daun Seledri (*Apium graveolens* L.)

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Abstract

Background: Free radicals are one of the causes of skin problems and premature aging, so antioxidants are needed to inhibit this process. Celery leaves (*Apium graveolens* L.) are known to contain flavonoids, tannins, saponins, and steroids that have antioxidant potential. **Objective:** To formulate celery leaf extract into a moisturizing gel, evaluate the physical properties of the formulation, and test its antioxidant activity. **Methods:** This study is a quantitative experimental study involving maceration extraction with 70% ethanol, formulation of the product, evaluation of physical properties, and antioxidant testing using the DPPH method. **Results:** The results showed that the celery leaf extract was successfully formulated into a moisturizing gel that met the physical requirements for gel formulations, as indicated by good organoleptic properties and homogeneity, a pH within the skin's pH range, spreadability and adhesion meeting standards, viscosity within the required range, and stability without phase separation. The IC_{50} value for vitamin C of 7,4 µg/mL is classified as very strong, whereas the IC_{50} values for formulations F0, F1, F2, and F3 were, respectively, 3968,6 µg/mL, 7607,1 µg/mL, 4179,3 µg/mL, and 3060,2 µg/mL. Based on these IC_{50} values, the antioxidant activity of the celery leaf extract moisturizing gel formulations is classified as weak (>200 µg/mL); however, an increase in extract concentration shows a trend toward increased antioxidant activity. **Conclusion:** This study demonstrates that celery leaf extract was successfully formulated into a moisturizing gel with good physical properties; however, further optimization of the formulation is needed to enhance its antioxidant activity so that it can provide optimal benefits as a skin care product.

Keywords: Celery leaves, Antioxidants, Moisturizing gel, DPPH.

Abstrak

Latar belakang: Radikal bebas adalah salah satu penyebab permasalahan kulit dan penuaan dini, sehingga dibutuhkan antioksidan untuk menghambat proses tersebut. Daun seledri (*Apium graveolens* L.) diketahui mengandung senyawa flavonoid, tanin, saponin, dan steroid yang berpotensi sebagai antioksidan. **Tujuan:** Untuk memformulasikan ekstrak daun seledri dalam bentuk sediaan gel pelembab, mengevaluasi sifat fisik sediaan, serta menguji aktivitas antioksidan. **Metode:** Penelitian ini merupakan penelitian eksperimental secara kuantitatif yang melibatkan ekstraksi maserasi dengan etanol 70%, formulasi sediaan, evaluasi sifat fisik, dan pengujian antioksidan menggunakan metode DPPH. **Hasil:** Hasil penelitian menunjukkan bahwa ekstrak daun seledri berhasil diformulasikan ke dalam sediaan gel pelembab yang memenuhi persyaratan fisik sediaan gel, ditandai dengan organoleptis dan homogenitas yang baik, pH sesuai rentang pH kulit, daya sebar dan daya lekat memenuhi standar, viskositas berada dalam rentang yang dipersyaratkan, serta stabil tanpa terjadi pemisahan fase. Nilai IC_{50} vitamin C sebesar 7,4 µg/mL tergolong sangat kuat, sedangkan nilai IC_{50} sediaan F0, F1, F2, F3 berturut-turut sebesar -3968,6 µg/mL, 7607,1 µg/mL, 4179,3 µg/mL, dan 3060,2 µg/mL. Berdasarkan nilai IC_{50} tersebut, aktivitas antioksidan sediaan gel pelembab ekstrak daun seledri tergolong lemah (>200 µg/mL), meskipun demikian peningkatan konsentrasi ekstrak menunjukkan kecenderungan peningkatan aktivitas antioksidan. **Kesimpulan:** Penelitian ini menunjukkan bahwa ekstrak daun seledri berhasil diformulasikan ke dalam sediaan gel pelembab dengan sifat fisik yang baik, namun diperlukan optimalisasi formula lebih lanjut untuk meningkatkan aktivitas antioksidannya sehingga dapat memberikan manfaat yang lebih optimal sebagai produk perawatan kulit.

Kata kunci* : *Daun seledri, Antioksidan, Moisturizer gel, DPPH.

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Introduction

Indonesia is one of the countries facing a never-ending air pollution problem, with air pollution levels increasing every year [1]. Air pollution encompasses various components such as PM2.5, PM10, NOx, SO_2, CO, and O_3. The WHO reports that approximately 90% of the world's population is exposed to polluted air, and an estimated 7 million people die each year as a result of air pollution [2]. In Southwest Papua, particularly in the city of Sorong, one of the main causes of air pollution is the increasing number of motor vehicles, where emissions from vehicles such as carbon monoxide (CO), hydrocarbons, and fine particulate matter contribute to the region's air pollution levels [3].

The formation of free radicals is one of the harmful effects of air pollution on the human body. When free radicals enter the body in abnormal amounts, they can attack vulnerable compounds such as proteins and lipids, potentially leading to various diseases. This condition can occur because the antioxidants in the body are unable to counterbalance the oxidants entering the body [4]. Some skin problems caused by oxidative stress include redness, darkening, premature aging, and cancer [5]. Premature aging is a significant problem and a major concern, particularly among women. It is estimated that approximately 76% of Indonesian women have experienced symptoms of premature aging [6]. Premature aging is characterized by uneven skin tone, cell death, hyperpigmentation, hypopigmentation, thinning skin, dry skin, and wrinkles caused by a lack of collagen synthesis and collagen damage [7].

Premature aging can be slowed by consuming foods rich in antioxidants, which combat free radicals entering the body, thereby delaying the premature aging process [6]. Antioxidants are compounds that slow down the oxidation process by binding to free radicals and highly reactive molecules, thus preventing cellular damage [8]. Plants are an abundant source of natural antioxidants. Generally, the antioxidant compounds found in plants include flavonoids, polyphenols, vitamin C, vitamin E, and carotenoids. These compounds work by neutralizing free radicals, thereby preventing cellular damage [9]. One plant that exhibits antioxidant activity is celery [10].

Celery (*Apium graveolens* L.) contains flavonoids, steroids, saponins, and tannins [11]. Water extract of celery prepared via the maceration method exhibits very strong antioxidant activity with an IC_{50} value of 23,713 ppm; thus, the water extract obtained through maceration holds great potential as a natural antioxidant source comparable to vitamin C. A lotion formulation of celery herb extract exhibited the highest antioxidant activity of 15,186 µg/mL at a 15% concentration [12].

Natural ingredients with antioxidant activity can be used in anti-aging products because they are believed to cause fewer side effects compared to synthetic chemicals [13]. Gels are one of the most commonly used cosmetic formulations. The advantage of gels over other topical formulations is their optimal spreadability on the skin without disrupting the skin's physiological functions, as they do not form an occlusive layer on the skin's surface and do not clog pores [14].

Based on the above explanation, the researchers intend to conduct a study on the formulation of a moisturizing gel containing celery leaf extract, the evaluation of its physical properties, and the testing of its antioxidant activity using the DPPH assay. This is based on the explanation above that celery leaf extract is known to contain compounds with antioxidant potential; therefore, new innovations are needed in the development of natural topical antioxidant formulations, one of which is through the formulation of a moisturizing gel containing celery leaf extract, the evaluation of its physical properties, and testing of its antioxidant activity.

Methods

Plant Sample Collection

The sample collection process was conducted in the Klasuluk Village area of Sorong Regency, Southwest Papua, Indonesia (1º00'01.7"S, 131º18'55.1"E). This study used leaf celery (*Apium graveolens* L., *Secalium* variety). Celery plants measuring approximately 25 cm were harvested in the morning, and the stems were separated from the leaves, which were in good condition, fresh, and green in color.

Materials and Equipment

The equipment used in this study included aluminum foil, a 60 mesh sieve, a stirring rod, a blender (Turbo, Indonesia), glass bottles, porcelain dishes, a glass funnel (Pyrex®, United States), beakers (Pyrex®, United States), measuring cups (Pyrex®, United States), hot plate (Faithful, China), vernier caliper, watch glass, microscope slide, filter paper, label paper, volumetric flask (Iwaki®, Japan), maserator, micropipette (DLAB, China), mortar and pestle, analytical balance (Fujitsu FSR-A220, Japan), Oven (Kenton 101-3, China), pH meter (ATC pH-009, China), Dropper pipette, Plastic wrap, Glass jar, Rotary evaporator (Buchi R-100, Switzerland), Centrifuge (Oregon, China), UV-Vis spectrophotometer-Vis (Thermo Genesys 150, United States), spatulas, tissues, vials, viscometer (Anton Paar viscoQC 100, Austria), and water bath (Memmert WNB 22, Germany). The materials used in this study were distilled water, DPPH (*2,2-diphenyl-2-picrylhydrazyl*), celery leaf extract (*Apium graveolens* L.), 70% ethanol, p.a. ethanol, glycerin, methylparaben, CMC-Na, and vitamin C.

Sample Preparation and Preparation of Crude Drug

Celery was harvested, and the leaves were separated from the stalks. A total of 5 kg of leaves was weighed, washed under running water, and dried for approximately 14 hours in an oven at 41°C. After the samples were dry, they were sorted, then blended and sieved using a 60 mesh sieve to ensure uniform particle size [15].

Preparation of Celery Leaf Extract

Extraction was performed using a 1:10 maceration method, in which 600 grams of celery leaf powder was soaked in 6.000 mL of 70% ethanol for 3×24 hours, with stirring every 8 hours [16]. Remaceration was performed once using a fresh solvent of the same volume, followed by filtration. The extract was evaporated and concentrated using a rotary evaporator and a water bath at 50°C until a thick extract was obtained [17]. The thick extract was weighed, and the yield was calculated using the following formula [18].

$$\% \text{ Yield } = \frac{Weight\ of\ thick\ extract}{Initial\ weight\ of\ simple\ materials} \times 100\%$$

Moisturizing Gel Formulation

Moisturizing gels were prepared using concentrations of 0%, 5%, 15%, and 25%. The selection of 5% and 15% concentrations was based on the study [12], which used concentrations of 5%, 10%, and 15%, while the 25% concentration was added to evaluate the effect of increased extract levels on physical properties and antioxidant activity. The preparation of the moisturizing gel began by weighing the ingredients according to the formula in **Table 1.** CMC-Na was swollen with distilled water in a mortar (mass 1). Methylparaben was dissolved in glycerin until completely dissolved (mass 2). The concentrated celery leaf extract was dissolved with a portion of distilled water until it reached a smooth and homogeneous texture (mass 3). After the CMC-Na swelled, mixture 1 was ground, then mixture 2, the remaining distilled water, and mixture 3 were added little by little while continuing to grind until a homogeneous gel formed. The gel was then packaged in glass jars [13].

Table 1. Formulation of Celery Leaf Extract Moisturizing Gel

Ingredient	Function	Ingredient Concentration (%)			
		F0	F1	F2	F3
Celery leaf extract	Active ingredient	0	5	15	25
CMC-Na	Gelling agent	1	1	1	1
Glycerin	Humectant	5	5	5	5
Methylparaben	Preservative	0,1	0,1	0,1	0,1
Distilled water	Solvent	100	100	100	100

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Evaluation of the Physical Properties of Moisturizing Gels

Organoleptic Test

Organoleptic testing was conducted to assess the physical characteristics of the formulation using the five senses by observing its texture, color, and odor [19].

Homogeneity Test

The preparation is spread on a glass slide or other transparent material; the preparation is considered homogeneous if no coarse particles are visible [20].

pH Test

A 0,5 gram sample was diluted with 5 mL of distilled water, and then a pH meter was immersed for 1 minute using the pH meter [21].

Spreadability Test

A 0,5 gram sample was placed on a standardized round glass plate; another glass plate was placed on top, and weights of 50 grams, 100 grams, and 150 grams were applied in stages. The sample was left for 1 minute, after which the spread diameter was measured [22].

Adhesion Test

A 0,25 gram sample is placed between two flat glass plates on an adhesion tester and pressed with a 250 gram load for 1 minute; the load is then removed, and an 80 gram load is gradually applied to the device, with the release time recorded [20].

Viscosity Test

The preparation is placed in a cylindrical container and then positioned under the viscometer. A size 6 spindle is attached and immersed into the preparation until it is fully submerged at a speed of 30 revolutions per minute at a temperature of 25 ± 1°C; the viscometer is then operated until the viscosity value is read [23].

Stability Test

Stability testing of the gel by centrifugation: 10 grams of the preparation are placed in a centrifuge tube and centrifuged at 3.800 rpm for 30 minutes [24].

Antioxidant Activity Test Using DPPH (*2,2-Diphenyl-2-Pycrylhydrazyl*)

Preparation of the DPPH Solution

Weigh 0,0157 grams of DPPH and dissolve it in a 100 mL volumetric flask with p.a. ethanol until the mark is reached [25].

Determination of the Maximum Wavelength of DPPH

A blank solution was prepared by pipetting 1 mL of the DPPH solution and adding p.a. ethanol to a total volume of 5 mL. The solution was left to stand in the dark for 30 minutes, after which its absorbance was measured using a UV-Vis spectrophotometer at wavelengths of 400–800 [26].

Determination of Operating Time

Pipette 1 mL each of the vitamin C solution and the sample into separate dark vials. Add 1 mL of the DPPH solution to each, then mix thoroughly and measure the absorbance over a 60 minute period at the maximum wavelength obtained using a UV-Vis spectrophotometer [27].

Antioxidant Activity Testing on the Positive Control

10 mg of vitamin C was dissolved in p.a. ethanol, and the volume was made up to 100 mL with p.a. ethanol to form a 100 ppm vitamin C stock solution. Working solutions of vitamin C at concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were prepared by pipetting 0,1 mL, 0,2 mL, 0,3 mL, 0,4 mL, and 0,5 mL of the stock solution into 5 mL volumetric flasks and adding p.a. ethanol to the mark [28].

A 2 mL sample of the vitamin C working solution at each concentration was transferred to a dark vial and mixed with 1 mL of DPPH solution. The solution was incubated for the specified operating time, and its absorbance was measured at the maximum wavelength obtained using a UV-Vis spectrophotometer.

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Testing the Antioxidant Activity of a Moisturizing Gel Formulation Containing Celery Leaf Extract (*Apium graveolens* L.)

Each 250 mg formulation was dissolved and homogenized using p.a. ethanol, then transferred to a 25 mL volumetric flask to form a 10.000 ppm moisturizing gel stock solution. Working solutions with concentrations of 4.500 ppm, 5.000 ppm, 5.500 ppm, 6.000 ppm, and 6.500 ppm were prepared by pipetting 2,25 mL, 2,5 mL, 2,75 mL, 3 mL, and 3,25 mL into a 5 mL volumetric flask and adding p.a. ethanol until the mark was reached [39]. For each concentration of the moisturizing gel working solution, 3 mL was taken, transferred to a dark vial, and 2 mL of DPPH solution was added. The solution was incubated for the specified operating time, and its absorbance was measured at the maximum wavelength obtained using a UV-Vis spectrophotometer.

Data Analysis Technique

Antioxidant activity was calculated using the percentage of DPPH absorption inhibition, according to the following formula: [40]

$$\% \text{ Inhibition} = \frac{(Blank\ absorbance - Sample\ absorbance)}{Blank\ absorbance} \times 100$$

The calculated results were plotted in a linear regression equation, with the preparation concentration (ppm) as the x-axis and the percentage inhibition value as the y-axis, yielding the r value (correlation coefficient). The IC_{50} value was calculated using the equation: [14].

$$y = bx + a$$

Data from the organoleptic test, homogeneity test, and stability test were analyzed visually using descriptive statistics, while the pH test, spreadability test, adhesion test, and viscosity test were analyzed using one-way ANOVA. If the significance value is >0,05, no significant difference is found; if the significance value is [19].

Results and Discussion

Results of Celery Leaf (*Apium graveolens* L.) Extraction

Extraction of 600 grams of celery leaf crude drug (*Apium graveolens* L.) yielded a concentrated extract with a yield of 44,2%. This result reflects the amount of extract successfully obtained from the crude drug, where a higher percentage indicates a greater yield. The value obtained in this study meets the criteria by exceeding the minimum threshold set by the Farmakope Herbal Indonesia, which is not less than 10% [31]. The resulting concentrated extract is a deep, almost blackish brown color and has the characteristic aroma of celery leaves. The yield results for the concentrated extract are shown in **Table 2**.

Table 2. Yield Results of Celery Leaf Extract

Sample Weight (kg)	Raw Herb Weight (gram)	Extract Weight (gram)	Yield (%)
5	600	294,985	44,2

Results of the Moisturizing Gel Formulation Evaluation

Organoleptic Test

The organoleptic test involved observing the texture, color, and odor of the moisturizing gel formulation. The organoleptic test was conducted to develop a gel formulation with an appealing color, an odor acceptable to users, and a texture that is comfortable to apply, given the topical nature of the formulation, which requires careful attention to aesthetic aspects [14]. The results of the organoleptic test are shown in **Table 3.** Based on these results, the intensity of the color is influenced by the extract concentration; the higher the extract concentration used, the more intense the color of the resulting gel formulation [13].

Homogeneity Test

Homogeneity testing of semisolid dosage forms is very important because it ensures the uniform distribution of the active ingredient in each [32]. A preparation is considered homogeneous if all particles observed under a microscope are evenly distributed without any clumping on any side [14]. The test results

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are shown in **Table 4.** Based on these results, it can be concluded that all ingredients and active ingredients used during the formulation process are evenly mixed [32].

Table 3. Results of Organoleptic Testing of the Celery Leaf Extract Moisturizing Gel Preparation

Formulation	Texture	Color	Odor
F0	Thick	Clear white	Characteristic of the material
F1	Semi-solid	Deep light green	Characteristic of celery extract
F2	Semi-solid	Deep dark green	Characteristic of celery extract
F3	Semi-solid	Deep dark green	Characteristic of celery extract

Table 4. Results of the Homogeneity Test for the Celery Leaf Extract Moisturizing Gel Preparation

Formulation	Homogeneity	Remarks
F0	No particles	Meets requirements
F1	No particles	
F2	No particles	
F3	No particles	

pH Test

The pH test was conducted to measure the alkalinity or acidity level of the moisturizing gel formulation. pH measurement is a key physicochemical parameter in gel formulations because it plays a crucial role in the effectiveness of the active ingredient, the stability of both the active ingredient and the formulation as a whole, as well as user comfort on the skin [33]. The pH test results are shown in **Table 5.**

Based on these results, all formulations of the celery leaf extract (*Apium graveolens* L.) moisturizing gel fall within the pH range considered suitable for the skin, namely 4,5–8. Topical formulations should have a pH close to that of the skin to facilitate diffusion into the skin layers. According to SNI 16-4399-1996, the normal pH range for skin is between 4,5 and 7,5 [34].

The pH test results for the celery leaf extract (*Apium graveolens* L.) moisturizing gel formulation were analyzed using one-way ANOVA. The analysis results showed a pH test significance value of 0,000 (<0,05), indicating a significant difference among the various concentrations of the moisturizing gel formulations.

Table 5. pH Test Results for the Celery Leaf Extract Moisturizing Gel Formulations

Formulation	Replication 1	Replication 2	Replication 3	Average	Standard
F0	7,1	6,8	6,6	6,8	4,5-8
F1	5	4,9	4,9	5	
F2	4,5	4,5	4,5	4,5	
F3	4,5	4,5	4,5	4,5	

Spreadability Test

The spreadability test aims to evaluate the speed of spread and the smoothness of the gel formulation on the skin's surface. Good spreadability is one indicator that a moisturizing gel formulation is easy to apply [33]. The results of the spreadability test are shown in **Table 6.**

Table 6. Spreadability Test Results for Celery Leaf Extract Moisturizing Gel Formulations

Formulation	50 gr Load	100 gr Load	150 gr Load	Average	Standard
F0	5,88	6,2	6,26	6,11	5-7 cm
F1	5,54	6,02	6,13	5,9	
F2	5,26	5,79	6,01	5,7	
F3	5,07	5,26	5,28	5,2	

Based on these results, all formulations of the moisturizing gel containing celery leaf extract (*Apium graveolens* L.) exhibit a spreadability within the standard range for semisolid formulations, namely 5-7 cm. Spreadability can be influenced by the formulation's viscosity; the higher the viscosity, the lower the spreadability. The relationship between spreadability and viscosity is inverse; thus, an increase in formulation viscosity will decrease the spreadability value [35].

The results of the spreadability test for the celery leaf extract (*Apium graveolens* L.) moisturizing gel formulation were analyzed using one-way ANOVA. The analysis results showed a significance value for the

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spreadability test of 0,019 (<0,05), indicating a significant difference among the various concentrations of the moisturizing gel formulations.

Adhesion Test

The adhesion test was conducted to determine the extent to which the gel formulation can adhere to the skin over a specific period of time so that it functions optimally [32]. The results of the formulation's adhesion test are shown in **Table 7.**

The results indicate that all formulations of the celery leaf extract (*Apium graveolens* L.) moisturizing gel met the adhesion criteria, namely not less than 1 second. The longer the gel formulation's adhesion time, the more effective it is at ensuring thorough absorption of the active ingredients. The results of the adhesion testing of the gel formulations are related to their spreadability; the lower the spreadability, the longer it takes for the gel formulation to adhere, whereas higher spreadability allows for faster adhesion due to the gel's thicker consistency [37].

The results of the adhesion test for the celery leaf extract (*Apium graveolens* L.) moisturizing gel formulation were analyzed using one-way ANOVA. The analysis results showed a significance value for the adhesion test of 0,029 (<0,05), indicating a significant difference among the various concentrations of the moisturizing gel formulations.

Table 7. Adhesion Test Results for Celery Leaf Extract Moisturizing Gel Formulations

Formulation	Replication 1	Replication 2	Replication 3	Average	Standard
F0	1,94	1,52	1,35	1,60	>1 second
F1	2,66	2,34	3	2,66	
F2	7,02	17,1	10,03	11,38	
F3	5,8	16,72	23,01	15,17	

Viscosity Test

Viscosity testing is used to determine the viscosity of gel formulations, where the viscosity value indicates the resistance of a liquid to flow [33]. Viscosity describes the thickness of a formulation, which is directly related to its spreadability and adhesion [34]. The viscosity test results are shown in **Table 8.**

These results indicate that all formulations of the celery leaf extract (*Apium graveolens* L.) moisturizing gel fall within the viscosity range for gel formulations, namely 3.000–50.000 mPa•s. An increase in extract concentration raises the solid content in the formulation, and interactions between these solid particles can increase the overall viscosity of the formulation [38].

The viscosity test results for the moisturizing gel formulation containing celery leaf extract (*Apium graveolens* L.) were analyzed using one-way ANOVA. The analysis results showed a significance value of 0,000 (<0,05), indicating a significant difference among the various concentrations of the moisturizing gel formulations.

Table 8. Viscosity Results of Celery Leaf Extract Moisturizing Gel Formulations

Formulation	Replication 1	Replication 2	Replication 3	Average	Standard
F0	4.000 mPa·s	3.966 mPa·s	2.100 mPa·s	3.355 mPa·s	3.000-50.000 mPa•s
F1	5.000 mPa·s	4.766 mPa·s	4.666 mPa·s	4.810 mPa·s	
F2	8.466 mPa·s	7.933 mPa·s	8.299 mPa·s	8.232 mPa·s	
F3	9.199 mPa·s	8.333 mPa·s	7.699 mPa·s	8.410 mPa·s	

Stability Test

Stability testing using a centrifuge is a method for assessing gel stability by observing the effect of mechanical forces on the phase separation process. These mechanical forces provide insight into the extent of the impact of gravitational forces on the storage stability of the gel over a one-year period [39]. The results of the stability test are shown in **Table 9.**

The results indicate that no phase separation occurred in any of the formulations of the moisturizing gel containing celery leaf extract (*Apium graveolens* L.) after centrifugation testing. It can therefore be concluded that the resulting moisturizing gel formulations are stable during storage for one year [24].

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Table 9. Stability Test Results for Celery Leaf Extract Moisturizing Gel Formulations

Formulation	Stability	Remarks
F0	No phase separation	Meets requirements
F1	No phase separation	
F2	No phase separation	
F3	No phase separation	

Antioxidant Activity Test Results

Antioxidant Activity in the Positive Control

Antioxidant activity testing of vitamin C as a positive control was conducted using the DPPH (*2,2 diphenyl-1-picrylhydrazyl*) method with a UV-Vis spectrophotometer. As shown in **Table 10**. the absorbance of vitamin C was measured at a wavelength of 515 nm at concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.

The DPPH method is based on the ability of antioxidant compounds to reduce DPPH free radicals through electron transfer (ET) or hydrogen atom transfer (HAT) mechanisms, so that DPPH which is originally purple with maximum absorption is converted into a pale yellow reduced form. A decrease in absorbance indicates the ability of phenolic and flavonoid compounds to scavenge free radicals, expressed as the IC_{50} value; the lower the IC_{50} value, the higher the antioxidant activity of the tested sample [40].

Based on **Table 10** and **Figure 1**. the IC_{50} value for vitamin C is 7,4 µg/mL, obtained from the linear regression equation y = 6,0285×−5,627 with a coefficient of determination R^2 = 0,9207. A low IC_{50} value indicates that vitamin C possesses very strong antioxidant activity, since a smaller IC_{50} value means a lower concentration of the compound is required to inhibit 50% of DPPH free radicals [41]. Antioxidant activity is categorized as very strong if the obtainedIC_{50} value is <50 µg/mL [42]. Thus, vitamin C, used as the positive control in this study, falls into the category of very strong antioxidants.

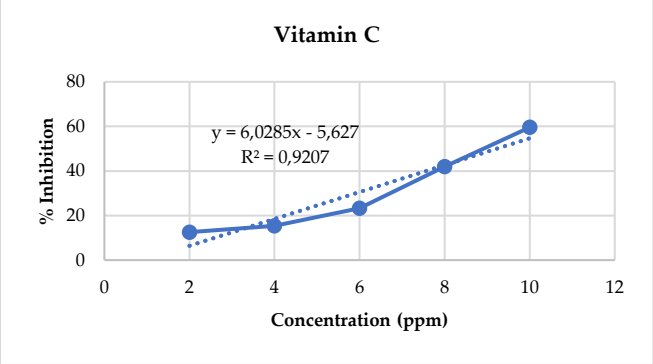

Figure 1. Graph of the Average Relationship Between % Inhibition and Vitamin C

Table 10. Results of Vitamin C Antioxidant Activity Testing

Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (µg/mL)
DPPH	**0,836**		
2	0,731	12,55	7,4
4	0,707	15,43	
6	0,642	23,20	
8	0,485	41,98	
10	0,338	59,56	

A comparison with the results of a study conducted by [43] shows that the IC_{50} value for vitamin C was 7,10 µg/mL, whereas the IC_{50} value from this study was 7,4 µg/mL; thus, the IC_{50} value in this study indicates slightly lower antioxidant activity. Nevertheless, the IC_{50} valuesfrom the previous study and this study still indicate very strong antioxidant activity, and this relatively small difference remains within the range of experimental tolerance. This indicates that the results of this study are consistent with previous research.

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Vitamin C is a nutrient that acts as an antioxidant and is effective in neutralizing free radicals that can damage cells or tissues, including protecting the lens from oxidative damage caused by radiation. As a natural antioxidant, it is frequently used as a reference compound in antioxidant activity testing because natural antioxidants are safer and do not cause toxicity [44]. Vitamin C is used as a reference (positive control) in antioxidant activity testing because it has been proven to possess high and stable antioxidant capacity [41]. Ascorbic acid (vitamin C) plays a role in neutralizing free radicals through an electron donating mechanism, thereby protecting cells from oxidative damage [45].

Antioxidant Activity of a Moisturizing Gel Formulation Containing Celery Leaf Extract (*Apium graveolens* L.)

The antioxidant activity of the moisturizing gel formulation containing celery leaf extract (*Apium graveolens* L.) was tested using the DPPH (*2,2-diphenyl-1-picrylhydrazyl*) method, a quantitative method for determining antioxidant capacity using a UV-Vis spectrophotometer. As shown in **Table 11**. the absorbance of the celery leaf extract (*Apium graveolens* L.) moisturizing gel samples was measured at a wavelength of 515 nm at concentrations of 4.500 ppm, 5.000 ppm, 5.500 ppm, 6.000 ppm, and 6.500 ppm.

The IC_{50} values of the four formulations indicated antioxidant activity, specifically -3968,6 µg/mL for F0, F1 at 7607,1 µg/mL, F2 at 4179,3 µg/mL, and F3 at 3060,2 µg/mL; thus, the antioxidant activity of the moisturizing gel formulation containing celery leaf extract (*Apium graveolens* L.) is considered weak because the IC_{50} value is >200 µg/mL. Specifically, a compound is considered a very strong antioxidant if the IC_{50} value is <50 µg/mL, strong if the IC_{50} value is 50-100 µg/mL, and moderate if the IC_{50} value is 151-200 µg/mL [42]. However, this indicates that as the concentration of celery extract in the moisturizing gel formulation increases, the IC_{50} value decreases, resulting in increased antioxidant activity.

In this study, the relationship between concentration and percentage of inhibition was analyzed using linear regression. Based on **Figure 2**. the regression results show that F0 has the linear equation y = -0,0023×-40,872 with a coefficient of determination R^2 = 0,7907, while F1 has the linear equation y = 0,0042×-18,05 with a coefficient of determination R^2 = 0,9242; F2 has the linear equation y = 0,0072×-19,909 with a coefficient of determination R^2 = 0,8877; and F3 has the linear equation y = 0,0126×-11,441 with a coefficient of determination R^2 = 0,9832. An R^2 value close to one indicates that the relationship between concentration and percentage of inhibition exhibits a very strong correlation [46].

In comparison, a previous study reported that celery ethanol extract had an IC_{50} value of 59,429 ppm, which is classified as having strong antioxidant activity [47], and another previous study also reported that all three concentrations of celery herb extract lotion formulations exhibited very strong antioxidant activity [12]. This study showed a decrease in antioxidant activity in moisturizing gel formulations containing celery leaf extract (*Apium graveolens* L.) across various concentrations, placing them in the "weak" category. This is attributed to the concentration of the test samples: as the concentration increases, absorbance decreases and the inhibition percentage increases; conversely, as the concentration decreases, absorbance increases and the inhibition percentage decreases [48]. This condition may also be attributed to CMC-Na, a hydrophilic polymer that swells upon contact with the medium, causing the active compounds to gradually diffuse out through the matrix pores, while some phenolic compounds and flavonoids remain trapped within the polymer network. As a result, the amount of antioxidant compounds available to react with DPPH radicals is lower compared to the pure extract, so the IC_{50} value ofthe gel formulation is higher and the antioxidant activity appears lower [45].

In qualitative observations, the mixture of DPPH solution with the moisturizing gel formulation of celery leaf extract (*Apium graveolens* L.) at concentration F3 showed a color change from purple to yellow, indicating the presence of antioxidant activity. The discrepancy between quantitative and qualitative observations is due to the gel system being a complex matrix that can retain active compounds, cause turbidity, and affect homogeneity and absorbance readings; thus, the visible color change does not necessarily reflect the magnitude of antioxidant activity quantitatively. Additionally, technical factors such as the calibration conditions of the spectrophotometer may also potentially influence the obtained absorbance values [50].

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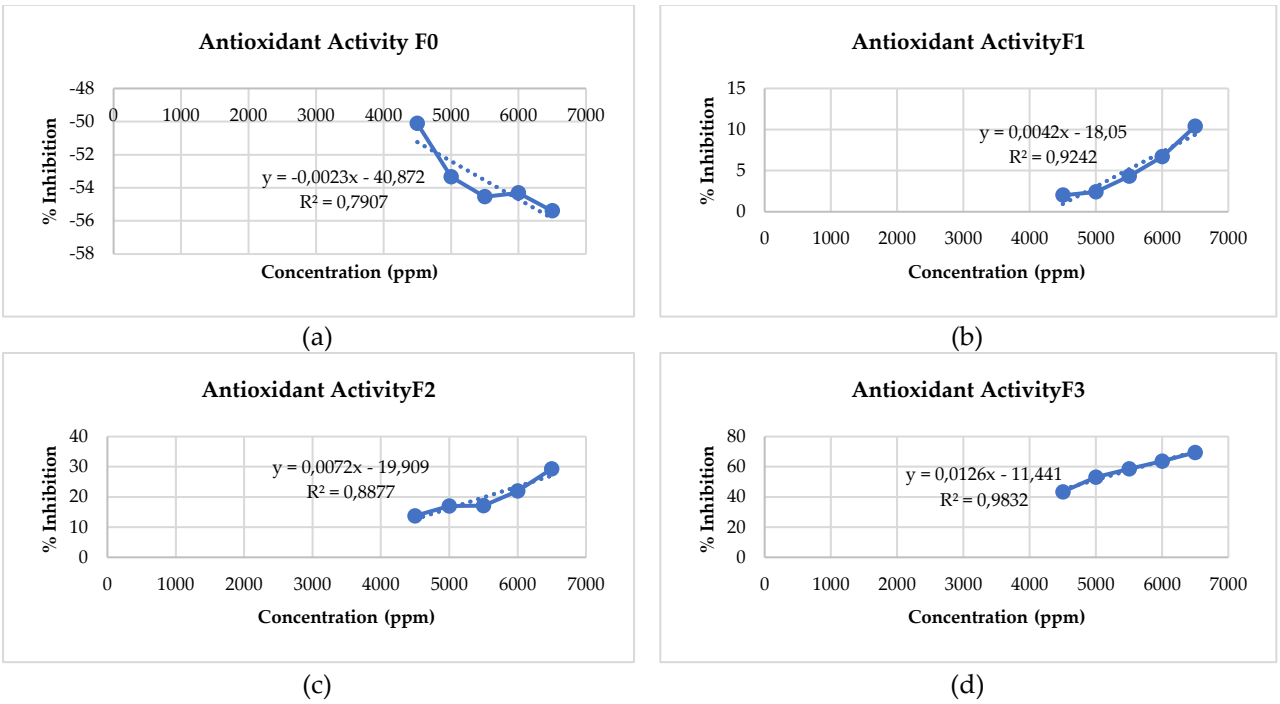

Figure 2. Graphs showing the average relationship between % inhibition and (a) Moisturizing Gel F0, (b) Moisturizing Gel F1, (c) Moisturizing Gel F2, and (d) Moisturizing Gel F3

Table 11. Results of the Antioxidant Activity Test for Moisturizing Gel Formulations F0, F1, F2, and F3

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (µg/mL)
	DPPH	**0,836**		
F0	4.500	1,255	-50,12	-3968,6
	5.000	1,282	-53,35	
	5.500	1,292	-54,54	
	6.000	1,290	-54,31	
	6.500	1,299	-55,4	
F1	4.500	0,820	2	7607,1
	5.000	0,816	2,4	
	5.500	0,800	4,3	
	6.000	0,780	6,7	
	6.500	0,749	10,4	
F2	4.500	0,721	13,75	4179,3
	5.000	0,694	17	
	5.500	0,693	17,10	
	6.000	0,652	22,01	
	6.500	0,591	29,31	
F3	4.500	0,473	43,42	3060,2
	5.000	0,393	53	
	5.500	0,347	58,49	
	6.000	0,304	63,63	
	6.500	0,255	69,49	

The concentration at which the antioxidant activity of the moisturizing gel formulation containing celery leaf extract (*Apium graveolens* L) was highest was greater than that of vitamin C as the positive control. This finding is consistent with previous studies that used higher sample concentrations compared to vitamin C as the positive control [51]. This is because the extract contains a complex mixture of several secondary metabolites, whereas vitamin C is a pure compound with strong antioxidant activity; therefore, a higher concentration of the extract is required to achieve a DPPH radical inhibition percentage equivalent to that of vitamin C [52].

Based on the results of the antioxidant activity of vitamin C as a positive control and the moisturizing gel formulation containing celery leaf extract (*Apium graveolens* L.), vitamin C was found to have a higher antioxidant level compared to the samples. This study is consistent with previous research, which found that

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vitamin C exhibited greater antioxidant activity than the samples [43]. This is because vitamin C is a pure antioxidant compound with a very high ability to scavenge free radicals, enabling it to demonstrate strong antioxidant activity, whereas plant extracts are complex mixtures consisting of several secondary metabolites, only some of which contribute to antioxidant activity [53].

Conclusion

Based on the results of the study, it can be concluded that celery leaf extract (*Apium graveolens* L.) can be formulated into a moisturizing gel preparation in four concentrations: F0 (0%), F1 (5%), F2 (15%), and F3 (25%), which demonstrated good physical properties such as organoleptic testing, homogeneity, pH, spreadability, adhesion, viscosity, and stability meeting general standards for gel formulations. Celery leaf extract influences the physical properties of the moisturizing gel formulation, particularly in terms of organoleptic testing, pH, spreadability, adhesion, and viscosity of the formulation. Antioxidant activity testing of the celery leaf extract-based moisturizing gel formulations showed that the IC_{50} were -3968,6 µg/mL for F0, 7607,1 µg/mL for F1, 4179,3 µg/mL for F2, and 3060,2 µg/mL for F3; thus, the antioxidant activity can be considered weak since the IC_{50} values were >200 µg/mL. For future research, stability tests such as cycling tests or freeze-thaw tests should be conducted to determine the effect of storage on the physical properties of the celery leaf extract moisturizing gel formulation; safety tests such as irritation or sensitization tests should be performed as a preliminary step before further product development; modifications should be made by increasing the extract concentration or adjusting the formulation to achieve meaningful antioxidant activity; it is necessary to conduct antioxidant activity tests on the celery leaf extract moisturizing gel formulation using other methods, such as the ABTS method to measure total antioxidant capacity or the FRAP method to assess reducing capacity; and it is necessary to conduct in vivo efficacy tests using volunteers to measure the moisturizing and antioxidant effects on the skin.

Conflict of Interest

The authors declare that there are no conflicts of interest, whether personal or financial, that could potentially influence or distort the results of this study.

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