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Research Article
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The Effects of Varying Saffron Extract Concentration on the Physical Quality, Stability and Antioxidant Activity Tests of Gel Dosage Form

Nadia Pramasari, Fenita Shoviantari, Yuliana Rumwarin, Ninis Yuliati, Ade Giriayu Anjani, Asih Imulda Hari Purwani, and Sugiyartono

Fakultas Farmasi, Institut Ilmu Kesehatan Bhakti Wiyata Kediri, Kediri, 64114, Indonesia

Correspondence: Nadia Pramasari
Email: nadia.pramasari@iik.ac.id

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ABSTRACT: *Saffron (Crocus sativus L) is a plant that contains antioxidants, which is known as "red gold" and belongs to the iris family (Iridaceae). Saffron stigmas are reported to have antioxidant activity to prevent free radicals. This study aimed to determine the effects of varying saffron extract concentrations on the physical quality, stability, and antioxidant activity of the gel dosage form. There were 3 gel preparation formulations containing 1% (F1), 2% (F2), and 3% (F3) saffron extract. Physical quality tests for the saffron extract gel were included organoleptic test, homogeneity test, pH test, adhesion test, and spreadability test. A stability test was conducted at 25 ± 2°C for 1 month. Antioxidant activity test was performed using DPPH. The results of the physical quality tests, stability tests, and antioxidant tests were analyzed using SPSS. The results of the saffron extracts in gel F1, F2, and F3 met the requirements, and no differences in F1, F2, and F3. The stability test revealed differences in adhesiveness among F1, F2, and F3. The antioxidant activity test of the saffron extract gel dosage form was included in the moderate antioxidant category, with the IC_{50} values of formulation 1 (195.25 ppm), formulation 2 (178.31 ppm), and formulation 3 (159.26 ppm). The results of the antioxidant activity test on saffron extract also showed a moderate category. This study showed that all physical quality tests met the requirements, the preparation was unstable during storage, and the antioxidant activity was categorized as moderate.*

Keywords: *antioxidant activity tests; gel; physical quality tests; saffron; stability tests*

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1. Introduction

Skin is an organ that covers the entire surface of the body and provides protection from the environment. Skin can experience aging, which has an impact on its physiological and functional decline. The factors that play a role in skin aging are internal factors, such as hormones, genetics, and cellular metabolism, while external factors come from UV exposure, pollution, and other chemicals [1]. The presence of these triggers can lead to the formation of free radicals, which contribute to premature skin aging.

Free radicals are molecules that contain unpaired electrons and are very reactive [2]. Free radicals can be prevented with antioxidants, which donate electrons to free radicals, stabilizing them and protecting cells [3]. The body can produce antioxidants, but the capacity of antioxidant production is lower than the speed of free radical production [4]. Some efforts are required to prevent the formation of free radicals through the application of additional antioxidants.

One plant that contains antioxidants is saffron, known as "red gold". One of the important parts of saffron, with a high antioxidant content, is the stigma. Saffron stigmas contain 150 components, including lipophilic and hydrophilic carbohydrates, amino acids, proteins, alkaloids, flavonoids, phenolic compounds, and other chemical compounds [5]. The main components of the stigmas are crocin, crocetin, picrocrocin, saffranal, and flavonoids, including quercetin and kaempferol [6].

Saffron stigmas are reported to have the highest antioxidant activity when compared to the leaves and corm of the saffron plant, which are indicated by the highest phenolic and flavonoid content [5]. The IC_{50} values for water extracts of stigmas 304 ± 1.79 ppm, corm 465 ± 3.52 ppm, and leaf could not be determined [5]. The research shows that saffron stigma can reduce oxidative stress in plants, fungi, and bacteria [7]. Safranal, contained in the saffron plant, has antioxidant activity and an effective sun protection factor (SPF) against premature skin aging because it has strong photoprotective abilities [8].

Saffron can also reduce the production of a pigment called melanin [4]. Formulations containing *C. sativus* extract cause significant depigmentation and anti-rhythmic effects on human skin. Saffron is known to reduce a skin condition called erythema, which is characterized by inflammation, redness, or rashes. Application of a formulation containing 3% of *C. sativus* extract to human skin may be helpful in the treatment of melanoma [4].

Saffron contains over 160 volatile and aromatic compounds, primarily terpenes, terpene alcohols, and their esters, requiring a formulation base that capable on protecting their stability [4]. The gel formulation was chosen because it provides better release of active ingredients and enhances penetration into the skin. Furthermore, the saffron extract in the gel formulation is expected to have good antioxidant activity, easy to apply, non-sticky, and provide a pleasant cooling sensation upon application.

In gel formulations, product quality, safety, and stability are essential. To ensure product quality and safety, the physical quality was tested, including organoleptic test, pH test, homogeneity test, spreadability test, and adhesion test. Drug stability is important because it impacts effectiveness, safety, and quality. Stability can be detected through changes in the physical quality of the product and the active ingredient content. This study also examined the antioxidant activity of saffron extract gel.

Based on this background, researchers made three gel preparation formulas with variations in saffron extract concentration, namely F1 (1%), F2 (2%), and F3 (3%), This research contributes to inform the effect of variations in saffron extract concentration on physical quality, stability, and antioxidant activity of saffron extract gel.

2. Material and methods

2.1. Materials

The ingredients used in this research were dried stigma of saffron (*Crocus sativus* L.) from

Nazanin (Iran), carbomer (Acrypol®, India), menthol (Polar Bear®, China), propylene glycol (PG Dow®, Indonesia), methyl paraben (Ueno Fine Chemicals Industry, Japan), propyl paraben (Ueno Fine Chemicals Industry, Japan), TEA (Petronas®, Malaysia), distilled water (Water One®, Indonesia), DPPH (Sigma Aldrich®, USA), and vitamin C (Merck®, China).

2.2. Instrument

The instruments used were analytical balance (Mettler Toledo® ME204, Switzerland), UV-VIS Spectrophotometer (Shimadzu® UV-1780, China), pH test (Mettler Toledo®, Swiss), adhesive test instrument, and spreadability test instrument.

2.3. Methods

2.3.1. Stigma saffron extract

Dried stigma saffron is made in three different concentrations in each formula: 1%, 2% and 3%. Formula 1 contains 1 gram, formula 2 contains 2 grams, and formula 3 contains 3 grams of dried stigma saffron. Each of them was macerated with 100 grams of water as a solvent for 24 hours. The stigma of saffron was then separated by filtration.

2.3.2. Phytochemical Screening

2.3.2.1. Flavonoid

One drop of concentrated HCl and methanol were added to the extract. Magnesium powder was then added to form an orange, red, or blue solution, indicating the flavonoids presence [7].

2.3.2.2. Phenolic

The extract was added with two drops of 10% $FeCl_3$ solution. If the sample turned into blackish blue, dark blue, greenish black, or brownish-black, it indicated that the sample contains phenolic groups [7].

2.3.3. Gel preparation

The formula used to make the gel is shown in Table 1. All ingredients were prepared and weighed according to the formula to make 100 grams of gel. Hot water (70°C) was added to a mortar, then carbomer was added to form a gel. The gel base was transferred into a beaker and placed on a hot plate at temperature of 50°C and stirring speed of 200 rpm using a magnetic stirrer. Methylparaben and propylparaben were dissolved in propylene glycol and added to the gel. Then, menthol was dissolved in saffron extract and mixed into the gel. After that, TEA was added to form the gel.

2.3.4. Physical quality test of gel

2.3.4.1. Organoleptic test

Visual observation of shape and color was performed by examining the preparation on a white background under lighting. Odor observation was carried out by smelling the formed gel [8].

Table 1. Formulation of saffron stigma extract gel

No.	Materials	Function	Formulation (% w/w)		
			F1	F2	F3
1.	Stigmas saffron extract 1%	Active ingredient	67	-	-
2.	Stigmas saffron extract 2%	Active ingredient	-	67	-
3.	Stigmas saffron extract 3%	Active ingredient	-	-	67
4.	Carbomer	Gelling agent	1	1	1
5.	Menthol	Enhancer	0.5	0.5	0.5
6.	Propylene glycol	Humectant	10	10	10
7.	TEA	Alkalizing agent	0.8	0.8	0.8
8.	Methyl paraben	Preservative	0.2	0.2	0.2
9.	Propil paraben	Preservative	0.5	0.5	0.5
10.	Aquadest	Solvent	20	20	20

2.3.4.2. Homogeneity test

The gel was weighed 0.1 grams on an object glass and then observed whether the particles or aggregates formed in the sample [8].

2.3.4.3. pH test

The pH meter electrode was washed, rinsed with distilled water, and dried [9]. The instrumen was calibrated using standard buffer solutions at pH 4 and 7. Then, the electrode was inserted into the prepared sample [8].

2.3.4.4. Adhesive test

The gel was placed on a object glass in the test equipment. The object glass was covered with another object glass, weighed for 1 kg, and then left for 5 minutes. The object glass was pulled with a freely attached lever of 80 g. The time was recorded when the object glass was released [10].

2.3.4.5. Spreadability test

0.5 grams of gel was placed on the test glass, covered with a cover glass for 1 minute, and the spread was measured. A 50-gram load was placed on the glass, and after 1 minute, the spread value was recalculated. The load was repeatedly added up to 250 grams [10].

2.3.5. Real-time stability test

The physical stability test of the gel was conducted by evaluating several test parameters, including organoleptic test, homogeneity test, pH test, adhesive power test, and spread power test, at 0, 2, and 4 weeks. The stability test was conducted at a temperature of 25 ± 2°C [11].

2.3.6. Antioxidant activity test using DPPH method

2.3.6.1. Determination of DPPH maximum wavelength

The DPPH reagent was made by weighing 15.8 mg of DPPH and dissolving it with ethanol pro analysis up to 100 mL in a volumetric flask to obtain a solution with a concentration of 0.4 mM [12]. 2 ml of 0.4 mM DPPH solution was pipetted, 2 ml of ethanol was added, homogenized, and incubated for 30 minutes in a dark place [1]. The absorbance was observed with a visible spectrophotometer at a wavelength of 517 nm [13].

2.3.6.2. Preparation of vitamin C solution 100 ppm (positive control)

Vitamin C (10 mg) was dissolved in 100 mL of ethanol pro analysis in a volumetric flask to obtain a 100 ppm solution. Solutions of 2, 3, 4, 5, and 6 ppm were prepared in 10 mL volumetric flasks, the volume then adjusted with ethanol pro analysis. 2 mL of each standard was mixed with 2 mL of 0.4 mM DPPH solution in a vial, shaken until homogeneous, and incubated for 30 minutes. Absorbance was measured at 517 nm with a visible spectrophotometer. Each measurement was performed in triplicate [14].

2.3.6.3. Gel 1000 ppm preparation

100 mg gel was dissolves with 100 ml ethanol pro analysis in a volumetric flask with a concentration of 1000 ppm. Series standard solutions were made at concentrations of 50, 100, 150, 200, and 250 ppm in a 10 mL volumetric flask; the volume was filled with ethanol pro analysis to the limit mark. The steps to test antioxidant activity were the same as vitamin C evaluation.

2.3.6.4. Saffron extract solution 1000 ppm preparation

100 mg saffron extract was disolved with 100 ml ethanol pro analysis in a volumetric flask, hence the concentration of 1000 ppm saffron extract was achieved. Series solutions of the sample with concentrations 50, 75, 100, 125, 150 ppm were prepared in a 10 ml volumetric flask, the volume was filled with ethanol pro analysis to the limit mark. The steps for testing the antioxidant activity were the same as for vitamin C. The percentage of DPPH inhibition was be used to determine the antioxidant activity of a compound and can be calculated using the following equation [15,16]:

$$\%\ \text{Inhibition} = \frac{\text{Blank absorbance} - \text{sample absorbance}}{\text{Blank absorbance}} \times 100\%$$

The linear regression equation can be used to determine the IC_{50} value of each sample concentra-

tion as written bellow [12]:

$$Y = a + bx$$

Y = % inhibition; x = concentration; a = intercept; b = slope

2.3.7. Data analysis

The statistical analysis was performed using SPSS with the one-way ANOVA method. The analysed data must be normally distributed and homogeneous when tested using the normality test and homogeneity test with a value of (sig>0.05), and if the significance value is p<0.05, continue with the Kruskal-Wallis test. The data for ANOVA were test results of the pH stability test, spreada–bility test, adhesion test, and antioxidant activity test. Homogeneity tests and organoleptic tests were analysed descriptively.

3. Result and discussion

3.1. Phytochemical screening

3.1.1. Flavonoid test

The results of the phytochemical screening test for stigma saffron water extract showed positive results with ethanol and HCl solutions, because they produced an orange color as presented in Figure 1. This is due to the formation of the $[Mg(OAr)_6)]^{4-}$ complex. The reaction equation can be seen as follows [7]:

$$Mg_{(s)} + 2HCl_{(l)} \rightarrow MgCl_{2(aq)} + H_{2(g)}$$

$$MgCl_{2(aq)} + 6\ ArOH_{(s)} \rightarrow [Mg(OAr)_6]^{4-}{}_{(aq)} + 6H^{+}{}_{(aq)} + 2Cl^{-}{}_{(aq)}$$

3.1.2. Phenolic test

The phytochemical screening test for phenolic compounds of stigma saffron water extract using $FeCl_3$ solution showed a brownish-black color, as displayed in Figure 2. This color change indicates that the saffron stigma water extract contains phenolic compounds. The extract solution, which changes to a brownish-black color, occurs due to the reaction, which is stated as follows [7]:

$$FeCl_{3(aq)} + 6ArOH_{(s)} \rightarrow 6H^{+}{}_{(aq)} + 3Cl^{-}{}_{(aq)} + [Fe(OAr)_6]^{3-}{}_{(aq)}$$

3.2. Evaluation of physical quality of gel

The results of the evaluation of gel physical quality include organoleptic, homogeneity, pH, adnesion, and spreadability, as shown in Table 2.

3.2.1. Organoleptic test

Organoleptic observations of gel preparations were carried out visually by observing shape,

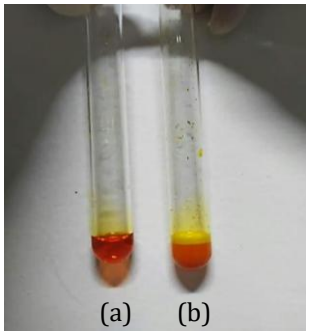


Figure 1. The result of flavonoid test (a) before tes–ting (b) after testing

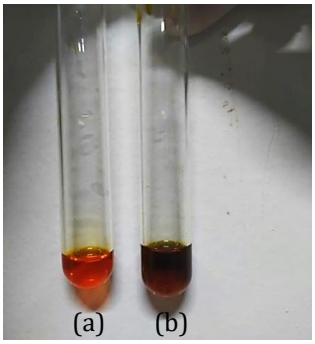


Figure 2. The result of phenolic test (a) before tes–ting (b) after testing

color, and odor. The results show that the three formulations have different colors because of differences in the concentration of saffron stigma extract. In the week-4, each formulation of gel experienced a color change caused by oxidation [17]. This is the same as previous research regarding the stability of bitter gourd fruit extract gel which experienced color changes after week-4 of storage [10].

3.2.2. Homogeneity test

The homogeneity test aimed to see the absence of particles or aggregates in the gel product. Homogeneity test is one of the factors that can influence the quality of the gel [8]. This was shown by all the gel particles being evenly dispersed on the glass object and did not show any agglomeration. This is in line with research evaluating *aloe vera* gel, which showed a homogeneous composition, absence of coarse granules, and no agglomeration [18].

3.2.3. pH test

The pH test aims to determine the suitability pH of the gel preparation in the range of 4.5-6.5 [18]. This pH test is considered important because a pH value less than this range can irritate the skin, while a pH value above this range can cause swelling and dry skin [18]. The results of the pH test during storage at week-0, week-2, and week-4, are shown in Table 2, all formulations are within the normal pH range of the skin. pH can be influenced by the environment, such as changes in temperature [19]. The statistical test results show a significant value of > 0.05, which means there was no significant difference during the storage period, so the pH of the gels were stable during the storage period.

3.2.4. Adhesive test

The adhesion test aimed to determine how long the preparation can adhere or stick to the skin so that it optimal in absorbing the drug content. The requirement for good adhesion is more than 4 seconds [8]. The long stickiness of the preparation on the skin surface can provide optimal time for the active ingredients to diffuse into the skin [10]. Increasing the concentration of saffron extract did not affect the adhesion test, because the percentage of saffron extract added between formulas was not significantly different. The results of the adhesive strength test during storage at week-0, week-2, and week-4 are shown in Table 2. The results show that the adhesive strength of all gel preparations during the storage period increased from week-0 to week-4. This instability was influenced by the consistency or thicker dosage form, so that the sticking time became longer. The results of data analysis obtained a significance value > 0.05, which means

Table 2. The result evaluation of the physical quality of saffron stigma extract in gel dosage form

Test	Formulation								
	I			II			III		
	Week-0	Week-2	Week-4	Week-0	Week-2	Week-4	Week-0	Week-2	Week-4
Shape	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel
Color	Light orange	Light orange	Yellow	Dark orange	Dark orange	Brownish orange	Brownish orange	Brownish orange	Dark orange
Odor	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol
Homogeneity	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen
pH	5.9 ±0.05	5.9 ±0.06	5.8 ±0.06	6.0 ±0.10	6.0 ±0.06	5.9 ±0.06	6.1 ±0.10	6.0 ±0.06	6.0 ±0.06
Adhesion test (second)	5.24 ±0.02	6.03 ±0.01	7.22 ±0.02	5.37 ±0.06	6.07 ±0.01	7.25 ±0.04	5.47 ±0.02	6.10 ±0.02	7.31 ±0.04
Spreadability (cm)	5.16 ±0.06	4.73 ±0.06	4.10 ±0.10	5.23 ±0.12	4.83 ±0.06	4.16 ±0.06	5.16 ±0.06	4.86 ±0.06	4.20 ±0.10

there is no significant difference during the storage period.

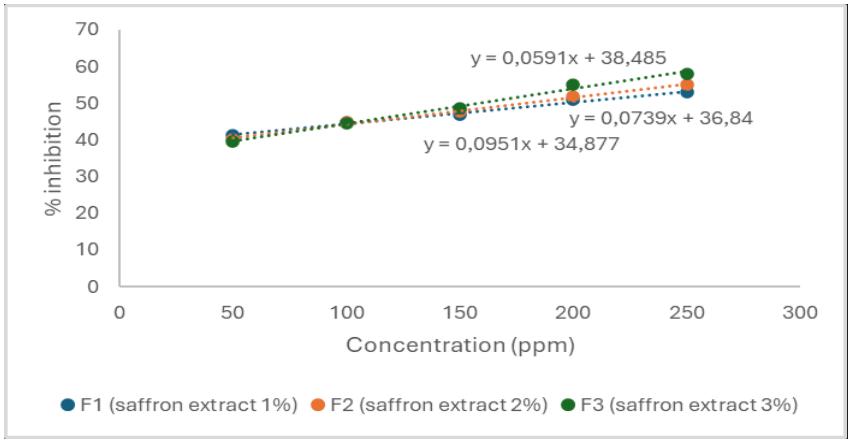
3.2.5. Spreadability test

The spreadability test was carried out to determine the ability of the gel to spread evenly on the skin [20]. The observation results at week-0, week-2, and week-4 were shown in Table 2. The result of a good gel spread is 5-7 cm [8]. An increase in saffron extract concentration in the gel showed no effect on the spreadability test, as the proportion of extract used in each formula was identical. In the week-2 and week-4, the spreadability of the gel decreased, because the consistency of the gel preparation became thicker during the storage period. The results of data analysis obtained a significant value for the spreadability test of > 0.05. There was no significant difference during the storage period. These results are in line with the stability test of bitter melon ethanol extract gel, which showed that the spreadability test decreased during storage from week-2 to week-4 [10].

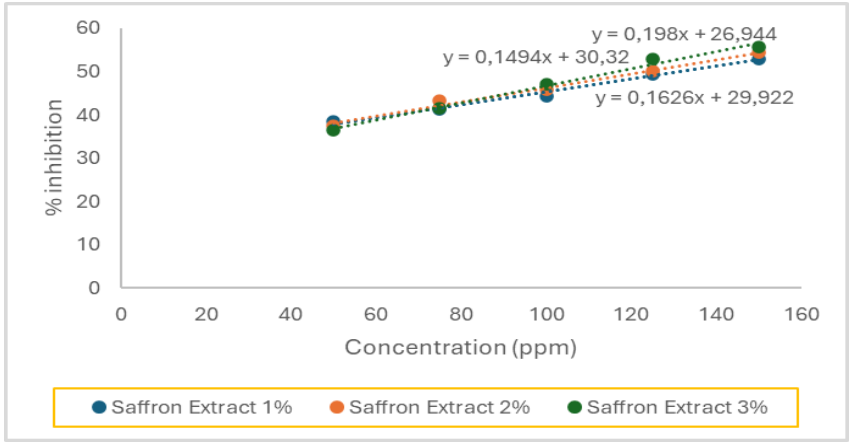
3.3. Antioxidant activity of DPPH

The regression curve to determine the IC_{50} values of saffron extract on gel dosage form and saffron extract is shown in Figure 3, and the IC_{50} value is shown in Table 3. The percent inhibition value increased with increasing the extract concentration. The inhibition value obtained was then used to obtain the regression curve.

Antioxidant activity testing was carried out using the DPPH method using a visible spectrophotometer [21]. DPPH is an organic nitrogen radical that is stable at room temperature. Electrons or hydrogen donated to DPPH form stable molecules. In this study, the maximum wave-



(a)



(b)

Figure 3. The regression curve between % inhibition and concentration of (a) saffron extract in gel dosage form, (b) saffron extract

Table 3. IC_{50} value of vitamin C and saffron extract in gel dosage form and extract form

Sample	Concentration	IC_{50} (ppm)
Vitamin C	Vitamin C 100 ppm	14.35 ± 0.03
Gel	Formula 1 (1% extract)	195.17 ± 2.22
	Formula 2 (2% extract)	178.62 ± 1.57
	Formula 3 (3% extract)	159.19 ± 0.96
Extract	Saffron stigma 1%	131.83 ± 2.38
	Saffron stigma 2%	123.69 ± 0.40
	Saffron stigma 3%	116.30 ± 0.40

length of DPPH was 517 nm. This is in accordance with previous research regarding the maximum wavelength of DPPH [13].

The positive control used in this research was vitamin C, because it is a strong antioxidant that effectively captures free radicals, especially ROS or reactive oxygen compounds [22]. Vitamin C is also a non-enzymatic antioxidant that is soluble in water and works as an electron donor to DPPH free radicals [22]. The IC_{50} value of vitamin C as a positive antioxidant control was 14.32 ppm, which was included in the strong antioxidant category (IC_{50} value ≤ 50 ppm) [7].

The IC_{50} values of saffron stigma extract and gel are shown in Table 3. The IC_{50} values of the three extracts and gel are included in the moderate antioxidant category according to the IC_{50} value parameters of moderate antioxidants (IC_{50} value 100-250 ppm) [7]. The results of this test were considered relatively high compared to the previous research on saffron extract with water solvent, with IC_{50} results of 304 ± 1.79 [5].

Based on the calculation results of the % inhibition listed in Figure 3 and Table 3, the greater extract concentration produces more significant antioxidant activity. Moreover, the antioxidant activity of the saffron in gel dosage form was slightly reduced compared to the pure extract. This was because the gel entrapment of the extract, resulting smaller release of active antioxidant substances compared to saffron extract. These results are in line with other studies, which show that the antioxidant activity of serum preparations is lower than that of plant extracts [23].

4. Conclusion

Phytochemical screening tests showed that saffron extract contains flavonoids and phenolics. For the saffron extracts in gel F1 (1%), F2 (2%), and F3 (3%), physical quality tests were conducted, including organoleptic, homogeneity, pH, adhesion, and spreadability tests, which met the requirements. During the stability test conducted at 25 °C in weeks 0, 2, and 4, physical quality tests were performed, including organoleptic, homogeneity, pH, adhesion, and spreadability tests. The results showed significant differences in the adhesive test among F1, F2, and F3. The results of the antioxidant activity test showed that the saffron extract in gel was in the medium category, with the IC_{50} values of 195.25 ppm (F1), 178.31 ppm (F2), and 159.26 ppm (F3). In the antioxidant activity test, the saffron extract also showed a medium category. Based on the results, all physical quality tests for the saffron gel dosage form met the requirements. The preparation was unstable during storage, and the antioxidant activity was categorized as moderate. In further research, the concentration of the gelling agent can be optimized to improve viscosity, which can affect the stability of adhesion tests during storage.

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