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FORMULATION AND TESTING OF ANTIOXIDANT ACTIVITY COMBINATION OF CALENDULA OIL AND SWEET ORANGE OIL WITH DPPH METHOD

Deni Firmansyah[1], Sulistiorini Indriaty[1*], Nina Karlina[1], Eka Mardiyani[1]
[1]Sekolah Tinggi Farmasi Muhammadiyah Cirebon Jl. Cideng Indah, Kertawinangun, Kedawung, Cirebon, Jawa Barat 45123
**Email Corresponding: s.indriaty82@gmail.com*

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ABSTRACT

Indonesia has many natural ingredients that can be used as primary ingredients for medicines with antioxidant effects. Calendula oil and sweet orange oil have the potential to be antioxidants. This study aimed to determine the stability of cream preparations based on stability test parameters and to determine antioxidant activity using the DPPH method. This study was conducted because the combination of calendula oil and sweet orange oil was tested for antioxidant activity and then formulated in cream dosage forms with ratio of 1:1, 1:2, and 2:1. Antioxidant activity tests were carried out using a combination of calendula oil and sweet orange oil and a cream preparation of a combination of calendula oil and sweet orange oil, which was tested for stability using the cycling test method. Based on the results of the study, the combination of calendula oil and sweet orange oil in a ratio of 1:1 and 1:2 had weak antioxidant activity, as can be seen from the IC50 value of 170.02 ppm, 150.43 ppm, whereas in a 2:1 ratio, it had moderate antioxidant activity because the IC50 value was 143.89 ppm. In the combination cream preparations of calendula oil and sweet orange oil, ratios of 1:1, 1:2, and 2:1 had weak antioxidant activity. It can be seen from the IC50 value of each preparation, which was 188.85 ppm, 170.01 ppm, and 189.61 ppm.

Keywords: Calendula Oil, Sweet Orange Oil, Antioxidant, Cream, DPPH

INTRODUCTION

Generally, radicals cause various diseases. Free radicals are molecules with several components, one of which is the unpaired electron. The negative impact of free radicals can damage cells excessively, accelerating premature aging (Handayani et al., 2018). One way to overcome the negative impact of free radicals is to use antioxidant compounds (Pogaga et al., 2020). Antioxidants are commonly found in plants. Plants with active ingredients such as flavonoids, alkaloids, and terpenoids are natural antioxidants (Purwanto et al., 2017). One of the natural ingredients that can be used in traditional medicine and can be found in Indonesia are sweet orange and calendula flowers.

According to research that has been done, sweet orange essential oil contains limonene (90.66%) and linalylacetate (2.80%) (Singh et al., 2010; Dalam Syarif, 2018). The antioxidant activity of sweet orange essential oil shows 50%-60% against free radicals at a concentration of 100 µg/mL–1000 µg/mL (Frassineti, 2018; dalam Syarif, 2018). The IC50 value affects antioxidant activity, where an IC50 value $<$ 200 µg/mL is considered a good antioxidant (Hanani, 2005; dalam Syarif, 2018).

Calendula flowers contain chemical compounds, including alkaloids, carotenoids, and flavonoids, such as quercetin, lupeol, protocatechuic acid, isorhamnetin, and triterpenoids

(Matysik et al., 2005; Dalam John et al., 2017). Calendula essential oil has moderate antioxidant activity, as shown by its IC50 value of 118.64 ppm (Ashwlayan et al., 2018).

This study used a combination of calendula oil and sweet orange oil because they have a synergistic antioxidant effect, so that they complement each other and improve the therapeutic effect. This study aimed to prepare a cream and determine the stability of the preparation and antioxidant activity of calendula oil and sweet orange oil combination cream preparation. Antioxidant testing was performed using a combination of calendula oil and sweet orange oil, as well as a combination cream preparation of calendula oil and sweet orange oil using the DPPH method.

RESEARCH METHODS

Tools and Materials

This study used tools such as glassware (Pyrex), mixer (Cosmos), analytical balance (OHAUS), pH meters (Metler Toledo), object glasses, stopwatches, glass plates, Brookfield viscometers RV models, cuvettes, water baths, UV-Vis spectrophotometry (Shimadzu Uvmini-1240), and a digital ultrasonic cleaner (Lensent). The main ingredients in this study were calendula oil and sweet orange oil (Rumah Atsiri Indonesia), DPPH (Himedia), Vitamin C (Merck), and cetyl alcohol (PT. Global) triethanolamine (PT. Global), stearate acid (PT. Global), glycerin (PT. Global), methyl parabens (PT. Global), propyl parabens (PT. Global), methanol (PT. Global), sodium metabisulfite (PT. Global), and aquades (PT. Brataco Indonesia).

Research Procedure

1. Provision of raw materials

   The ingredients used in this study were calendula oil and sweet orange oil obtained from Rumah Atsiri, Indonesia.

2. Manufacture of cream formula

Table I. Calendula Oil and Sweet Orange Oil Combination Cream Formula

No	Material	Concentration %		
		F1	F2	F3
1	Calendula oil	1	1	2
2	Sweet orange Oil	1	2	1
3	Cetyl alcohol	2	2	2
4	Triethanolamine	0,75	0,75	0,75
5	Stearate acid	6	6	6
6	Glycerine	10	10	10
7	Methyl paraben	0,1	0,1	0,1
8	Propyl paraben	0,05	0,05	0,05
9	Sodium metabisulfite	0,1	0,1	0,1
10	Aquades	Ad 100	Ad 100	Ad 100

Description: F1: Formula 1 (calendula oil and sweet orange oil 1:1)
F2: Formula 2 (calendula oil and sweet orange oil 1:2)
F3: Formula 3 (calendula oil and sweet orange oil 2:1)

How to make the cream formula:

a. Weigh all ingredients.
b. The oil phase was prepared by melting cetyl alcohol, stearic acid, and propylparaben at 70ºC.
c. The water phase was prepared using methyl parabens, glycerin, sodium metabisulfite, and triethanolamine, and equates heated at 70ºC.
d. The oil phase was mixed with the water phase while stirring until a homogeneous cream base was formed.
e. Calendula oil and sweet orange oil were added and stirred until they were homogeneous. Put in a cream container

3. Cream stability test

A stability test of the cream was performed using a cycling test. The test was conducted by storing the sample at 4 °C for 24 hours and then transferring it to a 40ºC oven for 24 hours. The treatment was performed in 1 cycle. The treatment was repeated for 6 cycles and observations were made with organoleptic parameters, homogeneity, pH, viscosity, flow properties, dispersion, and cream type (Mardikasari et al., 2017). Observations of organoleptic tests, homogeneity, pH, dispersion, and cream type were carried out in cycles 0 to 6. Viscosity tests and flow properties were carried out for 0 and 6 cycles.

a. Organoleptic test

Organoleptic tests are carried out by observing the shapes, odors, and colors of the object of study visually (Hamidah & Priatni, 2019).

b. Homogeneity test

The homogeneity test was performed using 1g of weighed cream applied to a glass plate. Cream preparations are said to be homogeneous if they do not show the presence of particles that clump or mix (Khumaidi, 2015).

c. pH Test

The pH of cream preparations was measured using a pH meter. The pH meter was first calibrated by inserting the electrode into the pH buffer 4 solution and pH 7 buffer solution; then, the electrode was washed and dried again. Take a preparation of 1 gram and diluted using aquadest 10 ml. Then, the electrode was dipped in diluted cream, waiting until letter A appeared on the display/full screen turned to √A. The measurement results were then recorded (Zamzam & Indawati, 2020). A good cream preparation must meet the pH criteria of topical preparations, namely in the interval 4.0-8.0 (Lumentut et al., 2020).

d. Dispersion test

Cream weighed 1 g, then placed on a glass plate covered with another glass plate, and loaded on the cover glass until a total weight of 150 g was allowed to stand for 1 minute. Then, the diameter of the cream spread was measured using a caliper at 4 corner points. Good dispersion was 5-7 cm (Lumentut et al., 2020).

e. Cream type test

This was carried out using the dilution method. The cream was weighed 0.5 grams and then diluted with aquadest as much as 10 ml in a beaker glass. If the cream cannot be diluted with aqua dest, it belongs to the type of water in oil (A/M); however, if the cream can be diluted with aqua dest, it belongs to the type of oil in water (M/A) (Mektildis, 2018).

f. Viscosity and flow properties tests

The viscosity and flow properties were determined using the Brookfield viscometer RV model. The test was performed by adjusting and changing the cream spindle inserted into a glass container, and the spindle that was installed was lowered so that the spindle boundary was dipped in the cream. Then, on the monitor set the rpm and spindle used press the motor on the button and wait until the viscosity and % torque numbers appear. The results of these measurements were then recorded. The viscosity of a good cream is in the range of 2000-50000 cps (Mailana et al., 2016). The flow properties can be obtained by making a curve between the shear stress and shear speed (Dewi et al., 2014).

4. Test the antioxidant activity of a combination of calendula oil and sweet orange oil and a combination cream preparation of calendula oil and sweet orange oil

a. Preparation of solution (preliminary preparation)

1) Preparation of DPPH solution (100 ppm)

Weigh, 10 mg DPPH was added to 100 mL methanol and shaken until homogeneous (Tw et al., 2018).

2) Preparation of Vitamin C master solution (100 ppm)

Weigh 10 mg of Vitamin C were added to 100 mL of methanol and shaken until homogeneous (Tw et al., 2018).

a) Making dilution of Vitamin C solution 2 ppm

Pipettes of 0.2 mL Vitamin C parent solution, and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Tw et al., 2018).

b) Making dilution of Vitamin C solution 4 ppm

Pipettes of 0.4 mL Vitamin C parent solution, and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Tw et al., 2018).

c) Making dilution of Vitamin C solution 6 ppm

Pipettes of 0.6 mL Vitamin C parent solution, and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Tw et al., 2018).

3) Making a solution stock combination of calendula oil and sweet orange oil ratio 1: 1 (1000 ppm)

Weigh 100 mg combination of calendula oil and sweet orange oil ratio 1 of1 was dissolve in methanol to the limit mark of 100 mL, and shaken until homogeneous (Syarif, 2018).

a) Making a dilution of a 40 ppm test solution

0.4 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

b) Making dilution of 80 ppm test solution

0.8 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

c) Making a dilution of a 120 ppm test solution 1.2 mL pipette of the parent solution and adding methanol to the limit mark of 10 mL, shake until homogeneous (Syarif, 2018).

4) Making a solution stock combination of calendula oil and sweet orange oil ratio 1: 2 (1000 ppm)

A 100 mg combination of calendula oil and sweet orange oil ratio of 1 was2 and dissolve in methanol to a limit mark of 100 mL and shaken until homogeneous (Syarif, 2018).

a) Making a dilution of a 40 ppm test solution

0.4 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

b) Making dilution of 80 ppm test solution

0.8 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

c) Making a dilution of a 120 ppm test solution

1.2 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

5) Making a solution stock combination of calendula oil and sweet orange oil ratio 2: 1 (1000 ppm)

Weigh 100 mg combination of calendula oil and sweet orange oil ratio 2 of1 was dissolve in methanol to the limit mark of 100 mL and shaken until homogeneous (Syarif, 2018).

a) Making a dilution of a 40 ppm test solution

0.4 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

b) Making dilution of 80 ppm test solution

0.8 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

c) Making a dilution of a 120 ppm test solution

1.2 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

6) Making a cream solution combination of calendula oil and sweet orange oil ratio 1: 1 (1000 ppm)

Weigh 100 mg of calendula oil and sweet orange oil were mixed in a cream ratio 1 of1 and dissolve in methanol to the limit mark of 100 mL, beat until homogeneous (Syarif, 2018).

a) Making a dilution of a 40 ppm test solution

0.4 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

b) Making dilution of 80 ppm test solution

0.8 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

c) Making a dilution of a 120 ppm test solution

1.2 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

7) Making a cream solution combination of calendula oil and sweet orange oil ratio 1: 2 (1000 ppm)

Weigh 100 mg of calendula oil and sweet orange oil combination cream ratio 1 of2 was dissolve in methanol to the limit mark of 100 mL, beat until homogeneous (Syarif, 2018).

a) Making a dilution of a 40 ppm test solution

0.4 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

b) Making dilution of 80 ppm test solution

0.8 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

c) Making a dilution of a 120 ppm test solution

1.2 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

8) Make a cream solution of calendula oil and sweet orange oil combination of 2: 1 (1000 ppm)

Weigh 100 mg of calendula oil and sweet orange oil combination cream in a ratio of 2:1 was dissolve with methanol to the limit mark of 100 mL, beat until homogeneous (Syarif, 2018).

a) Making a dilution of a 40 ppm test solution

0.4 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

b) Making dilution of 80 ppm test solution

0.8 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

c) Making a dilution of a 120 ppm test solution

1.2 mL pipette of the parent solution and methanol was added to the limit mark of 10 mL and shaken until homogeneous (Syarif, 2018).

b. Measurement of antioxidant activity

1) Determination of maximum wavelength

The solution was prepared using 2 mL of methanol, 1 mL of DPPH solution was added, and the mixture was shaken until homogeneous. The solutions were determined by absorption using a UV-Vis spectrophotometer measured at wavelengths of 400-800nm (Karlina et al., 2023).

2) Determination of operating time

The solution was prepared by adding 2 mL of methanol to 1 mL DPPH and then shaken until homogeneous. Uptake was measured at maximum wavelengths at minutes 0, 5, 10, 15, 20, 25, and 30 (Karlina et al., 2023).

3) Testing of antioxidant activity was performed at several concentrations:

a) Vitamin C solution testing

Vitamin C solutions with concentrations of 2, 4, and 6 ppm were prepared. Each Vitamin C solution (2 mL) was added to 1 mL DPPH solution, shaken until homogeneous, incubated for 10 minutes in the dark, and the absorbance was read at a wavelength of 516nm (Karlina et al., 2023).

b) Samples of calendula oil and sweet orange oil combination solutions at a ratio of 1 with1 concentrations of 40, 80, and 120 ppm.

   A combination solution of calendula oil and sweet orange oil ratio 1 of1 with concentrations of 40, 80, and 120 ppm. Then, 2 mL of each solution was added to 1 mL of DPPH solution, shaken until homogeneous, incubated for 10 minutes in the dark, and the absorbance was read at a wavelength of 516nm (Karlina et al., 2023).

c) Samples of calendula oil and sweet orange oil combination solutions with a ratio of 1:2 concentrations of 40, 80, and 120 ppm.

   A solution of calendula oil and sweet orange oil in a ratio of 1:2 with concentrations of 40, 80, and 120 ppm. Then, 2 mL of each solution was added to 1 mL of DPPH solution, shaken until homogeneous, incubated for 10 minutes in the dark, and the absorbance was read at a wavelength of 516nm (Karlina et al., 2023).

d) Samples of calendula oil and sweet orange oil combination solutions with a ratio of 2:1 concentrations of 40, 80, and 120 ppm.

   A combination solution of calendula oil and sweet orange oil ratio of 2:1 at concentrations of 40, 80, and 120 ppm. Then, 2 mL of each solution was added to 1 mL of DPPH solution, shaken until homogeneous, incubated for 10 minutes in the dark, and then the absorbance was read at a wavelength of 516nm (Karlina et al., 2023).

e) Samples of calendula oil and sweet orange oil combination cream solutions with a ratio of 1:1 concentrations of 40, 80, and 120 ppm.

   Make a cream solution combination of calendula oil and sweet orange oil ratio of 1:1 with concentrations of 40, 80, and 120 ppm. Then, 2 mL of each solution was added to 1 mL of DPPH solution, shaken until homogeneous, incubated for 10 minutes in the dark, and the absorbance was read at a wavelength of 516nm (Karlina et al., 2023).

f) Samples of calendula oil and sweet orange oil combination cream solutions with a ratio of 1:2 concentrations of 40, 80, and 120 ppm. Make a cream solution combination of calendula oil and sweet orange oil ratio of 1:2 with concentrations of 40, 80, and 120 ppm. Then, 2 mL of each solution was added to 1 mL of DPPH solution, shaken until homogeneous, incubated for 10 minutes in the dark, and the absorbance was read at a wavelength of 516nm (Karlina et al., 2023).

g) Samples of calendula oil and sweet orange oil combination cream solutions with a ratio of 2:1 concentrations of 40, 80, and 120 ppm.

   Make a cream solution combination of calendula oil and sweet orange oil ratio of 2:1 with concentrations of 40, 80, and 120 ppm. Then, 2 mL of each solution was added to 1 mL of DPPH solution, shaken until homogeneous, incubated for 10 minutes in the dark, and the absorbance was read at a wavelength of 516nm (Karlina et al., 2023).

4) Calculation of antioxidant activity

   Antioxidant activity was calculated using the DPPH method (2,2 diphenyl-1picrylhydrazyl) where the sample was reacted with a DPPH radical solution. Sample absorbance was measured using UV-Vis spectrophotometry, with methanol and DPPH as blank solutions. DPPH absorbance was measured at wavelengths of 511-517 nm (Rohmah et al., 2020).

   The inhibitory power of antioxidant compounds was expressed as % inhibition and was calculated using the following equation:

$$\text{nhibition} = \frac{Standard\ absorbance - sample\ absorbance}{Standard\ absorbance} \text{ X } 100\%$$

To test the activity of antioxidants using the DPPH method, the IC_{50} value was used as a parameter to determine the antioxidant activity of the sample. This value shows the magnitude of the concentration of the test sample, which can reduce the DPPH activity by 50%. The calculation results were entered into the regression equation with the oil concentration (ppm) as the abscissa (x-axis) and the % inhibition value as the ordinate (y-axis) (Rispita, 2018).

The formula for determining the value of IC_{50}:

$$y = bx + a$$

Description: y = bound variable (IC_{50})
x = independent variable (oil concentration)
a = intercept
b = regression coefficient

Data Analysis

The analysis data in this study were the antioxidant activity data obtained from the calculation of % inhibition. After calculating the % inhibition of each sample solution concentration, the next step was to use linear regression with the equation y = bx + a, where x is the concentration (ppm) and y is the percentage of inhibition (%). Antioxidant activity was expressed as IC_{50}, which is the concentration of samples that can reduce DPPH radicals by as much as 50% (Erviana et al., 2016).

The data obtained in this study will then be tested statistically using IBM SPSS Version 22. The data from the study were tested for normality using the Shapiro-Wilk test and homogeneity. When normal and homogeneous data were used to analyze the difference between the 0 cycle with the 1 cycle to the 6th cycle of the cream base and the cream formula of the combination of calendula oil and sweet orange oil at a ratio of 1:1, 1:2, and 2:1, the stability of the preparation was carried out with a t-test followed by post hoc LSD. If the data were abnormal or inhomogeneous, a non-parametric Kruskal Wallis analysis was carried out followed by the Mann Whitney test.

RESULTS AND DISCUSSION

The following are the results of the stability test of the combination of cream calendula oil and sweet orange oil in cycles 0 to 6.

Table II. Stability Test Results Of Calendula Oil And Sweet Orange Oil Combination Cream

Cycle to-	Product	Organoleptic			Homogeneity	Dispersion (cm)	pH	Cream Type
		Color	Smell	Texture				
0	Base	White	-	S	H	5,93	7,4	M/A
	F1	White	+	S, Sh	H	6,42	6,54	M/A
	F2	White	+++	S, Sh	H	6,35	6,79	M/A
	F3	White	++	S, Sh	H	7	6,74	M/A
1	Base	White	-	S	H	5,79*	7,46*	M/A
	F1	White	+	S, Sh	H	5,14*	7,66*	M/A
	F2	White	+++	S, Sh	H	6,35*	7,32*	M/A
	F3	White	++	S, Sh	H	6,25*	7,28*	M/A
2	Base	White	-	S	H	5,91*	7,38*	M/A
	F1	White	+	S, Sh	H	5,67*	7,59*	M/A
	F2	White	+++	S, Sh	H	5,88*	7,46*	M/A
	F3	White	++	S, Sh	H	5,87*	7,4*	M/A
3	Base	White	-	S	H	6,32*	7,4*	M/A

	F1	White	+	S, Sh	H	6,02*	7,05*	M/A
	F2	White	+++	S, Sh	H	6,5*	7,25*	M/A
	F3	White	++	S, Sh	H	6,7*	7,2*	M/A
4	Base	White	-	S	H	6,43*	7,19*	M/A
	F1	White	+	S, Sh	H	6,46*	7,16*	M/A
	F2	White	+++	S, Sh	H	6,22*	7,22*	M/A
	F3	White	++	S, Sh	H	6,38*	7*	M/A
5	Base	White	-	S	H	6,25*	7,27*	M/A
	F1	White	+	S, Sh	H	6,19*	7,57*	M/A
	F2	White	++	S, Sh	H	5,79*	7,46*	M/A
	F3	White	+	S, Sh	H	6,66*	7,62*	M/A
6	Base	White	-	S	H	6,21*	7,46*	M/A
	F1	White	+	S, Sh	H	6,3*	7,42*	M/A
	F2	White	++	S, Sh	H	6,74*	7,12*	M/A
	F3	White	+	S, Sh	H	6,69*	6,93*	M/A

Description: F1: Formula 1 (calendula oil and sweet orange oil 1:1)
F2: Formula 2 (calendula oil and sweet orange oil 1:2)
F3: Formula 3 (calendula oil and sweet orange oil 2:1)
-: No smell
+: Weak sweet orange oil aroma
++: Medium sweet orange oil aroma
+++: Strong sweet orange oil aroma
S: Soft
Sh: Shiny
H: Homogeneous
M/A: Oil in water
* : $p < 0.050$

The first stability test was the organoleptic test. The test aims to determine any changes in color, odor, and texture from the test results of the 0th to 6th cycles. In previous research that has been done, organoleptic test results for base preparations, formulas 1, 2, and 3 did not change in terms of color and texture. Meanwhile, the odor for formulas 2 and 3 changed from the 5th cycle to the 6th cycle.

A homogeneity test was performed to observe the presence of coarse particles in the glass object. The observations obtained showed that the preparations of cream base and Formulas 1, 2, and 3 carried out from the 0th to the 6th cycle were homogeneous during storage because there were no coarse particles on the glass object. This shows that the ingredients used to make the cream were evenly mixed.

The pH test aims to determine the degree of acidity of each preparation, which is based on the pH of the topical preparation. A good cream has a pH that matches the requirements of topical preparation, ranging from 4.5 to 8.0 (Lumetut et al., 2020). If the pH of the preparation is not the standard, it can irritate the facial skin (Lumetut et al., 2020). The pH test results of formulas 1, 2, and 3 decreased with the addition of a combination of calendula oil and sweet orange oil; the more significant the comparison between calendula oil and sweet orange oil, the more acidic the pH of the preparation. The pH test results from cycle 0 to cycle six have changed with values between 6.54 and 7.66, where the number was still within the range of pH requirements of good topical preparations. These changes could be caused by the storage process and the combination of calendula oil and sweet orange oil used for evaporation. Furthermore, the pH data were statistically analyzed using normality, homogeneity, one-way ANOVA, and LSD follow-up tests. If the data were not normally distributed or homogeneous, proceed with the Kruskal-Wallis test.

The results of the analysis of base preparations and Formulas 1, 2, and 3 show significance values from the normality test ($p > 0.050$) and homogeneity test ($p > 0.050$), which means the data are normally distributed and homogeneous. Because the resulting data were normally distributed and homogeneous, one-way analysis of variance (ANOVA) was performed. In the One-Way ANOVA test, a significance value of 0.000 ($p < 0.050$) was obtained, indicating a significant difference. Because the significance value of the One-Way ANOVA test was less than 0.050, it was continued with the LSD follow-up test to determine the significant difference between cycles 0 and 1 to 6. The results of the LSD test showed a significant difference in cycle 0 from cycle 1 to cycle 6 because a significance value of 0.000 ($p < 0.050$) was obtained, which means that there is a difference in pH from each cycle, but still within the range of pH requirements for good cream preparation. Therefore, it can be concluded that a combination of calendula oil and sweet orange oil can be formulated into cream preparations.

The dispersion test aimed to determine the ability of the cream preparation to spread to the surface of the skin when applied. The results of the observation of dispersion for each cream preparation can be said to be good because it obtained numbers ranging from 5.14-7 cm whereas good cream preparations have a dispersion value in the range of 5-7 cm, (Lumetut et al., 2020). The results of the research were then subjected to statistical analysis tests using normality, homogeneity, and one-way ANOVA tests.

The results of the analysis of base preparations and Formulas 1, 2, and 3 show significance values from the normality test ($p > 0.050$) and homogeneity test ($p > 0.050$), which means the data are usually distributed and homogeneous. Because the resulting data are typically distributed and homogeneous, a one-way analysis of variance (ANOVA) was performed. In the One-Way ANOVA, a significance value of 0.000 ($p < 0.050$) was obtained, indicating a significant difference. Because the significance value of the One-Way ANOVA test was less than 0.050, it was continued with the LSD follow-up test to determine the significant difference between cycles 0 and 1 to 6. The results of the LSD test showed a significant difference in cycle 0 from cycle 1 to cycle 6 because a significance value of 0.000 ($p < 0.050$) was obtained, which means that there was a difference in the dispersion of each cycle, but still within the range of reasonable cream preparation spreadability requirements. Therefore, calendula and sweet orange oils can be formulated into creams.

The cream-type test determined the cream to be prepared using a dilution. The results of the observations obtained starting from the base, Formulas 1, 2, and 3 are creams with oil-in-water types because after adding water, a homogeneous mixture is obtained so that it can be concluded that the cream preparations made include oil in water (M/A) (Mektildis, 2018).

The viscosity test was performed only in cycles 0 and 6 for each formula. The purpose of the viscosity test was to determine the viscosity of the cream preparations.
The results of the observations are shown in Table III.

Table III. Viscosity Test Results of Cycle 0 And 6 Cream Preparations

Cycle to -	Cream Preparations	Spindle	Replication	Rpm	Viscosity (Cps)	Average Viscosity ± SD
0	Base	07	1	50	9280	9066,67 ± 244,40
			2		9120	
			3		8800	
	F1	07	1	20	22000	21400 ± 600
			2		21400	
			3		20800	
	F2	07	1	12	35670	36670 ± 1000
			2		36670	
			3		37670	
	F3	07	1	20	23800	23200 ± 529,15
			2		23000	
			3		22800	
6	Base	07	1	50	11680*	11520 ±

		2 3		11440* 11440*	138,56
F1	07	1 2 3	30	15600* 15330* 14670*	15200 ± 478,43
F2	07	1 2 3	20	25000* 24000* 23600*	24200 ± 721,11
F3	07	1 2	30	16530* 17330*	16796,67 ± 461, 88

Description: * : p < 0,050

Based on the results of the viscosity tests in Table III, the preparations made for the base experienced an increase in viscosity from cycles 0 and 6. Formulas 1, 2, and 3 experienced a decrease in viscosity values from cycles 0 and 6. This indicates that the viscosity of the preparation is unstable, which can be caused by several factors such as pressure, storage temperature, and concentration of the material used. The viscosity results obtained are 8800-37670 cps whereas good cream preparations had viscosity values ranging from 2000-50000 cps (Mailana *et al., 2016)*. The data obtained were then subjected to statistical analysis using normality, homogeneity, and one-way ANOVA tests.

The results of the analysis of base preparations and Formulas 1, 2, and 3 show significance values from the normality test (p > 0.050) and homogeneity test (p > 0.050), which means the data are normally distributed and homogeneous. Because the resulting data were normally distributed and homogeneous, one-way analysis of variance (ANOVA) was performed. In the One-Way ANOVA test, a significance value of 0.000 (p < 0.050) was obtained, indicating a significant difference. Because the significance value of the One-Way ANOVA test was less than 0.050, it was continued with the LSD follow-up test to determine the significant difference between cycles 0 and 6. The results of the LSD test showed a significant difference between cycles 0 and 6 because a significance value of 0.000 (p < 0.050) was obtained, which means that there was a difference in viscosity from cycle 0 to cycle 6, but still within the range of viscosity requirements of a good cream preparation. Therefore, it can be concluded that a combination of calendula oil and sweet orange oil can be formulated into cream preparations.

The results of the dosage flow property tests in cycles 0 and 6 are shown in the figure below.

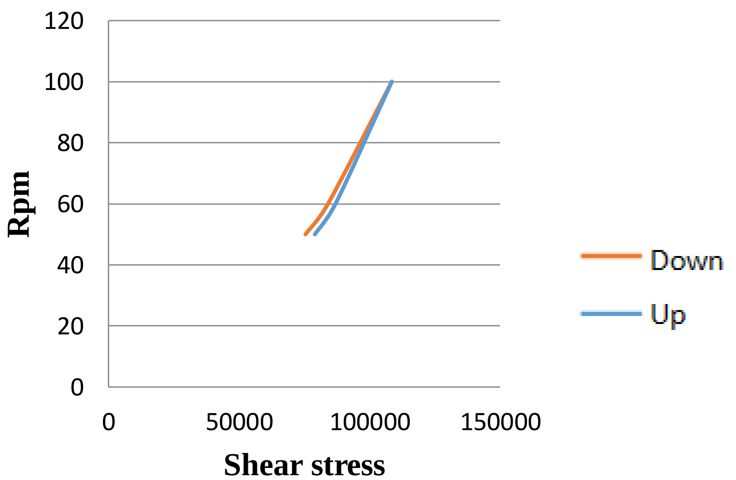

Figure 1. Cycle 0 Base Flow Properties

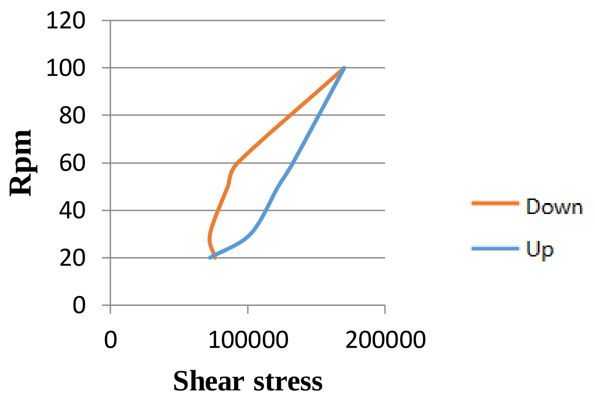

Figure 2. **Formula 1 Cycle 0 Flow Properties**

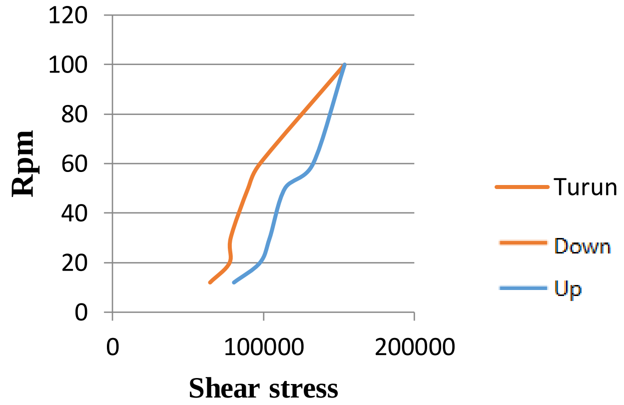

Figure 3. **Formula 2 Cycle 0 Flow Properties**

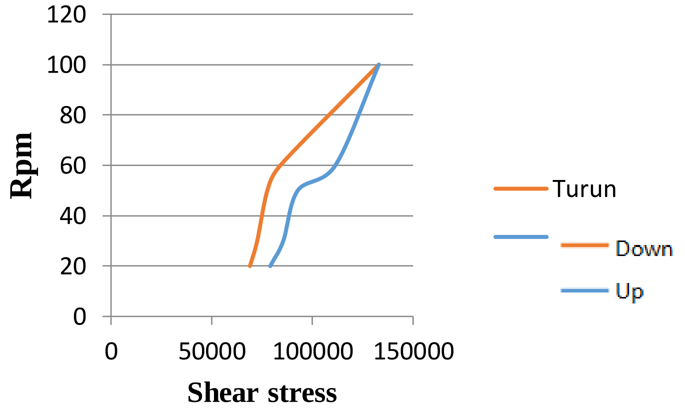

Figure 4. **Formula 3 Cycle 0 Flow Properties**

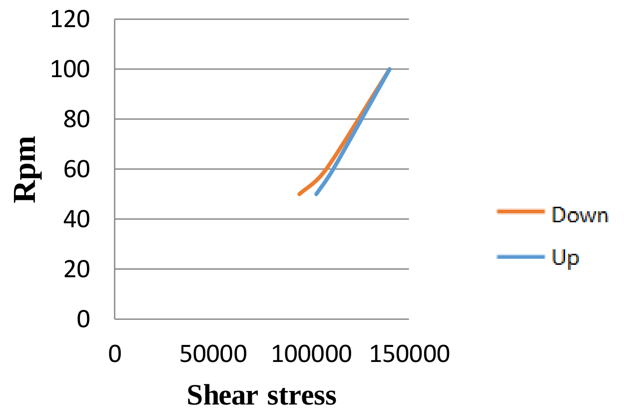

Figure 5. **Cycle 6 Base Flow Properties**

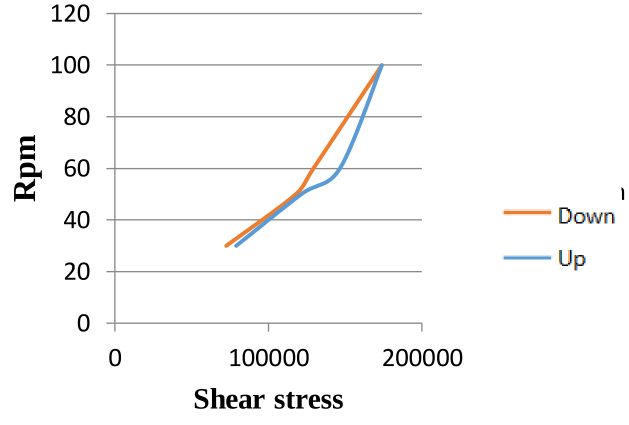

Figure 6. **Formula 1 Cycle 6 Flow Properties**

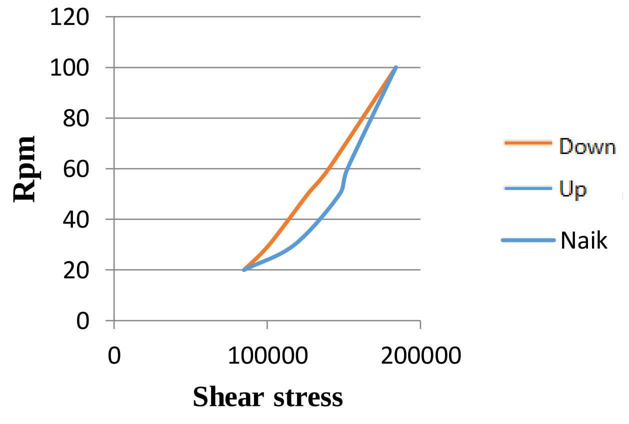

Figure 7. **Formula 2 Cycle 6 Flow Properties**

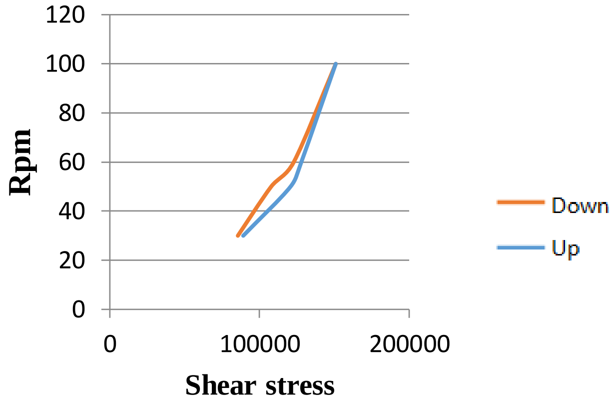

Figure 8. Formula 3 Cycle 6 Flow Properties

Based on the results of the observations presented in the form of curves and the flow properties for base preparations, formulas 1, 2, and 3 include non-Newton flow properties in the form of ticsotropic flow. The resulting curve indicates that the descending curve is to the left of the ascending curve. Semi-solid preparations are expected to have ticsotropic flow because they have a high concentration and are easily dispersed (Rahman et al., 2013).

The results of the antioxidant activity test are shown in

Table **IV**.

Table IV. Antioxidant Activity Test Results

Concentration (ppm)	ABS (Blanks)	ABS R1	ABS R2	ABS R3	% Inhibition R1	% Inhibition R2	% Inhibition R3	IC_{50} (ppm) R1	IC_{50} (ppm) R2	IC_{50} (ppm) R3	Average IC_{50} ± SD
Vitamin C											
2	0,955	0,729	0,729	0,729	23,66	23,66	23,66	5,24	5,24	5,24	5,24±0
4		0,611	0,611	0,611	36,02	36,02	36,02				
6		0,399	0,399	0,399	58,22	58,22	58,22				
Combination of Calendula Oil and Sweet Orange Oil Ratio 1:1											
40	0,955	0,758	0,758	0,758	20,63	20,63	20,63	170,02	170,02	170,02	170,02±0
80		0,630	0,630	0,630	34,03	34,03	34,03				
120		0,595	0,595	0,595	37,7	37,7	37,7				
Combination of Calendula Oil and Sweet Orange Oil Ratio 1:2											
40	0,955	0,761	0,761	0,761	20,31	20,31	20,31	150,43	150,43	150,43	150,43±0
80		0,617	0,617	0,617	35,39	35,39	35,39				
120		0,567	0,567	0,567	40,63	40,63	40,63				

Combination of Calendula Oil and Sweet Orange Oil Ratio 2:1

Concentration (ppm)	ABS (Blanks)	ABS			% Inhibition			IC_{50} (ppm)			Average IC_{50} ± SD
		R1	R2	R3	R1	R2	R3	R1	R2	R3	
40		0,785	0,785	0,785	17,8	17,8	17,8				
80	0,955	0,603	0,603	0,603	36,86	36,86	36,86	143,89	143,89	143,89	143,89±0
120		0,567	0,567	0,567	40,63	40,63	40,63				

Calendula Oil and Sweet Orange Oil Combination Cream Ratio 1:1

Concentration (ppm)	ABS (Blanks)	ABS			% Inhibition			IC_{50} (ppm)			Average IC_{50} ± SD
		R1	R2	R3	R1	R2	R3	R1	R2	R3	
40		0,628	0,628	0,628	34,24	34,24	34,24				
80	0,955	0,578	0,578	0,578	39,48	39,48	39,48	188,85	188,85	188,85	188,85±0
120		0,549	0,549	0,549	42,51	42,51	42,51				

Calendula Oil and Sweet Orange Oil Combination Cream Ratio 1:2

Concentration (ppm)	ABS (Blanks)	ABS			% Inhibition			IC_{50} (ppm)			Average IC_{50} ± SD
		R1	R2	R3	R1	R2	R3	R1	R2	R3	
40		0,594	0,594	0,594	37,8	37,8	37,8				
80	0,955	0,564	0,564	0,564	40,94	40,94	40,94	170,01	170,01	170,01	170,01±0
120		0,521	0,521	0,521	45,45	45,45	45,45				

Calendula Oil and Sweet Orange Oil Combination Cream Ratio 2:1

Concentration (ppm)	ABS (Blanks)	ABS			% Inhibition			IC_{50} (ppm)			Average IC_{50} ± SD
		R1	R2	R3	R1	R2	R3	R1	R2	R3	
40		0,593	0,593	0,593	37,91	37,91	37,91				
80	0,955	0,573	0,573	0,573	40	40	40	189,61	189,61	189,61	189,61±0
120		0,517	0,517	0,517	45,86	45,86	45,86				

Based on the results of this study, the % inhibition value of Vitamin C samples ranged from 23.66%-58.22%, the combination of calendula oil and sweet orange oil in the ratio of 1:1 ranged from 20.63%-37.7%, the combination of calendula oil and sweet orange oil in the ratio of 1:2 ranged from 20.31%-40.63%, the combination of calendula oil and sweet orange oil in the ratio of 2:1 ranged from 17.8%-40.63%, the combination cream of calendula oil and sweet orange oil in the ratio of 1:1 ranged from 34.24%-42.51%, the Calendula Oil and Sweet Orange Oil combination cream ratio of 1:2 ranges from 37.8%-45.45%, and the combination cream calendula oil and sweet orange oil ratio of 2:1 ranges from 37.91%-45.86%. The result of % inhibition results were used to calculate the values a, b, and r to obtain the equation that was used to calculate the IC50 value. The r value obtained for Vitamin C replication 1, 2, and 3 was 0.9737; the combination of calendula oil and sweet orange oil ratio 1:1 replication 1, 2, and 3 was 0.9023; the combination of calendula oil and sweet orange oil ratio 1:2 replication 1, 2, and 3 was 0.9275; the combination of calendula oil and sweet orange oil ratio 2:1 replication 1, 2, and 3 was 0.8699; calendula oil and sweet orange oil combination cream of 1:1 replication of 1, 2, and 3 was 0.9767; calendula oil and sweet orange oil combination cream of 1:2 replication of 1, 2, and 3 was 0.9894; and calendula oil and sweet orange oil combination cream of 2:1 replication of 1, 2, and 3 was 0.9303. The results of the research that have been carried out obtained quite good data because the r value obtained is close to 1, whereas if the calculation results have an r value close to 1 or equal to 1, the research data obtained is quite good (Khofifah, 2021). The r value illustrates that the higher the concentration, the greater the number of antioxidant active compounds and the greater the inhibitory ability.

The IC_{50} was calculated using a linear regression equation for each sample. The IC_{50} Vitamin C result of 5.24 ppm which indicating that vitamin C is included in the very strong antioxidant category because its IC_{50} value is less than 50 ppm. The results of IC_{50} combination of calendula oil and sweet orange oil ratio of 1:1 of 170.02 ppm which is included in the weak category because the IC_{50} value ranges from 150-200 ppm as well as the combination of calendula oil and sweet orange oil ratio of 1:2 of 150.43 ppm. A cream combination of calendula oil and sweet orange oil ratio of 1:1 of 188.85 ppm, Calendula Oil and Sweet Orange Oil combination cream with a ratio of 1:2 of 170.01 ppm, and a combination cream of Calendula Oil and Sweet Orange Oil in a ratio of 2: 1 of 189.61 ppm. The IC_{50} results of a combination of calendula oil and sweet orange oil ratio of 2:1 amounted to 143.89 ppm which is included in the medium category because the IC_{50} value ranges from to 100-150 ppm.

CONCLUSION

1. The combination of calendula oil and sweet orange oil in ratio of 1:1, 1:2, and 2:1 can be formulated into a cream preparation.
2. a. % inhibition of the combination of calendula oil and sweet orange oil ratio of 1:1 ppm ranged from 20.63% to-37.7% and an IC_{50} value of 170.02 ppm.
   b. % inhibition of the combination of calendula oil and sweet orange oil ratio of 1:2 ranges from 20.31%-40.63% and IC_{50} value of 150.43 ppm.
   c. % inhibition of the combination of calendula oil and sweet orange oil ratio of 2:1 ranges from 17.8%-40.63% and IC_{50} value of 143.89 ppm.
3. a. % inhibition of calendula oil and sweet orange oil combination cream ratio of 1:1 ranged from 34.24% to-42.51% and an IC_{50} value of 188.85 ppm.
   b. % inhibition of calendula oil and sweet orange oil combination cream ratio of 1:2 ranges from 37.8%-45.45% and IC_{50} value of 170.01 ppm.
   c. % inhibition of calendula oil and sweet orange oil combination cream ratio of 2:1 ranges from 37.91%-45.86% and IC_{50} value of 189.61 ppm.

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