Formulation And Characterization Of Gels Of Telang Flower Extract (Clitoria Ternatea L) With Variations Of Carbopol Concentration And Antioxidant Activity Test Using Dpph Methods

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Abstract

Telang flower (Clitoria ternatea L) has antioxidant activity. Formulation in gel form provides convenience in the use of telang flower extract. This study aims to determine the effect of carbopol as a gelling agent on the physical properties of the gel and to determine the antioxidant activity of telang flower extract gel. Telang flower extract was obtained by maceration method. The gel was formulated with 3 variations of carbopol concentration, namely 0.5%, 1% and 2%. Characterization of Clitoria ternatea L. gel includes: organoleptic, homogeneity, pH, viscosity, spreadability and stickiness. Antioxidant activity test of telang flower (Clitoria ternatea L) using DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The gel formulation of telang flower extract (Clitoria ternatea L.) from 3 formulas met the requirements. Gel with 2% carbopol concentration was the gel with the best characteristics: pH 5.82; viscosity 2,666 cP; spreadability 4.33 cm; stickiness 2.2 s. The Antioxidant activity test of telang flower extract gel showed strong category with an IC50 value of 97.72 ppm. Carbopol has an influence on the physical properties of telang flower extract (Clitoria ternatea L) gel. Telang flower extract gel has strong antioxidant activity.

Keywords*: antioxidant activity, carbopol, gel, telang flower.*

INTRODUCTION

Telang flower is a traditional plant that is usually used as food and traditional medicine. Phytochemicals in telang flowers are anthocyanins that can form colors in this flower and have antioxidant properties (Apriani and Pratiwi, 2021). The antioxidant activity of telang flower ethanol extract obtained IC_{50} value of 26.10 ppm which is included in the very strong category (Jayanti *et al.*, 2021). Telang flower extract can also protect skin cells from oxidative stress derived from ultraviolet light so that it has potential as a cosmetic to slow down skin wrinkles (Marpaung, 2020).

Gel preparations are widely chosen because they have the advantages of good skin spreadability, good drug release, clear physical appearance, easy washing and stability in storage (Putri and Anindhita, 2022).

In this study, the gelling agent used was carbopol. Carbopol is a strong gelling agent, so with a small concentration it can form a gel (Kusuma *et al.*, 2018). Carbopol has the best characteristics at a concentration of 0.5%, produces a clear transparent gel, has good stability because it can bind water quickly while the release of liquid is slow and has good viscosity, and does not irritate the skin (Ida and Noer, 2012).

RESEARCH METHODOLOGY

The materials used in this study were telang flowers (Surakarta, Indonesia) that had been determined at the UPT Laboratorium of Setia Budi University, Mojosongo-Solo with determination letter number 76E/DET/UPT-LAB/18.08/2023, 96% ethanol (Cipta Kimia, Indonesia), carbopol (Griya Mandiri, Indonesia), propylene glycol, methyl paraben, triethanolamine (TEA), DPPH, Magnesium

Table 1. Gel Formula of Telang Flower Extract

Ingredient	Formula			Function
	F1	F2	F3	
Telang flower extract (g)	1	1	1	Active substance
Carbopol (g)	0.5	1 g	2	Gelling agent
TEA (g)	0.6	0.6	0.6	Organizer alkaline atmosphere
Propylene glycol (g)	15	15	15	Humectant
Methyl paraben (g)	0.2	0.2	0.2	Preservative
Aquadest ad (mL)	100	100	100	Solvent

(Mg), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), $FeCl_3$ 5% and distilled water (UMS Pharmacy Laboratory, pharmaceutical grade).

Preparation of Telang Flower Extract

Dried telang flowers were pulverized with a blender (Miyako), then weighed as much as 500 grams. The solvent used was 96% ethanol in a ratio of 1:10. Extraction using maceration method. This process was carried out for 4 days and stirred every 1x24 hours. The extract was filtered using whatman filter paper to obtain the filtrate. The dregs obtained were re-macerated once with 96% ethanol as much as 3.5 l. The filtart obtained was evaporated using a rotary evaporator (Heidolph) with a temperature of 60 ^oC. Then the process continued using a waterbath (Memmert) with a temperature of 60 ^oC to obtain a thick extract and calculate the % yield.

Phytochemical Screening

Phytochemical tests were carried out to determine the presence or absence of flavonoids, saponins, terpenoids, and tannins in the extract.

Gel Formulation of Telang Flower Extract

To make the gel (**Table 1**), carbopol was first developed with 50 ml of hot water. The expanded carbopol was added with triethanolamine (mixture 1). Methyl paraben was dissolved in propylene glycol and telang flower extract was added (mixture 2). The result of mixture 2 was added little by little to mixture 1, stirred until homogeneous and the remaining water was added.

Gel Characteristics of Telang Flower Extract

The characteristics carried out include organoleptic, homogeneity, pH, spreadability, adhesion, and viscosity of telang flower extract gel preparations.

Antioxidant Activity Test

Preparation of 0.1 mM DPPH Solution

An amount 3.9432 mg of DPPH powder was dissolved with ethanol p.a in a 100.0 ml volumetric flask wrapped in aluminum foil. The solution was stored in a dark place until it was use.

Maximum Wavelenght Measurement

A total of 3.0 ml of 0.1 mM DPPH solution was put into a 5.0 ml volumetric flask, ethanol p.a was added until the limit was reached and homogenized. Then the solution was poured into a cuvette and measured at a wavelength of 515-520 nm with a UV-Vis spectrophotometer (Shimadzu UV-1280).

Preparation of DPPH Blank Solution

3.0 ml of 0.1 mM DPPH solution was put into a 5.0 ml volumetric flask, ethanol p.a was added to the limit and homogenized. Then the solution was incubated for 30 min. Then the solution was poured into a cuvette and measured at a wavelength of 516 nm using a UV-Vis spectrophotometer (Shimadzu UV-1280).

Preparation of Stock Solution of Telang Flower Extract and Gel

As much as 10.0 mg of telang flower extract/gel stock solution was then put into a 10.0 ml volumetric flask and 96% ethanol was added until the limit was reached and shaken homogeneously. Stocks of ethanol extract was taken in amounts of 100; 200; 300; 400 and 500 µl. A DPPH solution of 3.0 ml and 96% ethanol up to the limit mark were added in a 5.0 ml volumetric flask. The solution was incubated for 30 min, and then the absorbance was measured a wavelength of 516 nm using a UV-VIS spectrophotometer (Shimadzu UV-1280). The final concentrations of telang flower extract and gel were 20; 40; 60; 80 and 100 ppm.

Data Analysis

The data obtained from the characterization results were analyzed statistically with one-way ANOVA test with 95% confidence level.

Calculation of Inhibitory Concentration

The absorbance results of the sample are then calculated the percentage value of immersion with formula:

$$\% \text{ inhibition} = \frac{DPPH\ abs - abs\ sample}{DPPH\ abs} x100\% \quad (1)$$

The percentage of inhibition or %inhibition is used to determine the IC_{50} value. Values IC_{50} is the sample concentration that can immerse DPPH free radicals by 50% of the initial concentration. The calculation of the IC_{50} value shows the relationship curve between the concentration of the test sample and the % immersion, so that a linear regression equation is obtained, namely y = bx + a. The IC50 value is obtained with the x value after replacing y with 50 (Ambari et al., 2021).

Specifically, antioxidants with IC50 values of less than 50 ppm are categorized as very strong, IC50 values of 50-100 ppm are categorized as strong, IC50 values of 100-150 ppm are categorized as moderate, and if IC50 values of 150-200 ppm are categorized as weak. The stronger the antioxidant activity, the smaller the IC50 value (Molyneux, 2004; Andriani and Murtisiwi, 2020)

RESULT AND DISCUSSION

The telang flower extract has a dark blue characterization, a distinctive smell of telang flowers, and a thick form. The weight of the yield obtained is 40.47% (**Table 2**). In the extraction process, the solvent used is 96% ethanol because it is polar and can extract polar and nonpolar compounds (Agustin and Ismiyati, 2015).

Table 3. Extraction Result of Telang Flower *(Clitoria ternatea L)*

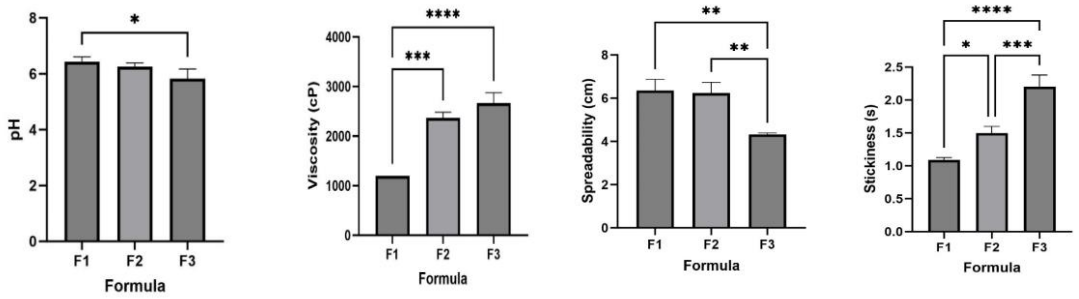
Identification		Result
Characteristics of telang flower extract	Color	Dark blue
	Smell	Typical telang flower
	Shape	Viscous
Weight of telang flower extract		202.39 g
% yield		40.47 %

Phytochemical results showed that telang flower extract contains flavonoids, saponins, terpenoids and tannin compounds (Table 3). This is in line with other studies that showed the presence of flavonoids, tannins, saponins, terpenoids, anthracinones, and alkaloids in telang flower extract (Apriani and Pratiwi, 2021; Cahyaningsih *et al.*, 2019). Telang flower extract has a strong flavonoid content (Jayanti *et al.*, 2021). Organoleptical

Table 2. Phytochemical Screening Result of Telang Flower Extracts

Sampel	Identification	Observations	Result
Telang flower	Flavonoid	Yellow	+
	Saponin	Stable foam	+
	Terpenoid	Red lust	+
	Tannin	Green black	+

+ Contained the identified compound
-no identification of the compound



(a) (b) (c) (d)

*there is a significant difference

Figure 1. Histogram of Physical Properties of Gel of Telang Flower Extract (Clitoria ternatea L); (a) pH; (b) Viscosity; (c) Spreadability; (d) Stickiness

characterization of telang flower extract gel preparation has a distinctive smell of telang flower, semisolid and homogeneous shape, and a green color that fades in different formulas. The pH test of each formula has met the requirements (**Error! Reference source not found.**. For topical preparations, the pH range is 4.5-6.5. This pH value is safe for the skin and, does not cause irritation or scaly skin (Kusuma *et al.*, 2018). Data analysis using the anova test obtained a p-value of 0.04 (< 0.05), which means there is a significant difference in pH between formulas. In F1 and F3 there was a significant decrease in pH with increasing carbopol concentrations. The decrease in pH was caused by carbopol which is acidic so that an increase in carbopol concentration and the addition of the same amount of TEA will reduce the pH of the gel preparation (Nurlely *et al.*, 2021). Carbopol with a higher concentration will increase the pH of the preparation (Shabrina *et al.*, 2023).

The results of the viscosity test showed that the telang flower extract gel was in accordance with the requirements (**Figure 1**). A good gel has a viscosity in the range of 2000- 4000 cP (Senja and Amelia, 2018). The viscosity test results showed an increase in viscosity along with an increase in carbopol concentration. The results of the data analysis with the anova test obtained a p-value of 0.00002 (<0.05) that there was a significant difference in viscosity between the formulas. The viscosity of the gel can be influenced by the concentration of carbopol used. The greater the concentration of gelling agent used, the viscosity of the preparation will. Another study reported that the amount of carbopol in each formula affected the variation in viscosity between preparations (Imanto *et al.*, 2019)

The spreadability of a good semisolid preparation for topical use ranges from 5-7 cm in diameter (Senja and Amelia, 2018; Eka Valentina and Saryanti, 2023). The telang flower extract gel F1 and F2 have met the requirements, while F3 did not meet the requirements (**Figure 1**). However, other studies stated that good spreadability for semisolid preparations ranges from 3-5 cm in diameter, while spreadability with a diameter of 5-7 cm shows a consistency that is comfortable to use (Satolom *et al.*, 2023). Based on this research, F3 is still in the range of good gel spreadability. The anova test results obtained a p-value of 0.0017 (<0.05), that there is a significant difference in spreadability between formulas. The spreadability of each formula decreased with increasing carbopol concentration (**Error! Reference source not found.**. The spreadability of carbopol will be greater at low concentrations. This is influenced by the viscosity of the preparation. The higher the viscosity, the lower the spreadability (Setiyadi and Qonitah, 2020). In another

study, it was stated that the thicker the preparation, the smaller its ability to spread (Rachmawati *et al.*, 2018). This is also in line with the increase in carbopol concentration which will increase pH value, viscosity, adhesion and decrease spreadability (Afifah and Nurwaini, 2019).

The gel adhesion test is said to be good if it has high adhesion. The results of the gel adhesion test of telang flower extract have met the requirements where the adhesion of the preparation semisolid > 1 second (Nurlely et al., 2021) (**Figure 1**). The standard of good adhesion is less than 4 seconds (Yusuf et al., 2022). The p-value obtained from the anova test is 0.00008 <0.05, indicating there is a significant difference in adhesion between formulas (Error! Reference source not found.). Increasing the carbopol content can increase the adhesion (Sumule et al., 2020). The nature of carbopol base that can expand

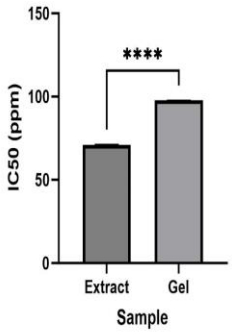


Figure 1. Histogram of IC_{50} Comparison of Extract and Gel of Telang Flower Extract (Clitoria ternatea L) with Gelling Agent Carbopol

and the more colloids formed causes an increase in carbopol concentration to increase the adhesion of the gel (Adnan et al., 2021). Characterization results from 3 formulas, showing F3 (carbopol 2%) has good physical properties.

In this study, telang flower extract has a strong IC_{50} value of 71.12 ppm with a linear regression equation y = 0.582x + 12.446. Another study stateds the antioxidant activity of telang flower extract obtained an IC50 value of 41.36 ± 1.191 µg/ml which is included in the very strong category as an antioxidant (Andriani and Murtisiwi, 2020). Vitamin C as a positive control aims to test antioxidant activity and determine the level of antioxidant potential of the extract. From this study, telang flower extract has an IC50 value of 356.65 ppm which is included in the category of very weak antioxidant activity compared to the IC50 value of vitamin C which is 4.74 ppm including a very strong category (Phongpaichit et al., 2018).

In this study, the antioxidant activity test of telang flower extract gel in F3, obtained strong IC50 measurement results of 97.72 ppm with a linear regression equation y = 0.6082x – 9.4347. Furthermore, data analysis was conducted between the IC50 value of the extract and the gel using unpaired t-test. The results of the analysis obtained the value of R2 which is 1.000. The R2 value indicates a significant relationship between the solvent concentration and the observed silencing percentage with a degree of closeness of 1.000. The value of R2 close to (+1) means that the data obtained is very good (Cahyaningsih et al., 2019). The IC50 value of carbopol-based telang flower extract gel has a significant difference with telang flower extract (**Figure 1**). There are several things that can cause the IC50 values to be different in each study. These include the concentration of DPPH, the wavelenght used, the type of preparation being tested, the extract solvent, and storing the samples at the wrong temperature (Mudrikatin, 2022).

CONCLUSIONS

Telang flower extract can be formulated in gel form with the gelling agent carbopol. Carbopol affects the physical properties of telang flower extract gel. The IC50 value of extract and gel of telang flower extract showed strong antioxidant activity.

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