

## Gel Formulation of *Clitoria ternatea* L. Flower Extract on Total Flavonoid Content

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### Abstract

*Clitoria ternatea* L., or *telang* flower, contains antioxidants that are beneficial for protecting cells from free radicals, maintaining the immune system, preventing aging, and supporting skin health. The aim of this research was to formulate and characterize a topical gel containing *C. ternatea* flower extract, evaluating its physicochemical properties and total flavonoid content. The formulation was subjected to physical quality tests and total flavonoid content analysis. The physical quality parameters included organoleptic tests, homogeneity tests, pH measurement, spreadability, hedonic tests, moisture content, and irritation tests. Higher extract concentrations increased the flavonoid content in the gel formulation and also made the preparation's color more intense (purplish blue), which was less preferred by users. Based on the hedonic test results, the most preferred gel formulation by users was the one containing 1% *C. ternatea* condensed extract. The best formulation was found to have balanced, complete components and optimal gelling agent consistency. The final formulation demonstrated a total flavonoid content of 63.21 µg/g, as measured by UV-Vis spectrophotometry. This flavonoid content confirms that the gel formulation contains antioxidants beneficial for skin health.

**Keywords:** *Clitoria ternatea* L., gel formulation, total flavonoid

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## Introduction

*Clitoria ternatea* L. is a herbaceous perennial plant belonging to the Fabaceae family. This plant has recently attracted considerable attention due to its potential applications in herbal medicine, particularly for its rich antioxidant content (Oguis et al., 2019).

*C. ternatea* produces blue flowers that contain anthocyanins with high medicinal value. A distinctive characteristic of *C. ternatea* flowers is their high content of polyacylated anthocyanins, known as ternatins, which are polyacylated derivatives of delphinidin 3,3',5'-triglucoside (Vidana Gamage et al., 2021).

Various studies have demonstrated that anthocyanins exhibit a wide range of biological activities, including antimicrobial, antioxidant, cardioprotective, antidiabetic, and anticancer effects (Rajamanickam et al., 2015).

Anthocyanins in these plants are present as glycosides, where the anthocyanidin is bound to a sugar group, such as glucose, galactose, rhamnose, xylose, or arabinose attached to the aglycone (Pojer et al., 2013). The six main types of anthocyanidins commonly found in plants are cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin (Choo, 2019). Anthocyanins belong to the flavonoid group of polyphenolic compounds, which produce red and blue colors in vegetables, fruits, flowers, and leaves (Jeyaraj et al., 2022).

Flavonoids, a group of natural substances with diverse phenolic structures, are present in fruits, vegetables, seeds, bark, roots, stems, flowers, tea, and wine. These compounds are well known for their beneficial effects on health, and ongoing research aims to isolate flavonoids for

various uses. Flavonoids are now considered indispensable components in numerous nutraceutical, pharmaceutical, medicinal, and cosmetic applications, owing to their antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, as well as their ability to modulate key cellular enzymes (Panche et al., 2016).

Flavonoids are a large family of polyphenols comprising several classes based on their basic structure: flavanones, flavones, isoflavones, flavonols, flavanols, and anthocyanins. They exhibit numerous biological properties, such as anti-inflammatory, antioxidant, antiviral, antimicrobial, anticancer, cardioprotective, and neuroprotective effects. Current research and development on flavonoids focus on their identification, extraction, isolation, physicochemical characterization, and health applications (Liga et al., 2023).

The types of flavonoids found in *C. ternatea* include anthocyanins, quercetin glycosides, flavonol glycosides, kaempferol glycosides, and myricetin glycosides (Sakdiah et al., 2022). *C. ternatea* is a plant with high antioxidant content. Additionally, the phenolic compounds in its flower extract show the highest total phenol content when compared to its flavonoid and anthocyanin levels (Cheewabanthoeng et al., 2021).

Extracts of *C. ternatea* are widely used as natural food coloring, herbal tea, and as an active ingredient in pharmacological products. However, to date, the extract has not been used as an active ingredient in gel preparations. Gel is a semisolid system consisting of a suspension of small inorganic particles or large organic molecules penetrated by a liquid. Gels are sometimes referred to as jellies (Kementarian Kesehatan RI, 2020). They are soft mass

preparations, formed as suspensions of small particles or macromolecules of organic compounds, each encased and mutually absorbed by a liquid (Kementarian Kesehatan RI, 2022).

Some advantages of gel preparations include: 1) good spreadability on the skin, 2) a cooling effect resulting from slow evaporation, 3) no physiological inhibition of hair function, and 4) easy washing with water (Ghiffari et al., 2024). Studies have shown that gels are easy to use, more homogeneous, provide a cooling sensation when applied, and are soluble in water when rinsed (Marlina et al., 2023).

Based on this background, it is important to determine the total flavonoid content in *C. ternatea* flowers when formulated as a gel, as this application has not yet been widely developed or utilized.

## Research Methods

The ingredients used in this study included *C. ternatea* flower simplicia sourced from Kalasan, Sleman, Yogyakarta (latitude  $-7.750^{\circ}$  S, longitude  $110.450^{\circ}$  E); solvents consisting of distilled water and tartaric acid (Hartono et al., 2013); humectants such as pure rose hydrosol, panthenol, glycerin, hyaluronic acid (HA); gel agents in the form of CMC Na; and preservatives in the form of phenoxyethanol.

### Extraction

The extraction of *C. ternatea* flowers was performed using the maceration method with solvents (distilled water and tartaric acid). The volume of distilled water used was 300 mL (200 mL for initial maceration and 100 mL for remaceration), while tartaric acid was used at 2.25 grams (equivalent to 0.75% of the total volume of distilled water). The amount of *C. ternatea* simplicia used was

30 grams. Maceration was carried out for 24 hours, followed by remaceration for another 24 hours. The resulting extract was then concentrated using a rotary evaporator at a temperature of  $80^{\circ}\text{C}$ .

## Physical Quality Tests

Physical quality tests for the gel included organoleptic tests, homogeneity tests, pH tests, spreadability tests, hedonic tests, moisture tests, and irritation tests.

### Organoleptic Test

Observations were made of the odor, color, and texture of each gel preparation using the five senses.

### Homogeneity Test

The homogeneity test was performed using two glass slides. The sample was placed on one slide and spread evenly. A good preparation must be homogeneous and free from visible clumps or particles.

### pH Test

The pH test aimed to determine the safety, stability, and effectiveness of the lotion preparations. A 25 mL sample was placed in a 100 mL beaker, and the pH was measured using a pH meter. Calibration was performed using pH 4 and pH 7 buffer solutions. The pH value was recorded, and the gel was considered acceptable if the pH ranged from 4.5 to 6.5 (Ifaya et al., 2024).

### Spreadability test

For the spreadability test, 0.5 grams of gel was placed in the center of a transparent glass plate. Another glass plate was placed on top, followed by weights of 50 grams, 100 grams, and 200 grams. After standing for 1 minute, the diameter of the spread was recorded.

### Hedonic Test

The hedonic test was conducted on 20 users to assess their preference for texture, color, and aroma using a scale from 1 to 5: (5) Really Like, (4) Like, (3) Neutral, (2) Dislike, (1) Really Dislike (Ode et al., 2024).

### Moisture Test

The moisture test was conducted on users who did not use other products on the test area. The test lasted for 7 days and used a skin analyzer. The gel was applied to a 2 × 5 cm area of skin, and baseline moisture was measured beforehand. The resulting moisture percentages were categorized as follows: dry (0%–45%), normal/moist (46%–55%), and very moist (56%–100%) (Iskandar et al., 2019).

### Irritation Test

The irritation test was performed on volunteers' skin using an open patch test. The preparation was applied to a 2.5 × 2.5 cm area of skin and left uncovered for 30 minutes, then observed for any reaction. This procedure was repeated for three consecutive days. A positive irritation reaction was indicated by redness, itching, or swelling of the treated area on the inner forearm.

### Total Flavonoid Content

The total flavonoid content was measured using the UV-Vis spectrophotometry method at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada.

## Research Results and Discussion

This study represents an advanced stage of research following the *in silico* tests

previously conducted by the researchers. This stage is part of the Design and Development phase of gel formulation research. Prior to evaluating the total flavonoid content, *C. ternatea* flower extract and gel-based product formulations were prepared.

The second stage involved the preparation of *C. ternatea* flower extract using the maceration method with distilled water (*aquades*) and tartaric acid as solvents. The total volume of distilled water used was 300 mL (200 mL for initial maceration and 100 mL for remaceration), while the amount of tartaric acid was 2.25 g (0.75 g or 75% of the distilled water volume). A total of 30 g of *C. ternatea* flower simplisia was used. Maceration was carried out for 1 × 24 hours, followed by remaceration for another 1 × 24 hours. The resulting extract was then heated at 80°C to obtain a thick extract, as shown in Figure 1.

The use of the maceration method with distilled water and 75% tartaric acid aimed to obtain extracts with a high antioxidant content, particularly flavonoids. The resulting *C. ternatea* flower extract was subsequently formulated into a gel-based preparation, specifically a serum gel. The formulation and reformulation results are presented in **Table 1**. Physical quality evaluations included organoleptic, homogeneity, pH, spreadability, hedonic, moisture, and irritation tests.

**Table 1***Formulation and reformulation of Clitoria ternatea gel preparations*

Formulation 1	%	Formulation 2	%	Formulation 3	%
Pure rose hydrosol	90	Pure rose hydrosol	88	Pure rose hydrosol	44
Panthenol	1	Panthenol	1	Distilled water	46
Extract	1	Extract	3	Panthenol	1
Glycerin	6	Glycerin	6	Extract	1
Hyaluronic acid (HA)	1	Hyaluronic acid (HA)	1	Glycerin	6
Phenoxyethanol	1	Phenoxyethanol	1	Hyaluronic acid (HA)	0.1
				CMC Na	0.9
				Phenoxyethanol	1

**Organoleptic Test**

Organoleptic observations included aroma, color, and texture of each gel formulation, assessed using the five senses.

The results of the organoleptic evaluation are shown in **Table 2**.

**Table 2***Organoleptic test results of Clitoria ternatea gel preparations*

Formulation	Odor	Color	Texture
F1	++ Fragrant (rose)	+++ Deep purplish blue	+++ Viscous (semi-solid)
F2	++ Fragrant (rose)	+ Very intense purplish blue	+++ Viscous (semi-solid)
F3	++ Fragrant (rose)	++ Clear purplish	+++ Viscous (semi-solid)

**Homogeneity Test**

The homogeneity test showed that all gel formulations were homogeneous and free from clumping particles.

The pH of each gel preparation was measured using a pH meter by immersing the electrode directly into the sample. The pH values were required to fall within the acceptable skin pH range of 4.5–6.5. The results are presented in **Table 3**.

**pH Test****Table 3***pH values of Clitoria ternatea gel preparations*

Formulation	pH
Formulation 1	7.5
Formulation 2	7.8
Formulation 3	6.5

**Spreadability Test**

The spreadability test was conducted by placing 0.5 g of gel between two transparent

glass plates. Loads of 50 g, 100 g, and 200 g were applied sequentially, and the diameter of the spread was measured after 1 minute. The results are shown in Table 4.

**Table 4**  
*Spreadability test results of Clitoria ternatea gel preparations*

Load	F1 (cm x cm)	F2 (cm x cm)	F3 (cm x cm)
50 g	5.0 x 4.8	5.5 x 5.4	6.5 x 6.6
100 g	6.2 x 6.0	7.2 x 7.0	7.1 x 7.2
200 g	6.9 x 6.4	7.3 x 7.2	7.4 x 7.2

**Hedonic Test**

The hedonic test results showed that 80% of respondents liked the *C. ternatea* extract gel preparation (Formulation 3), while the remaining 20% reported that they really liked the formulation.

**Humidity Test**

Based on the moisture test, 90% of users reported that their skin felt very moisturized (56%–100%), while 10% reported normal to moisturized skin conditions (46%–55%) after using the *C. ternatea* extract gel preparation (Formulation 3).

**Irritation Test**

The irritation test was conducted over three consecutive days. A positive irritation reaction was indicated by redness, itching, or swelling on the inner forearm. The results showed that 100% of users experienced no irritation after using the gel (Formulation 3).

**Total Flavonoid Content of *Clitoria ternatea* Gel Preparation**

Based on the results presented in Table 5, the *C. ternatea* extract gel formulation exhibited a total flavonoid content of 63.21 µg/g. This flavonoid content indicates that the formulation contains antioxidants that are beneficial for neutralizing free radicals, supporting anti-aging effects, and maintaining skin health.

**Table 5***Total flavonoid content (quercetin equivalent) determined by the spectrophotometric method.*

Replication	Sample Weight	Added Solution	Dilution Factor	Reading Results	Results
	g	mL	fp	(ppm)	µg/g
Rep1	0.8968	10.0	1.0	5.3851	60.05
Rep2	0.9537	10.0	1.0	6.2130	65.15
Rep3	0.9813	10.0	1.0	6.3229	64.43

The gel preparation product underwent repeated formulation and reformulation stages. The final reformulated product was subjected to physical quality testing and total flavonoid analysis. Physical quality test parameters included organoleptic, homogeneity, pH, spreadability, hedonic, moisture, and irritation tests.

Based on the organoleptic test, the observed parameters of the preparation included aroma, color, and texture, which were evaluated using the five senses. Furthermore, the organoleptic test results presented in **Table 2** indicate that the best formulation was gel formulation 3, which contained pure rose hydrosol, aquades, panthenol, *C. ternatea* extract, glycerin, hyaluronic acid (HA), CMC-Na, and phenoxyethanol. After two weeks of observation, no changes in shape, color, or odor were observed.

The homogeneity test results showed that all formulations were homogeneous and free of clumpy particles, particularly formulation 3. The presence of clumpy particles may be caused by incomplete dissolution of CMC-Na or hyaluronic acid; in such cases, the preparation can be re-stirred or gently heated at room temperature.

The pH test results are presented in **Table 3** and indicate that each gel formulation had a pH of 6.5. This value complies with the skin moisturizer pH quality requirements specified in SNI 16-4399-1996, which range from 4.0 to 8.0 (Suryana, 2021). Other studies have also reported that acceptable pH values for topical preparations should correspond to normal skin pH, ranging from 4.5 to 6.5. Therefore, the *C. ternatea* gel preparation meets the standard pH

quality requirements for gel products. The pH test aimed to evaluate the safety of the gel during use to prevent skin irritation, as well as to assess formulation stability and effectiveness.

Regarding the spreadability test, the results of this study are consistent with theoretical explanations stating that gel preparations possess good spreadability due to their high water content (Megantara et al., 2017). Other studies have reported that spreadability testing was conducted to ensure uniform distribution of the gel upon application to the skin. Good gel spreadability ranges from 5 to 7 cm (Garg et al., 2002; Sharma & Singh, 2018; Nurlaela & Ikhsanudin, 2012).

The hedonic test was conducted on 20 users and focused on gel formulation 3, as this formulation was more stable and did not produce a warming sensation on the skin. Based on the hedonic test results, users preferred formulation 3 because the purple color was not overly intense. The purple coloration of the gel is attributed to the *C. ternatea* flower extract; higher extract concentrations result in a more intense color. This finding is consistent with Luthfiyana et al. (2019), who stated that product color is influenced by the color of its constituent ingredients.

The results showed that 80% of users liked the *C. ternatea* extract gel (formulation 3), while the remaining 20% reported that they really liked it. Although higher extract concentrations increase the content of active compounds (particularly flavonoids), they also intensify the gel color, which may reduce user preference. Therefore, based on the hedonic test results, the formulation preferred by users contained 1% *C. ternatea* condensed extract.

Based on the overall data, formulation 3 was identified as the best formulation. This was due to (1) balanced and complete components: formulation 3 contained pure rose hydrosol (44%), aquades (46%), extract (1%), panthenol (1%), glycerin (6%), hyaluronic acid (0.1%), and phenoxyethanol (1%), totaling approximately 100%. The formulation had a dominant water base (90% hydrosol and aquades), making it ideal for hydrophilic cosmetic gels, along with humectants (glycerin and HA), a moisturizer (panthenol), a preservative, and an active extract; (2) Optimal gelling agent consistency: the formulation contained 0.9% CMC-Na as a thickening agent, producing ideal viscosity (2,000–50,000 cps) and good spreadability (5–7 cm), in accordance with SNI standards for stable, non-irritating gels. This formulation met key physical evaluation criteria for cosmetic gels, including homogeneity, skin-compatible pH (4–7), viscosity, and spreadability. In comparison, formulation 1 lacked extract and a thickening agent, resulting in a thin consistency, while formulation 2 contained no gelling agent and failed to form a proper gel structure.

The moisture test results indicated that the gel preparation effectively moisturized the skin. This effect can be attributed to the presence of humectant ingredients in the formulation. Humectants are hygroscopic substances that draw water from the dermis to the outer skin layers. In this study, hyaluronic acid, glycerin, and panthenol functioned synergistically to maintain skin moisture and hydration. Additionally, the flavonoid content in *C. ternatea*, which acts as a natural antioxidant and collagenase enzyme inhibitor, may help inhibit collagen degradation caused by free radicals. This finding aligns with Cahnia et al. (2022), who stated that sagging skin results from reduced moisture, collagen, and elastin levels, as well as exposure to UV radiation and pollution. The use of antioxidant-containing cosmetics can neutralize free radicals and enhance collagen production, thereby improving skin elasticity.

These findings are further supported by the total flavonoid content analysis, which showed that the *C. ternatea* gel contained 63.21 µg/g of total flavonoids. The high flavonoid content

suggests strong collagenase inhibition, thereby helping to maintain collagen levels in the skin.

The use of cosmetics containing harmful ingredients may cause skin irritation, such as peeling, redness, burning sensations, and, in severe cases, systemic effects including organ damage. Irritation typically occurs upon first use when one or more ingredients act as irritants, particularly in individuals with compromised skin barriers. Therefore, irritation testing is a critical component of product safety evaluation. One commonly used method is the patch test, in which the test preparation is applied to normal skin, covered, and left in place for at least 24 hours. In addition to patch testing, safety evaluations may include tests for metal content and hazardous substances (Ramli & Fadhila, 2022).

Based on the irritation test results, 100% of users reported no skin irritation after using the *C. ternatea* gel (formulation 3). Qualitative tests for hazardous metal content, including mercury, copper, iron, chromium, sulfite, chlorine, bromine, nitrate, nitrite, and total alkalinity, indicated that the gel preparation was free from heavy metals and hazardous chemicals. Therefore, the *C. ternatea* gel preparation is considered safe for topical use.

These findings are supported by previous studies demonstrating that *C. ternatea* flower extract gels do not cause erythema or edema in human volunteer skin irritation tests, indicating non-toxicity. *In vitro* OECD-validated assays, including sensitization, cytotoxicity, and phototoxicity tests, also confirmed favorable dermal safety profiles without systemic exposure risks. Furthermore, animal models exposed to UVB radiation showed enhanced skin recovery following application of *C. ternatea* gels, supporting their non-irritating and protective effects on the skin (Fu et al., 2026).

## Conclusion

In conclusion, a thick extract of *C. ternatea* flowers was successfully produced, exhibiting a concentrated purplish-blue color, which indicates a high anthocyanin content. Anthocyanin pigments belong to the flavonoid



class. The best formulation was Formulation 3, as it demonstrated balanced component composition and optimal gelling agent consistency. The total flavonoid content test showed that the *C. ternatea* gel preparation contained 63.21 µg/g of flavonoids, as determined by UV-Vis spectrophotometry. The gel preparation underwent repeated formulation and reformulation stages. The final reformulation was subjected to physical quality tests, heavy metal content tests, and total flavonoid content analysis. Overall, the results of all tests indicated that the gel preparation met the required quality standards.

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