

## The Effect of Kitolod (*Isotoma Longiflora*) Leaf Extract Concentration in Gel Formula on Total Phenolic and Flavonoid Levels

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### ABSTRACT

Belonging to the Campanulaceae family, Kitolod (*Isotoma longiflora*) possesses antioxidant properties crucial for wound healing; presenting a case for its formulation into a gel and investigating the total phenolic and flavonoid content of kitolod leaf extract when in gel form is essential to understand its capabilities as an anti-inflammatory and wound-healing gel, as well as to examine the possible correlation between the extract's concentration and its phenolic and flavonoid content. This study assessed how different concentrations of kitolod leaf extract affect the total phenolic and flavonoid levels in a gel formulation. Using 96% ethanol, kitolod leaves were macerated. The gel formulations contained varying concentrations of kitolod leaf extract: F1(5%), F2(10%), and F3(20%). The presence of phenols and flavonoids was confirmed through qualitative tests using FeCl<sub>3</sub> 1% for phenols and the Shinoda test for flavonoids. The findings confirmed that the total levels of phenolics and flavonoids increased with the concentration of kitolod leaf extract in the gel. SPSS analysis showed a strong correlation between extract concentration in the gel and the amounts of total phenolics and flavonoids, highlighting a positive relationship between these two chemical compounds since they are in the same category.

**Keyword:** Kitolod, Gel, Total Phenolic Level, Total Flavonoid Level

### ABSTRAK

Kitolod (*Isotoma longiflora*) termasuk dalam famili Campanulaceae dan memiliki aktivitas antioksidan yang berperan dalam penyembuhan luka sehingga berpotensi untuk diformulasikan dalam bentuk gel. Kadar total fenolik dan flavonoid ekstrak daun kitolod dalam bentuk gel perlu dikaji untuk mengetahui potensinya sebagai gel anti inflamasi dan penyembuhan luka, serta mengetahui potensi korelasi antara konsentrasi ekstrak daun kitolod dalam gel dan kadar total fenolik dan flavonoidnya. Penelitian ini bertujuan untuk mengetahui pengaruh konsentrasi ekstrak daun kitolod dalam formula gel terhadap kadar total fenolik dan flavonoid. Daun kitolod dimaserasi menggunakan etanol 96%. Konsentrasi ekstrak daun kitolod pada formula gel adalah F1(5%), F2(10%), dan F3(20%). Uji kualitatif fenolik dilakukan dengan menggunakan pereaksi FeCl<sub>3</sub> 1%, sedangkan uji kualitatif flavonoid dilakukan dengan metode uji Shinoda. Hasil uji kualitatif ketiga formula gel tersebut positif fenol dan flavonoid. Hasil penelitian ini menunjukkan bahwa kadar total fenolik dan flavonoid meningkat seiring dengan peningkatan konsentrasi ekstrak daun kitolod dalam formulasi gel. Berdasarkan analisis data SPSS terdapat hubungan yang cukup besar antara kadar total fenolik dan flavonoid dengan konsentrasi ekstrak dalam gel. Terdapat hubungan positif antara senyawa kimia fenolik dengan senyawa flavonoid karena keduanya termasuk dalam golongan yang sama

**Kata Kunci :** Kitolod, Gel, Kadar Fenolik Total, Kadar Flavonoid Total

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## Introduction:

Kitolod (*Isotoma longiflora*), belonging to the Campanulaceae family, is a common weed found in aquatic environments, rice fields, and fences. The plant is rich in phenolic and flavonoid compounds, known for their antioxidant properties. Among the various parts of the kitolod plant, the leaves have the highest levels of total phenolic and flavonoid content, with measurements of 1.46 ppm and 10.48 ppm, respectively, outperforming other plant parts (Grahita et al., 2020). These high levels correlate with the potent antioxidant activity observed in kitolod leaves, measured at 52.70 ppm, categorizing it as vital (Egarani et al., 2020).

Given its antioxidant capabilities, kitolod leaf extract aids wound healing by managing oxidative stress, thereby speeding up the healing process (Geethalakshmi et al., 2013). A higher leaf extract concentration enhances this wound-healing effect (Awwaliyah, 2021), suggesting its suitability for gel formulation, which additionally provides a cooling effect on wounded skin.

The total phenolic and flavonoid levels of kitolod leaf extract in a gel form need to be studied to understand its potential as an anti-inflammatory and wound-healing gel and also to know the potential correlation between the concentration of kitolod leaf extract in gel and its total phenolic and flavonoid levels. This study evaluates how different concentrations of kitolod leaf extract in a gel formulation influence the total phenolic and flavonoid contents.

## Methods:

### Extraction of Kitolod Leaves

To extract Kitolod leaves, 120 grams of the leaf powder were combined with 600 mL of 96% ethanol, stirred for 6 hours, and then left to macerate for 18 hours. The solution was then filtered, with the remaining solid matter subjected to a second round of maceration under the same conditions using another 600 mL of 96% ethanol. The resulting filtrate was

evaporated at 50° Celsius until it formed a thick extract.

### Formulation of Kitolod Leaf Extract Gel

The gel was created in three different concentrations of kitolod leaf extract: F1 (5%), F2 (10%), and F3 (20%), with each formulation weighing 100 grams. To create each gel, 3.5 grams of HPMC was dissolved in hot distilled water and methylparaben until it turned transparent. Glycerin (7.5 grams), propylene glycol (5 grams), and TEA (2 grams) were mixed in thoroughly. Then, kitolod leaf extract was added in quantities of 2.5 grams (F1), 5 grams (F2), and 10 grams (F3), along with the necessary volume of distilled water to achieve a uniform mixture.

### Qualitative Analysis of Kitolod Leaf Extract Gel

For the qualitative analysis, 0.5 grams of each gel formulation was dissolved in 10 mL of 96% ethanol. To test for phenolics, a 1% FeCl<sub>3</sub> solution was added. The presence of flavonoids was determined using the Shinoda test method (Hanani, 2016), with each test involving 2 mL of the gel solution.

### Quantitative Test of Kitolod Leaf Extract Gel

a. Phenolic compounds

1 mL of gallic acid solution (at concentrations of 20, 30, 40, 50, and 60 ppm) and the kitolod leaf extract gel solution (at 5000 ppm) were combined in a test tube with 5 milliliters of a 1:10 diluted Folin-Ciocalteu reagent. This mixture was stirred well and remixed by adding 4.0 mL of a 1M Na<sub>2</sub>CO<sub>3</sub> solution. After waiting for 61 minutes, the absorbance was measured at a wavelength of 762 nm. The level of total phenolics was then calculated based on a linear regression value (Ramayani et al., 2021).

$$\text{Total Phenolic Levels} = \frac{C \times V \times Fp}{g}$$

C = Phenolic concentration (x value)

V = Volume of extract used (mL)

Fp = Dilution Factor

g = Sample weight used (g)

b. Flavonoid Compounds

Quercetin solutions (20, 40, 60, 80, and 100 ppm) and 1.0 mL of kitolod leaf extract gel solution (5000 ppm) was put in a test tube, added with 1 mL of AlCl<sub>3</sub> 10% solution and 8 mL of CH<sub>3</sub>COOH 5%, and stirred homogeneously. After 46 minutes, the absorbance was measured at wavelength 415 nm. The total flavonoid level was calculated using a linear regression value (Ramayani, Nugraheni, et al., 2021).

$$\text{Total Flavonoid Levels} = \frac{C \times V \times Fp}{g}$$

- C = Quercetin equivalence (mg/mL)
- V = Volume of extract used (mL)
- Fp = Dilution Factor
- g = Sample weight used (g)

Data Analysis

The study analyzed the levels of total phenolics and flavonoids using SPSS, employing the Levene Test to assess data normality and the Shapiro-Wilk Test for homogeneity. Data are considered normal and homogeneous if the *p* value exceeds 0.05. Conversely, a *P* value below 0.05 indicates non-normal or non-homogeneous data, necessitating a non-parametric method, the Kruskal-Wallis test, at a 95% significance level. Significant results (*p* value < 0.05) lead to further analysis with the Mann-Whitney test to pinpoint significant differences, maintaining a 95% confidence level.

Results:

The maceration-produced Kitolod leaf extract was viscous, with a blackish-green hue and a distinct Kitolod scent, yielding 10.26% with a 3.00% drying shrinkage. Qualitative analysis of the Kitolod leaf extract gel revealed the presence of phenolic compounds in all gel formulations (F1, F2, F3). During the flavonoid qualitative test, indicated by the Shinoda test, the gel's color transitioned from yellow-green to moss-green after adding FeCl<sub>3</sub> solution and from yellowish-green to orange-red, confirming flavonoid presence.

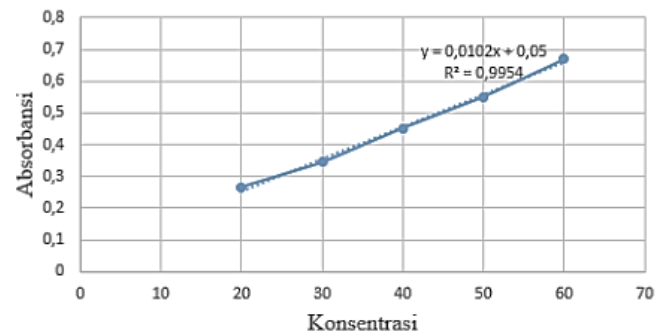


Figure 1. A gallic acid standard curve

Quantitative analysis for total phenolic content began with establishing a gallic acid standard curve, which demonstrated linearity with  $y = 0.0102x + 0.05$ ;  $R^2 = 0.9954$ . Among the gels, F3 exhibited the highest phenolic content, with F2 and F1 following in descending order. This aligns with the principle that higher extract concentrations correlate with increased total phenolic content.

Table 1. Total Phenolic Level of Kitolod Leaf Extract gel

Formula	Total Phenolic Level (mg GAE/g gel) ± SD
F1	0,395 ± 0,01 <sup>a</sup>
F2	0,563 ± 0,01 <sup>b</sup>
F3	0,703 ± 0,02 <sup>c</sup>

Different subscript denotes significant difference ( $p < 0.05$ )

The total flavonoid content was quantified using a quercetin standard curve equation,  $y = 0.0056x + 0.1005$ ;  $R^2 = 0.9985$ .

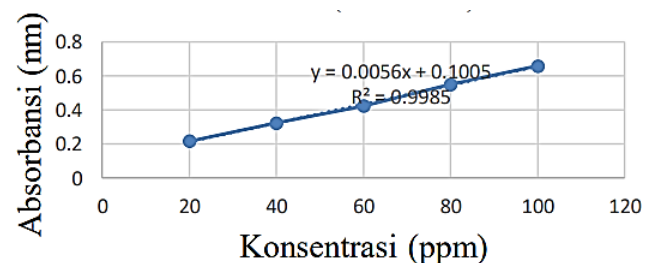


Figure 2. Quercetin standard curve

F3 also topped the chart for flavonoid content in Kitolod leaf extract gel, with F1 recording the lowest. This trend mirrors the



extract concentration effect, where higher concentrations increase flavonoid content.

**Table 2.** Total Flavonoid Level of Kitolod Leaf Extract gel

Formula	Total Flavonoid Level (mg KE/g gel) ± SD
F1	0,828 ± 0,05 <sup>a</sup>
F2	1,164 ± 0,03 <sup>b</sup>
F3	1,625 ± 0,06 <sup>c</sup>

Different *subscript* shows a significant difference (p<0.05)

Statistical analysis via SPSS revealed a *p* value < 0.05, indicating a significant correlation between the extract concentration and the total phenolic and flavonoid contents in the Kitolod leaf extract gel.

### Discussion:

Kitolod leaf powder was macerated using 96% ethanol as the solvent. Previous studies, such as the one conducted by Munte et al. (2015), have shown that extraction of Prasman (*Ayapana triplinervis* Vahl.) leaves with 96% ethanol yields a higher total phenolic level than extractions using 60% ethanol. Similarly, extracting flavonoids from *Stachytarpheta jamaicensis* (L.) Vahl flowers is more efficient with 96% ethanol than 70% or 50% ethanol. The growth of *Staphylococcus aureus* and *Escherichia coli* was more inhibited by *Stachytarpheta jamaicensis* leaf extract extracted with 96% ethanol than by 70% or 50% ethanol (Ramayani, Hastuti, et al., 2021). In the case of freeze-dried *Limnophila aromatica*, ethanol has proven to be the most effective solvent for extracting the highest levels of total phenolics and flavonoids. The highest level of total phenolic content of freeze-dried *Limnophila aromatica* belongs to the ethanol, followed by acetone, 75% aqueous acetone, 75% methanol, methanol, 75% ethanol, 50% ethanol, 50% acetone, 50% methanol, and water. The highest level of total phenolic content of freeze-dried *Limnophila aromatica* belongs to the ethanol, followed by acetone, 75% aqueous methanol, 75% aqueous ethanol, 50% aqueous acetone, 50% aqueous ethanol, methanol, 50% aqueous

methanol and the lowest is the water extract (Do et al., 2014).

Kitolod leaves, rich in thermolabile phenolic compounds and flavonoids, are best extracted through the maceration method. This approach has been shown to yield the highest total phenol content and superior antioxidant activity in extracts of *Syzygium polyanthum* compared to Soxhlet and infusion methods (Luliana et al., 2019). Heat extraction methods such as Soxhletation and infundation can degrade sensitive phenolic compounds due to the involved heat (Putri et al., 2022). The amount of phenolic content and temperature are related. Phenolic levels rise with rising temperatures until they reach a particular point, after which they fall as temperatures rise. A phenolic substance with conjugated aromatic systems exists, and it is called a flavonoid. High temperatures are easily damaging to conjugated aromatic systems. A phenolic substance with conjugated aromatic systems is called a flavonoid. High temperatures are easily damaging to conjugated aromatic systems. Many

The flavonoid group is linked to sugar molecules by glycoside linkages. High heat can easily break or degrade bond bonds. The heating process might cause a 15–78% reduction in flavonoid amounts (Sàadah et al., 2017).

The dark green color of Kitolod leaf extract is attributable to its chlorophyll content. Gels, as polymers, exhibit superior water absorption, tissue hydration, and moisturizing properties compared to ointments. Therefore, the Kitolod leaf extract has been formulated into a gel. This form benefits wound management, enhancing healing by improving tissue moisturization (Rüther & Voss, 2021). Gel structures serve as encapsulating mediums for bioactive ingredients and aroma compounds, allowing for controlled release. Given these components' instability yet high value for human health, incorporating phenolic compounds into hydrogels and other forms can mitigate undesirable oxidative reactions. The stability of these sensitive compounds under various conditions can be ensured by encapsulating them within a hydrogel network,

highlighting the importance of selecting suitable polymers. Due to their polarity, phenolic and flavonoid compounds quickly disperse in water-based gel formulations (Ćorković et al., 2021).

In phenolic qualitative assays, a visible color change occurs when  $\text{Fe}^{3+}$  ions form complexes, which indicates the presence of phenolic compounds due to electron transitions facilitated by ligands (Kejík et al., 2021). The Shinoda test for flavonoids changes the color of the gel solution from yellowish-green to orange-red, signalling a positive result for flavonoids through the formation of flavylum salts (Ramayani, et al., 2021).

Quantitative assessment of total phenolic levels is performed using the colourimetric method and Folin-Ciocalteu reagent. This reagent forms a blue-coloured complex with phenols, detectable by UV-Vis spectrophotometry within a 600-800 nm wavelength (Aminah et al., 2020).  $\text{AlCl}_3$  reagent is used in the colourimetric analysis of total flavonoid level. This method detects and measures flavonoids by creating a stable acid complex with an ortho dihydroxyl group on the A or B ring of the flavonoid (Da Silva et al., 2015).

Tables 2 and 3 illustrate that increased extract concentration within the gel correlates with higher total flavonoid and phenolic levels, suggesting a concentration-dependent effect. The greater the extract concentration inside the preparation, the more bulk and chemicals the extract has. The statistical analysis showed a significant influence between extract concentration and total phenolic and flavonoid levels of the kitolod leaf extract gel. The Kruskal-Wallis test revealed a substantial difference in each formula's total phenolics and flavonoids. Suwandi et al. (2012), which examined the impact of increasing the extract's concentration as an active ingredient on the flavonoid content of leaf extract cream kepel, produced similar findings. A higher quantity of kepel leaf extract was followed by a higher flavonoid content in the cream. This result also conforms to previous research by Octasari et al. (2021), which explained that increasing the extract concentration in a particular

pharmaceutical dosage form will also increase its total phenolic and flavonoid levels.

There is a positive link between phenolic chemicals and flavonoid compounds because they belong to the same class. The phenolic content increases with the amounts of flavonoids present. Ramayani et al., (2021) research showed similar results, demonstrating that phenolic content increased with increasing flavonoid content in noni and taro leaf extract. Phenolic chemicals and flavonoids have a similar (directly proportional) connection, meaning that the flavonoid value rises with increasing phenolic value and vice versa. They are significant since one component of phenolic compounds is flavonoid compounds. Therefore, the total phenolics in the bamboo leaf extract will similarly increase with the number of flavonoids present (Wahyuni et al., 2021). The lack of physical attributes testing on each preparation is the research's shortcoming.

### Conclusions:

This research confirms that total phenolic and flavonoid levels increase with kitolod leaf extract concentration in the gel formulation. Based on SPSS data analysis, there is a substantial relationship between the total phenolic and flavonoid levels and the extract concentration in the gel. There is a positive link between phenolic chemicals and flavonoid compounds because they belong to the same class.

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