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Phytochemical Screening and Quality of *Crescentia cujete* L. Leaf Extract

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Abstract

Crescentia cujete L., known as maja, has traditionally been used as a medicinal plant in several countries. The fruit and other parts of this plant contain bioactive compounds with antioxidant, antibacterial, antidiabetic, anti-inflammatory, and cytotoxic activities related to their secondary metabolites. The purpose of this study was to determine the quality of Crescentia cujete L. leaf extract obtained from Gajahan Hamlet, Pasuruan. This study used an experimental method, which included organoleptic tests, water- and ethanol-soluble extract levels, water content, ash content, and phytochemical screening. The results showed that the yield of Crescentia cujete L. leaf extract was 13.2939%. The quality parameters of Crescentia cujete L. leaf extract included organoleptic tests such as thick extract, odor according to the plant of origin, brown color, water-soluble extract content of 11.54%, ethanol-soluble extract content of 12.49%, water content of 8.44%, and ash content of 4.12%. These results indicate that Crescentia cujete L. leaf extract meets the requirements according to the Indonesian Herbal Pharmacopoeia. The results of phytochemical screening of Crescentia cujete L. leaf extract indicate that Crescentia cujete L. leaf extract contains flavonoids, alkaloids, saponins, tannins, and steroids.

Keywords: *phytochemical, screening, Crescentia cujete* L., *leaf, extract.*

INTRODUCTION

The use of natural ingredients as a source of bioactive compounds continues to increase along with the development of green pharmaceuticals and functional foods (Morales, 2025), (Khan, Anwar, Ghazali, & Mahyudin, 2024), (Messinese et al., 2024). Analysis of the quality of natural ingredient extracts is an important aspect to ensure product safety and standardization before application on an industrial scale (Edo et al., 2024), (Jamilatun, Lukito, & Kumalasari, 2026). One plant that has this potential is *Crescentia cujete* L., known as maja, which has traditionally been used as a medicinal plant in several countries (Gonzales, Sevilla, & Tsai, 2023). The fruit and other parts of this plant contain bioactive compounds with antioxidant,

antibacterial, antidiabetic, anti-inflammatory, and cytotoxic activities related to the content of its secondary metabolites (Balogun & Sabiu, 2021).

In the development of natural raw materials, extract quality parameters such as yield, water content, ash content, water-soluble and ethanol content, active compound content, and organoleptic characteristics are basic indicators that determine compliance with regulatory requirements. Extract quality standardization is also an important prerequisite for ensuring consistent raw material quality in the development of herbal medicines, supplements (Jamilatun & Lukito, 2025), functional foods (Jamilatun, Purnamasari, & Tolkhah, 2024), (Jamilatun & Lukito, 2022), and natural food additives. Extract quality analysis is a step to determine the characteristics of the extract and support its use as a raw material that meets quality standards. The quality of plant extracts can be influenced by geographic factors and the environmental conditions where the plant grows (Ren et al., 2023). This indicates that *Crescentia cujete* L. grown in different regions can have different phytochemical profiles.

Based on the description above, research was conducted on the quality analysis of *Crescentia cujete* L. leaf extract, which comes from Gajahan Hamlet, Pasuruan, with the aim of determining the quality of *Crescentia cujete* L. extract, with quality parameters including organoleptic tests, water-soluble extract content, ethanol extract content, water content, and ash content, as well as phytochemical tests.

Literature Review

Extract quality is a key parameter for ensuring the quality of raw materials used in the pharmaceutical and food industries. According to the herbal ingredient standardization guidelines, extract quality evaluation includes organoleptic characteristics, phytochemical content, water content, ash content, and other parameters that contribute to product quality (Kementerian Kesehatan RI, 2023). Organoleptic characteristics serve as an initial indicator for assessing the uniformity of raw materials and detecting potential degradation during extraction and storage. Water-soluble extract and ethanol-soluble extract levels indicate the amount of chemical compounds that can be extracted by solvents based on their polarity. Water-soluble extract levels reflect the content of polar compounds, while ethanol-soluble extract levels reflect the content of semi-polar to non-polar compounds. Furthermore, low water content can inhibit microbial growth and hydrolysis reactions. Ash content reflects mineral content and the possibility of inorganic contamination during processing. These parameters are crucial for controlling extract quality according to standards (Edo et al., 2024).

Crescentia cujete L. is known to contain various secondary metabolites, which contribute to various activities, such as antioxidant, antimicrobial, and anti-inflammatory (Gaspersz, Kapelle, Hattu, Sangadji, & Arpipi, 2024), (Balogun & Sabiu, 2021). Phytochemical analysis indicates the presence of various bioactive compounds that have the potential to be developed as natural raw materials for health products. In formulation, the quality of the extract affects the stability of the preparation and product safety. The quality of the fruit extract is a step in ensuring the consistency of raw material quality and supporting the development of herbal, cosmetic, and food products that meet regulatory requirementsPhytochemical analysis indicates the presence of various bioactive compounds that have the potential to be developed as natural raw materials for health products. In formulation, the quality of the extract affects the stability of the preparation and product safety. The quality of the fruit extract is a step in ensuring the consistency of raw material quality and supporting the development of herbal, cosmetic, and food products that meet regulatory requirements (Edo et al., 2024), (Lukman, 2020), (Kementerian Kesehatan RI, 2023).

MATERIALS AND METHODS

Materials

The materials used include *Crescentia cujete* L. leaves, ethyl acetate (Merck), HCl (Merck), magnesium powder (Mg), H2SO4 (Merck), FeCl3 (Merck), Chloroform (Merck), anhydrous acetic acid (Merck), BaCl2 (Merck), Dragendorf reagent (Merck), distilled water, 96% ethanol. The tools used include a grinder (Maksindo), aluminum pan, beaker (Iwaki), dropper pipette, volume pipette, glass funnel (Pyrex), test tube (Pyrex), stirring rod (Pyrex), Erlenmeyer flask (Pyrex), knife, jar, porcelain cup (Pyrex), porcelain crucible (Pyrex), Whatman filter paper, measuring cup (Iwaki), desiccator, oven (Memmert), analytical balance (Ohaous), maceration equipment set, water bath (Equitron).

Preparation of Raw Materials

Crescentia cujete L. plants were obtained from Gajahan Hamlet, Pasuruan. The *Crescentia cujete* L. leaves used were fresh, dark green, and located on the leaf stalk without any spots (Rahmawati, 2022).

Plant Determination

The plant identification process is carried out to determine the correctness of the plant's identity and avoid errors in the material being studied (Ulandari & Sani, 2023). Determination of Crescentia cujete L. was carried out at the UPT Materia Medika Batu, East Java.

Preparation of *Crescentia cujete* L. Leaves

Weigh 2 kg of fresh, mature *Crescentia cujete* L. leaves. They are washed with water to remove any dirt. Then, they are cut into small pieces to reduce the surface area of the leaves, allowing the solvent to more easily extract the compounds contained within. They are then dried in an oven at 50°C for 2 days to reduce the water content and facilitate storage. The dried leaves are ground using a grinder to form a powder (Rahmawati, 2022).

Preparation of *Crescentia cujete* L. Leaf Extract

Maja leaf powder was weighed at 150 grams and then macerated by soaking in 1.5 liters of ethyl acetate solvent for 3 × 24 hours at room temperature and protected from light. Then, the macerate was separated by filtration using Whatman No. 1 filter paper, and the residue was discarded. The filtrate was rotary evaporated at 60°C and then concentrated with a water bath at 50°C until a thick extract was obtained (Jamilatun, Utami, & Tolkhah, 2025). The yield (%) of the thick extract was calculated using the following formula:

$$\text{Yield (\%)} \frac{Extract\ weight}{Initial\ Weight\ of\ Simplex} \text{ x } 100\%$$

Quality Testing of *Crescentia cujete* L Leaf Extract.

1) Organoleptic

   Extracts are observed simply using the five senses to describe the shape, color, odor, and taste (Jamilatun, Lukito, & Zahra, 2025).

2) Water-Soluble Extract Content

   A 0.5 g extract was macerated for 18 hours with 20 mL of chloroform-saturated water (1:1) using a stoppered flask, shaken repeatedly for 6 hours. 5 mL of the filtrate was evaporated to dryness in a porcelain dish preheated to 105ºC and tared. The residue was heated at 105ºC until a constant weight was achieved (Khairi, Hapiwaty, Yusuf, & Indrisari, 2023). The water-soluble extract content was calculated as follows:

   $$\text{Water-Soluble Extract Content (\%)} = \frac{(W2-W0)}{W1} \text{ x } 100\%$$

   Description: W0 = weight of empty dish (g); W1 = weight of initial extract (g);

W2 = constant weight of dish + residue (g)

3) Ethanol-Soluble Extract Content

A 0.5 g extract was macerated in 20 mL of 96% ethanol for 18 hours using a stoppered flask, shaking frequently for the first 6 hours, then rapidly filtered to prevent ethanol evaporation. Twenty mL of the filtrate was evaporated to dryness in a porcelain dish preheated to 105ºC and tared. The residue was heated at 105ºC until a constant weight was reached (Khairi et al., 2023). The ethanol-soluble extract content was calculated as follows:

Ethanol-Soluble Extract Content (%) = $\frac{(W2-W0)}{W1}$ x 100%

Description: W0 = weight of empty dish (g); W1 = weight of initial extract (g);

W2 = constant weight of dish + residue (g)

4) Water Content

Water content was measured using the gravimetric method by accurately weighing 1 g of extract in a pre-weighed dish. The sample was then oven-dried at 105°C for 4 hours. The sample was then cooled in a desiccator for 10 minutes and weighed until a constant weight was achieved (Jamilatun et al., 2026). The formula for determining water content is as follows:

Water Content (%) = $\frac{(W1-W0)-(W2-W0)}{W1-W0}$ x 100%

Description: W0 = weight of empty cup (g); W1 = weight of cup + sample (g);

W2 = cup weight + drying yield (g)

5) Ash Content

1 g of the extract is placed in a heated and tared crucible, then mixed evenly. Heat slowly until the charcoal is completely gone, then weigh. If this method does not remove the charcoal, filter through ash-free filter paper and add hot water. Place the residue and filter paper in the same crucible. Add the filtrate to the crucible, evaporate, and heat until the weight is constant, then weigh again (Jamilatun et al., 2026). The formula for determining ash content is as follows:

Total Ash Content (%) $\frac{W2-W0}{W1-W0}$ x 100%

Description: W0 = weight of empty crucible (g); W1 = weight of crucible + sample (g);

W2 = weight of crucible + heating result (g)

6) Phytochemical Testing Phytochemical Testing
   a. Flavonoid identification: Dissolve 0.5 grams of extract in 2 mL of methanol, add 2 mg of Mg powder and 1 mL of concentrated HCl, and shake vigorously. The formation of a red, orange, or yellow color indicates the presence of flavonoids (Jamilatun, 2023).
   b. Alkaloid identification: Add 5 drops of 2M H2SO4 and 3 drops of Dragendorf's reagent to 0.5 g of extract. The formation of an orange or red precipitate indicates the presence of alkaloids (Manongko, Sangi, & Momuat, 2020).
   c. Saponin identification: Dissolve 0.5 g of extract in hot distilled water, add 1 mL of concentrated HCl, and shake vigorously. The formation of foam that persists for 30 seconds indicates the presence of saponins (Manongko et al., 2020).
   d. The Tannin Test is performed by adding 0.5 g of extract to 1 mL of 1% FeCl3 solution. The formation of a blackish-blue to blackish-green color indicates a positive tannin content (Manongko et al., 2020).
   e. The Steroid Test is performed by adding 2 mL of chloroform, 1 mL of anhydrous acetic acid, and finally 2 drops of concentrated H2SO4 to the 0.5 g extract. The mixture is gently shaken and allowed to stand for several minutes. The formation of a brownish ring and two layers (Royani, Febri Fatwami, Islamiyati, & Stasiana Yunarti, 2024), or a blue or green color, indicates a positive steroid content (Rahmatia, Nasrudin, & Nurlansi, 2022).

RESULTS AND DISCUSSION

This study was conducted with the aim of determining the quality of *Crescentia cujete* L. leaf extract obtained from Gajahan Hamlet, Pasuruan, East Java. Determination was carried out at the UPT Materia Medika Batu, East Java, to determine the authenticity of the plants studied to avoid errors in the plants used (Jamilatun, Lukito, & Zahra, 2025). The results of the determination of *Crescentia cujete* L. were obtained with the following determination key: 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-13b-14b-15b-16b-286a-287a:Bignoniaceae-1b-3a: Crescentia3:C.cujete.Maja is included in the Bignoniaceae family with the genus Crescentia (Materia Medica, 2025) .

The *Crescentia cujete* L. leaves used were fresh, dark green, and old leaves located on the leaf stalks without any spots (Rahmawati, 2022). The selection of fresh, dark green old leaves indicates that the leaves have optimal active compound components, while the absence of spots also ensures that the samples used are not contaminated or damaged. The collected fresh leaves were wet-sorted, then cut and dried in an oven at 50°C, and then ground into

powder for maceration. After that, the sample was filtered, the filtrate was concentrated to obtain a thick extract, and the yield was calculated as presented in Table 1.

Table 1. Yield of *Crescentia cujete* L. Leaf Extract

Fresh Leaves (g)	Simple Powder (g)	Thick Extract (g)	Yield (%)
2000	150,0150	19,9429	13,2939%

Based on the results of the *Crescentia cujete* L. leaf extract in Table 1, the yield was 13.2939%, and it meets the requirements according to the Indonesian Ministry of Health (2022), which is not less than 7.12%. The yield value is directly proportional to the active compound content in the extract. The higher the yield value obtained, the greater the active compound content concentrated in the sample. (Pratiwi, 2019).

Table 2. Organoleptic Test Results, Water and Ethanol Soluble Essence Content, Water Content, Ash Content of *Crescentia cujete* L. Leaf Extract

Parameter	Results	Standard	Explanation
Organoleptic	Thick extract, distinctive smell of *Crescentia cujete* L. leaves, blackish brown color	Thick extract, smells like the plant of origin, brown color	Qualify
Water-Soluble Extract Content (%)	11.54	$\geq$ 8.3	Qualify
3) Ethanol-Soluble Extract Content (%)	12.49	$\geq$ 11.9	Qualify
Water Content (%)	8.44	$\leq$ 16.6	Qualify
Ash Content (%)	4.12	$\leq$ 7.5	Qualify

Organoleptic testing of *Crescentia cujete* L. leaf extract included testing of shape, odor, and color. The test results are shown in Table 2. The *Crescentia cujete* L. leaf extract was thick, had a distinctive odor of *Crescentia cujete* L. leaves, and was blackish brown in color. The results obtained met the requirements for good organoleptic testing of extracts according to the Indonesian Herbal Pharmacopoeia, Edition II. (Kementerian Kesehatan RI, 2023).

The extract content test was conducted to determine the number of compounds contained in the crude drug. In the water-soluble and ethanol extract content tests, the extract was macerated using chloroform-saturated water solvent for the water-soluble extract content, while in the ethanol extract content test, it was macerated using ethanol solvent. This was done so that the solvent could attract and dissolve the chemical compounds in the extract (Nabila Nur Latifa, Lanny Mulqie, & Siti Hazar, 2022). Based on the results of the tests carried out, the determination of the water-soluble extract content of *Crescentia cujete* L. leaf extract in table 2 was obtained at 11.54% and the ethanol-soluble extract content in table 4.4 was 12.49%, both of which have met the requirements for good water-soluble extract content and ethanol-

soluble extract content according to the Herbal Pharmacopoeia Edition II, namely less than 8.3% and more than 11.9%. These results provide an illustration that *Crescentia cujete* L. leaves are more soluble in ethanol solvents or less polar solvents than polar solvents such as water.

Moisture content determination was carried out to determine the water content in the extract. Moisture content is a parameter that provides the maximum limit of water content in the extract. High water concentrations in the extract can become a medium for bacterial and fungal growth, which can damage the compound and reduce the biological activity of the extract during storage. Based on the test results in Table 2, the moisture content of *Crescentia cujete* L. leaf extract was 8.44%. This value has met the requirements for good moisture content based on the Indonesian Herbal Pharmacopoeia II edition, which is less than 16.6% (Kementerian Kesehatan RI, 2023). Determination of ash content aims to determine the content of inorganic compounds (mineral content) contained in the extract. Ash content testing is carried out to measure the total content of inorganic compounds, especially in the form of metal oxides, and to identify the characteristics of non-organic ash residue after the ashing process (Supriningrum, Sundu, Sentat, Niah, & Kumalasari, 2021). Based on the test results in Table 2, the ash content of *Crescentia cujete* L. leaf extract was 4.12%. This value has fulfilled the requirements for good ash content based on the Indonesian Herbal Pharmacopoeia, edition II, which is less than 7.5% (Kementerian Kesehatan RI, 2023).

Table 3. Phytochemical Screening Results of *Crescentia cujete* L Leaf Extract

Phytochemical Test	Test Results	Test Results
Flavonoids	A red, yellow, or orange solution forms	Positive (+)
Alkaloids	An orange precipitate forms	Positive (+)
Saponins	Foam forms, stable for 30 seconds	Positive (+)
Tannin	A green solution forms	Positive (+)
Steroids	A green solution forms	Positive (+)

Phytochemical screening was conducted to determine the compounds contained in *Crescentia cujete* L. leaf extract, with the following results. *Crescentia cujete* L. leaf extract positively contained flavonoids, alkaloids, saponins, and tannins. These results are in accordance with research (Rahmawati, 2022) which stated that *Crescentia cujete* L. leaves contain these compounds. Crescentia cujete L. leaf extract also positively contained steroid compounds, which is in accordance with research (Nurhasanah, Harlina, & Adhitiyawarman, 2014). The presence of flavonoids, alkaloids, saponins, tannins, and steroids in *Crescentia*

cujete L. leaf extract shows its potential as a raw material for herbal medicines, cosmetics, and functional foods. Flavonoids and tannins act as antioxidants, while alkaloids, saponins, and steroids have antimicrobial and anti-inflammatory activities that support the development of pharmaceutical and food preparations (Jamilatun, Utami, et al., 2025), (Jamilatun, Lukito, & Rayhanissa, 2025), (Jamilatun & Lukito, 2025), while still paying attention to safety evaluations, as well as stability and effectiveness testing in the final formulation to meet regulatory requirements and ensure consistent product quality (Kementerian Kesehatan RI, 2023).

CONCLUSION

The yield of *Crescentia cujete* L. leaf extract was 13.2939%. The quality parameters of *Crescentia cujete* L. leaf extract included organoleptic tests in the form of a thick extract, odor according to the original plant, brown color, water-soluble extract content of 11.54%, ethanol-soluble extract content of 12.49%, water content of 8.44%, and ash content of 4.12%. These results indicate that *Crescentia cujete* L. leaf extract meets the requirements according to the Indonesian Herbal Pharmacopoeia. The results of phytochemical screening of *Crescentia cujete* L. leaf extract showed that *Crescentia cujete* L. leaf extract contains flavonoids, alkaloids, saponins, tannins, and steroids.

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