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Identification Of Bioactive Compounds In Butterfly Pea (*Clitoria ternatea* L.) Flower Simplicia: A Macroscopic, Microscopic, And Phytochemical Study

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ABSTRACT
The butterfly pea flower (*Clitoria ternatea* L.) is known for its distinctive purple petals. This plant typically grows as an ornamental and can be used as a natural eye remedy and food coloring, safe for human consumption. This study aims to investigate the characteristics and perform a phytochemical screening of the butterfly pea flower (*Clitoria ternatea* L.). The characteristics of the flower simplicia were examined through macroscopic and microscopic observations, followed by a phytochemical screening process, which included testing for alkaloids, flavonoids, saponins, tannins, and terpenoids/steroids using specific reagents. Additionally, non-specific characteristics, such as moisture content, were analyzed. The results indicate that the flower simplicia has a distinct odor, is tasteless, blue in color, and appears in a dry form. Microscopically, the flower simplicia revealed single grains and thin slices, showing epidermal cell fragments with stomata and crystal fibers with vascular bundles. The phytochemical screening confirmed the presence of alkaloids, saponins, and flavonoids, while tannins and terpenoids/steroids were absent.

Key words: *Clitoria ternatea* L, phytochemical, macroscopic, microscopic

INTRODUCTION
Indonesia is known as a country with one of the most extensive biodiversities in the world. There are approximately 90,000 plant species that grow in Indonesia (Fitmawati, et al., 2016). This biodiversity is utilized by the Indonesian people for various purposes, such as food, medicinal plants, traditional customs, ornamental use, and local technology (Endang, 2020). The butterfly pea flower, characterized by its distinctive blue color, is believed to have originated in tropical Asia, although some sources suggest it came from Central South America and spread to tropical regions, including Indonesia, in the 19th century (Anto, 2021; Ulimaz, et al., 2020). Parts of the butterfly pea plant commonly used for medicinal purposes include the leaves, seeds, bark, fruit, sprouts, flower stem, and roots (Tabeo, et al., 2019).

Simplicia characterization is conducted by observing and examining both the macroscopic and microscopic properties, determining moisture content, levels of water-soluble extract, ethanol-soluble extract, total ash content, acid-insoluble ash, and performing phytochemical screening (Indonesian Ministry of Health, 1989). Simplicia, or herbal material, refers to natural substances that have been dried for medicinal use and have not undergone processing. Unless otherwise specified, simplicia drying temperatures should not exceed 60°C (Ditjen POM, 2008). To ensure the uniformity of active compounds, safety, and efficacy, simplicia must meet minimum quality requirements. Characterization of simplicia involves examining both macroscopic and microscopic properties, determining moisture content, levels of water-soluble and ethanol-soluble extracts, total ash content, acid-insoluble ash, and conducting phytochemical screening (Indonesian Ministry of Health, 1989).

Phytochemical screening is a method used to study the active compound components in a sample, including their chemical structure, biosynthesis, natural distribution, biological function, as well as isolation and comparison of chemical compositions across various plant species. Factors such as geographical location, temperature, climate, and soil fertility significantly influence the chemical content of a plant. Plant samples for phytochemical testing can include leaves, stems, fruits, flowers, and roots, which possess medicinal properties and are used as raw materials in the production of both modern and traditional medicines (Agustina, et al., 2016). According to Kristianti, et al. (2018), phytochemical screening is one method that can be used to identify the secondary metabolite content of a natural substance. This preliminary screening stage provides an overview of specific compounds within the natural material under study. Phytochemicals found in butterfly pea flowers include tannins, saponins, triterpenoids, alkaloids, flavonoids, and steroids. The seeds of the butterfly pea flower contain cinnamic acid, phenotin, and beta-sitosterol (Budiasih, 2017).

The butterfly pea flower (*Clitoria ternatea*) with its unique bioactive compounds, is a plant traditionally used for its medicinal properties, yet much remains to be understood about its phytochemical profile and potential applications. Phytochemical screening and simplicia characterization are crucial steps in ensuring the quality, safety, and efficacy of medicinal plants, particularly as they provide insights into the active compounds that may vary based on geographical, climatic, and environmental factors. This research not only supports the sustainable use of local plant resources but also contributes to the advancement of natural product-based medicines, which have long been an integral part of Indonesia's cultural and health practices.

MATERIAL AND METHODS

Material

Butterfly pea flower simplicia, $FeCl_3$ reagent, Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, magnesium powder, 96%

ethanol, 2N hydrochloric acid, anhydrous acetic acid, concentrated sulfuric acid, n-hexane, amyl alcohol, chloral hydrate, chloroform, and distilled water.

Methods

Preparation of Butterfly Pea Flower Simplicia

The collected butterfly pea flowers are first wet-sorted, then washed under clean running water and drained. Prepare a place for drying the flowers under direct sunlight for 48 hours. After drying, sort the plant parts to select those that will be used in the dried simplicia. The dried simplicia is then stored in a clean, dry, and tightly sealed container.

Organoleptic

The organoleptic examination of butterfly pea flower simplicia is conducted visually, covering aspects such as shape, taste, color, and aroma (Ministry of Health, 2017).

Microscopic

Microscopic examination is conducted on butterfly pea flower simplicia, which is first ground into a fine powder. Then, the general characteristic fragments of the butterfly pea flower are observed using a microscope with magnification up to 400x (Eliyanoor, 2012; Indonesian Ministry of Health, 2000).

Determination of Ethanol-Soluble Extract Content

Place 5 grams of butterfly pea flower extract in a stoppered flask, add 100 mL of 96% ethanol, and shake frequently for the first 6 hours. Let the mixture sit for an additional 18 hours, then filter to obtain the filtrate. Evaporate 20 mL of the filtrate at 105°C until a constant weight is achieved (Ministry of Health, 2017). The percentage of ethanol-soluble extract content is calculated similarly:

$$Ethanol - soluble\ extract = \frac{weight\ of\ extrac\ (g)}{weight\ of\ simplicia\ (g)} x \frac{100}{20} x\ 100\%$$

Determination of Water-Soluble Extract Content

Place 5 grams of butterfly pea flower extract in a stoppered flask, add 100 mL of chloroform-saturated water (prepared by adding 3 drops of chloroform to 100 mL distilled water), and shake frequently for the first 6 hours. Let the mixture sit for an additional 18 hours, then filter to obtain the filtrate. Evaporate 20 mL of the filtrate at 105°C until a constant weight is achieved (Ministry of Health, 2017). The percentage of water-soluble extract content is calculated using the formula:

$$Water - soluble\ extract = \frac{weight\ of\ extract\ (g)}{weight\ of\ simplicia\ (g)} x \frac{100}{20} x\ 100\%$$

Phytochemical Screening

Alkaloids

Treat 1 gram of fresh butterfly pea flower with 25% ammonia, then add chloroform and filter the mixture. Shake the resulting filtrate vigorously with 2N HCl until two layers form. Take the acidic layer (top layer) and divide it into two parts. The first part is used as a blank, and the second part is tested with 4-5 drops of Mayer's reagent and Dragendorff's reagent, respectively. A positive result for alkaloids is indicated by a white precipitate with Mayer's reagent and a yellowish-red precipitate with Dragendorff's reagent (Tiwari et al., 2011; J.B. Harborne, 1996; Farnsworth, 1966).

Saponins

Place 0.5 grams of butterfly pea flower simplicia powder into a beaker containing 10 mL of distilled water, then heat until boiling for 10 minutes and filter the solution. Pour the resulting filtrate into a test tube, shake vigorously for 10 seconds, and then add 2N HCl. A positive result for saponins is indicated by the formation of foam between 1-10 cm in height that remains stable for 10 minutes (Harborne, 1996).

Flavonoids

Place 0.5 grams of butterfly pea flower simplicia powder into a beaker containing 10 mL of distilled water, then heat until boiling for 10 minutes. Filter the solution, then take 5 mL of the filtrate and add 0.1 grams of magnesium powder (Mg), 1 mL of concentrated HCl, and 2 mL of amyl alcohol. Shake the mixture vigorously and let it sit until it separates into two layers. A positive result for flavonoids is indicated by a red, yellow, or orange color in the amyl alcohol layer (Harborne, 1996).

Tannins

Place 0.5 grams of butterfly pea flower simplicia powder into a beaker containing 10 mL of distilled water, then heat until boiling for 10 minutes and filter the solution. Take 2 mL of the resulting filtrate and add 2 drops of 1% $FeCl_3$ reagent. A positive result for tannins is indicated by the formation of a blue-black or greenish-black color (Harborne, 1996).

Terpenoids/Steroids

Add 10 mL of chloroform to 0.5 grams of butterfly pea flower simplicia powder and let it sit for 30 minutes, then filter the mixture. Evaporate the resulting filtrate in a porcelain dish. After evaporation, add a drop of Liebermann-Burchard reagent (LB) to the residue. A positive result for steroids is indicated by the formation of a blue-green ring, while a purple or orange color signifies the presence of triterpenoids (Harborne,1996).

RESULT AND DISCUSSION

Standardization is conducted as a reference to determine whether the butterfly pea flower simplicia and extract meet established requirements. The standards and requirements currently applied to butterfly pea flowers are still

general, as there is no specific official guideline for this plant to date (Ramdhini, 2023). The organoleptic examination aims to provide an initial assessment of raw materials (simplisia and extracts) using sensory perception, covering shape, color, aroma, and taste. The results of the organoleptic examination on Table 1. The blue color in butterfly pea flowers is due to the presence of anthocyanins, which possess antioxidant properties and can produce colors ranging from red to blue (Makasana et al., 2017).

Table 1 Organoleptic of butterfly pea flower simplicial

	Result
Organoleptic	Shape: dry form Taste: sweet Color: blue Aroma: distinctive aroma

Table 2 Water-soluble and ethanol-soluble extract content

	Content (%)			Average (%)
	I	II	III	
water-soluble extract content	36	34	38	36
ethanol-soluble extract content	30	26	31	29

The determination of water-soluble and ethanol-soluble extract content is a classic approach to estimate the active compound levels based on polarity. This method allows for calculating the percentage of polar and semi-nonpolar to nonpolar compounds in the extract. The results provide a rough estimate of polar compounds (water-soluble) and semi-polar to nonpolar compounds (ethanol-soluble). The combined percentages of water- and ethanol-soluble extracts in an extract should not exceed 100% (Saifudin et al., 2011). The characterization results of butterfly pea flower simplicia powder indicate that the ethanol-soluble extract content is 29% and water-soluble extract content is 38% (Table 2).

Microscopic observation of Butterfly Pea Flower (Clitoria ternatea) simplisia, as shown in Figure 1, reveals the presence of single granules and thin slices, indicating the existence of (1)epidermis, (2)covering trichomes, calcium oxalate crystals, and (3) pollen grains. In the powdered form of the simplisia, several cellular structures and tissues can be identified, including epidermal cells, covering trichomes, calcium oxalate crystals, and fragments of yellowish oil glands. The abundance of covering trichomes scattered throughout the sample is a distinguishing feature for microscopic

identification of the leaves and flowers. These observations align with findings by Helma N., et al. (2021), emphasizing their significance in the authentication and quality assessment of the plant material.

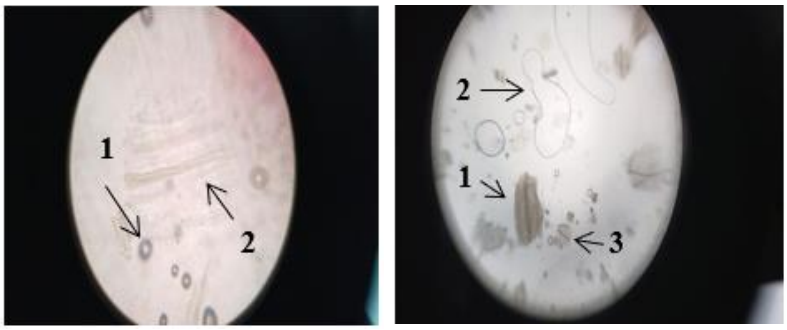

Figure 1 Microscopic Examination Results of Butterfly Pea Flower (Clitoria ternatea) Simplisia

Table 3 Phytochemical screening of the butterfly pea flower (*Clitoria ternatea* L.)

Compound	Result	Description
Alkaloid	Formation of white precipitate	+
Saponin	Produces foam	+
flavanoid	Red-colored layer	+
Tannin	Does not produce dark blue color	-
terpenoid	Does not produce red color	-

Phytochemical screening is a method used to study the active compound components in a sample, including their chemical structure, biosynthesis, natural distribution, biological functions, isolation, and comparison of chemical compositions across various plant species. The results of the phytochemical screening on Table 3 indicate that butterfly pea flower simplicia contains alkaloids, saponins, and flavonoids, but does not contain tannins or terpenoids/steroids. In the alkaloid test, the formation of a precipitate indicates the presence of alkaloids in the solution. A white precipitate forms with Mayer's reagent, an orange-red precipitate forms with Dragendorff's reagent, and a brown precipitate forms with Wagner's reagent. The saponin test results indicate that the simplicia powder contains saponin compounds, as evidenced by the formation of a stable foam approximately 3.5 cm high when the solution is left to stand for about 10 minutes. According to Ningsih et al. (2016), the foam produced in the saponin test is due to glycosides, which can form foam in water and hydrolyze into glucose and other compounds. The butterfly pea flower simplicia contains flavonoid

compounds, as indicated by the color change to yellow-red in the filtrate or orange-red in the amyl alcohol layer. The reaction between flavonoids, HCl, and magnesium (Mg) metal can be seen in the following image:

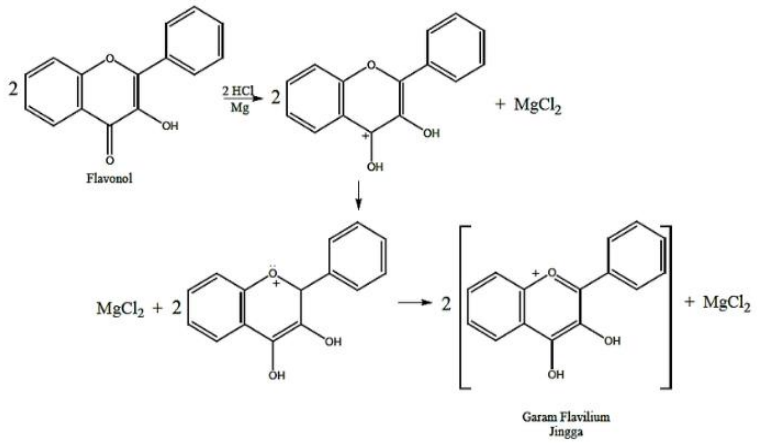

Figure 2 Flavonoid Reaction with Mg Metal and HCl (Parbuntari, 2018)

In the tannin test, the resulting color change produced a purple solution. This indicates that the butterfly pea flower simplicia does not contain tannins, as the diluted filtrate turned purple rather than the dark blue or greenish-black color typical of a positive tannin reaction. In the terpenoid or steroid test, the solution changed to a clear color and formed a yellow precipitate. Therefore, the solution is considered negative and does not contain terpenoid or steroid compounds. According to Anjar Purba A. (2017), a positive result in the terpenoid test is indicated by a color change to red, purple, or brown, which is due to the oxidation of terpenoid compounds that produces chromophore groups.

CONCLUSION

Microscopic observation of butterfly pea flower simplicia revealed granules and thin sections identified as epidermal cells with stomata, as well as crystal fibers with vascular bundles. The simplicia also possesses a characteristic butterfly pea flower scent, a neutral (tasteless) flavor, a blue color, and a dry form. The ethanol-soluble extract content and water-soluble extract content were determined to be an average of 29% and 36%, respectively. Butterfly pea flower simplicia contains alkaloids, saponins, and flavonoids, but does not contain tannins or terpenoids/steroids.

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