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Phytochemical Screening of *Strobilanthes crispa* Leaf Extract Obtained by Ultrasound-Assisted Extraction (UAE) Method

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Abstract
The study was conducted to determine of secondary metabolite compounds in the ethanol extract of keji beling leaves (*Strobilanthes crispa* (L.). Keji beling leaves are known to possess pharmacological effects such as antioxidant, antibacterial, and anticancer. These pharmacological effects are due to the presence of secondary metabolites. This study aims to investigate the secondary metabolite compounds contained in keji beling (*S. crispa*). Fresh keji beling leaves were dried, ground into powder, and extracted using 70% ethanol solvent using the Ultrasonic Assisted Extraction (UAE) method. Phytochemical screening was carried out by adding reagents specific for secondary metabolites. The test results showed that the ethanol extract of keji beling leaves contained positive flavonoids, alkaloids, saponins, and tannins, while the terpenoid test showed negative results. The presence of these metabolites indicates that keji beling leaves have the potential as a source of bioactive compounds and can be developed into raw materials for herbal medicine preparations.

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INTRODUCTION

Keji beling (Strobilanthes crispa (L.) Blume) is a medicinal plant widely used traditionally in Indonesia to treat various diseases, such as kidney stones, diabetes, and hypertension (Adibim et al., 2017; Nuraqidah et al., 2025). Previous research has been conducted and it is known that administering Kejibeling Leaf Extract Infusion (S. crispa) can reduce blood sugar levels in male Wistar rats (Rafli et al., 2023). Ethanol extract of keji beling leaves has been tested on alloxan-induced rats and demonstrated antidiabetic effects (Palit et al., 2018). Keji beling has pharmacological effects due to its content of secondary metabolites that act as antioxidants, antibacterials, and anticancer (Widowati et al., 2018). Secondary metabolites originate from the biogenesis process of primary metabolites such as amino acids, proteins, and carbohydrates (Saputra et al., 2022).

Secondary metabolites are produced by living things for various purposes, such as to defend themselves from the environment or threats from other organisms. The production of secondary metabolites occurs in different metabolic pathways. Secondary metabolites differ from primary metabolites, although not as vital as primary metabolites, they are necessary (Pury, 2020). The content of plant metabolites is influenced by geographic location, temperature, climate, and soil fertility where they grow (Wardhani and Akhyar, 2018).

Several studies have shown that S. crispa contains secondary metabolites such as flavonoids, tannins, alkaloids, and saponins. However, phytochemical screening is still needed to obtain information on the composition of specific bioactive compounds. Phytochemical screening is

carried out to evaluate the composition of secondary metabolites from the plant (Ikbal, 2024). Phytochemical screening uses specific chemical reagents. Keji beling leaf extract is obtained through extraction using the Ultrasonic Assisted Extraction (UAE) method. The Ultrasonic-Assisted Extraction (UAE) method is an extraction method that utilizes the effects of ultrasonic waves which can increase the rate of mass transfer and can break cell walls with a lot of wave resonances so that it will shorten the extraction process time (Lukmayani et al., 2024). UAE is a modern extraction method often used by researchers to obtain natural compounds because modern extraction methods in their application do not require a lot of solvents so they can minimize waste and the time used is relatively short (Suhendar et al., 2020). The 70% ethanol solvent was chosen because 70% ethanol is a more polar solvent so it can dissolve secondary metabolites from the sample (Pramudita Riwanti, Farizah Izazih, A., 2020). Ethanol is also non-toxic, safe for all secondary metabolites, and volatile

METHOD

The research was conducted experimentally in the laboratory. Pharmacognosy-Phytochemistry Faculty of Pharmacy, Muslim University of Indonesia, Makassar. The tools used are glassware such as: Erlenmeyer flasks, measuring cups, stirring rods, funnels, porcelain dishes, dropper pipettes, UAE. The materials used are Keji beling leaf extract (*Strobilanthes crispa* L.), 70% ethanol, ethyl acetate pa ($C_4H_8O_2$), aluminum chloride ($AlCl_3$), Magnesium Powder, Chloroform reagent, distilled water, Mayer reagent, Wagner, Dragendroff, iron (III) chlorida, 2 N HCl, Lieberman-Burchard reagent, concentrated sulfuric acid.

Sample Preparation

Samples were determined in the Phytochemical Pharmacognosy Laboratory, Faculty of Pharmacy, Muslim University of Indonesia. Determination number 0027/C/UD-FF/UMI/IV/2023 (Hasnaeni et al., 2025). 200 grams of powdered leaf simplicia keji beling was extracted with 70% ethanol at a ratio of 1:10. The extraction used the Ultrasonic Assisted Extraction (UAE) method, a frequency of 38 kHz, a temperature of 30 ° C for 60 minutes. The extract was filtered and evaporated using a rotary evaporator.

Ultrasonic-Assisted Extraction (UAE)

200 grams of crushed keji beling leaves (*Strobilanthes crispa* (L.) Blume) were placed in a beaker. Enough 70% ethanol solvent was added to cover the leaves, then extracted for 60 minutes at a temperature of 30 ^{o}C, frequency 38 kHz (Hasnaeni, et al 2023).

Flavonoid Screening

Phytochemical screening was conducted to determine the secondary metabolite content of keji beling extract. The screening was subject to several modifications (Masriani et al., 2023; Wangloan et al., 2025; Dedek Fitriani & Melindra Mulia).

10 mg of thick ethanol extract was dissolved in 1 mL of 96% ethanol. Magnesium powder and 2 drops of 2 N HCl were added.

Alkaloid Screening

Ethanol extract was dissolved in 1 mL of 96% ethanol, added with 2N HCl. The solution was divided into 3 parts. The first tube was added with 2N HCl which served as a blank. The first tube was added with 3 drops of Dragedroff's reagent, the second test tube was added with Wagner's reagent and the third test tube was added with 3 drops of Mayer's reagent. The formation of an orange precipitate in the first tube indicated the presence of alkaloids. The second test tube was yellow with a yellow precipitate and the third test tube formed a brownish-yellow precipitate indicating the presence of alkaloids (Wangloan et al., 2025

Saponin Screening

The extract in the test tube was added with 1 ml of distilled water and then shaken vigorously for 10 minutes. Positive saponin content is indicated by the formation of a stable foam 1-10 cm high. (Masriani et al., 2023; Wangloan et al., 2025).

Terpenoid Screening

H_2SO_4 were added . Positive terpenoid content was indicated if a reddish brown color formed on the surface (Masriani et al., 2023; Dedek Fitriani & Melindra Mulia).

Tannin Screening

The extract of keji beling leaves was dissolved in distilled water and then added with a 1% $FeCl_3$ solution .Positive tannin content was indicated by the formation of a brownish-green or bluish-green color (Masriani et al., 2023).

RESULTS AND DISCUSSION

The extraction of 200 grams of keji beling leaves (*Strobilanthes crispa) using the Ultrasonic-Assisted Extraction* **(** UAE) method using 70% ethanol solvent, obtained a thick extract of 30 grams, the yield calculation was carried out as follows:

$$\%\text{Yield} = \frac{weight\ of\ extract)}{weight\ fowder}\ x\ 100\%$$

Phytochemical screening shows that the ethanol extract of keji beling leaves (*S. crispa*) contains secondary metabolites of alkaloids, flavonoids, tannins and saponins. Several studies on phytochemical screening of keji beling leaves conducted tests on the water fraction and ethyl acetate fraction containing alkaloids, flavonoids, tannins, saponins and triterpenoids and steroids (Wangloan et al., 2025). In other studies it was also stated that keji beling contains polyphenol compounds, caffeine, saponins, alkaloids, flavonoids, tannins, β-sitosterol, and stigmasterol (Sukendi et al., 2025). Research conducted by Dedek Fitriani & Melindra Mulia 2025, phytochemical screening tests of keji beling leaf extract contain saponins, tannins, flavonoids, phenolics, Triterpenoids but the results of the study found negative results for alkaloids and steroids. In this study, phytochemical screening was carried out on the extract obtained from the UAE extraction method (Table 1).

Table 1. Phytochemical Screening Results of Keji Beling Leaves Extract (*Strobilanthes crispa* (L.) Blume) *Ultrasonic-Assisted Extraction* (UAE) method

Test	Reagent	Results
Flavonoid	Mg + concentrated HCl	+
Alkaloid	Mayer	+
	Dragendorff	+
	Wagner	+
Saponin	Aquades + HCl 2N	+
Terpenoid	Chloroform _ H_2SO_4	-
Tannin	FeCl3	+

Flavonoid Screening

Phytochemical screening of keji beling leaf extract (*S. crispa*) using concentrated sulfuric acid and 2 N HCl reagents. In flavonoid screening, there is a reduction of polyhydroxy compounds from the extract flavonoid compounds by magnesium metal in hydrochloric acid to form benzopyryllium salts (Manongko et al., 2021). This test produces an orange color which

indicates the flavonoid content in the keji beling leaf extract. Tests have been carried out by Masriani et al., 2023; Wangloan et al., 2025).

$$MgCl_{(s)} + 2HCl_{(aq)} \rightarrow MgCl_{2(aq)} + H_{2(g)}$$

Figure1.Shinoda Reaction in Flavonoid Test

Alkaloid Screening

The results of the secondary metabolite test of alkaloids from the keji beling leaf extract showed a positive result for the presence of alkaloids. The presence of alkaloids was indicated by the presence of an orange precipitate with Dragendorff's reagent, a white precipitate with Mayer's reagent, and a brown precipitate with Wagner's reagent. Alkaloid testing is based on a precipitation reaction between alkaloid compounds and heavy metal ions. Alkaloids are nitrogen-containing organic compounds found in many plants. Nitrogen atoms in alkaloids are able to interact with metal ions, the lone electron pair on the nitrogen atom is reactive to metal ions (Azizi et al., 2013). In the following reaction, a complex reaction is seen. When Mayer's reagent is added, a potassium-alkaloid complex reaction occurs, namely a reaction between the nitrogen contained in the alkaloid and potassium ions from tetraiodomercurate (II) (Riwanti & Izazih, 2019).

$$HgCl_{2(aq)} + 2KI_{(aq)} \rightarrow HgI_{2(aq)} + 2\,KCl_{(aq)}$$
$$HgI_{2\,(aq)} + 2\,KI_{(aq)} \rightarrow K_2[HgI_4]_{(aq)}$$

Figure 2. Reaction of alkaloids with Mayer Reagent

The nitrogen atoms in the alkaloids will form a precipitate through interaction with iodine ions. Iodine in comparison with ions I^- from potassium iodide gives ions I_3^- which are brown. In the Wagner test, K^+metal ions will form coordinate covalent bonds with nitrogen in alkaloids to form potassium alkaloid complexes that precipitate (Fajrin & Susila, 2019) (Figure 3).

$$I_2(aq) + KI\,(aq) \rightarrow KI_3(aq)$$

Figure 3. Alkaloid test reaction with Wagner reagent

Saponin Screening

Saponins are secondary metabolites of the glycoside group that can form colloidal solutions in water and produce foam when shaken (Akasia et al., 2021). Phytochemical screening results indicate that keji beling leaf extract contains saponins. Adding 10 mL of distilled water and heating it, then shaking it, produces a stable foam for 10 minutes. The formation of foam after shaking is due to the hydrolysis of saponins in water into glycosides and other compounds (Abu Hayullah, Mohammad Basyuni, 2012).

Terpenoid Screening

Terpenoids are compounds composed of isoprene units. Testing for these secondary metabolites is performed using the Liebermann-Burchard reagent. Adding this reagent to the keji beling leaf extract produces two red phases, indicating the presence of terpenoids. The Liebermann-Burchard reagent is a mixture of chloroform and concentrated sulfuric acid (Dhurhania & Novianto, 2019).

Tannin Screening

Testing of secondary metabolites of tannins in the extract of keji beling leaves (*S. crispa*) was carried out by adding $FeCl_3$ reagent. The test results showed the presence of tannins in the keji beling leaf extract, which was indicated by the formation of a blackish green color. In this test reaction, a condensation reaction of the hydroxyl group of the tannin compound occurred (Noer et al., 2018).

CONCLUSION

Based on the phytochemical screening results conducted in this study, it was concluded that the ethanol extract of keji beling (S.crispa) leaves tested contained secondary metabolites such as flavonoids, alkaloids, saponins, and tannins. The results of the study showed that keji beling extract can be applied to the development of natural ingredient-based drugs.

RECOMMENDATION

Based on the results of this study, it is recommended that further research conduct quantitative testing of secondary metabolites and the activity of Keji beling leaves. Such data will be invaluable in understanding the compounds' bioactivity and potential therapeutic benefits, as well as providing additional insights into their potential applications in medicine.

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