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Phytochemical Screening and Determination of Total Flavonoid Content of Keji Beling Leaves Ethanol Extract (*Strobilanthes crispa* Bl.)

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Abstract

This study aims to determine the total flavonoid content in the ethanol extract of keji beling (Strobilanthes crispa) leaves using the UV-Vis spectrophotometric method with AlCl3 reagent. The research process includes maximum wavelength testing, preparation of quercetin standard solution, and analysis of total flavonoid levels. The results showed that the total flavonoid content in the extract was 144.347 ± 134.854 mg QE/g extract, with an extract yield of 0.4% and extract moisture content of 5.256%, which showed good potential in preventing the growth of microorganisms. Phytochemical screening revealed the presence of alkaloids, flavonoids, and tannins, while saponins, triterpenoids, and steroids were not detected. These findings confirm the importance of secondary metabolite compounds in plants and the potential of keji beling leaf extract as a source of active compounds with health benefits, including antioxidant and antibacterial activities. This study provides a basis for further research into the medical applications of this plant extract.

Keywords: Herbal plant; strobilanthes crispa; phytochemical screening; flavonoids.

INTRODUCTION

Secondary metabolites are chemical compounds that generally exhibit bioactivity and function as protective agents for the plants against pests and diseases, either for the plants themselves or their surrounding environment. As secondary metabolite products, these chemical compounds have been widely used as dyes, poisons, food aromas, medicines, and other applications. Numerous plants with medicinal properties are used in traditional medicine, highlighting the need for research on the utilization of medicinal plants and the identification of chemical compounds with medicinal properties (Aksara et al., 2013).

The keji beling plant (*Strobilanthes crispa Bl.*) is widely recognized in society as a traditional remedy for addressing various health issues. Its leaves contain several active compounds, such as alkaloids, flavonoids, saponins, terpenoids, and tannins (Fardiyah et al., 2020). Keji beling has been extensively used in traditional medicine for its antibacterial, antioxidant, antilithiasis, antidiabetic, antiangiogenic, anticancer, and wound healing properties (Ng et al., 2021).

Dried leaves of the keji beling plant contain a high total ash content (21.6%), including abundant minerals such as potassium (51%), calcium (24%), sodium (13%), iron (1%), and phosphorus (1%) (Ismail et al., 2000). Research indicates that the leaf position significantly affects the catechin content in the water fraction. The highest catechin content in the water fraction is found in the basal leaves, with a concentration of 11.813%, compared to the apical leaves (8.173%) and the middle leaves (11.453%) (Nurraihana & Hanoon, 2013).

One method commonly employed in discovering traditional medicines is extraction. Extraction is a process of separating compounds from mixtures using suitable solvents. One example of an extraction technique is maceration, a cold extraction method that does not require high temperatures. Maceration involves soaking the crude drug (simplisia) in a solvent with occasional stirring at room temperature (Meydia, 2016).

Keji beling leaves (*Strobilanthes crispa Bl.*) are extracted using 96% ethanol. The use of 96% ethanol as a solvent in the maceration process is due to its higher selectivity, non-toxicity, good absorption properties, and ability to inhibit bacterial and fungal growth. Ethanol at 96% concentration penetrates the simplisia cells more effectively compared to ethanol with lower concentrations, resulting in a more concentrated extract (Suhendar, 2019).

This study aims to determine the active compounds present in the ethanol extract of keji beling leaves using the maceration method.

MATERIALS AND METHODS

Study area

This study was conducted in October 2024 at the Microbiology Laboratory, Integrated Laboratory, Universitas Islam Malang, East Java, Indonesia.

Procedures

Sampling

The Keji Beling leaves were collected from Malang City, East Java. The leaves underwent a wet sorting process, washing, chopping, drying, and dry sorting. Then, the leaves were dried using an oven at 80°C until the sample was completely dry (Wijaya, 2022; Fachriza, 2023), after which the dry weight of the sample was measured and the moisture content was calculated to ensure it was below 10%. Once the sample was dry, the Keji Beling leaves were blended into a powder form (Januarista, 2024; Rafsanjani, 2024). The powder was then sieved using an 80-mesh sieve to increase the surface area, facilitating the penetration of the solvent during the extraction process (Siregar et al., 2020; Nurjannah et al., 2022).

Extraction

Keji Beling leaves were extracted using the maceration method with a solvent ratio of 1:10, with 96% ethanol as the solvent. The reason for using 96% ethanol is that it can dissolve active compounds such as alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids. Secondary metabolites and 96% ethanol have similar polarities, making it easier for the ethanol solvent to dissolve in the sample. This follows the principle of "like dissolves like," meaning that compounds will dissolve in solvents with similar properties. Additionally, ethanol is easily accessible and affordable compared to other solvents (Haryani et al., 2021; Nurjannah et al., 2022). In the initial step, 100 grams of powdered simplicia were weighed and placed into a 1-liter glass bottle (reagent bottle). A 1:7 ratio was used in the first stage, and in the second stage, a 1:3 ratio was applied (Tatang, 2019; Fakhruzy, 2020). The first and second maceration stages were homogenized in a 1000 ml Erlenmeyer flask. The solution was then transferred to a rotary evaporator at 40 rpm at a temperature of 60°C - 70°C for 1 hour until it became a paste (Kemenkes RI, 2020; Januarista, 2024). The maceration process involves soaking the sample in an organic solvent at room temperature (Nurjannah et al., 2022) to extract the secondary metabolites contained in the plant (Endarini, 2016; Agung, 2017; Aini, 2023).

Screening Fitokimia

The phytochemical screening test method refers to the research of Ramadhan et al. (2019) carried out qualitatively with color testing using a reagent that aims to determine the secondary metabolite compounds contained in plants:

- ***Alkaloid Test***

a) Mayer's Test
   A total of 20 drops of liquid extract from each sample were put into a test tube. Next, 10 drops of Mayer Reagent were added slowly (drop by drop) into the test tube and then shaken gently. The observed changes indicate a positive result for alkaloids if the formation of a white precipitate accompanies a yellowish color change.
b) Wagner's Test
   A total of 20 drops of liquid extract from each sample were put into a test tube. After that, 10 drops of Wagner's Reagent were added gradually (drop by drop) into the test tube and then shaken gently. The formation of a reddish-brown precipitate characterizes the positive results of alkaloids.
c) Bouchardat Test
   A total of 20 drops of liquid extract from each sample was put into a test tube. Then, 10 drops of Dragendorff's Reagent were added slowly (drop by drop) into the test tube and shaken carefully. The formation of an orange or yellow precipitate characterizes positive results for alkaloids.

- ***Flavonoid Test***

a) Shinoda Test
   A total of 20 drops of liquid extract (isopropyl alcohol) from each sample were put into a test tube. Next, 20 drops of absolute ethanol and three drops of concentrated hydrochloric acid were added to the test tube and shaken gently. A color change to red indicates the presence of aurones and chalcones. If no color change occurs, add 1 small spatula of magnesium to the test tube containing the liquid extract, then shake gently. A positive result for flavonoids is indicated by a pink to red color change (such as orange, red, or magenta, which indicates the presence of flavones and flavonols).
b) 10% Sodium Hydroxide Test
   A total of 20 drops of liquid extract from each sample was put into a test tube. Next, 4 drops of 10% NaOH solution were added to the test tube and then shaken gently. Observed color changes, such as reddish yellow, blackish orange, reddish-purple, or blue, indicate the presence of santone/flavone, flavonol, chalcone, or anthocyanin compounds.

- ***Saponin Test***

a) Foam Test
   A total of 20 drops of liquid extract from each sample was put into a test tube. After that, 20 drops of hot distilled water were added to the test tube and then shaken gently continuously for 15 minutes. The positive result of saponin is characterized by the formation of a consistent foam layer as high as ± 1 cm.Uji Tannin (Polifenol)

b) Base Solution Test
A total of 20 drops of liquid extract from each sample were put into a test tube. Then, 20 drops of 10% ammonium hydroxide (NH4OH) solution were added to the test tube and shaken gently. The observed changes indicate a positive result for tannin (polyphenols) when a fluorescent yellow color is formed.

- ***Steroid/Triterpenoid Test***

a) Salkowski Test
A total of 20 drops of liquid extract (isopropyl alcohol) from each sample was put into a test tube. After that, 20 drops of chloroform were added, followed by 10 drops of Salkowski's solution slowly into the tube. The mixture was then shaken carefully. The appearance of a brown ring in the center of the mixture indicated the presence of steroids.
b) Lieberman Bourchard Test
A total of 20 drops of liquid extract (isopropyl alcohol) from each sample were poured into a test tube. Next, 10 drops of Lieberman Bourchard solution were added slowly, and the mixture was shaken gently. After incubating for 5 minutes, a color change was observed. A blue-green color indicates the presence of sterols, while a pink to red-violet color indicates the presence of terpenoids.

Determination of Total Flavonoid Content by Colorimetric Method

Determination of total flavonoid content in ethanol extract of tiger milk fungus was carried out using UV-Vis spectrophotometry with the colorimetric method (AlCl3) at a wavelength of 410 nm, and the results were expressed as total flavonoids in quercetin equivalents (EQ) (Rebaya et al., 2014).

Determining the Highest Wavelength

A total of 0.5 mL of quercetin solution with a concentration of 100 ppm was taken, then added 0.1 mL of 10% AlCl3, 0.1 mL of 1M CH3COONa, and 2.5 mL of distilled water. The solution was homogenized using a vortex and incubated for 40 minutes in a place protected from light. The highest wavelength test was carried out on a spectrophotometer with an absorbance range between 415-440 nm.

Preparation of Quercetin Standard Solution

A total of 10 mg of quercetin was dissolved in 96% PA ethanol to a volume of 10 mL. From the mother solution with a concentration of 1000 ppm, 1 mL was taken and then 96% PA ethanol was added to a volume of 10 mL, resulting in a solution with a concentration of 100 ppm. Next, pipette 2, 4, 6, 8, and 10 mL of this solution, and add 96% PA ethanol to reach a volume of 10 mL, resulted in solutions with final concentrations of 20, 40, 60, 80, and 100 ppm. At each concentration, 0.5 mL of quercetin solution was taken, and 0.1 mL of 10% AlCl3, 0.1 mL of 1M CH3COONa and 2.5 mL of distilled water were added. The solution was homogenized using a vortex and incubated for 40 minutes in a place protected from light. The test was performed on a spectrophotometer with a maximum wavelength of 434 nm.

Determination of Total Flavonoids

A total of 10 mg of the tested sample was dissolved in 96% PA ethanol with a volume of 10 mL. From the mother solution which has a concentration of 1000 ppm, 1 mL was taken, and 96% PA ethanol was added to a volume of 10 mL, resulting in a solution with a concentration of 100 ppm. Pipette 5 mL of this solution and add 96% PA ethanol to a volume of 10 mL to obtain a final concentration of 50 ppm. At each concentration, 0.5 mL of sample solution was taken, then 0.1 mL of 10% AlCl3, 0.1 mL of 1M CH3COONa, and 2.5 mL of distilled water were added. The solution was homogenized using a vortex and incubated for 40 minutes in a place protected from light. The assay was performed on a spectrophotometer with a maximum wavelength of 434 nm. The total flavonoid content in the extract was calculated and expressed as mg of quercetin equivalent (QE) per gram of fresh sample.

Data analysis

The results of the study were analyzed qualitatively descriptively, and then conclusions were drawn based on the results obtained.

RESULTS AND DISCUSSION

Water Content of Keji Beling Leaf Extract Simplisia (*Strobilanthes crispa*)

Table 1. Results of Water Content of Keji Beling Leaf Extract (Strobilanthes crispa).

Simplisia Weight (gram)	Extract Weight (gram)	Water Content (%)
100	40.071	2.060

Yield of Keji Beling Leaf Extract (*Strobilanthes crispa*)

Table 2. Yield of Keji Beling Leaf Extract (*Strobilanthes crispa*).

Simplisia Weight (gram)	Solvent (mL)	Extract Weight (gram)	Yield (%)
100	1000	40.071	0,4

Phytochemical screening

Table 3. Qualitative Test Results of Keji Beling Leaf Extract (*Strobilanthes crispa*).

No	Test	Reagents	Results
1	Alkaloid	Mayer's	+
		Wagner's	+
		Dragendroff's	+
		Bourchardat	+
2	Flavonoid	Shinoda	+
		NaOH 10%	+
3	Saponin	Uji Busa	-
4	Tanin	Braymer's	+
		Larutan Basa	+
5	Triterpenoid / Steroid	Salkouski	-
		Liebermen Bourchard	-

Description:
(+) = contains compounds
(-) = does not contain compounds

Total Flavonoid Activity of Keji Beling Leaf Extract (*Strobilanthes crispa*)

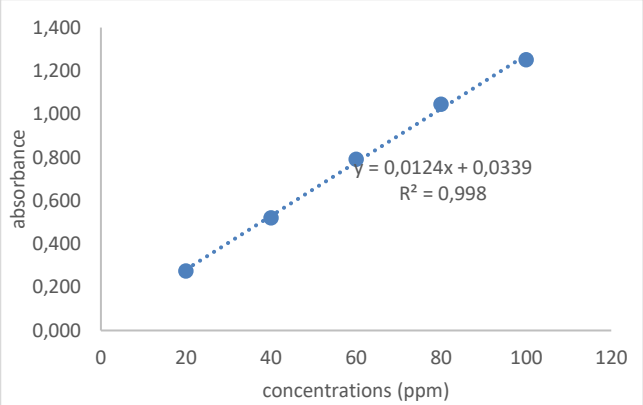

Figure 1. Regression value graph of total flavonoid assay

Table 4. Total Flavonoid Test Result of Keji Beling Leaf Extract (*Strobilanthes crispa*).

Sample	Replay	Concentrations (ppm)	Average Total Flavonoid Content (mgEQ/g extract)
Ethanol Extract of Keji Beling Leaf (*Strobilanthes crispa*)	1	1000	144.347 ± 134.854
	2	500	

Discussion

Simplisia, with a moisture content that is too high, is at risk of the growth of microorganisms such as fungi and bacteria. This condition can cause damage to active compounds and reduce the quality of simplisia. Therefore, testing water content is essential to maintain the active compounds in the simplisia. Based on the results of the study, the moisture content of keji beling leaf simplisia is 5.256%, which is classified as low and meets the standard of simplisia moisture content (not more than 10%). This low moisture content also helps extend the shelf life of the simplisia because it reduces the risk of microorganism growth and degradation of active ingredients.

The yield of keji beling leaf extract in this study was 0.4%. This value is low when compared to the positive standard of yield, which is considered good (>10%). Factors such as solvent type, extraction time, temperature, solvent to material ratio, and sample particle size can affect extract yield (Bustan et al., 2008, cited in Hasim et al., 2016). Low yields may indicate that the concentration of active compounds in the raw material is not very high or that there are obstacles during the extraction process, such as suboptimal solvent selection.

Phytochemical screening showed that the ethanol extract of keji beling leaves contained secondary metabolite compounds in the form of alkaloids, flavonoids, and tannins, while saponins, triterpenoids, and steroids were not detected. Flavonoids in the extract have strong antibacterial activity, which damages the bacterial cell wall by binding compounds to peptidoglycan, resulting in cell lysis. Tannins damage polypeptides in the bacterial cell wall and prevent enzyme adhesion and transport proteins, causing cell leakage and bacterial death. Steroids and triterpenoids, although not detected in keji beling leaf extract, are known to have a mechanism of action by damaging the bacterial cell membrane, increasing membrane permeability, and causing leakage of intracellular material.

The results of the total flavonoid content test showed that keji beling leaf extract has a flavonoid content of 144.347 ± 134.854 mg qe/g extract. The flavonoid content was determined using the UV-vis spectrophotometric method with a maximum wavelength of 434 nm. This result is in line with the research of Werdiningsih et al. (2023), which showed that the uv-vis spectrophotometric method was able to provide accurate results for determining total flavonoid levels. The total flavonoid content in this study is high and indicates that keji beling leaves have the potential as a source of bioactive compounds.

In addition, the relationship between extract concentration and absorbance value showed good linearity, with a linear regression equation y = 0.0124x and a r^2 value of 0.998. This linearity indicates that the flavonoid concentration is directly proportional to the resulting absorbance intensity. The high flavonoid content in keji beling leaf extract indicates great therapeutic potential, especially as an antibacterial and antioxidant, which makes it a promising base material for herbal medicine formulations.

Overall, the results of this study indicate that keji beling leaves contain significant active compounds and

have the potential to be developed as raw materials for pharmaceutical or phytopharmaceutical products. However, optimization of the extraction process and further research are needed to increase the yield and study its pharmacological activity in more depth.

CONCLUSIONS

This study showed that ethanol extract of keji beling (Strobilanthes crispa) leaves contained total flavonoids of 144.347 ± 134.854 mg QE/g extract was measured using UV-Vis spectrophotometric method with AlCl3 reagent. The extract yield of 0.4% and moisture content of 5.256% indicated the good quality of the simplisia, with low potential for microorganism growth. Phytochemical screening identified the presence of secondary metabolite compounds such as alkaloids, flavonoids, and tannins, while saponins, triterpenoids, and steroids were not detected. These findings support the utilization of ethanol extract from keji beling leaves as a source of active compounds that have the potential to be used in the development of health products, especially for antioxidant and antibacterial activities, with recommendations for further research to explore its potential applications.

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Competing Interests: The authors declare that there are no competing interests.

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