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Kelakai (*Stenochlaena palustris* (Burm.) Bedd) Extract from Kalimantan: Determination of Total Phenol and Alkaloid Content Potential as Nutraceuticals

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Abstract: Degenerative diseases such as diabetes, hypertension, and cancer have become health problems in the world. Currently, plants that have bioactive compounds are an alternative for preventing and treating health problems. Kelakai plants are one of the plants that are commonly consumed as a vegetable by Kalimantan. In addition to having nutritional content, kelakai also has bioactive compounds, namely phenols and alkaloids. However, thus far, the total content in the extract of kelakai leaves and stems remains unknown. Therefore, in this study, a test was conducted on the total phenols and alkaloids of the extract of kelakai leaves and stems from Central Kalimantan, Indonesia. This study is a quantitative study with a laboratory experimental method. The extraction method used is maceration with 70% ethanol solvent. Measurement of alkaloids and phenols utilized the spectrophotometric method. The study showed that the extract of kelakai leaves and stems has a total phenol content of 56,180+0.81 mg GAE/g and alkaloids of 86,524+0.522 ppm so it has the potential as a nutraceutical. The results of kelakai extract test can be a reference for pre-clinical research as a plant that not only has nutritional content but also bioactive compounds.

Keywords: Kelakai extract; Total alkaloids; Total phenol

Introduction

Indonesia's biodiversity has the potential to be a nutraceutical. Nutraceuticals are foods that provide health effects for the prevention and cure of diseases (Daliu et al., 2019). The use of nutraceuticals is growing rapidly and is well accepted by the public because of its natural origin (Chandra et al., 2022). Plants that have the potential to be nutraceuticals, in addition to having nutritional content, also contain bioactive compounds such as flavonoids, alkaloids, tannins, and saponins (Benvenga et al., 2019; Khair et al., 2017; Nugrahani et al., 2016). Plants need to be evaluated to determine their biological, chemical and bioactive compound properties so that their health benefits can be known. One of the plants that can be used as a nutraceutical is Stenochlaena palustris (Burm.) Bedd or kelakai plants.

Kelakai plants are a type of fern typical of Kalimantan which are widely found in residential areas where the leaves and stems are usually taken to be consumed as vegetables (Pandiangan et al., 2022). Kelakai plants are also easy to find and are included in the category of organic vegetables. Kelakai plants have various nutritional contents, such as energy, protein, fat, carbohydrates, dietary fiber, calcium, phosphorus, Fe, and vitamin C (Amanda et al., 2021; TKPI, 2018). In addition to nutrients, kelakai plants also contain bioactive compounds. The presence of bioactive compounds in plants such as alkaloids and phenols, according to several studies, has health benefits.

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Developing research alkaloid compounds can be antidiabetic, anticancer, anti-cholestrol and anti-inflammatory agents (Berampu et al., 2024; Dafriani et al., 2024; Hariyati et al., 2015; Huan et al., 2023).

In addition, phenol compounds also have a protective effect on heart health, protect the skin from UV radiation, and improve the immune system (Oluwole et al., 2022; Sun & Shahrajabian, 2023). Previous studies have revealed that kelakai leaf extract positively contains flavonoids, steroids, alkaloids, saponins, and tannins (Fitriyanti et al., 2023; Ndanusa et al., 2020; Rostinawati et al., 2017; Widayati et al., 2022). The presence of bioactive compounds that are beneficial for health makes the kelakai plant potentially a nutraceutical. However, previous studies were still limited to qualitative tests by investigating and revealing the presence or absence of bioactive compounds in kelakai extract using kelakai leaf or root samples. Therefore, further research is needed regarding the total content of bioactive compounds for the development of its use a (Magfirah et al., 2024).

Accordingly, in this study, the researchers conducted further tests by investigating the levels of phenol and alkaloids in kelakai plants using leaf and stem samples because these plant parts are commonly used by the community in processing the plants. Testing the levels of bioactive compounds is important to increase the effectiveness of one of Kalimantan's natural resources so that it can be a reference for further pre-clinical research by utilizing the parts of kelakai plant that are commonly consumed.

Method

This is quantitative research using laboratory experimental methods. Total alkaloids and phenols were analyzed using UV-Vis Spectrophotometry. Determination was carried out before the study to ensure the type and accuracy of the plants used. Determination was carried out in the Biology Learning Laboratory of the Faculty of Applied Science and Technology, Ahmad Dahlan University (UAD).

Kelakai plants were obtained from Jalan G. Obos, Palangkaraya, Central Kalimantan, Indonesia. The kelakai plants were then sorted from dirt that stuck to them, washed with running water, dried by airing them and not directly exposed to sunlight, then blended into a coarse powder and sieved to produce simplicia. Kelakai simplicia was weighed and then extracted by the maceration method using 70% ethanol solvent for 24 hours. The step was repeated 3 times. The results obtained were macerated, concentrated using a rotary evaporator, and evaporated until a thick extract was formed.

Alkaloid testing was determined using a caffeine standard curve. The testing process began by weighing a sample of 40 mg dissolved in ethanol with a dilution of 5, 20, 60, 80, 100 mg/dL. In each dilution. 2 ml of phosphate buffer and 2 ml of bromocresol green was added. After 10 ml of chloroform was added, the absorbance was measured using a UV-Vis T90 + PG Spectrophotometer (Putra, 2024).

In this study, the total phenol content was determined using the Folin-Ciocalteau method. The initial process was carried out by dissolving gallic acid in ethanol. After that, Aquabides was added and homogenized. Next, the absorbance measurement was carried out using a Spectrophotometer at a wavelength of 747 nm with a time range of 0-90 minutes. The total amount of alkaloids and phenols was determined by finding the regression of the linear regression variation coefficient, where the sample testing was carried out three times. The results obtained were expressed as an average + standard deviation (Qoriasmadillah et al., 2024).

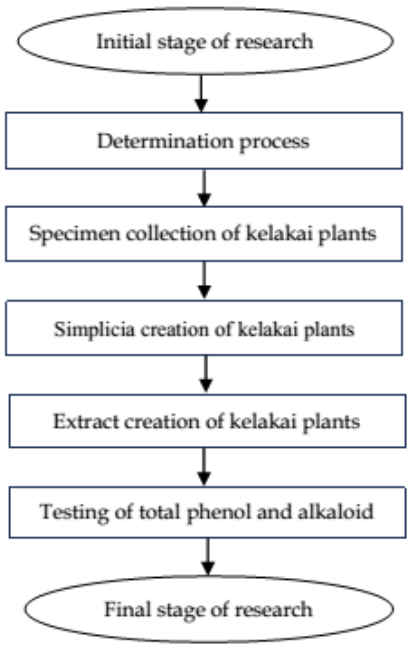

Figure 1. The flow diagram

Result and Discussion

The simplicia obtained from the drying results was 1,023 g of powder, then macerated using 70% ethanol solvent to obtain 96.86 g of thick kelakai extract.

Analysis of Total Alkaloid

The total amount of alkaloids was determined by measuring the standard curve at a maximum wavelength of 430 nm with concentrations of 50, 100, 125, 150, 175, 200, and 250 ppm. The alkaloid content was

calculated using linear regression, and the results of the equation were calculated to measure the total alkaloid content in the kelakai extract by entering the sample absorbance obtained from spectrophotometry as the Y value (Figure 2), so that the average analysis results of the total alkaloids were 86.524 ppm, with a standard deviation value of + 0.522 ppm (Table 1).

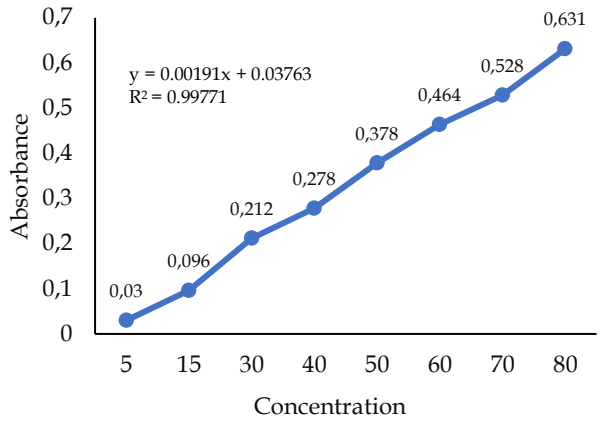

Figure 2. The standard curve calibration for alkaloid

Table 1. Total Alkaloid of Kelakai Extract

Result	Alkaloid (ppm)	Total	SD
Replica 1	86.275	86.524	+0.522 ppm
Replica 2	87.240		
Replica 3	85.836		

Analysis of Total Phenol

The total amount of phenol was determined by measuring the gallic acid curve at concentrations of 5, 15, 30, 40, 50, 60, and 70 mcg/mL. Total phenol measurements were carried out three times in replication. Replication of measurements of the kelakai extract produced absorbance values in the range of 0.434-0.436 (Table 2) obtained from the absorbance y = ax-b (Figure 3). Thus, the average analysis results of total phenol were obtained at 56,008 + 0.81 mg GAE/g (Table 2).

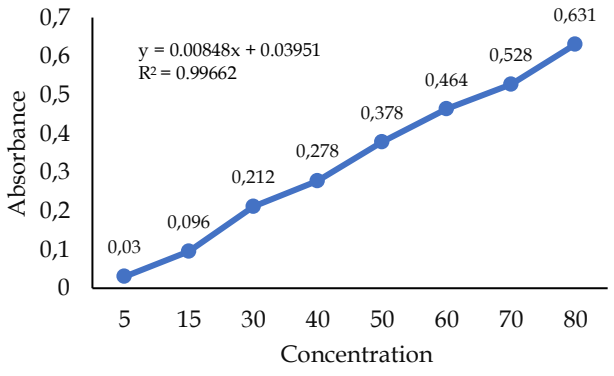

Figure 3. The standard curve calibration for phenol

Table 2. Total Phenol of Kelakai Extract

Result	Optical Density	Phenol (mg GAE)	Total	SD
Replica 1	0.437	56.360	56.180	+0.81 mg GAE/g
Replica 2	0.436	56.194		
Replica 3	0.434	56.008		

Discussion

The kelakai plant (Stenochlaena palustris (Burm.) Bedd) is a type of fern that is commonly consumed by people, especially the Dayak tribe in Kalimantan (Puspitasari et al., 2022). Based on the Indonesian Food Composition Table, 100 g of kelakai plants contain nutrients including 2.4 grams of protein, 0.2 gram of fat, 6.6 grams of carbohydrates, 18 milligrams of calcium, 9-milligrams of phosphorus, 1.1 milligrams of Fe and 8 milligrams of vitamin C (Achamad et al., 2019). In addition to the nutritional content, based on research, kelakai has a bioactive compound content that is good for health (Puspitasari et al., 2022).

The total components of bioactive compounds such as polyphenols, alkaloids, and carotenoids can be determined by carrying out an extraction process to extract these compounds using solvents based on the solubility of the materials used (Komal et al., 2019). In this study, the kelakai simplicia was extracted using 70% ethanol solvent using the maceration method to obtain alkaloid and phenol compounds. These results are in line with those of previous research studies conducted by Syamsul et al. (2019) who used kelakai leaf extract samples with 70% ethanol solvents and revealed that they can bind alkaloid, flavonoid, and tannin compounds. Research conducted by Jamilah et al. (2022) unveiled that kelakai leaf and stem extracts with 70% ethanol solvents positively contained alkaloids, flavonoids, and tannins. Furthermore, Forestryana et al. (2022) investigated kelakai root extract with 70% ethanol solvents and found that they contained phenols and flavonoids. The results of this study are different from research findings revealed by Ikrommuslimin et al. (2024) who used kelakai leaf extract samples with 96% ethanol solvent and did not find alkaloid compounds, but were positive for flavonoid and tannin content. This means that ethanol with a concentration of 70% is more effective in obtaining bioactive alkaloid and phenol compounds.

This study also analyzed the total alkaloids contained in the extract of kelakai leaves and stems. The analysis used a comparison of the caffeine standard curve. The results of absorbance measurements show that there was a relationship between caffeine concentration and the resulting absorbance which is directly proportional, where the higher the caffeine concentration was, the higher the resulting absorbance value would be. From the results of standard curve

measurements, a linear regression value of y = 0.00191x + 0.03763 was obtained and a coefficient value of 0.099771 which is close to 1 indicates a strong relationship between the two variables by forming a linear curve so that a total alkaloid content of 86.524 + 0.522 ppm was obtained.

This research is in line with previous studies conducted by Rostinawati et al. (2017) and Widayati et al. (2022) who revealed that the extract of kelakai leaf from Central Kalimantan contained alkaloids obtained. Research conducted by Fahruni et al. (2018) resulted in the extract kelakai root obtained from Central Kalimantan containing alkaloids. Other studies done by Rahmawati et al. (2017) and Saniah et al. (2023) produced a positive extract of kelakai from South Kalimantan containing alkaloids. However, the results of this study differ from research results conducted by Roanisca et al. (2017) who did not find alkaloid compounds in the extract of Stenochlaena palustris from Bangka Regency, and research conducted by Sulasmi et al. (2018) did not produce alkaloid content in kelakai extract obtained from Baluran National Park. This means that differences in growth places affect the content of bioactive compounds in kelakai extract. According to Mahmud et al. (2024) differences in environmental conditions such as differences in temperature and soil pH cause differences in the amount of bioactive compounds contained in a food ingredient.

This study also analyzed the total phenol content of the extract of kelakai leaves and stems. The analysis used a comparison of the galat acid standard curve. In the study of the standard curve of the correlation coefficient, there was a relationship between the concentration of gallic acid and the resulting absorbance. Based on the calibration curve, y = 0.00848x + 0.03951 was obtained with a coefficient of 0.99662 approaching the value of 1. Based on the R2 value obtained, the correlation coefficient shows a linear result because it meets the acceptable criteria of 0.99 so that the total phenol in the extract of kelakai leaves and stems is obtained. These results are in line with previous research by Saniah et al. (2023) which resulted in the extract of kelakai leaf obtained from Central Kalimantan containing phenolics. Research by Kusmardiyani et al. (2016) produced a total phenol in the ethanol extract of kelakai roots obtained from Central Kalimantan of 24.22 g GAE/100 gr. Another study done by Ndanusa et al. (2020) resulted in kelakai leaf ethanol extract of 3.80+0.22 mg GAE/g obtained from Kuala Belait, Brunei Darussalam. The results of the research carried out had a higher total phenol content of 56,180 + 0.81 mg GAE/g.

This study produced extracts of kelakai leaves and stems using a solvent that was 70% more effective in binding alkaloid and phenol compounds, and obtained a higher total phenol content compared to previous studies, while the total alkaloids could not be compared due to limited literature regarding the total alkaloid content in kelakai extract. The known bioactive compounds in kelakai leaf and stem extracts can potentially be used as nutraceuticals in addition to utilizing the nutritional content in them.

Several studies have shown that phenol and alkaloid compounds can increase the immune system and can be agents of anticancer, anti-inflammatory, antidiabetic, anti-inflammatory, antiparasitic, and antigenotoxic (Borsoi et al., 2024; Dey et al., 2020; Oluwole et al., 2022; Sun & Shahrajabian, 2023; Wulandari & Yuniarti, 2023). These bioactive compounds work by inhibiting free radical reactions caused by ROS (Safrida et al., 2023; Tandi et al., 2023; Zagoskina et al., 2023).

Conclusion

The extract of kelakai (*Stenochlaena palustris* (Burm.) Bedd) produced a total of phenols of 56,180+0.81 mg GAE/g and alkaloids of 86,524+0.522 ppm. The results of kelakai extract test can be a reference for pre-clinical research as a plant that not only has nutritional content but also bioactive compounds.

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Conflict of Interest
The authors report no conflicts of interest.

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