Journal of Tropical Pharmacy and Chemistry

Journal homepage: https://jtpc.farmasi.unmul.ac.id

Analysis of Flavonoids and Terpenoids in Ethanol Extract of *Colocasia esculenta* L. (Schoot) Stalk and Leaves

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Abstract

Colocasia esculenta L. (Schoot) is a widely used plant in developing countries in Asia, Africa, and Central America. The parts of the Colocasia plant that can be used are the tubers, stalks, and leaves. There has not been much research on the content of this plant. The study aims to determine the flavonoids and terpenoids in the ethanol extract of Colocasia stalk and leaves. The maceration method with 70% ethanol solvent and drying with vacuum evaporator. Determination of flavonoid levels based on quercetin marker compounds using $AlCl_3$ reagent by UV-Vis spectrophotometry. The terpenoid levels were carried out gravimetrically with petroleum ether as a solvent. The results showed that the ethanol extract of Colocasia stalk and leaf contained flavonoids, respectively 3.18±0.0581% and 4.33±0.0285%, while the results of the terpenoid levels for stalks were 7.10±0.0676% and leaves were 8.39±0.0023%.

Keywords: Colocasia esculenta, Stalk, Leaves, Flavonoids, Terpenoids

Submitted: 21 May 2021 **Accepted**: 24 December 2021 **DOI**: https://doi.org/10.25026/jtpc.v5i4.349

1 Introduction

Colocasia plants have chemical contents, including flavonoids, steroids, glycosides, and other micronutrients. Prajapati et al. [1], it assumed that Colocasia plants act as an analgesic, anti-inflammatory, anti-cancer, anti-diarrheal, astringent, nerve tonic moreover hypolipidemic activity. Herwin et al. [2] and Wijaya et al. [3] state the Colocasia leaf stalk contains flavonoids, saponins, tannins, alkaloids, and steroids. Fadilla et al. [4] stated that Colocasia leaf stalks have flavonoid compounds that act as antibacterials based on the chemical. Dalimartha [5] says that the saponins in Colocasia stalks and leaves have potential as a wound medicine. According to Hibai et al. [6] in the research of Biren et al. [7] and Eddy [8], it affirmed that colocasia leaves have chemical content, including saponins,

tannins, terpenes, flavonoids, flobatin, anthraquinone, cardiac glycosides, and alkaloids [9].

In this study, only analysis of flavonoid and terpenoid levels was carried out because the content of flavonoids and terpenoids can be an alternative to wound medicine. In research, Atmaja [10] flavonoids function as antioxidants to inhibit toxic substances, while according to Anggraini [11], flavonoids also have an antibacterial and anti-inflammatory effect, which functions as an anti-inflammatory and can prevent stiffness and pain. Apart from flavonoids, terpenoids also function as antimicrobials and antioxidants responsible for wound contraction and increased speed of epithelialization [12]. The analytical methods used were UV-Vis spectrophotometry and gravimetry.

2 Materials and Methods

2.1 Materials and Tools

The materials used in the research, namely fresh Colocasia stalk and leaves were obtained from Bantargadung, Sukabumi (Indonesia), $AlCl_3$ (Merck), aluminum foil, NH_3 (Merck), CH_3COOH (Merck), HCl (Merck), H_2SO_4 (Merck), Ethanol (Merck), Quercetin(Sigma Aldrich), $FeCl_3$ (Merck), CH_3COONa (Merck), Petroleum ether (Merck), Dragendroff reagent,Mayer reagent, Magnesium powder (Merck).

The equipment used in this study included glassware (Pyrex®, Indonesia), grinder (Miyako®, Japan), watch glass (Germany), filter paper (Japan), silicate crucible (Indonesia), pumpkin measuring (Pyrex®, Indonesia), oven (Memmert®, Germany), dropper pipette (Indonesia), UV-Vis spectrophotometry (Jasco V-730®, LTD, Tokyo, Japan), sonicator (Shanghai), furnace (Daihan Scientific®, Korea), test tubes (Pyrex®, Indonesia), analytical scales (LabPRO®, Indonesia), *vaccum evaporator (OGAWA*®, Shanghai).

2.2 Making SimpliciaColocasia Stalks and Leaves

Colocasia stalks and leaves that are sorted from impurities. The material is washed with running water until it is clean, then weighed down. After that, the ingredients are cut into small pieces to speed up drying. Colocasia leaf pieces dried in the oven at 50°C for the leaves for 20 hours while for the Colocasia stalks for 16 hours so that the ingredients are dehydrated. The dry material is weighed, the dry simplicia of Colocasia leaves are mashed by grinding to a powder with a 40 mesh sieve.

2.3 Making Ethanol Extract of Colocasia Stalk and Leaves

The ethanol extract of Colocasia stalk and leaf made by the maceration method, where each stalk and Colocasia leaf weighed approximately 1 kg, then macerated with 70% ethanol solvent with a ratio of 1:10. The maceration process is carried out for 72 hours using a brown bottle protected from light with occasional stirring every 6 hours. After 24 hours, the mass was filtered and poured until the filtrate was obtained. The filtrate obtained was then made dry extract using a vacuum evaporator. Then the yield obtained is calculated.

2.4 Determination of Water Content

The extract carried out using the gravimetric method by weighing 2 grams of the sample in a tared container. The extract was dried for 5 hours in an oven at 105°C, and after that, weighed. Drying is continued and weighed at a distance of 1 hour until the difference between 2 consecutive weights is not more than 0.25%. The moisture content is calculated as a percent of the initial sample weight until the weight is constant. The water content requirement of the simplicia extract is not more than 22.3% [13].

2.5 Determination of Ash Content

The extract was carried out by weighing 2-3 grams of the sample and carefully weighed. It was put into a silicate crucible that had been incandescent and tared. The sample is then flattened and then slowly annealed until the charcoal runs out, then it is cooled and weighed. The ash content is calculated on the material that has been dried in the air. The ash content requirement of the simplicia extract is not more than 16.6% [13].

2.6 Qualitative Analysis

Flavonoid test: A total of 1 gram of Colocasia stalk and leaf extract each was weighed, then put into a test tube, then added 2-3 drops of ethanol, then added sufficient Mg powder and ten drops of 5M hydrochloric acid. The presence of flavonoids characterized by forming a reddish black color in the solution [14].

Tannin test: As much as 1 gram of Colocasia stalk and leaf extract, each weighed, then put in a test tube and added with hot water then added a few drops of iron (III) chloride. The presence of tannins is indicated by forming a blackish green color in the solution [3].

Alkaloid test: Weighed 1 gram of Colocasia stalk and leaf extract, each weighed then shaken with 20 ml of ethanol and 3 ml of NH_3, heated at 60 °C while shaking for 15 minutes. The solution is filtered, then the filtrate is concentrated to approximately 3 ml, then 5 ml of 1N hydrochloric acid is added. The solution dropped on three watch glasses, three drops each then added with dragendorff and Mayer reagents. Alkaloids are characterized by the formation of white deposits and orange deposits to brown in solution [14].

Saponin test: As much as 1 gram of Colocasia stalk and leaf extract put into a test tube, 10 ml of hot water added, cooled, and shaken vigorously for 10 seconds. The presence of saponins characterized by forming a stable foam for not less than 1 minute [14].

Terpenoid test: A total of 10 mg of Colocasia stalk and leaf extract added five drops of anhydrous acetic acid, then three drops of sulfuric acid are added, the formation of red color indicates the presence of terpenes, and the presence of steroids indicated by the formation of a green color [15].

2.7 Quantitative Analysis

2.7.1 Analysis of Flavonoid Levels Using the Uv Vis Spectrophotometer Method

A total of 50 mg of ethanol extract of Colocasia stalk and leaf each weighed then dissolved with ethanol solvent into a 50 mL (1000 ppm) volumetric flask, then 0.5 mL pipette was put into a 5 mL volumetric flask, then added 0.1 mL. Aluminum chloride 10% and 0.1 mL of sodium acetate 1 M, then add distilled water to the limit mark, then shake until homogeneous. Furthermore, the solution was incubated at room temperature for 15 minutes. The absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 430 nm. A test on the quercetin standard precedes testing then the concentration and absorption data are plotted into a linear graph. From the linear graph, a linear regression equation will be obtained from the quercetin calibration curve, which is used to determine the levels of flavonoids in the extracted sample. From this linear equation, the total flavonoid content of the extract can be calculated using the following formula [16].

2.7.2 Analysis of Terpenoid Levels Using the Gravimetric Method

A total of 100 mg of dry extract of Colocasia stalk and leaf was put into a bottle and then immersed in 9 ml of 70% ethanol for 24 hours, then filtered and then obtained the filtrate. The filtrate is separated and then extracted using 10 ml of petroleum ether solvent using a separating funnel for 10 minutes. The petroleum ether extract obtained was evaporated in a steam cup then weighed [17].

3 Results and Discussion

3.1 Extract of Colocasia Stalk and Leaves

Extracts of Colocasia stalks and leaves were obtained by extraction using the maceration method. The characteristics of the dry extract of Colocasia stalk are purplish-brown and have a distinctive odor, while the dry extract of Colocasia leaves has a blackish brown color and has a distinctive odor.

The average yield of Colocasia stalk extract water content was 6.50±0.4183%, and Colocasia leaves were 6.38±0.0096%. These results have met the general requirements that the dry extract moisture content is not more than 10% [18]. The average yield of Colocasia stalk extract ash content was 3.90±0.0055%, and Colocasia leaves were 3.75±0.0201%. These results have met the requirements of the applicable parameters that the dry extract ash content is not more than 5% [13].

3.2 Qualitative Analysis Results

The results of the qualitative analysis showed that the active compounds contained in the extract of Colocasia stalks and leaves were flavonoids, saponins, tannins, phenolics, and terpenoids. The results of the phytochemical test can be seen in Table 1.

Table 1. Phytochemical test result

Sample	Flavonoids	Saponins	Tanins	Alkaloids	Terpenoids	Steroids
Stalks	+	+	+	+	+	-
Leaves	+	+	+	+	+	-

This result is not the same as previous research, in the study of Wijaya et al. [3] using the same type of Colocasia plant, namely Colocasiaesculenta L. stated that the stalks and leaves of Colocasia contain flavonoids, saponins, tannins, terpenoids, and steroids. In contrast, in this study, there were no steroids. This study did not use chloroform, while in the Wijaya study, the addition of chloroform was carried out. Chloroform functions as a solvent for non-polar compounds because steroids are non-polar and form acetyl derivatives when anhydrous acetic acid is added. The anhydrous acetic acid in the steroid test serves to absorb water and oxidize the acid by sulfuric acid because the oxidation reaction will not occur if water is still contained in the compound reacted. The use of solvents during the extraction process is a semipolar solvent, and steroid compounds are lipid derivative compounds that are not hydrolyzed so that the compound is not extracted completely [19].

3.3 Flavonoid Levels of Ethanol Extract of Colocasia Stalk and Leaves

The determination of flavonoid levels was carried out by the aluminum chloride method using UV-Vis spectrophotometry. The principle of aluminum chloride occurs in the formation of a stable complex with C-4 keto groups and adjacent hydroxyl groups and at C-3 or C-4. The addition of aluminum chloride forms a stable complex with ortho hydroxyl groups on the A- or B- rings of flavonoid compounds that can shift wavelengths towards visible, which indicated by a solution that produces a yellow color, and the purpose of adding sodium acetate is to maintain the long waves in the visible area [20].

Quercetin was used as a marker for extract analysis. The maximum wavelength measurement result is 430 nm with an optimum incubation time of 15 minutes. In this study, a standard series of quercetin is used, namely 2, 4, 6, 8, and 10 ppm. The results obtained are plotted between the concentration and the absorbance so that the linear regression equation is obtained, namely $y = 0.0677x + 0.1114$ with the R2 value obtained is 0.9992, and the r value is 0.9996. The value of r, which is close to 1 with a strong level of confidence, shows a linear calibration curve, and there is a relationship between the concentration of quercetin solution and the absorption value [21]. The absorbance measurement was carried out three times to obtain the accuracy of the data, namely for the taro stalk, the average absorbance was 0.3279±0.0028, while for Colocasia leaves, it was 0.3998±0.0016. It is following the literature according to [22], the range of flavonoid levels based on the absorbance value ranges from 0.2 to 0.8.

The results showed that the flavonoids in Colocasia leaves were more significant than that of Colocasia stalks; namely, the average level of flavonoids for Colocasia stalks was 3.18±0.0581%, while for Colocasia leaves was 4.33±0.0285%. Whereas previous research conducted by Lindawati et al. [23] stated, the total flavonoid levels in Colocasia leaf stalks were 0.30±0.0080%. This research shows that Colocasia leaves contain good flavonoids. There are differences in the results of this study with previous studies due to several factors that can affect the results of flavonoid levels, one of which is the difference in extraction methods. Settharaksa et al. [24] stated that the temperature and length of heating time in the extraction process could affect the flavonoid compounds' levels. The amount of solvent in the extraction process also affects the amount of secondary metabolite that is interested. The drying process can also affect the active ingredient content, where during the drying process, some of the ingredients experience evaporation, so that it affects the amount of content obtained. The research of Safrina et al. [25] stated that the height of the growing area

and the drying process affected the active ingredient content.

3.4 Total Terpenoid Levels of Ethanol Extract of Colocasia Stalk and Leaves

The determination of terpenoid levels was carried out using the gravimetric method. This method is one of the simplest and easiest quantitative analysis methods to do, and the results obtained are more specific and accurate because the determination is done by measuring the weight of the component in a pure state after going through the separation process. The use of petroleum ether because is non-polar and can attract terpenoid compounds. Because terpenoids are non-polar and dissolve in non-polar solvents or fats, non-polar solvents are used. Terpenoids are secondary metabolites that have potential as antifungal, insecticide, antiviral, antioxidant, and antibacterial properties [26].

Terpenoids are secondary metabolites that have potential as antifungal, insecticide, antiviral, antioxidant, and antibacterial properties [26]. The results showed that the total terpenoid levels in Colocasia leaves were more significant than that of Colocasia stalks; namely, the average terpenoid levels for Colocasia stalks were 7.10±0.0676%, while the average terpenoid levels for taro leaves were 8.39%±0.0023%. Research conducted by Singh et al. [27] on the Tanjung plant (Mimusopselengi L.) obtained 2% levels of terpenoids in the leaves and 2.8% in the bark of the trees. In Theng and Kopenwar's [28] research on Pueraria tuberose plants or typical Thai plants, the terpenoid levels were 1.32%. This research shows that taro leaves contain good terpenoids.

4 Conclusions

The results of the qualitative analysis showed that the ethanol extract of Colocasia stalks and leaves contained flavonoids, saponins, alkaloids, phenolic tannins, and terpenoids. The levels of flavonoids in the ethanol extract of Colocasia stalk were 3.18±0.0581%, the levels of flavonoids in Colocasia leaves were 4.33±0.0285%. The terpenoid content for the ethanol extract of Colocasia stalk was 7.10±0.0676% while the terpenoid content for Colocasia leaves was 8.39±0.0023%.,

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