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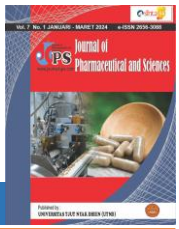

The Effect of Different Extraction Methods on the Total Flavonoid and Alkaloid Content of Red Fruit Stalk (*Pandanus conoideus* L.)

Pengaruh Perbedaan Metode Ekstraksi Terhadap Kadar Total Flavonoid dan Alkaloid Bonggol Buah Merah (*Pandanus conoideus* L.)

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Abstract

Background: Red fruit (*Pandanus conoideus* L.) is an endemic plant of Papua that has been traditionally used to treat various diseases. Its biological activities are associated with the presence of secondary metabolites, particularly flavonoids and alkaloids. **Objective:** This study aimed to identify the secondary metabolites and evaluate the effect of different extraction methods on the total flavonoid and alkaloid contents of ethanolic extracts of red fruit (*Pandanus conoideus* L.) stem core. **Methods:** Extraction was performed using three different methods, namely maceration, Soxhlet extraction, and reflux extraction with ethanol as the solvent. Secondary metabolites were identified qualitatively, while total flavonoid and alkaloid contents were determined spectrophotometrically. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, steroids, and tannins in the ethanolic extract of red fruit stem core. The total flavonoid contents obtained by maceration, Soxhlet extraction, and reflux extraction were 218.966 mgQE/g (21.89%), 104.023 mgQE/g (10.40%), and 247.701 mgQE/g (24.77%), respectively. The total alkaloid contents obtained by maceration, Soxhlet extraction, and reflux extraction were 145.770 mgAE/g (14.58%), 65.767 mgAE/g (6.58%), and 76.433 mgAE/g (7.64%), respectively. **Conclusion:** The extraction method affected the total flavonoid and alkaloid contents obtained from the sample. Reflux extraction produced the highest total flavonoid content (247.701 mgQE/g), whereas maceration produced the highest total alkaloid content (145.770 mgAE/g). Therefore, reflux was the most effective method for flavonoid extraction, while maceration was the most effective method for alkaloid extraction from the stem core of red fruit (*Pandanus conoideus* L.).

Keywords: Extraction Method, Flavonoids, Alkaloids, Pandanus conoideus L., FTIR

Abstrak

Latar Belakang: Buah merah (*Pandanus conoideus* L.) merupakan tanaman endemik Papua yang telah dimanfaatkan secara tradisional untuk pengobatan berbagai penyakit. Aktivitas biologis buah merah diduga berkaitan dengan kandungan metabolit sekundernya, terutama flavonoid dan alkaloid. Tujuan: Penelitian ini bertujuan untuk mengidentifikasi kandungan metabolit sekunder serta membandingkan pengaruh metode ekstraksi terhadap kadar total flavonoid dan alkaloid dalam ekstrak etanol bonggol buah merah (*Pandanus conoideus* L.). Metode: Ekstraksi dilakukan menggunakan tiga metode, yaitu maserasi, soxhletasi, dan refluks dengan pelarut etanol. Identifikasi metabolit sekunder dilakukan secara kualitatif, sedangkan kadar total flavonoid dan alkaloid ditentukan secara spektrofotometri. Hasil: Hasil skrining fitokimia menunjukkan bahwa ekstrak etanol bonggol buah merah mengandung alkaloid, flavonoid, terpenoid, steroid, dan tanin. Kadar total flavonoid yang diperoleh pada metode maserasi, soxhletasi, dan refluks berturut-turut sebesar 218.966 mgQE/g (21.89%), 104.023 mgQE/g (10.40%), dan 247.701 mgQE/g (24.77%). Kadar total alkaloid yang diperoleh pada metode maserasi, soxhletasi, dan refluks berturut-turut sebesar 145.770 mgAE/g (14.58%), 65.767 mgAE/g (6.58%), dan 76.433 mgAE/g (7.64%). Kesimpulan: Perbedaan metode ekstraksi memengaruhi kadar total flavonoid dan alkaloid yang diperoleh. Metode refluks menghasilkan kadar total flavonoid tertinggi sebesar 247.701 mgQE/g, sedangkan metode maserasi menghasilkan kadar total alkaloid tertinggi sebesar 145.770 mgAE/g. Dengan demikian, refluks merupakan metode paling efektif untuk ekstraksi flavonoid, sedangkan maserasi merupakan metode paling efektif untuk ekstraksi alkaloid dari bonggol buah merah (*Pandanus conoideus* L.).

Kata Kunci: Metode Ekstraksi, Flavonoid, Alkaloid, Pandanus conoideus L., FTIR

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Introduction

Indonesia is a country that has abundant and diverse natural resources. The diversity of natural resources, such as plants, has the potential to be studied regarding the potential of medicinal plants [1]. In general, a plant can be used as a medicine because it contains secondary metabolite compounds, such as flavonoids, alkaloids, steroids, tannins, saponins, and others [2]. Red fruit (*Pandanus conoideus* L.) is one of the endemic plants of Papua. This genus pandanus plant, ecologically, grows distributed almost throughout Papua, both in lowlands and highlands, but red fruit is a type of seasonal plant so it cannot be found all the time [3]. The people of Papua use the fruit of this plant as a food ingredient that can increase stamina and physical fitness and is also used to treat worm infections and skin disease [4].

Red fruit is used in the treatment of various diseases such as cancer, diabetes mellitus, gout, osteoporosis, hypertension, heart disease, cholesterol, stroke, and various diseases caused by bacteria, fungi, and viruses [5]. However, the part of the red fruit that is usually used is the seed, but in this study, the identification of compounds will be carried out on another part of the red fruit, namely its core, to observe the potential of its compounds. The process of scientific validation is very important so that the use of traditional medicine is not based solely on experience, but has scientific evidence so that it can be used in the formal modern healthcare system. Therefore, further identification of the contents of a plant is necessary. The identification of secondary metabolite compounds contained in the plant can be carried out through phytochemical screening and can also be done using instruments, for example through checks using instruments such as FTIR (Fourier Transform Infrared) and UV-Vis Spectrophotometer. These two instruments can be an efficient alternative for quality control of natural materials in terms of identifying species characteristics and compounds in a plant. Data processing from UV-Vis spectrophotometry results is done by identifying the absorbance of the tested sample, while data processing from FTIR results is done by observing the wavelength of the generated data and interpreting the results of the functional groups [6].

Based on research conducted by Asrianto [5] with the title "Screening and Bioactivity of Red Fruit (*Pandanus conoideus* Lamk.) Extract from Pegunungan Bintang Regency against Candida Albicans Fungi: Randomized Study", red fruit extract (*Pandanus conoideus* L.) contains chemical compounds such as flavonoids, tannins, alkaloids, steroids, and triterpenoids. The results of the bioactivity test of red fruit extract were not sensitive to Candida albicans 10231 fungi. Research conducted by Wabula [7] with the title "Antioxidant Activity of Ethanol Extract of Red Fruit (*Pandanus conoideus* Lam.) Using the Ferric Reducing Antioxidant Power (FRAP) Method", showed that red fruit (*Pandanus conoideus* L.) positively contains phenolic compounds, flavonoids, and tannins and has antioxidant activity with a capacity of 1.392x10−3g ATE/ g extract. Furthermore, according to research on red fruit (*Pandanus conoideus* L.) [8] with the research title "Identification of Phytochemical Compounds in Red Fruit Oil (*Pandanus conoideus* Lam.)", red fruit oil (*Pandanus conoideus* Lam.) contains phytochemical compounds including flavonoids, terpenoids, steroids, and alkaloids.

Flavonoids are secondary metabolite compounds that belong to the phenolic compound group, in which the benzene structure is substituted with an OH group. Flavonoids have pharmacological effects as antioxidants, anti-aging, anti-inflammatory, antiviral, and others [9]. Alkaloids are the most abundant secondary metabolite compounds containing nitrogen atoms, found in plant tissues. Alkaloids in plants function as toxins to protect them from insects and herbivores, as growth regulators, and as storage compounds capable of supplying nitrogen and other elements required by plants. Alkaloid compounds act as antifungal agents because these compounds work by disrupting the peptidoglycan components in fungal cells

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so that they so that it causes the failure of the process of forming the cell wall completely and will cause the cell to die [10].

The solvent used in this study is 95% ethanol because it has the same polarity as the compound to be extracted. Ethanol solvent is effective for obtaining alkaloid, flavonoid, saponin, and tannin compounds because the polarity of the solvent is the same as the polarity of these chemical compounds [11]. Maceration is the process of extracting simplicia using a solvent with several soaking and stirring repetitions at room temperature (Athaillah et al., 2024). Its working principle is based on the ability of the extracting solution to penetrate the cell wall and enter the cell cavity containing various active components [12].

Soxhletation is an extraction method using a fresh solvent, usually carried out using special equipment so that constant extraction occurs with reflux cooling. Reflux is an extraction method that uses the boiling point of a certain solvent over a limited time and amount of solvent that remains relatively constant with the presence of a condenser to achieve better or more complete extraction results [13]. From several previous studies, it can be concluded that red fruit (*Pandanus conoideus* L.) has potential as a medicinal plant due to the secondary metabolites it contains. The researchers want to showcase novelty in the study regarding red fruit (*Pandanus conoideus* L.) because the research conducted so far on the compounds and benefits of the red fruit itself will, in this study, involve identification to determine whether other parts of the plant also have the potential for the same compounds. Therefore, further research was conducted to identify the content of secondary metabolite compounds and to determine the total flavonoid and alkaloid levels found in the corm of red fruit (*Pandanus conoideus* L.). using 95% ethanol solvent and using different extraction methods, namely maceration, soxhlet, and reflux methods. This research is expected to be an inspiration and can serve as a new foundation for other research to develop medicines, especially those derived from natural materials.

Method

Materials and Apparatus

All chemicals and reagents used in this study were of analytical grade (p.a.) unless otherwise specified, and were obtained from commercial suppliers (Merck, Darmstadt, Germany; Sigma-Aldrich, St. Louis, MO, USA). The plant material consisted of red fruit stalks (Pandanus conoideus L.) collected from the Papua region, Indonesia. The chemicals employed included 95% ethanol for extraction purposes, absolute ethanol (p.a.), chloroform ($CHCl_3$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), acetic acid (CH_3COOH), ammonia, sodium hydroxide (NaOH), ferric chloride ($FeCl_3$) 1% solution, aluminum chloride ($AlCl_3$) 10% solution, potassium acetate (KCH_3COO) 1 M solution, Bromocresol Green (BCG) solution, phosphate buffer solution (pH 4.7), Dragendorff's reagent, Mayer's reagent, Wagner's reagent, and magnesium powder (Mg). Distilled water was used throughout the experiments for all aqueous solution preparations. The instrumentation utilized in this study included a Fourier-Transform Infrared (FTIR) spectrometer (Shimadzu IRTracer-100, Kyoto, Japan) equipped with a single-reflection attenuated total reflectance (ATR) accessory, operating within the spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per spectrum. A UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) was employed for quantitative analysis of alkaloid and flavonoid content, with absorbance measurements conducted at wavelengths of 275 nm and 436 nm, respectively, using quartz cuvettes with a 1 cm path length.

The extraction and fractionation processes were facilitated by a series of reflux and Soxhlet apparatus (Pyrex, USA), a percolator, a heating mantle (Thermo Scientific, Waltham, MA, USA), a rotary evaporator (Büchi R-300, Flawil, Switzerland) for solvent removal, and a water bath maintained at controlled temperatures. Other glassware and auxiliary equipment utilized throughout the study included beakers (Pyrex, 50–1000 mL), Erlenmeyer flasks (Pyrex, 100–500 mL), volumetric flasks (Pyrex, 10 mL and 25 mL), graduated measuring cylinders, separatory funnels, conical funnels, round-bottom flasks, condensers, dropping pipettes, volumetric pipettes, micropipettes (Eppendorf, Hamburg, Germany) with various volume ranges, test tubes (13 × 100 mm), boiling flasks, porcelain dishes, glass bowls, stirring rods, spatulas, stands and clamps, connectors, hoses, aluminum foil, plastic wrap, cotton, filter paper (Whatman No. 1, GE Healthcare, UK), scissors, hand scoops, masks, and an analytical balance (Mettler Toledo AB204-S, Greifensee, Switzerland) with a precision of 0.0001 g. All glassware was thoroughly cleaned and rinsed with distilled water and dried prior to use.

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Sample Preparation

Samples of red fruit cores (*Pandanus conoideus* L.) were taken from the Sorong Regency area, Southwest Papua, totaling 3 kg. The part used was the core, which was then cleaned of dirt using running water. After that, it was weighed to obtain the initial weight. Next, the sample was sliced and dried using an oven at a temperature of 45-50℃ until it was obtained in the form of simplisia, then dry sorting was carried out to remove any remaining dirt, and then the simplisia was ground using a blender to obtain a fine powder and the powder was then sieved to obtain a finer powder.

Sample Extraction

The extraction of red fruit cores (*Pandanus conoideus* L.) was carried out using three extraction methods, namely maceration, soxhletation, and reflux. In the maceration process, the simplisia powder was weighed 50 grams, then placed in a maceration container (glass jar), Add 95% ethanol solvent as much as 500 mL (1:10), let it sit for 3 days, then filter. Perform remaceration for 1 day with the same treatment, then filter again. After that, the obtained filtrate is evaporated over a water bath until it becomes a thick extract. In the Soxhlet extraction method, weigh 50 grams of red fruit corm powder (*Pandanus conoideus* L.), then wrap it using filter paper and tie it, then put it into a Soxhlet tube (thimble), add 350 mL of 95% ethanol solvent into a round-bottom flask (1:7). The extraction process is carried out until the drops become clear. After that, the obtained filtrate is evaporated over a water bath until it becomes a thick extract [14]. In the reflux extraction method, weigh 50 grams of red fruit corm powder (*Pandanus conoideus* L.) and put it into a round-bottom flask, then add ethanol solvent as much as 400 mL (1:8) and heated for 2-3 hours, then filtered using a funnel. The macerate was concentrated using a rotary evaporator at 40°C and 180 mbar until a viscous extract was obtained. Operationally, the extract was considered viscous when it exhibited a semi-solid consistency, showed no free-flowing properties at room temperature, and did not drip when the container was tilted at an angle of approximately 45° for 30 s. The concentrated extract was subsequently weighed for yield determination and stored at 4°C prior to further analyses. [15].

Phytochemical Screening of Ethanolic Extract of Red Fruit Corm

Qualitative phytochemical screening of the ethanolic extract of red fruit corm was conducted to identify the presence of major secondary metabolite classes, including flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids, using established colorimetric and precipitation methods [11,16–20]. For the flavonoid test, 1 mL of the test solution was distributed into three test tubes; the first tube was treated with hydrochloric acid (HCl), the second with 10% lead acetate solution, and the third with 20% sodium hydroxide (NaOH), wherein the formation of a yellow coloration indicated a positive result for flavonoids, while a positive reaction with HCl reagent was specifically characterized by the development of red, yellow, or orange colors [16,17]. Alkaloid detection was performed by pipetting approximately 1 mL of the sample, divided into three equal portions in separate test tubes, and each was treated with Dragendorff's, Mayer's, and Bouchardat's reagents, respectively; a positive result was indicated by the formation of a red precipitate with Dragendorff's reagent, a yellowish-white precipitate with Mayer's reagent, and a brown to black precipitate with Bouchardat's reagent [18,19]. The tannin test was carried out by pipetting approximately 1 mL of the sample and adding a few drops of 1% ferric chloride ($FeCl_3$) solution, where the appearance of a brownish-green, bluish-black, or blackish-green coloration signified the presence of tannins [18]. For saponin analysis, 1 gram of the extract was placed into a test tube and mixed with 10 mL of distilled water, followed by vigorous shaking for 1 minute and the subsequent addition of 2 drops of HCl; the persistence of stable foam for approximately 10 minutes was considered a positive indication of saponin content [11]. The detection of steroids and terpenoids was conducted using the Liebermann-Burchard reaction, wherein 1 mL of the sample was mixed with chloroform, followed by the sequential addition of acetic anhydride and concentrated sulfuric acid (H_2SO_4); a positive result for steroids was indicated by a blue to green coloration, whereas terpenoids were characterized by a red to purple coloration, while the formation of a brown ring at the interface was not considered a specific positive indicator [18,20].

FTIR Test (Fourier Transform Infrared)

This FTIR test will display the functional groups present in the sample. The test is carried out by taking a sample formed into a pellet and placed in a tablet holder that has been coated with a KBr pellet. Next, the sample is inserted into the FTIR instrument and then recorded using infrared [21].

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Spectrophotometric Determination and Quantification of Total Flavonoid Content

The determination of total flavonoid content was performed using a UV-Vis spectrophotometric method based on the formation of a flavonoid-aluminum chloride complex [11]. A quercetin standard solution at a concentration of 100 ppm was prepared by dissolving 1 mg of quercetin in ethanol within a 10 mL volumetric flask adjusted to the mark, and subsequent dilutions were made by transferring 0.1, 0.3, 0.5, 0.7, and 0.9 mL of the stock solution into separate 10 mL volumetric flasks, each brought to volume with ethanol to obtain working standard concentrations of 1, 3, 5, 7, and 9 ppm, respectively [11]. The calibration curve was constructed by plotting the absorbance of these quercetin standard solutions against their respective concentrations, with measurements conducted using a UV-Vis spectrophotometer at a wavelength of 436 nm [11]. For sample analysis, 10 mg of the ethanolic extract of red fruit corm was dissolved in 10 mL of ethanol, and 1 mL of this solution was transferred into a 25 mL volumetric flask, to which 1 mL of 2% aluminum chloride ($AlCl_3$) and 1 mL of potassium acetate were added, and the volume was made up to the mark with ethanol [11]. The test solution was prepared in triplicate, and its absorbance was measured at the same wavelength of 436 nm [11]. The concentration of flavonoids in the sample was determined by substituting the measured absorbance into the linear regression equation obtained from the quercetin standard curve, and the total flavonoid content was subsequently calculated by multiplying the concentration value by the volume of the sample solution in liters and dividing by the sample weight in grams, with the results expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

Spectrophotometric Determination and Quantification of Total Alkaloid Content

The absorbance values obtained from the alkaloid content determination were subsequently substituted into the linear regression equation derived from the caffeine standard curve, which conforms to the general formula Y = a + Bx, wherein Y denotes the measured absorbance and x represents the concentration of the alkaloid compound within the sample solution [11]. The calibration curve was constructed by measuring the absorbance of caffeine standard solutions at concentrations of 1, 3, 5, 7, and 9 ppm using a UV-Vis spectrophotometer set at a wavelength of 275 nm [11]. For sample preparation, 10 mg of the ethanolic extract of red fruit bulb was dissolved in ethanol within a 10 mL volumetric flask, after which 1 mL of this solution was transferred and mixed with phosphate buffer solution at pH 4.7 and Bromocresol Green (BCG) solution, followed by liquid-liquid extraction using chloroform, performed in triplicate [11]. The chloroform phase containing the alkaloid-BCG ion-pair complex was separated and collected into a 25 mL volumetric flask, adjusted to volume with chloroform, and its absorbance was measured at the identical wavelength of 275 nm [11]. The resulting concentration value (x) obtained from the regression equation was then multiplied by the volume of the sample solution in liters and divided by the sample weight in grams to calculate the total alkaloid content, expressed as milligrams of caffeine equivalent per gram of extract (mg CE/g extract)

Data Analysis

The absorbance results of alkaloid and flavonoid compounds were inserted into the linear regression equation from the standard caffeine solution for alkaloid compounds and quercetin for flavonoid compounds, then inserted into the formula Y = a + Bx to obtain the concentration value (x) of each compound in the sample solution. Furthermore, the total alkaloid and flavonoid content of the ethanol extract of red fruit corm was calculated using the formula: total content equals the concentration value (x) multiplied by the volume of the sample solution (in liters), then divided by the weight of the sample (in grams). This calculation yields the total content expressed as milligrams of equivalent standard compound per gram of sample extract, providing a quantitative measure of the alkaloid and flavonoid compounds present in the red fruit corm ethanol extract.

Results and Discussion

Extraction Results

Table 1. Extraction Results by Maceration, Soxhlet, Reflux Methods

Sample	Method	Sample Weight	Extract Weight	Solvent Volume	Yield
Red Fruit Core (*Pandanus conoideus* L.)	Maceration	50 g	5 g	500 mL	10%
Red Fruit Core (*Pandanus conoideus* L.)	Soxhlet Extraction	50 g	12 g	350 mL	24%
Red Fruit Core (*Pandanus conoideus* L.)	Reflux Extraction	50 g	7 g	800 mL	14%

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Screening Results

Table 2. Phytochemical Screening of Secondary Metabolites

Sample	Extraction Method	Metabolite Secondary	Reagent	Description
Red Fruit Stump (*Pandanus conoieus* L.)	Maserasi	Alkaloid	Meyer	Positive (+) White Sediment
			Dragendorff	Positive (+) Brick Red Sediment
			Bouchardat	Positive (+) Brown Sediment
		Flavonoid	NaOH	Positive (+) Yellow Sediment
			Pb2 Asetat	Positive (+) Yellowish Brown Sediment
			HCl	Positive (+) Yellow Sediment
		Tanin	FeCl3	Negative (-) No Sediment
		Saponin	Aquadest	Negative (-) No Stable Foam
		Terpenoid	Kloroform+Asam Asetat+Asam Sulfat	Positive (+) Brown Ring
		Steroid		Negative (-) No Sediment
	Soxhletasi	Alkaloid	Mayer	Positive (+) White Sediment
			Dragendorff	Positive (+) Brick Red Sediment
			Bouchardat	Positive (+) Brown Sediment
		Flavonoid	NaOH	Positive (+) Yellow Sediment
			Pb2 Asetat	Positive (+) Yellowish Brown Sediment
			HCl	Positive (+) Yellow Sediment
		Tanin	FeCl3	Positive (+) Dark Green Sediment
		Saponin	Aquadest	Negative (-) No Stable Foam
		Terpenoid	Kloroform+Asam Asetat+Asam Sulfat	Positive (+) Brown Ring
		Steroid		
	Refluks	Alkaloid	Mayer	Negative (-) No Sediment
			Dragendorff	Positive (+) White Sediment
			Bouchardat	Positive (+) Brick Red Sediment
		Flavonoid	NaOH	Positive (+) Brown Sediment
			Pb2 Asetat	Positive (+) Yellow Sediment
			HCl	Positive (+) Yellowish Brown Sediment
		Tanin	FeCl3	Positive (+) Yellow Sediment
		Saponin	Aquadest	Positive (+) Stable Foam
		Terpenoid	Kloroform+Asam Asetat+Asam Sulfat	Negative (-) No Sediment
		Steroid		Positive (+) Brown Ring

FTIR (Fourier Transform Infrared) Results

Maceration

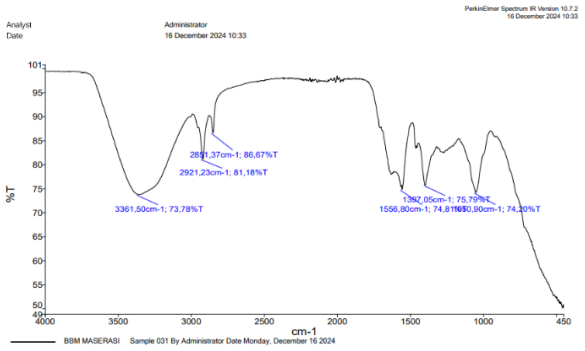

Figure 1. FTIR Spectrum of Red Fruit Bulb Extract by Maceration Method

Table 3. Interpretation of FTIR Spectrum Results of Ethanol Extract of Red Fruit Stem (*Pandanus conoideus* L.) by Maceration Method

Functional Group	Wavenumber (cm^{-1})	Frequency	Intensity
C-H (Alkana)	2851,37	3000-2850	Strong
C-H (Alkana)	2921,23	3000-2850	Strong
O-H (Alkohol, Fenol)	3361,50	3500-3100	Moderate
C-H (Alkana)	1397,05	1470-1340	Strong
N-H (Amina)	1556,80	1640-1550	Moderate
C-O (Alkohol, eter, ester, asam karboksilat, anhidrida)	1050,90	1300-1000	Strong

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Soxhletasi

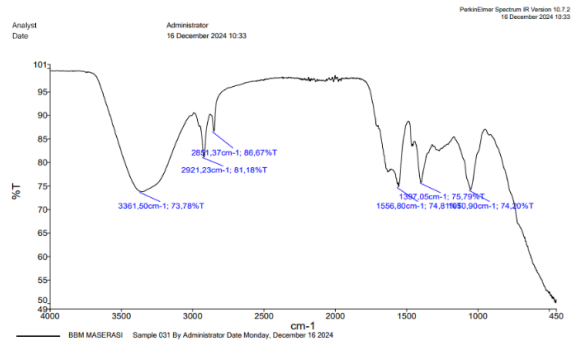

Figure 2. FTIR Spectrum of Red Fruit Bulb Extract by Soxhlet Method

Tabel 4. Interpretasi Hasil Spektrum FTIR Ekstrak Etanol Bonggol Buah Merah (*Pandanus conoideus* L.) Metode Soxhletasi

Functional Group	Wavenumber (cm^{-1})	Frequency	Intensity
O-H (Alkohol, Fenol)	3360,35	3400-3200	Medium
C-H (Alkana)	2918,54	3000-2850	Strong
N-H (Amina)	1556,75	1640-1550	Medium
C-H (Alkana)	1396,61	1470-1340	Strong
C-O (Alkohol, eter, ester, asam karboksilat, anhidrida)	1050,08	1300-1000	Strong

Refluks

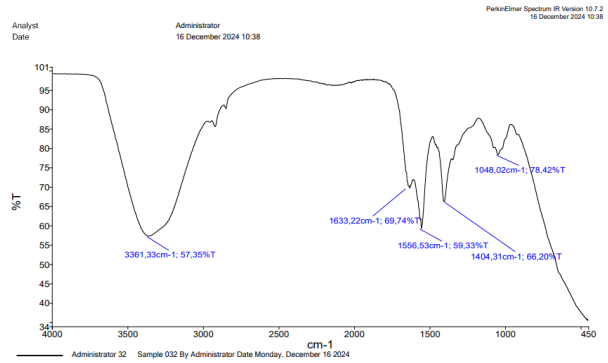

Figure 3. FTIR Spectrum of Red Fruit Core Extract by Reflux Method

Table 5. Interpretation of FTIR Spectrum Results of Ethanol Extract of Red Fruit Core (*Pandanus conoideus* L.) by Reflux Method

Functional Group	Wavenumber (cm^{-1})	Frequency	Intensity
O-H (Alkohol, Fenol)	3361,33	3400-3200	Medium
C=O (Amida)	1633,22	1700-1640	Strong
N-H (Amina)	1556,53	1640-1550	Strong
C-H (Alkana)	1404,31	1470-1340	Strong
C-O (Alkohol, eter, ester, asam karboksilat, anhidrida)	1048,02	1300-1000	Strong

Determination of Flavonoid Content

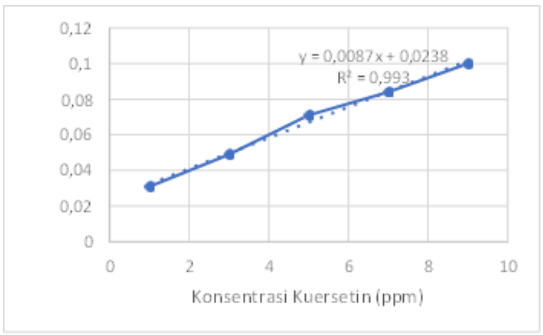

Figure 4. Linear Regression Curve of Quercetin Standard Solution

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Maceration

Table 6. Results of Flavonoid Content Determination in Ethanol Extract of Red Fruit Pulp by Maceration, Soxhlet Extraction, Reflux Methods

Test Extract	Absorbance			Average	KTF (mgEQ/g)	KTF %
	1	2	3			
Maceration	0,100	0,100	0,100	0,100	218,97	21,9
Soxhletation	0,070	0,060	0,050	0,060	104,023	10,40
Reflux	0,110	0,110	0,110	0,110	247,701	24,77

Determination of Alkaloid Content

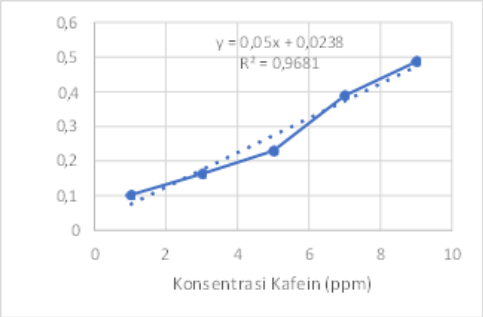

Figure 5. Linear Regression Curve of Standard Caffeine Solution

Table 7. Results of Alkaloid Content Determination in Ethanol Extract of Red Fruit Peduncle by Maceration, Soxhlet, and Reflux Methods

Test Extract	Absorbance			Average	KTA (mgAE/g)	KTA %
	1	2	3			
Maceration	0,395	0,297	0,254	0,315	145,77	14,5
Soxhletation	0,172	0,148	0,146	0,155	65,77	6,57
Reflux	0,207	0,162	0,163	0,177	76,433	7,64

Discussion

In this study, red fruit plants were used, and the part utilized was the fruit core. In the extraction process, two extraction methods were employed, namely cold extraction (maceration) and hot extraction (Soxhlet and reflux). The selection of these three different extraction types was due to the aim of this study, which was to identify the extraction method suitable for extracting secondary metabolite compounds contained in the red fruit core (*Pandanus conoideus* L.). The maceration method itself is one of the simplest cold extraction methods, as are Soxhlet and reflux, which are generally used to extract secondary metabolite compounds that are heat-resistant.

In the maceration method, an extract weight of 5 grams was obtained with a total yield of 10%, in the Soxhlet extraction method, an extract weight of 12 grams was obtained with a total yield of 24%, whereas in the reflux method an extract weight of 7 grams was obtained with a total yield of 14%. Based on these results, it can be concluded that the most effective method for extracting compounds from red fruit corm (*Pandanus conoideus* L.) extract is by using a hot extraction method, namely Soxhlet extraction. This is because the yield value is related to the amount of bioactive content contained in the plant. Therefore, the higher the extract yield, the higher the content of substances that are extracted from a raw material [22]. This is also in accordance with the statement that the Soxhlet method uses repeated percolation with a relatively constant amount of solvent, causing the components or chemical compounds in the sample to be well isolated [14]. In the testing of alkaloid compounds in the results of maceration, soxhletation, and reflux extracts of red fruit bulbs (*Pandanus conoideus* L.), positive results (+) were shown to contain alkaloids using Mayer's reagent with parameters or indications being the presence of a white precipitate, positive results (+) were shown to contain alkaloids using Dragendorff's reagent with parameters or indications being the presence of a brick-red precipitate, and positive results (+) were shown to contain alkaloids using Bouchardat's reagent with parameters or indications being the presence of a brown precipitate. Alkaloid compounds will interact with

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tetraiodomercurate (II) ions to form a complex compound and precipitate. This is because mercury ions are heavy metal ions capable of precipitating basic alkaloid compounds [23].

In the testing of flavonoid compounds in the maceration, soxhlet, and reflux extracts of red fruit corms (*Pandanus conoideus* L.), positive results were observed for flavonoid content using sodium hydroxide (NaOH) reagent, indicated by a yellow color change due to decomposition and the cleavage of the isoprene structure by the base in flavonoid derivatives such as flavone or flavonol, resulting in the formation of acetophenone molecules. Positive results for flavonoid content were also obtained using lead II acetate (Pb(CH3COO)2) reagent, indicated by a yellowish-brown precipitate due to the cleavage of the bond at the C3 atom [24]. Positive results for flavonoid content were also observed using hydrochloric acid (HCl) reagent, indicated by a yellow color change. The addition of HCl causes a redox reaction between magnesium as a reducing agent and the flavonoid compounds [25].

In the testing of terpenoid and steroid compounds in maceration, soxhlet, and reflux extract results, positive (+) results were obtained for the presence of terpenoids using a reagent of 2 mL chloroform + 1 mL acetic acid + 2 mL sulfuric acid, indicated by the presence of a brown ring. This occurs due to the oxidation of triterpenoid terpenoid compounds through the formation of conjugated double bonds, while for steroid compounds, negative (-) results were obtained for the presence of steroids because no color change occurred [23].

In testing for saponin compounds in the maceration and soxhlet extraction results of red fruit corm (*Pandanus conoideus* L.), the results were negative (-) for saponin content as no stable foam was found, whereas in the reflux extraction results, the test showed positive (+) for saponin content with the indication of stable foam after being dropped with 1 drop of HCl. Saponins have glycosyl groups as polar groups and steroid or triterpenoid groups as nonpolar groups, making them surface-active and forming micelles when shaken with water [26]. In testing for tannin compounds in the maceration and reflux extraction results of red fruit corm (*Pandanus conoideus* L.), the results were negative (-) for tannin content as no color change occurred, whereas in the soxhlet extraction results of red fruit corm (*Pandanus conoideus* L.), the test was positive (+) for tannin content using FeCl3 reagent with the indication of a greenish-black color change. The blackish-green color formed after FeCl3 is added is caused by tannins that are excitted with Fe3+ ions to form complex compounds [27]. The results of the ethanol spectrum of red fruit hump (*Pandanus conoideus* L.) maceration method, showed the presence of absorption bands in the areas of 3000-2850 cm-1 and 1470-1340 cm-1 showed the presence of C-H groups at wave numbers 2851.37 cm-1, 2921.23 cm-1, and 1397.05 cm-1 with strong intensity, in accordance with the results of positive phytochemical screening containing terpenoids and steroids. There were no steroid compounds on the screening results, but there were on the FTIR results. This is due to the possibility that the amount of steroids contained in red fruit hump extract (*Pandanus conoideus* L.) is small, making it difficult to react and illegible. According to [28]. The presence of vibrations from the C–H group, which is slightly detected, reinforces this statement because the C–H group indicates sufficient intensity to induce nonpolar compounds such as terpenoids and steroids to appear as vibrational bands. Absorption bands in the region of 3500-3100 cm-1 and 1640-1550 cm-1 indicate the presence of O-H and N-H groups at the wavenumbers of 3361.50 cm-1 and 1556.80 cm-1 with moderate intensity, in accordance with the positive phytochemical screening results showing the presence of alkaloids. Absorption bands in the region of 1300-1000 cm-1 indicate the presence of C-O groups at the wavenumber of 1050.90 cm-1 with strong intensity, consistent with the positive phytochemical screening results showing the presence of flavonoids. The ethanol extract spectrum of red fruit corm (*Pandanus conoideus* L.) using the Soxhlet method shows absorption bands in the region of 3400-3200 cm-1 indicating the presence of O-H groups at the wavenumber of 3360.35 cm-1 with moderate intensity, in accordance with the positive phytochemical screening results showing the presence of alkaloids. According to [28], the broadened absorption band of the O–H group indicates the presence of alkaloid compounds containing an N–H group. The absorption bands in the regions of 3000-2850 cm-1 and 1470-1340 cm-1 indicate the presence of C-H groups at the wave numbers of 2918.54 cm-1 and 1396.61 cm-1 with strong intensity, which is consistent with the positive phytochemical screening results showing the presence of terpenoids and steroids. No steroid compounds were found in the screening results, but they were present in the FTIR results. This is due to the possibility that the amount of steroids contained in the extract of the red fruit corm (*Pandanus conoideus* L.) is small, making it difficult to react and undetectable.

According to [28], the presence of vibrations from the C–H group that are slightly detected reinforces this statement because the C–H group indicates sufficient intensity to induce nonpolar compounds such as terpenoids and steroids to appear as vibration bands. Absorption bands in the 1640-1550 cm-1 region indicate the presence of N-H groups at a wavenumber of 1556.75 cm-1 with moderate intensity, corresponding to

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positive phytochemical screening results for containing alkaloids. Absorption bands in the 1300-1000 cm-1 region indicate the presence of C-O groups at a wavenumber of 1050.08 cm-1 with strong intensity, corresponding to positive phytochemical screening results for containing flavonoids. The spectral results of ethanol extract from the red fruit corm (*Pandanus conoideus* L.) using the reflux method show an absorption band in the 3400-3200 cm-1 region indicating the presence of O-H groups at a wavenumber of 3361.33 cm-1 with moderate intensity, corresponding to positive phytochemical screening results for containing alkaloids.

According to [28], the broadened absorption band of the O–H group indicates the presence of alkaloid compounds. The absorption band in the region of 1700-1640 cm-1 indicates the presence of the C=O group at a wavenumber of 1633.22 cm-1 with strong intensity, positively containing tannins. The C=O group is commonly found in tannin compounds. However, it was not detected during the phytochemical screening process due to the possibility that the amount of tannins present was small, making it difficult to react and undetectable. The absorption band in the region of 1640-1550 cm-1 indicates the presence of the N-H group at a wavenumber of 1556.53 cm-1 with strong intensity, consistent with the phytochemical screening results which were positive for alkaloids. The absorption band in the region of 1470-1340 cm-1 indicates the presence of the C-H group at a wavenumber of 1404.31 cm-1 with strong intensity, consistent with the phytochemical screening results that were positive for terpenoids and steroids. No steroid compounds were detected in the screening results, but they were present in the FTIR results.

This is due to the possibility that the amount of steroids contained is small, making it difficult to react and undetectable. According [28], the presence of vibrations from the C–H group being slightly detected strengthens this statement because the C–H group indicates sufficient intensity to induce nonpolar compounds such as terpenoids and steroids to appear as vibrational bands. The absorption bands in the 1300-1000 cm-1 region indicate the presence of a C-O group at a wavenumber of 1048.02 cm-1 with strong intensity, consistent with the positive phytochemical screening results showing the presence of flavonoids. Analysis of the flavonoid content in the ethanol extract of the red fruit bulb (*Pandanus conoideus* L.) was carried out with AlCl3. The formation of complexes between AlCl3 and the ketone group at the C-4 atom and the hydroxyl group at the C-3 or C-5 atom is the principle of AlCl3.

The reference solution used for this study was quercetin, because quercetin is the most widely distributed compound in plants and is one of the flavonoid compounds that can react with AlCl3 to form a complex [29]. The quercetin standard curve was made with concentration variations of 1, 3, 5, 7, and 9 ppm, measured at a maximum wavelength of 436 nm. From the absorbance measurements of the quercetin concentration series solutions, a linear regression equation can be made as Y = 0.0087x + 0.0238 with R2 = 0.993, and r = 0.996. A linear calibration curve is indicated by an r value close to 1. An r value close to 1 indicates a relationship between quercetin and absorbance values, as the higher the concentration, the higher the absorbance [29].

The results of the total flavonoid compound content obtained from the ethanolic extract of red fruit corm (*Pandanus conoideus* L.) using the maceration method were 218.966 mgQE/g with a percentage of 21.89%, using the Soxhlet method it was 104.023 mgQE/g with a percentage of 10.40%, and using the reflux method it was 247.701 mgQE/g with a percentage of 24.77%. The determination of alkaloid compound content was carried out by measuring the maximum wavelength of the caffeine standard solution. The purpose of determining the maximum wavelength is to achieve maximum absorption strength and to minimize the reading error as much as possible. The caffeine standard curve was prepared with concentrations of 1, 3, 5, 7, and 9 ppm measured at a maximum wavelength of 275 nm. In measuring the total alkaloid content of the ethanolic extract of red fruit corm (*Pandanus conoideus* L.), The sample was added with a phosphate buffer pH 4.7 so that the basic alkaloid compounds become acidic and will give optimal results when BCG reacts with the alkaloid [29]. Next, the BCG solution was added, forming a yellow-colored compound due to the formation of a complex between the alkaloid and the BCG reagent, which is the principle of BCG. Chloroform was added to re-extract the alkaloid compounds that were already free from their salts so that only the alkaloids remain in the final solution [29]. From the absorbance measurements of the quercetin concentration series solution, a linear regression equation can be made where Y = 0.05x + 0.0238 and R2 = 0.9681, with a value of r = 0.9839. The total alkaloid content obtained from the ethanol extract of red fruit corm (*Pandanus conoideus* L.) In the maceration method, it was obtained as much as 145.77 mgAE/g with a percentage of 14.5%, in the soxhletation method it was obtained as much as 65.767 mgAE/g with a percentage of 6.57%, in the reflux method it was obtained as much as 76.433 mgAE/g with a percentage of 7.64%.

The differences in flavonoid and alkaloid levels with various extraction methods can be explained by the mass transfer mechanisms that occur during the extraction process. Extraction basically involves the

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diffusion of bioactive compounds from the plant cell matrix into the solvent, which is influenced by concentration gradients, temperature, and solvent characteristics. In heat-based methods like reflux and Soxhlet extraction, higher temperatures speed up the diffusion rate by increasing the kinetic energy of molecules and weakening the bonds between bioactive compounds and cellular components like the cell wall and membrane. This causes more flavonoid compounds to be released from plant tissue, resulting in higher levels in the reflux method [30].

Flavonoids generally have relatively good thermal stability, especially in their aglycone form, so they don't easily break down at moderate heating temperatures like those used in reflux. This condition allows for improved extraction efficiency by increasing solubility and speeding up diffusion without causing significant damage to their chemical structure [31].

On the other side, alkaloids show different responses to extraction conditions. In this study, the maceration method produced the highest alkaloid content compared to the heat method. This can be linked to the fact that some alkaloids are more sensitive to heat, especially alkaloids in salt form or complex compounds that can degrade or change structure at high temperatures. Maceration, done at room temperature, allows the extraction process to occur through osmosis and gradual diffusion without the risk of thermal degradation, making the alkaloid compounds more stable during their transfer from plant cells to the solvent. Mechanistically, in maceration, the solvent penetrates the cell walls through osmosis, then dissolves the active compounds and carries them out along the concentration gradient. This process is slower compared to the heat method, but it's more suitable for compounds that aren't stable at high temperatures [32].

Meanwhile, the Soxhlet method in this study produced the highest extract yield, but this didn't come with an increase in flavonoid or alkaloid content. This shows that total yield doesn't always reflect the amount of specific active compounds, because Soxhlet also extracts non-polar components and other impurities like lipids, chlorophyll, and resins. So, it's more accurate to assess the effectiveness of an extraction method based on its ability to selectively extract the target compounds, not just on how much extract it produces. [32], [33].

Overall, these differences in results show that choosing an extraction method should match the physicochemical properties of the target compounds, especially regarding thermal stability and how they release from the plant matrix. Reflux works better for flavonoids that are relatively heat-stable, while maceration is more suitable for alkaloids that are more sensitive to heat [34].

Conclusions

Based on these results, it can be concluded that the most effective method to extract compounds from the red fruit core extract (*Pandanus conoideus* L.) is by using the hot extraction method, namely soxhletation, compared to maceration and reflux methods. The weight of the soxhletation extract is 12 grams, maceration 10 grams, and reflux 7 grams. The total yields sequentially are 24%, 10%, and 14%.

In the testing of alkaloid compounds from maceration, soxhlet, and reflux extracts, the results were positive for alkaloids (Mayer, Dragendorff, and Bouchardat reagents). Flavonoids were positive in maceration, soxhlet, and reflux extracts with reagents (NaOH, Pb2 acetate, and HCl). Saponins were positive in maceration and soxhlet extracts showing negative results for containing saponins, whereas in the reflux extract results, it showed positive for containing saponins. Tannins were positive in maceration and reflux extracts showing negative results for containing tannins, whereas in the soxhlet extract test results, it was positive.

Based on the results from the FTIR spectrum interpretation of ethanol extract of red fruit corm (*Pandanus conoideus* L.), in the maceration method there are functional groups C-H, N-H, C-H, and C-O; in the soxhletation method, there are functional groups O-H, C-H, N-H, C-H, and C-O; in the reflux method, there are functional groups O-H, C=O, N-H, C-H, C-O. From these results, it can be concluded that the ethanol extract of red fruit corm (*Pandanus conoideus* L.) contains functional groups indicating the presence of secondary metabolite compounds of the flavonoid, alkaloid, tannin, steroid, and terpenoid groups.

The total flavonoid content obtained from ethanol extracts of red fruit corms (*Pandanus conoideus* L.) using the maceration method was 218.966 mgQE/g with a percentage of 21.89%, the Soxhletation method was 104.023 mgQE/g with a percentage of 10.40%, and the reflux method was 247.701 mgQE/g with a percentage of 24.77%. The total alkaloid content obtained from ethanol extracts of red fruit corms (*Pandanus conoideus* L.) using the maceration method was 145.77 mgAE/g with a percentage of 14.5%, the Soxhletation method was 65.767 mgAE/g with a percentage of 6.57%, and the reflux method was 76.433 mgAE/g with a percentage of

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7.64%. From these results, it was found that the best method to obtain flavonoid compounds is the reflux method, while for alkaloids, the maceration method is used.

The results of this study demonstrated that the extraction method significantly influenced the total flavonoid and alkaloid contents obtained from the sample. The reflux extraction method was the most effective for flavonoid extraction, as evidenced by the highest total flavonoid content compared to the other extraction methods. In contrast, the maceration method showed the highest effectiveness for alkaloid extraction, yielding the highest total alkaloid content. These findings indicate that the selection of an extraction method should be tailored to the target class of bioactive compounds. Although the Soxhlet extraction method produced the highest extraction yield, a higher yield did not necessarily correspond to higher flavonoid or alkaloid contents. Therefore, the effectiveness of an extraction method should be evaluated based on its ability to recover the target bioactive compounds rather than solely on the extraction yield obtained.

Conflict of Interest

The authors declare no conflict of interest.

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