

Determination of Total Flavonoid Content and Antioxidant Activity of Ethanolic Extract of Salak Pondoh Leaves (*Salacca zalacca* (Gaertn.) Voss) Using UV-Vis Spectrophotometry

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ABSTRACT: Salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss) have the potential as a natural source of antioxidants due to their secondary metabolite content. This study aimed to determine the total flavonoid content and antioxidant activity of the ethanolic extract of salak pondoh leaves. Extraction was performed using the *Ultrasound-Assisted Extraction* (UAE) method with 70% ethanol as the solvent. Flavonoids were qualitatively analyzed using color reactions and *Thin Layer Chromatography* (TLC), and quantitatively determined by UV-Vis spectrophotometry using quercetin as a standard. Antioxidant activity was evaluated using the DPPH method by determining the IC₅₀ value. The results indicated the presence of flavonoids, as evidenced by an orange-red color and TLC spots with an R_f value of 0.62, consistent with the standard. The total flavonoid content was 2.66%, and the extract exhibited very weak antioxidant activity with an IC₅₀ value of 196.4690 µg/mL. In contrast, quercetin showed very strong antioxidant activity with an IC₅₀ value of 18.5399 µg/mL.

KEYWORDS: *Salacca zalacca* leaves, total flavonoid content, antioxidant activity, DPPH assay, UV-Vis spectrophotometry.

I. INTRODUCTION

Indonesia is an agrarian country with high biodiversity, including a wide variety of endemic fruits and local cultivars. This is supported by strong consumption and cultivation practices, making Indonesia have great potential in the horticultural sector, particularly in fruit commodities for both domestic consumption and export [3].

Salak plants are not only found in Indonesia but also in several Asian countries such as Burma, Thailand, the Philippines, and Malaysia. This plant is known to contain bioactive compounds with antioxidant potential, which play an important role in protecting cells from damage caused by free radicals that may lead to various degenerative diseases [6].

Salak leaves (*Salacca zalacca* (Gaertn.) Voss) are known to contain various secondary metabolites such as tannins, flavonoids, alkaloids, saponins, phenolics, and terpenoids that are beneficial for health. Previous studies reported the presence of tannins and flavonoids using a decoction method [15], as well as the presence of alkaloids, phenolics, saponins, tannins, and terpenoids using maceration, although flavonoids were not detected in that study [8].

Flavonoids are polyphenolic compounds that act as natural antioxidants and exhibit various biological activities such as antiviral, anti-inflammatory, antidiabetic, and anticancer effects. These compounds also protect plants from ultraviolet (UV) radiation and pathogen attacks [2].

Antioxidants are compounds capable of neutralizing free radicals by donating or accepting electrons, thereby inhibiting oxidation processes. Various natural compounds such as flavonoids, phenolic acids, tocopherols, and ascorbic acid are known to possess antioxidant, anticancer, and antibacterial activities [7].

One commonly used method to evaluate antioxidant activity is the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) assay, which is simple, rapid, and cost-effective. DPPH shows maximum absorbance at a wavelength of 517 nm. Previous studies reported that salak leaf beverages exhibited antioxidant activity with percent inhibition ranging from 24.431% to 41.511% [5], while salak peel extract showed very weak antioxidant activity with an IC₅₀ value of 202.89 µg/mL [4].

Based on the above background, this study aims to determine the total flavonoid content and antioxidant activity of ethanol extract of salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss) using UV-Vis spectrophotometric method, in order to provide important information regarding the potential of natural products for disease prevention, drug development, and health product formulation.

II. METHOD

Research Tools

The instruments used in this study included a UV-Vis spectrophotometer (Shimadzu UV-1800), rotary evaporator (Heidolph Hei-VAP), analytical balance (BIOBASE), oven (Mettler UN 55), furnace (Carbolite Gero ELF 1100), blender (Miyako), Ultrasound-Assisted Extraction (UAE) device (Sonics), and other supporting glassware.

Research Materials

The materials used in this study included salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss), 70% ethanol (PT Kisbiokim), methanol p.a. (Merck), hydrochloric acid (HCl) (Merck), chloroform, ethyl acetate, quercetin (Sigma-Aldrich), sodium acetate (Merck), aluminum chloride (AlCl₃) (Merck), distilled water (PT Kisbiokim), and DPPH (1,1-diphenyl-2-picrylhydrazyl) (TCI).

Sample Collection and Preparation

Salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss) used in this study were collected directly from a plantation located in Puhun Pintu Kabun Village, Mandiangin Koto Selayan District, Bukittinggi City, West Sumatra Province. The samples used were fresh leaves, with a total weight of 2 kg, selected based on green color and intact physical condition.

The collected leaves were first subjected to wet sorting to remove foreign materials such as attached twigs and other impurities, ensuring that only suitable leaves were used as samples. The leaves were then washed with clean running water to remove adhering dirt, with the washing process conducted as quickly as possible to prevent the loss of active compounds.

After washing, the leaves were cut into smaller pieces using a sharp stainless-steel knife to facilitate the drying process. The sliced leaves were then air-dried at room temperature until completely dry. The dried materials were subsequently ground using a blender and sieved through a 40-mesh sieve to obtain a uniform powder. The resulting simplicia powder was then stored in a container protected from direct sunlight.

Extraction Procedure

The extraction of salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss) was carried out using the Ultrasound-Assisted Extraction (UAE) method with 70% ethanol as the solvent. A total of

200 g of salak pondoh leaf simplicia (1:5) was extracted using 1000 mL of ethanol solvent. The mixture was extracted using ultrasonic waves at a frequency of 40 kHz for 35 minutes and then filtered. The same procedure was repeated three times. After that, the filtrate was evaporated using a rotary evaporator at a temperature of 50°C until a viscous extract was obtained [13].

Phytochemical Screening

Qualitative Test of Flavonoid Compounds

A total of 10 mg of salak pondoh leaf extract was weighed and diluted in a 10 mL volumetric flask using 70% ethanol up to the mark. One milliliter of the solution was pipetted, then 5 mL of 2 N HCl was added and the mixture was boiled for 5 minutes, followed by filtration. Subsequently, 0.05 g of magnesium powder and 1 mL of concentrated hydrochloric acid were added, and the mixture was allowed to stand for 1 minute. A positive result for flavonoids is indicated by the formation of a yellow-orange to red color.

Thin Layer Chromatography (TLC) Analysis

- **Stationary phase:** Silica gel plate
- **Mobile phase:** Ethyl acetate : methanol : chloroform (5:1:4, v/v)
- **Chamber saturation:** Saturated using filter paper immersed in the mobile phase
- **Test solution:** 10 mg extract dissolved in 10 mL of 70% ethanol
- **Standard solution:** 10 mg quercetin dissolved in 10 mL of 70% ethanol.
- **Procedure:** The test and standard solutions were spotted onto the activated silica gel plate with small spot diameters and allowed to dry. The plate was developed in a saturated chamber until the solvent front reached an appropriate distance, then removed and air-dried. The spots were observed, and the retardation factor (R_f) values were calculated.

Determination of Total Flavonoid Content

A stock solution of quercetin (1000 µg/mL) was prepared by dissolving 10 mg of quercetin in 96% ethanol and diluting to 10 mL. The maximum wavelength was determined by diluting the stock solution to 40 µg/mL, followed by the addition of 0.1 mL of 10% AlCl₃, 0.1 mL of 1 M sodium acetate, and dilution with ethanol and distilled water. The absorbance was measured using a UV-Vis spectrophotometer in the range of 400–800 nm.

A calibration curve was prepared at concentrations of 20, 30, 40, 50, and 60 µg/mL. Each

solution (0.5 mL) was treated with 0.1 mL of 10% AlCl_3 , 0.1 mL of 1 M sodium acetate, and diluted with ethanol and distilled water. The absorbance was measured at the maximum wavelength.

For sample analysis, 10 mg of extract was dissolved in 96% ethanol and diluted to 100 mL (100 $\mu\text{g/mL}$), then further diluted to 40 $\mu\text{g/mL}$. An aliquot of 0.5 mL was treated with the same reagents as the standard solution. The absorbance was measured at the maximum wavelength, and the analysis was performed in triplicate.

Antioxidant Activity (DPPH Assay)

Antioxidant activity was evaluated using the DPPH radical scavenging method (Molyneux, 2004; Andayani et al., 2008). A DPPH solution (30 $\mu\text{g/mL}$) was prepared in methanol and kept in a dark condition. The maximum absorbance wavelength of DPPH was determined using a UV-Vis spectrophotometer in the range of 400–800 nm.

Quercetin was used as a standard by preparing a stock solution (100 $\mu\text{g/mL}$) and diluted to obtain a series of concentrations (4, 8, 12, 16, and 20 $\mu\text{g/mL}$). Each concentration (0.2 mL) was mixed with 3.8 mL of DPPH solution, homogenized, and incubated in the dark for 30 minutes. The absorbance was measured at the maximum wavelength.

The sample solution was prepared by dissolving the extract in methanol to obtain a stock solution (1000 $\mu\text{g/mL}$), followed by dilution to 100 $\mu\text{g/mL}$ and further prepared into a series of concentrations (4, 8, 12, 16, and 20 $\mu\text{g/mL}$). Each solution (0.2 mL) was treated with 3.8 mL of DPPH solution, incubated for 30 minutes in the dark, and the absorbance was measured at the maximum wavelength.

The percentage of inhibition was calculated, and the IC_{50} value was determined from the linear regression equation of inhibition percentage versus concentration.

III. RESULT AND DISCUSSION

The simplicia powder of salak pondoh leaves was extracted using the Ultrasound-Assisted Extraction (UAE) method, which was selected due to its efficiency in reducing extraction time and solvent consumption compared to conventional methods, as well as its environmentally friendly nature. Prior to extraction, the dried simplicia was ground to reduce particle size, thereby enhancing solvent penetration and improving the extraction efficiency of active compounds. A 70% ethanol solvent was used due to its polarity and suitability for extracting polar compounds such as flavonoids. In addition, ethanol is relatively safe, less toxic, and more environmentally friendly compared to other organic solvents such as acetone and methanol [14].

The qualitative test for flavonoid compounds in the ethanol extract of salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss) showed a positive result, indicated by the formation of an orange to reddish color after the addition of magnesium powder and hydrochloric acid. This color change occurs due to the reduction of the benzopyrone structure of flavonoids under acidic conditions [14], thereby confirming the presence of flavonoid compounds in the extract.



Fig 1. Presence of Flavonoid Compounds (+)

Thin-layer chromatography (TLC) analysis was performed to provide a preliminary profile of the chemical composition based on chromatogram patterns. The separation principle in TLC is based on adsorption and partition mechanisms influenced by the stationary and mobile phases. Prior to analysis, eluent optimization was conducted to obtain a solvent system capable of producing well-resolved spots.

The eluent used was a mixture of ethyl acetate–methanol–chloroform (5:1:4, v/v). Observation under UV light at 366 nm showed spots

with an Rf value of 0.62 for the ethanol extract of salak pondoh leaves, which was identical to that of the quercetin standard. This similarity indicates the presence of flavonoid compounds in the extract. The obtained Rf value falls within the ideal range (0.2–0.8), suggesting good separation quality.



Fig 2. Chromatographic Results of the Ethanol Extract of Salak Pondoh Leaves (*Salacca zalacca* (Gaertn.) Voss)

Description:

1. Stationary phase: Silica gel 60 F254 plate
 2. Mobile phase: Ethyl acetate P – Methanol P – Chloroform (5:1:4)
 3. Standard: 0.1% quercetin in 70% ethanol
 4. Detection: UV light at 254 nm
- S = sample
P = Standart

In this study, quercetin was used as a reference standard due to its classification as a flavonoid compound. Prior to sample determination, the maximum wavelength was determined within the range of 400–800 nm. The results showed that the

maximum absorption wavelength of quercetin was 431.5 nm at a concentration of 40 µg/mL, with an absorbance value of 0.480, which falls within the optimal absorbance range (0.2–0.8) [10]

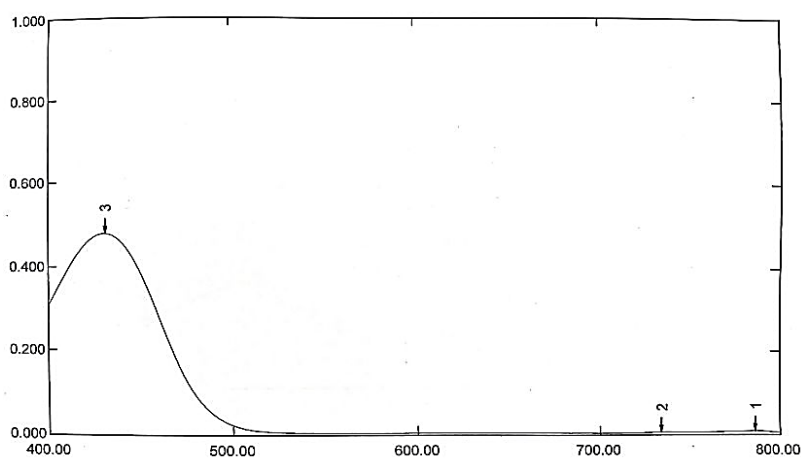


Fig 3. Absorption Spectrum of Quercetin at Maximum Wavelength (40 µg/mL)

Table 1. Determination of the Calibration Curve of Quercetin Standard Solution.

No	Concentration ($\mu\text{g/mL}$)	Absorbance
1.	20	0,275
2.	30	0,386
3.	40	0,503
4.	50	0,632
5.	60	0,756

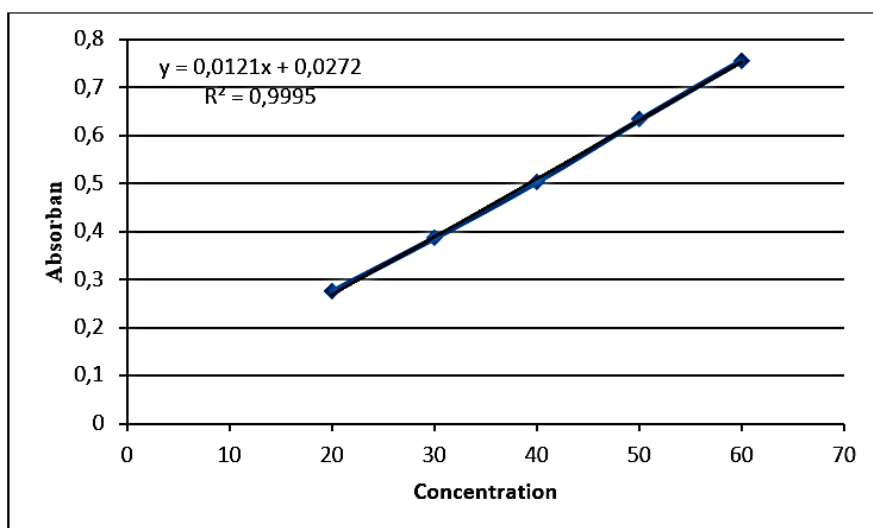


fig 4. Calibration Curve of Quercetin Standard Solution

The calibration curve was prepared using quercetin solutions at concentrations of 20, 30, 40, 50, and 60 $\mu\text{g/mL}$, derived from a 1000 $\mu\text{g/mL}$ stock solution. The regression equation obtained was $y = 0.01208x - 0.0272$ with a correlation coefficient (r^2) = 0.9995, indicating excellent linearity between absorbance and concentration. Based on this calibration curve, the flavonoid content in the ethanol extract of salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss) was determined to be 2.66%.

Antioxidant activity was determined using the DPPH method with a UV-Vis double beam spectrophotometer. Antioxidants act as electron donors or reducing agents that inhibit oxidation reactions by neutralizing free radicals. The reaction between DPPH and antioxidant compounds is indicated by a color change from violet to a lighter color due to the formation of its reduced form. This method is widely used due to its simplicity, rapid analysis, and minimal sample requirement.

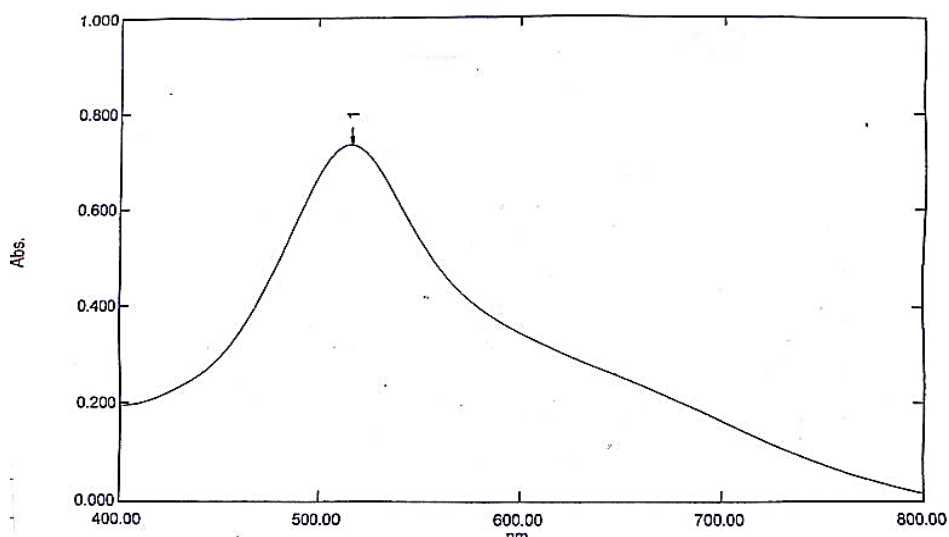


Fig 5. Maximum Wavelength of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) at 30 µg/mL

The antioxidant activity of a compound is indicated by the inhibition of DPPH absorbance at a wavelength of 515–517 nm. DPPH exhibits strong absorption in this range, characterized by a deep

violet color [9]. In this study, the maximum wavelength of DPPH was obtained at 516 nm with an absorbance of 0.735.

Table 2. Absorbance Measurement Results of DPPH 30 µg/mL with Quercetin Solution

No	Concentration (µg/mL)	Absorbance	% Inhibition	Regression Equation	IC ₅₀ (µg/mL)
1.	4	0,545	25,85	y= 1,6626x + 19,1725	18,5399 µg/mL
2.	8	0,496	32,51		
3.	12	0,450	38,77		
4.	16	0,395	46,25		
5.	20	0,351	52,24		

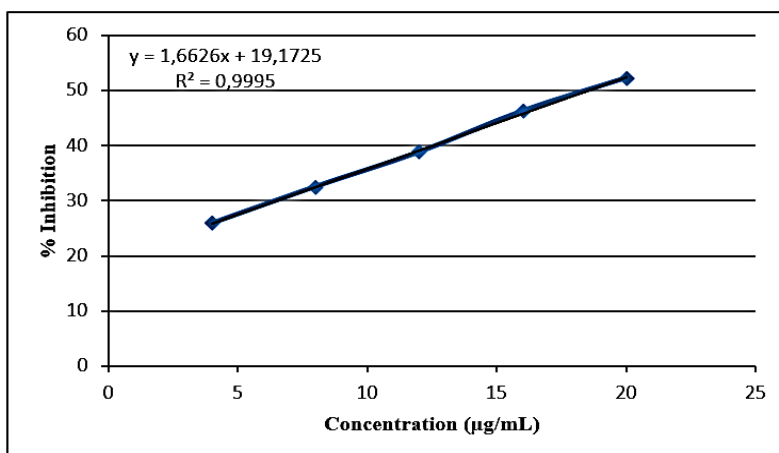


Fig 6 . Concentration vs. % Inhibition Curve of Quercetin.

In the antioxidant activity assay using quercetin as a reference standard, absorbance values decreased with increasing concentration, while the percentage of inhibition increased up to 52.24%. This

indicates that higher concentrations enhance free radical scavenging activity. The IC₅₀ value obtained was 18.5399 µg/mL, indicating very strong antioxidant activity.

Table 3. Absorbance of DPPH (30 µg/mL) with Ethanol Extract of Salak Pondoh Leaves

No	Concentration (µg/mL)	Absorbance	% Inhibition	Regression Equation	IC ₅₀ (µg/mL)
1.	4	0,699	4,89	y=0,2345x+3,928	196,4690
2.	8	0,692	5,85		
3.	12	0,686	6,66		
4.	16	0,679	7,61		
5.	20	0,671	8,70		

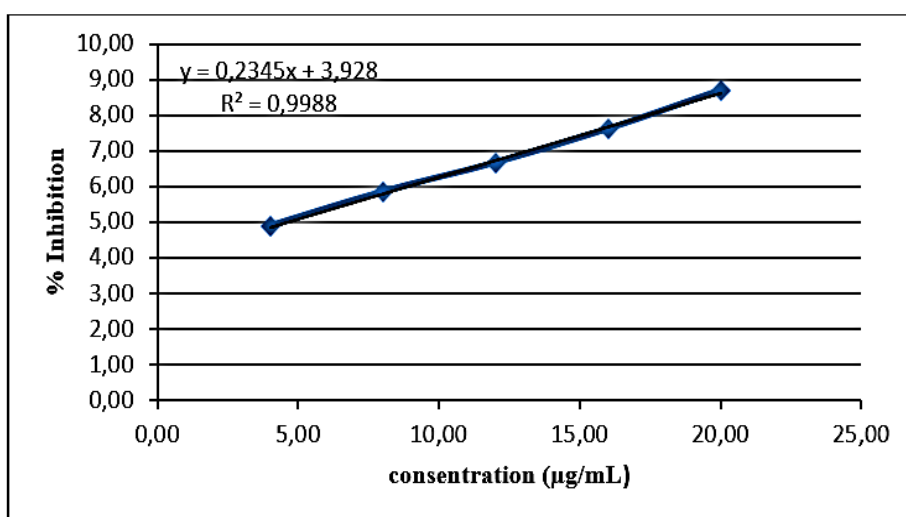


Fig 7. Concentration vs. % Inhibition Curve of 70% Ethanol Extract

The results showed that the ethanol extract of salak pondoh leaves exhibited very weak antioxidant activity, with an IC₅₀ value of 196.4690 µg/mL. The high IC₅₀ value may be attributed to the presence of multiple compounds in the extract that can interact or compete, thereby reducing antioxidant effectiveness. In addition, the percentage of inhibition at all tested concentrations did not reach

50%, indicating that the concentrations used were relatively low. Compounds such as phenols, flavonoids, alkaloids, and tannins are known to contribute to antioxidant activity [12]. These findings are consistent with previous studies reporting low to moderate antioxidant activity in salak pondoh leaf preparations [5].

IV. CONCLUSION

Based on the research conducted on the determination of total flavonoid content and antioxidant activity of the ethanol extract of salak pondoh leaves (*Salacca zalacca* (Gaertn.) Voss), it can be concluded that:

1. The ethanol extract of salak pondoh leaves contains flavonoid compounds as secondary metabolites.
2. The total flavonoid content obtained was 2.66%.
3. The antioxidant activity of the ethanol extract of salak pondoh leaves was

categorized as very weak, with an IC₅₀ value of 196.4690 µg/mL.

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