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RESEARCH PAPER

Determination of total flavonoid content in the ethanol extract of Renggak fruit (*Amomum dealbatum* roxb.) using uv–vis spectrophotometry

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Abstract: The local plant, *Amomum dealbatum* Roxb. (*A. dealbatum*), is native to Lombok Island. This plant is a member of the Zingiberaceae family, which is recognised to have antifungal and antibacterial properties due to the presence of secondary metabolites, particularly flavonoids. Nevertheless, there is still little information and few reports regarding the overall flavonoid content of *A. dealbatum* fruit. As a chemical marker to support an extract's quality control and pharmacological effects, Total Flavonoid Content (TFC) determination is crucial. The purpose of this work was to use UV-Vis spectrophotometry to measure the TFC in the ethanol extract of *A. dealbatum* fruit. Using a 1:10 solvent: simplicia ratio and maceration with 96% ethanol, the extraction procedure was completed. Thin Layer Chromatography (TLC) and the Wilstatter test were then used to identify the extracted material qualitatively. Using a UV-Vis spectrophotometer and a colourimetric approach, TFC was measured. The ethanol extract of *A. dealbatum* fruit contained flavonoids, as indicated by the qualitative analysis. The TFC values were 3.0833 ± 0.1439 mg rutin equivalent (RE)/g extract and 0.4858 ± 0.0014 mg quercetin equivalent (QE)/g extract. These findings suggest that the ethanol extract of *A. dealbatum* contains more flavonoid glycosides than flavonoid aglycones. This finding shows potential for further development. As this study is still limited to TFC at the extract level, further research on TFC at the fraction, sub-fraction, and isolate levels is needed to obtain more specific information about the active compounds contributing to its biological activities. In addition, further pharmacological testing is required to confirm the therapeutic potential of *A. dealbatum* and support its development as a candidate raw material for traditional medicine industries.

Keywords: *Amomum dealbatum* Roxb., Total Flavonoid Content, Quercetin, Rutin, UV-Vis Spectrophotometry

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INTRODUCTION

A. dealbatum is a local plant from Lombok Island, West Nusa Tenggara. It belongs to the Zingiberaceae family [1]. Several ethnomedicine and ethnobotanical studies report that *A. dealbatum* has long been used traditionally for medicinal purposes. In the local community of Lombok, a decoction of the leaves is used to reduce the unpleasant odor of postpartum blood[2]. In India, the rhizome is used to relieve joint pain[3]. In Bangladesh, people use rhizome extract to treat abscesses[4], while in the Philippines, the fruit is used to treat diarrhea and the rhizome is applied for rheumatism and joint inflammation[5]. These traditional uses are believed to be related to the secondary metabolites present in *A. dealbatum*.

Secondary metabolites such as flavonoids, alkaloids, tannins, and triterpenoids have been

found in ethanol leaf extract by phytochemical screening[2]. Terpenoids have been found in the n-hexane extract of the rhizome and in the ethyl acetate and ethanol extracts of the rhizome[6], while polyphenols, flavonoids, quinones, monoterpenoids, sesquiterpenoids, alkaloids, steroids, and saponins[7,8]. These secondary metabolites provide support for the pharmacological activities of *A. dealbatum.*

According to reports, the fruit of *A. dealbatum* exhibits antifungal properties against Pyricularia oryzae and antibacterial properties against Bacillus cereus ATCC 11778 and Xanthomonas oryzae. The fruit's secondary metabolites, especially flavonoids, are believed to be connected to these activities[7,8]. Flavonoids are a significant class of secondary plant metabolites[9] with additional bioactivities, including anti-inflammatory[10], antihypertensive[11], antioxidant[12] and insulin receptor activation[13]. Given these pharmacological effects, investigating flavonoid compounds in *A. dealbatum* is necessary to provide scientific evidence on the active components that support its medicinal potential.

Few studies on the TFC of *A. dealbatum* fruit have been published to date. Therefore, the purpose of this work is to use UV-Vis spectrophotometry to ascertain the TFC in *A. dealbatum* fruit. The data gathered can be used as a specific quality criterion and chemical marker for the standardization of extracts pertaining to pharmacological effects.

MATERIALS AND METHODS

Materials

The fruits of *A. dealbatum* were harvested from Merembu, West Lombok, West Nusa Tenggara. The materials that were used in this study included *A. dealbatum* fruit, 96% ethanol, analytical grade ethanol (Merck®), sodium acetate (Merck®), HCl (Merck®), Mg powder (Merck®), quercetin standard (Sigma Aldrich®), rutin standard (Sigma Aldrich®), 10% $AlCl_3$, silica gel 60 F254 (Merck®), ethyl acetate (Merck®), , glacial acetic acid (Merck®), n-hexane (Merck®), n-butanol (Merck®), distilled water, a rotary evaporator (RV 10 Basic V®), hot plate (Labnet®), TLC chamber, (Labnet®), a UV-Visible spectrophotometer (Specord 200®), TLC chamber (Camag®), and UV light at 254 nm and 366 nm (Camag®).

Ethanolic Extract *A. dealbatum*

Fruits of *A. dealbatum* were identified at the University of Mataram's Faculty of Mathematics and Natural Sciences' Advanced Biological Laboratory. After being cleaned with purified water, the fruits were baked to dryness. After that, the dried fruits were ground into a powder and macerated for a whole day in 96% ethanol; this procedure was repeated twice. A thick extract was obtained by filtering the macerate and evaporating the solvent.

Flavonoid Identification of *A. dealbatum*

TLC and the Wilstatter reaction were used to identify the flavonoids. In the Wilstater test, hydrochloric acid and magnesium powder were added to the extract, and the resulting colour change was noted. Additional investigation was conducted via TLC, employing a silica gel 60 F_{254} plate as the stationary phase with several mobile phases. The identification of quercetin was conducted utilising a solvent mixture of ethyl acetate and n-hexane in a ratio of 7:3. In contrast, Rutin was assessed using a solvent system including glacial acetic acid, n-butanol, and water in a ratio of 1:4:5. Rutin and quercetin were observed under UV light at 254 nm and 366 nm, prior to and after spraying. An $AlCl_3$ spray reagent was utilised to verify the presence of flavonoids.

Preparation of Standard Solutions

Rutin and quercetin standard solutions were made by dissolving each standard in ethanol to produce stock concentrations of 200 µg/mL and 100 µg/mL, respectively. A set of standard working solutions was generated through dilution. Quercetin standards were produced at values between 10 and 60 µg/mL, whilst rutin standards were formulated at concentrations between 20 and 140 µg/mL. Each standard solution (0.5 mL) was combined with 0.10 mL of 10% $AlCl_3$, 0.10 mL of 1 M sodium acetate, and 2.80 mL of distilled water, then incubated at room temperature for 15 minutes for quercetin and 24 minutes for rutin. The absorbance of each standard solution was quantified at the peak wavelengths of 435 nm and 417 nm, respectively.

Preparation of Sample Solution

In a volumetric flask, 20 mg of the ethanolic extract was dissolved in ethanol and diluted to 10 mL to create the sample solution. 0.5 mL of the sample solution was transferred to a test tube and reacted with 0.10 mL of 10% $AlCl_3$, 0.10 mL of 1 M sodium acetate, and 2.80 mL of distilled water. The solution was incubated under identical conditions to the standard solutions. The absorbance was

quantified at the peak wavelength with a UV-Vis spectrophotometer.

Measurment Procedure

A UV-Vis spectrophotometer was used to measure the absorbance of both standard and sample solutions at the maximum wavelengths of 417 nm for rutin and 435 nm for quercetin. All measurements were conducted in duplicates. The TFC in the extract was computed utilizing equations[14]:

$$TFC = \frac{C.V}{g}$$

TFC= Total quantity of flavonoid in mg quercetin and mg rutin equivalent per gram of extract.
C= The quantity of flavonoids derived from the calibration curve
V= The amount of extract utilized
g= Mass of the extract sample

RESULTS AND DISCUSSION

Phytochemical Screening

Following the maceration process, a 12.7% extract yield was obtained. Wilstater test findings for the ethanolic extract of *A. dealbatum* are shown in (Figure 1), while the elution results with quercetin standard are presented in (Figure 2), and with rutin standard in (Figure 4).

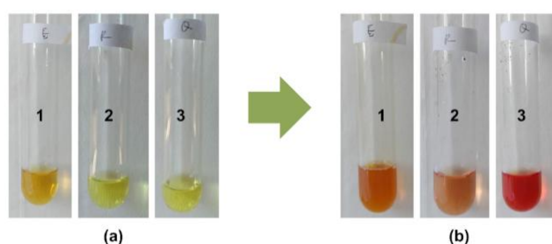

Figure 1. Wilstater Test Results of the Ethanolic Extract of *A. dealbatum*. (a) Initial coloration, (b) Result of the Wilstater assay, (1) Extract sample, (2) Rutin standard, (3) Quercetin standard.

To determine whether flavonoids were present, the Wilstatter test was used. The presence of flavonoids with a γ-benzopyrone structure, such as flavones, flavonols, and isoflavones, as well as the presence of C=O and –OH functional groups at particular locations in the molecular structure, is suggested by a colour shift to orange, red, or pink, which indicates a positive result [15,16].

The results of the Wilstater test shown in (Figure 1) demonstrated an orange coloration in the extract sample and the rutin standard, while a red coloration was observed in the quercetin standard, indicating the presence of flavonoids. The orange to red coloration confirmed flavonoid content[17]. The similar color intensity between the extract and rutin standard suggests that the extract may contain flavonoids belonging to a similar group. To further verify the flavonoid class contained in *A. dealbatum*, additional analysis using TLC was conducted, which can be observed in (Figure 2 and 4)

TLC profile of the ethanolic extract of *A. dealbatum* in comparison with the quercetin standard

In TLC optimization using different solvent systems, phytochemical compounds may exhibit different retention factor (Rf) values. These variations provide important information regarding compound polarity and assist in selecting the appropriate mobile phase for separation and isolation. The distance travelled by the chemical divided by the distance travelled by the solvent is represented by the Rf value[18].

The TLC test results (Figure 2) showed the presence of spots in the extract and the quercetin standard. The ethanol extract produced three spots with different colors. The Rf values obtained were 0.93 for spot 1, 0.87 for spot 2, and 0.68 for spot 3. The quercetin standard showed a single yellow spot with an Rf value of 0.68. The extract and quercetin standard exhibited similar color and Rf values, indicating that *A. dealbatum* fruit may contain flavonoids of the same group. Based on visual comparison with the TLC study conducted by other study spot 1 exhibiting blue fluorescence was assumed to be a flavone (chrysin), and spot 2 exhibiting turquoise fluorescence was assumed to be a flavonol (galangin)[19]. The observed color differences suggest the presence of flavonoids in the ethanolic extract, attributed to the formation of a complex with $AlCl_3$[20], as illustrated in Figure 3.

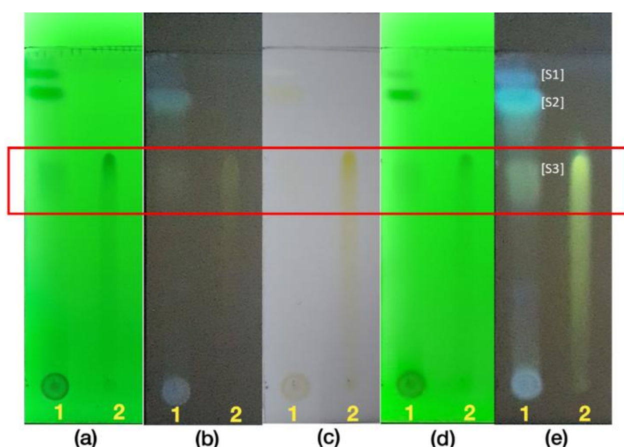

Figure 2. TLC profile of *A. Dealbatum* ethanol extract in comparison to the quercetin standard. (a) under UV 254 nm, (b) under UV 366 nm, (c) under room light (d) observed under UV 254 nm following application of AlCl3, (e) observed under UV 366 nm following application of AlCl3, (1) *A. Dealbatum* ethanol extract, (2) Quercetin standard, (S1) spot 1, (S2) spot 2, and (S3) spot 3.

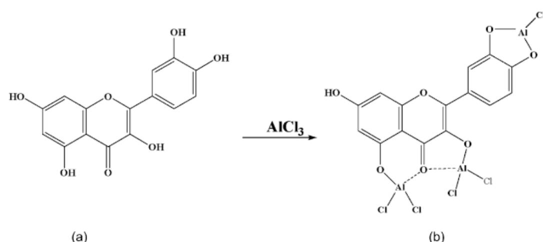

Figure 3. Reaction of the Quercetin–$AlCl_3$ Complex [21]
(a) Quercetin, (b) Quercetin–$AlCl_3$ complex.

TLC profile of the ethanolic extract of *A. dealbatum* in comparison with the rutin standard

The TLC test results (Figure 4) showed the presence of spots in both the extract and the rutin standard. The elution of the ethanolic extract of *A. dealbatum* produced one blue fluorescent spot. The Rf values obtained were 0.81 for the extract spot and 0.56 for the rutin standard spot. The blue fluorescent spot in the extract was presumed to indicate the presence of flavonoid compounds[22]. Meanwhile, the rutin standard showed a yellow fluorescent spot as a result of the reaction between $AlCl_3$ and the flavonoid group forming a complex, as illustrated in (Figure 5).

In (Figure 4), the extract and the rutin standard showed different spot colors and Rf values. This condition may be caused by an incomplete optimization of the mobile phase system. Additional factors that influence spot movement in thin-layer chromatography and affect Rf values include the chemical structure of the separated compounds, the nature of the adsorbent, the level of adsorbent activity, layer thickness, vapor saturation, and the applied sample volume[23]. The flavonoid screening using TLC in this study was limited to confirming the presence or absence of flavonoids. Standard compounds to confirm whether flavonoids from the same group can be separated using the mobile phase system still need to be examined. Additional confirmation using TLC-densitometry, High-Performance Liquid Chromatography (HPLC), or Liquid Chromatography–Mass Spectrometry (LC–MS) is necessary to confirm the presence of quercetin and rutin.

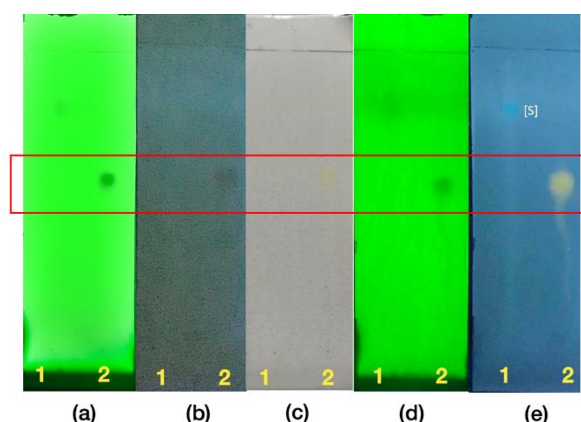

Figure 4. TLC profile of the ethanol extract of *A. dealbatum* in comparison to the rutin standard. (a) under UV 254 nm, (b) under UV 366 nm, (c) under room light (d) observed under UV 254 nm following application of AlCl3, (e) observed under UV 366 nm following application of AlCl3. Spots consist of (1) *A. Dealbatum* ethanol extract, (2) Rutin standard, and (S) spot.

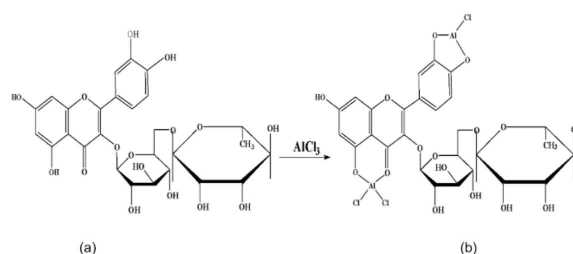

Figure 3. Reaction of the Quercetin–$AlCl_3$ Complex[24]
(a) Rutin, (b) Rutin–$AlCl_3$ complex.

TFC Expressed as Quercetin Equivalent

To quantify the total flavonoid compounds, the total flavonoid content was determined. This study quantified the total flavonoid content as milligrams of quercetin equivalent per gram of extract. The

determination of flavonoid content via spectrophotometry relies on the capacity of flavonoids to form complexes with $AlCl_3$. The regression Equation derived from the standard calibration series is y = 0.0108x + 0.0928, exhibiting an R^2 value of 0.9992 (r = 0.9996). The findings of TFC are displayed in (Table 1).

The extracted flavonoid concentration *A.dealbatum* was 0.4858 ± 0.0014 mg QE/g. The quercetin TFC value in this study is in line with earlier findings when compared to many studies on TFC in the Amomum genus. According to a study, there were 0.39 ± 0.01 mg of QE/g of fruit from *Amomum compactum* in the ethanolic extract[25]. While most flavonoids in plants are typically found in glycoside forms like rutin, quercetin is categorised as an aglycone flavonoid.

Table 1. Total Flavonoid Content Ethanolic Extract *A. dealbatum* fruit expressed quercetin equivalent

Replication	TFC (mg RE/g extract)	x̄ ± SD (mgKE/g extract)
1	0.4875	0.4858 ± 0.0014
2	0.4850	
3	0.4850	

TFC Expressed as Rutin Equivalent

The regression equation derived from the standard calibration curve is y = 0.0045x + 0.1274, with a R^2 value of 0.994 (r = 0.9970). The total flavonoid concentration was quantified as milligrams of rutin equivalent per gram of extract.

The total flavonoid content obtained *A. dealbatum* was 3.0833 ± 0.1439 mg RE/g extract (Table 2). This value is substantially higher than the reported rutin TFC value for other Amomum species, where the ethanolic leaf extract of *Amomum chinense* C. contained 1.75 mg RE/g extract [26]. The rutin TFC value was significantly greater than the quercetin TFC value. This result is associated with the fact that rutin is classified as a flavonoid glycoside[27], and in plants, the majority of flavonoids naturally occur in glycoside forms[28].

In this study, the TFC of *A. dealbatum* fruit has been determined. This TFC information may serve as a scientific basis for further development toward pharmacological activity evaluation, supporting the potential utilisation of *A. dealbatum* fruit as a candidate raw material for traditional medicinal industries.

Table 2. Total Flavonoid Content Ethanolic Extract *A. dealbatum* fruit expressed rutin equivalent

Replication	TFC (mg RE/g extract)	x̄ ± SD (mgKE/g extract)
1	2.9525	3.0833 ± 0.1439
2	3.0600	
3	3.2375	

Several limitations were identified in this study. First, the TFC analysis was limited to the extract level; therefore, further studies are required at the fraction, subfraction, and isolate levels to obtain more specific information regarding active compounds contributing to biological activities. Second, the mobile phase used in TLC did not provide optimal separation for the extract, indicating the need for further optimisation. Third, the TFC determination was conducted solely using UV-Vis spectrophotometry; thus, future research is recommended to apply TLC-densitometry, HPLC, or LC/MS for more precise profiling and quantification.

CONCLUSION

The phytochemical screening demonstrated that flavonoid compounds were present in the ethanolic fruit extract of *A. dealbatum*. Quantitative evaluation showed that the total flavonoid content reached 0.4858 ± 0.0014 mg QE/g extract and 3.0833 ± 0.1439 mg RE/g extract, indicating the existence of both glycoside and aglycone flavonoid forms. These findings provide a scientific basis for the developing of extract quality parameters associated with pharmacological potential.

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