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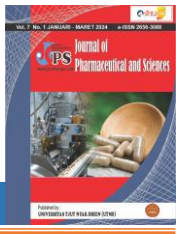

Quantitative analysis of quercetin in tea mistletoe (*Scurrula oortiana* Dans.)

Analisis kuersetin pada ekstrak benalu teh (*Scurrula oortiana* Dans.) secara kuantitatif

Annysa Ellycornia Silvyana [a], Yonathan Tri Atmodjo Reubun [a]*, Lia Warti [a], Lidia Octaviani Simangunsong [a]

[a] *Department of Pharmacy, Medistra Indonesia College of Health Sciences, West Java, Indonesia*
**Corresponding Authors: yonathanreubun94@gmail.com*

Abstract

Indonesia has a variety of plants that are useful as medicine. One is the Tea Mistletoe plant (*Scurrula oortiana* Dans.). This plant contains flavonoid compounds that are effective as antioxidants, especially in the form of quercetin. Quercetin in the tea mistletoe plant can prevent cell damage caused by free radicals, where cells need this antioxidant to avoid the harmful effects of excessive reactive oxygen species (ROS) production and prevent damage to immune cells. This study aims to analyze the levels of quercetin in Tea Mistletoe extract using quantitative analysis methods such as thin-layer chromatography and UV-Vis spectrophotometry. Tea Mistletoe powder was extracted by maceration with 96% ethanol solvent and then concentrated using a rotary evaporator and water bath. The thick extract obtained was then tested for phytochemical screening and specific and non-specific parameters. Analysis of flavonoid quercetin compounds was carried out using a thin layer chromatography method using a mobile phase of a mixture of chloroform, ethyl acetate, and n-butanol with a ratio of 5:4:1 and a stationary phase of Silica Gel GF_{254}. Determination of flavonoid levels was carried out using UV-Vis spectrophotometry at a wavelength of 428 nm. The results of this study indicate that the Mistletoe Tea plant contains flavonoids, alkaloids, steroids, tannins, saponins, phenolics, and glycosides. All test parameters meet the specified requirements. The thin layer chromatography test showed the Rf value of the Mistletoe Tea extract of 0.78. The linearity test showed a correlation value (r) = 0.9988 with a total flavonoid compound content of 372.250 mg QE/g extract, or a percentage of 37.225%.

Keywords: Tea Mistletoe, Total Flavonoid Content, Quercetin, Flavonoid Bioactive, UV-Vis Spectrophotometry

Abstrak

Indonesia memiliki beragam tanaman yang bermanfaat sebagai obat. Salah satunya adalah tanaman Benalu Teh (*Scurrula oortiana* Dans.). Tanaman ini mengandung senyawa flavonoid yang berkhasiat sebagai antioksidan, terutama berupa kuersetin. Kuersetin pada tanaman benalu teh berpotensi dalam mencegah kerusakan sel yang disebabkan karena radikal bebas dimana sel yang membutuhkan antioksidan ini untuk menghindari efek berbahaya dari produksi *reactive oxygen species* (ROS) yang berlebih serta mencegah kerusakan sel kekebalan. Penelitian ini bertujuan untuk menganalisis kadar kuersetin dalam ekstrak Benalu Teh menggunakan metode analisis kuantitatif seperti kromatografi lapis tipis dan spektrofotometri UV-Vis. Serbuk Benalu Teh diekstraksi secara maserasi dengan pelarut etanol 96%, lalu dipekatkan menggunakan rotary evaporator dan waterbath. Ekstrak kental yang diperoleh kemudian diuji untuk skrining fitokimia serta parameter spesifik dan non-spesifik. Analisis senyawa flavonoid kuersetin dilakukan dengan metode kromatografi lapis tipis menggunakan fase gerak campuran kloroform, etil asetat, dan n-butanol dengan perbandingan 5:4:1, serta fase diam Silika Gel GF_{254}. Penetapan kadar flavonoid dilakukan menggunakan spektrofotometri UV-Vis pada panjang gelombang 428 nm. Hasil penelitian ini menunjukkan bahwa tanaman Benalu Teh mengandung senyawa flavonoid, alkaloid, steroid, tanin, saponin, fenolik, dan glikosida. Semua parameter pengujian memenuhi syarat yang ditetapkan. Uji kromatografi lapis tipis menunjukkan nilai Rf

ekstrak Benalu Teh sebesar 0,78. Uji linearitas menunjukkan nilai korelasi (r) = 0,9988 dengan kadar total senyawa flavonoid sebesar 372,250 mg QE/g ekstrak, atau persentase sebesar 37,225%.

Kata Kunci: Benalu Teh, Kadar Flavonoid Total, Kuersetin, Bioaktif flavonoid, Spektrofotometri UV-Vis

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Introduction

Indonesia is a country rich in natural resources. Different plants grow and have good benefits in terms of nutrition and health. This plant can be used as medicine in phytotherapy and is expected to be used in official health services. Mistletoe tea is one type of plant that is interesting to study from the many medicinal plants in Indonesia. Mistletoe is the name of a group of plants that grow in the forest, are sticky, and interfere with other trees. This is because this plant grows on different trees, and is often given an additional name according to its owner, for example mistletoe tea [2].

Mistletoe Tea (*Scurulla oortiana* Dans.) is a parasitic plant on the tea tree. Mistletoe tea is a parasitic plant used as a traditional herbal medicine since ancient times. The community has traditionally used mistletoe tea to maintain health [16]. Traditionally, this plant is used to make mistletoe tea, which is dried and boiled in water. Mistletoe tea leaves and stems contain flavonoids, glycosides, alkaloids, saponins, triterpenoids, and tannins. In addition, other derivative compounds are often found in mistletoe tea, namely quercetin [17].

Quercetin is a group of flavonoids that has been reported to exhibit several biological activities. These activities include antitumor and antiproliferative properties in many human cancer cells and can inhibit glycolysis, macromolecular synthesis, and enzymatic activity. These activities are associated with quercetin's antioxidant properties, which allow it to suppress free radicals and reactive oxygen species such as superoxide anions and hydroxyl radicals [2].

The study from Yulva reported that mistletoe tea leaves dried using the sun drying method have strong antioxidant activity, with an IC_{50} value of 20.11. Mistletoe tea stems dried using the oven drying method have antioxidant activity, with an IC_{50} value of 20.74, and are included in the strongest group [18].

The study aims to determine the quercetin content in tea mistletoe extract using thin-layer chromatography (TLC) and UV-Vis spectrophotometry. TLC is appreciated for its simplicity, affordability, and efficiency in analyzing small sample volumes. UV-Vis spectrophotometry is selected for its rapid processing, ease of use, and effectiveness in handling multiple samples [2].

Experimental Section

Materials and Apparatus

The materials employed in this study include quercetin (Merck, Germany), distilled water, 96% ethanol (Merck, Germany), silica gel 60 GF_{254}, chloroform (Merck, Germany), n-butanol, ethyl acetate (Merck, Germany), tea mistletoe plant (Scurrula oortiana Dans.), aluminum chloride (Merck, Germany), and potassium acetate.

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The equipment utilized comprises glassware (Pyrex), a rotary evaporator (Buchi, India), a water bath (B-one), a UV-Vis spectrophotometer (B-one), an oven (Memmert), a light microscope (Olympus), a UV viewing cabinet (Camag), TLC plates, a chamber, a micropipette (Boeco, Germany), an analytical balance (Biobase, China), and a grinder (KMS, India).

Plant determination

The tea mistletoe, sourced from a sengon tree plantation in West Java, was identified at the Herbarium Bogoriensis, Center for Biological Research of the National Research and Innovation Agency (BRIN) in Cibinong, West Java. This plant identification was performed to ensure the accuracy of the samples used in the research [2].

Extract preparation

The dried tea mistletoe plant was ground using a grinder. The resulting powder was sifted through a 40-mesh sieve for fine consistency [2]. The extraction process involved macerating the tea mistletoe powder with 96% ethanol as the solvent. The macerated extract was then concentrated with a rotary evaporator and a water bath. The final concentrated extract was subjected to a yield calculation [19].

Phytochemical screening

Phytochemical screening is conducted to identify the secondary metabolite compounds present in the tea mistletoe extract. This screening includes tests for flavonoids [20], alkaloids [20], steroids [11], saponins [11], tannins [11], glycosides [7], and phenolics [7].

Organoleptic test

Organoleptic testing was performed to assess the extract's characteristics, including its color, odor, taste, and appearance [6].

Water content test

Weigh 10 grams of the extract in a pre-weighed container. Dry it at 105°C for 5 hours, then measure its weight. Continue drying and weighing at 1-hour intervals until the difference between two consecutive weighings is less than or equal to 0.25% [10].

Drying shrinkage test

Spread the extract evenly in a weighing bottle by shaking until the layer is 5 to 10 mm thick. Weigh 1-2 grams of the extract in a pre-weighed, pre-heated (at 105°C for 30 minutes), and taped-closed weighing bottle. Allow the bottle to cool in a desiccator to room temperature while keeping it closed. Then, please place it in a drying chamber with the lid open and dry at 105°C until a constant weight is reached [10].

Testing the ethanol-soluble essence content

Weigh 5 grams of the extract and dissolve it in 100 mL of 96% ethanol. Allow it to stand for 24 hours in a stoppered flask. Shake the mixture several times during the first 6 hours, then let it rest for 18 hours. Filter the solution through filter paper, then evaporate the filtrate in a pre-weighed evaporating dish until only the residue is left. Finally, heat the residue at 105°C until a constant weight is obtained [10].

Thin layer chromatography test

The identification of flavonoids in the sample's ethanol extract was carried out using thin-layer chromatography (TLC) with a silica gel 60 GF254 plate (2.5 x 8.5 cm) as the stationary phase and a mobile phase consisting of chloroform, ethyl acetate, and n-butanol in a 5:4:1 ratio. Quercetin was used as the standard reference [1].

First, the silica gel GF254 TLC plate was activated in an oven at 115°C for 30 minutes. The plate was then marked with a top line (0.5 cm) and a bottom line (1 cm). Twenty milligrams of the extract were dissolved in 10 mL of 96% ethanol and spotted onto the stationary phase starting from the bottom edge of the TLC plate. The plate was placed in a saturated chamber and eluted until the solvent front reached the marked line. Afterward, the TLC plate was allowed to air-dry [1].

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The elution results were examined under UV light at 254 nm and 366 nm. The TLC plate was then sprayed with a 10% $AlCl_3$ reagent and re-examined under UV light at 366 nm. If yellow or purple-green spots appeared, the sample was considered positive for flavonoids. The Rf (retardation factor) values for each secondary metabolite were calculated using the following formula [1]:

$$Rf = \frac{Distance\ traveled\ by\ the\ substance}{Distance\ traveled\ by\ the\ solvent} \qquad (1)$$

UV-Vis Spectrophotometric test

1) **Preparation of Reagents**
   a) **Preparation of 10% Aluminum Chloride Solution**
   Weigh 1 gram of aluminum chloride ($AlCl_3$) powder and dissolve it in a portion of distilled water until fully dissolved. Transfer the solution to a 10.0 mL volumetric flask and fill it with distilled water up to the mark [3].
   b) **Preparation of 1 M Potassium Acetate Solution**
   Weigh 0.9814 grams of potassium acetate (CH_3COOK) powder and dissolve it in a portion of distilled water until completely dissolved. Transfer the solution to a 10.0 mL volumetric flask and add distilled water to the mark [3].
   c) **Blank Solution**
   Combine 0.4 mL of 10% aluminum chloride ($AlCl_3$) solution, 0.4 mL of 1 M potassium acetate (CH_3COOK) solution, and distilled water to a total volume of 10 mL [3].
2) **Preparation of Quercetin Standard Solution**
   Weigh 10 mg of quercetin and dissolve it in a 100-mL volumetric flask filled with 96% ethanol up to the mark [9].
3) **Preparation of Sample Solution**
   Weigh 5 mg of tea mistletoe extract and dissolve it in 10 mL of 96% ethanol [3].
4) **Preparation of Calibration Curve Series**
   Prepare a calibration curve series from a 100 ppm stock solution by diluting it to 20, 30, 40, 50, and 60 ppm concentrations. Transfer each concentration to a 10 mL volumetric flask with ethanol. Then, transfer 2 mL of each concentration to a 10 mL volumetric flask. Add 0.4 mL of 10% aluminum chloride ($AlCl_3$) solution and 0.4 mL of 1 M potassium acetate (CH_3COOK) solution, and fill to the mark with distilled water. Shake until homogeneous and incubate at room temperature for 30 minutes. Measure the absorbance using a UV-Vis spectrophotometer [13].
5) **Determination of Wavelength**
   Place 2 mL of a 60 ppm quercetin solution into a 10 mL volumetric flask. Add 0.4 mL of 10% aluminum chloride ($AlCl_3$) solution, 0.4 mL of 1 M potassium acetate (CH_3COOK) solution, and fill with distilled water up to 10 mL. Shake until homogeneous and measure the absorbance at wavelengths ranging from 400–480 nm [3].
6) **Determination of Optimal Operating Time**
   Place 2 mL of a 60 ppm quercetin solution into a 10 mL volumetric flask. Add 0.4 mL of 10% aluminum chloride ($AlCl_3$) solution, 0.4 mL of 1 M potassium acetate (CH_3COOK) solution, and fill with distilled water up to 10 mL. Homogenize the mixture and determine the optimal stable time of 60 minutes [13].
7) **Measurement of Flavonoid Content**
   Add 2 mL of the test sample solution to a 10 mL volumetric flask. Add 0.4 mL of 10% aluminum chloride ($AlCl_3$) solution, 0.4 mL of 1 M potassium acetate (CH_3COOK) solution, and fill with distilled water up to 10 mL. Allow the solution to stand for the optimal time and measure the absorbance at the maximum wavelength for quercetin, performing the repetitions [3].

Results and Discussion

Plant identification confirmed that the sample is tea mistletoe (*Scurrula oortiana* Dans.) from the *Loranthaceae* family. The extraction was performed using the maceration method with 96% ethanol, chosen for its simplicity, minimal microbial growth, and effectiveness in dissolving a broad spectrum of secondary metabolites without heating [12]. The yield of the extract is detailed in Table 1.

Table 1. Extract yield of the extract result

Sample (g)	Extraction Result (g)	Percentage (%)
1028	382.8	37.237

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The concentrated extract of tea mistletoe (*Scurrula oortiana* Dans.) weighed 382.8 grams, with a yield of 37.237%, which exceeds the literature minimum of 10%. A higher yield indicates a greater concentration of active compounds [12]. Phytochemical screening was performed to identify secondary metabolites, including flavonoids, alkaloids, tannins, saponins, steroids, phenolics, and glycosides. The results are shown in Table 2.

Table 2. Phytochemical screening result of mistletoe tea extract

Sample	Testing	Result
Mistletoe tea (*Scurrula oortiana* Dans.)	Flavonoids	+
	Alkaloids	+
	Tannin	+
	Saponin	+
	Steroids	+
	Phenolik	+
	Glycosides	+

Information:
(+) : indicates the presence of secondary metabolite compounds
(-) : indicates the absence of secondary metabolite compounds

In this study, phytochemical screening of tea mistletoe (*Scurrula oortiana* Dans.) extract was performed, revealing the presence of several secondary metabolites:

1. Flavonoids: Positive results were obtained using magnesium powder and hydrochloric acid. The solution turned orange, indicating the presence of flavonols and flavones [11].
2. Alkaloids: Positive results were indicated by a color change to bright red, red, and orange with Dragendorff's reagent after acidifying the sample with hydrochloric acid [11].
3. Saponins: Positive results were shown by foam formation that lasted 10 minutes and did not dissipate with 2N hydrochloric acid [15].
4. Tannins: Positive results were indicated by a greenish-black or dark blue color after adding ferric chloride, suggesting the presence of phenolic compounds [11].
5. Steroids: Positive results were indicated by a green-blue color after reaction with glacial acetic acid and sulfuric acid [15].
6. Glycosides: Positive results were shown by a reddish-brown ring formation [7].
7. Phenolics: Positive results were indicated by the solution's color change to black [7].

Parameter testing ensures natural drug products' uniformity, consistency, stability, and safety. This includes both specific and non-specific tests. The organoleptic properties, determined through sensory evaluation, provide initial recognition and are documented in Table 3.

Table 3. Specific parameter test result

Sample	Organoleptic test	
	Information	Result
Mistletoe tea extract (*Scurrula oortiana* Dans.)	Color	blackish green
	Smell	Typical taste of mistletoe tea
	Flavor	Bitter
	Texture	Thick

The non-specific parameter tests performed include drying loss, moisture content, and ethanol-soluble extractive content tests. The results for these non-specific parameters in the ethanol extract of tea mistletoe are presented in Table 4.

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Table 4. Nonspecific parameter test result

No.	Test name	Result (%)	Criteria (%)	Literature
1.	Drying loss tests	4.32	≤ 10	FHI
2.	Moisture content test	4.94	≤ 12.1	FHI
3.	Ethanol-soluble extractive test	59.20	≥ 4.8	FHI

In this study, several non-specific parameters were measured for the tea mistletoe extract:

1. Drying Loss: This parameter assesses the percentage of compound loss during drying at 105°C for 30 minutes. The tea mistletoe extract exhibited a drying loss of 4.32%, below the 10% threshold specified in the literature [8].
2. Moisture Content: This measurement is crucial for preventing microbial growth. The moisture content of the tea mistletoe extract, determined by the gravimetric method through heating at 105°C for 5 hours, was 4.94%, well below the 12.1% limit [8].
3. Ethanol-Soluble Extractive Content: This parameter indicates the quantity of active compounds that dissolve in ethanol. The tea mistletoe extract had an ethanol-soluble extractive content of 59.20%, surpassing the minimum requirement of 4.8% [8].

All measured parameters met or exceeded the specified standards.

Thin-layer chromatography (TLC) is used to identify the secondary metabolite compounds in the ethanol extract of tea mistletoe (*Scurrula oortiana* Dans.) by analyzing the spots developed on the TLC plate. These spots are observed for color and measured to calculate the Rf values. The Rf values for quercetin and the ethanol extract of tea mistletoe are presented in Table 5.

Table 5. The Rf result

Chloroform: Ethyl acetate: n-Butanol (5:4:1)				
Substance	**Analite Migration Distance (cm)**	**Eluent Migration Distance (cm)**	**Rf**	**Spot Color under UV 366 nm**
Tea mistletoe extract (Scurrula oortiana Dans.)	5.5	7	0.78	Yellow fluorescence
Quercetin standard	5.5	7	0.78	Yellow fluorescence

This study analyzed secondary metabolite compounds in tea mistletoe (Scurrula oortiana Dans.) using Thin Layer Chromatography (TLC) with silica gel as the stationary phase. The optimal mobile phase was identified as a mixture of chloroform, ethyl acetate, and n-butanol in a 5:4:1 ratio [1]. The TLC plates were activated at 115°C for 30 minutes, and the chamber was saturated with the mobile phase to ensure adequate separation [5].

Under UV light at 366 nm, the tea mistletoe extract revealed five fluorescent spots (blue, red, and yellow-green) after spraying with 10% $AlCl_3$, indicating the presence of flavonoids [14]. Quercetin used as a standard, exhibited a yellow fluorescence with $AlCl_3$, confirming the presence of flavonoid compounds [9]. Both the tea mistletoe extract and quercetin displayed an Rf value of 0.78, suggesting the presence of quercetin in the extract. The tea mistletoe extract's maximum wavelength was 428 nm, with the highest absorbance recorded at 0.463. The results are shown in Figure 1.

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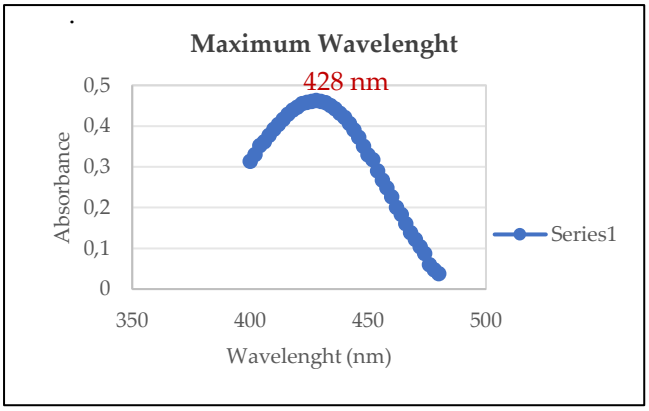

Figure 1. The result of the maximum wavelength

Quercetin was utilized as the standard solution, and it was prepared in five different concentrations. Each concentration was measured for absorbance in triplicate. The results of the standard curve are displayed in Figure 2.

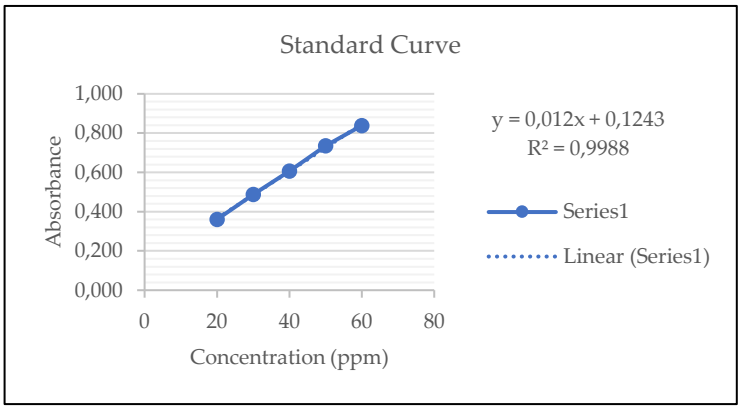

Figure 2. The result of the standard curve

The standard curve was created using quercetin solutions at concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, adhering to Lambert-Beer law to ensure absorbance within the range of 0.2–0.8 and analytical errors between 0.5–1%. The standard curve, depicted in Figure 2, shows a direct proportionality between quercetin concentration and absorbance. The regression equation obtained is y = 0.012x + 0.1243, with a correlation coefficient (r) of 0.9988, indicating a strong correlation between absorbance and concentration [4].

To determine the optimal operating time, the measurement was performed with a 60 ppm concentration at a wavelength of 428 nm for 60 minutes [4]. The results of this operating time determination are shown in Figure 3.

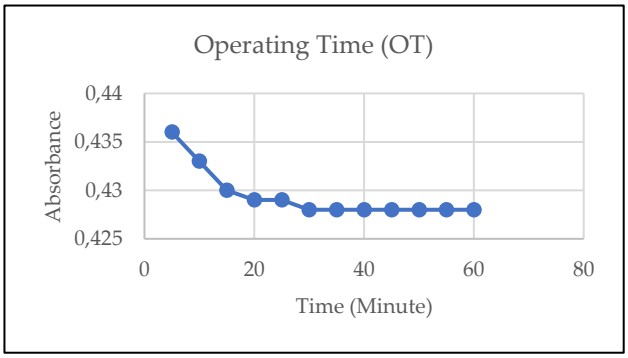

Figure 3. The result of the operating time

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The operating time (OT) was established as 30 minutes, the duration needed for quercetin to form a stable complex with aluminum chloride ($AlCl_3$). The absorbance measurements were taken at the maximum wavelength of 428.0 nm, where the absorbance was 0.463. This wavelength was chosen because it provides the most significant change in absorbance with concentration, ensuring minimal error and compliance with Lambert-Beer law, which supports a linear standard curve [4].

The tea mistletoe (*Scurrula oortiana* Dans.) extract was measured in triplicate, yielding an average absorbance of 0.571. The linear regression equation obtained was y = 0.012x + 0.1243, with a correlation coefficient (r) of 0.9988, indicating a strong correlation [4]. The results for the total flavonoid content of the tea mistletoe extract are detailed in Table 6.

Table 6. The result of the total flavonoid content of the tea mistletoe extract

Weight (g)	Volume (V)	Dilution factor (Fp)	Absorbance	Absorbance average	Total Flavonoid content (mg QE/g ekstrak)	Kadar Total Flavonoid (%)
0,005	0,01	5	0.571 0.571 0.571	0.571	372.250	37.225

In this study, quercetin content was analyzed using UV-Vis spectrophotometry. Quercetin, a flavonoid belonging to the flavonol group, shows strong absorption in both the ultraviolet and visible light regions due to its conjugated aromatic system. The maximum wavelength measurements were used to determine the wavelength at which quercetin exhibits peak absorption and stable absorbance [12].

The total flavonoid content in the tea mistletoe (*Scurrula oortiana* Dans.) extract was determined using colorimetry. The extract was reacted with aluminum chloride ($AlCl_3$) and potassium acetate (CH_3COOK) and then kept in the dark to prevent degradation of the light-sensitive complex. $AlCl_3$ forms a colored complex with flavonoids, shifting absorption to the visible region and enhancing the yellow color. Potassium acetate stabilizes this complex and maintains its visible wavelength [4]. The total flavonoid content in the tea mistletoe extract, measured in triplicate, was found to be 37.225% expressed as quercetin equivalents (QE).

Conclusions

Based on the research results, qualitative testing using the Thin Layer Chromatography (TLC) method showed an Rf value of 0.78. The quercetin content determined by the total flavonoid content formula was calculated at 372.250 mg QE/g extract or equivalent to a percentage of 37.225%. Based on these results, tea mistletoe extract has the potential as an antioxidant because the active compound quercetin can ward off free radicals. The need for development into preparations with active ingredients must be developed into pharmaceutical preparations such as tablets.

Conflict of Interest

The authors declare that this research is free from any conflicts of interest. The entire research process and article writing were conducted independently, without external intervention, and no personal, financial, or professional interests influenced the objectivity or integrity of the research.

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Supplementary Materials

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