JPPIPA 11(6) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Analysis of Absorbance Value of Flavonoid Level in Brotowali Stem Extract Using UV-VIS Spectrophotometer

Yunike Kurnia Unda'[1*], Elisabet Sa Wulo[1], Suparno[1]

[1] Physics Education, Universitas Negeri Yogyakarta, Yogyakarta, Indonesia.

Received: December 16, 2024
Revised: May 10, 2025
Accepted: June 25, 2025
Published: June 30, 2025

Corresponding Author:
Yunike Kurnia Unda'
yunikekurnia.2024@student.uny.ac.id

DOI: 10.29303/jppipa.v11i6.10066

Abstract: Flavonoids are polyphenolic secondary metabolites with various biological activities, including antioxidant and anti-inflammatory effects. This study aimed to determine the total flavonoid content in *Tinospora crispa* (brotowali) stem extract using the $AlCl_3$ colorimetric method and UV-Vis spectrophotometry. The brotowali stems were extracted using a decoction method with distilled water as the solvent. The formation of a yellow complex between flavonoids and $AlCl_3$ was measured at a wavelength of 435 nm using a UV-Vis spectrophotometer. A standard calibration curve was prepared using quercetin as the reference compound. The absorbance values of the sample were compared against the quercetin standard curve to determine the flavonoid concentration, expressed as mg quercetin equivalent (QE) per 100 g of extract. The total flavonoid content in the brotowali extract was found to be approximately 0.40%, indicating a moderate level of flavonoids relative to similar medicinal plants. These results suggest that *Tinospora crispa* stems may serve as a potential source of natural flavonoids with therapeutic applications.

Keywords: Brotowali extract; Flavonoid; UV-Vis sprectophotometer

Introduction

Flavonoids are secondary metabolites from the polyphenol group that are widely found in nature, especially in plants, and are known to possess various biological activities such as antioxidant, anti-inflammatory, and antibacterial effects (Silalahi et al., 2016; Widiastuti et al., 2019). These activities play a key role in neutralizing free radicals that cause cellular damage and contribute to the prevention of degenerative diseases. Due to their bioactive properties, flavonoids are widely used in both traditional medicine and modern pharmaceuticals (Wang et al., 2016). One plant with a high flavonoid content and significant therapeutic potential is Tinospora crispa or brotowali, which has been traditionally used across several Asian countries, including Indonesia (Hasanah et al., 2022).

The stem of Tinospora crispa contains various bioactive compounds, including flavonoids, which are believed to exhibit antioxidant and anti-inflammatory activities (Neldawati et al., 2013; Purnamasari et al., 2022). Scientifically determining the flavonoid content in brotowali stem extract is essential to evaluate its therapeutic potential. One of the common analytical methods used for this purpose is UV-Visible (UV-Vis) spectrophotometry. This method is widely applied due to its simplicity, relatively low cost, and adequate sensitivity for detecting compounds that absorb in the UV-visible spectrum (Sharma et al., 2025). Although alternative techniques such as High-Performance Liquid Chromatography (HPLC) are available, UV-Vis spectrophotometry offers a practical and efficient option for early-stage or laboratory-scale studies (De Araújo et al., 2022).

The technique is based on the Lambert-Beer law, which states that absorbance is directly proportional to the concentration of a substance in solution. In this context, the formation of a yellow complex between flavonoids and aluminum chloride ($AlCl_3$) exhibits a maximum absorbance at a specific wavelength,

How to Cite:
Unda, Y. K., Wulo, E. S., & Suparno. (2025). Analysis of Absorbance Value of Flavonoid Level in Brotowali Stem Extract Using UV-VIS Spectrophotometer. *Jurnal Penelitian Pendidikan IPA*, *11*(6), 284–289. https://doi.org/10.29303/jppipa.v11i6.10066

generally around 435 nm (Bhandari et al., 2015). The present study applies the $AlCl_3$ colorimetric method combined with UV-Vis spectrophotometry, using quercetin as a reference standard, to quantify the total flavonoid content in Tinospora crispa stem extract. Nabi et al. (2017) reported similar total flavonoid content analysis using UV-Vis spectrophotometry in Zingiberaceae species.

Although studies on flavonoid determination are widely reported, the novelty of this research lies in the application of a decoction extraction method using distilled water, a traditional approach that is underrepresented in quantitative flavonoid research. This makes the research important, as it provides a scientific basis for validating conventional herbal preparation techniques. Therefore, this study aims to determine the total flavonoid content of brotowali stem extract using UV-Vis spectrophotometry as a rapid, accessible, and reliable analytical method. This aligns with Harjono et al. (2022), who emphasized the relevance of contextual experiments like flavonoid analysis in education.

Method

This study aimed to determine the total flavonoid content in Tinospora crispa (brotowali) stem extract using the aluminum chloride ($AlCl_3$) colorimetric method combined with UV-Vis spectrophotometry. The $AlCl_3$ method was selected due to its specificity for flavonoids with hydroxyl groups, which form a yellow complex with maximum absorbance at 435 nm (Chang et al., 2004; Harborne, 1989). The UV-Vis spectrophotometer used was Go Direct SpectroVis Plus (Vernier). This method was chosen because of its simplicity, cost-effectiveness, and adequate sensitivity compared to more advanced techniques such as HPLC (Mansour et al., 2025; Pawar, 2024; Rani et al., 2024).

The principle of UV-Vis spectrophotometry is based on measuring the amount of light absorbed by a solution at a specific wavelength. According to the Lambert-Beer law, absorbance (A) is directly proportional to the concentration of the absorbing compound in the solution, where $A = \varepsilon \times c \times l$. In this equation, A is absorbance, ε is the molar absorptivity coefficient, c is the concentration of the solution in mol/L, and l is the path length of the cuvette, typically 1 cm. Cong-Hau et al. (2021) demonstrated how aqueous extraction and brewing conditions significantly affect flavonoid solubility.

The equipment used in this study included a UV-Vis spectrophotometer, analytical balance with ±0.01 mg precision, 50 mL beaker glass, measuring pipettes (1 mL and 5 mL), a centrifuge, and a water bath or incubator. The materials used were 96% ethanol (pro analysis grade), 10% aluminum chloride solution prepared by dissolving 1 gram of $AlCl_3$ in 10 mL distilled water, 1 M potassium acetate prepared by dissolving 98.15 grams of potassium acetate in 1000 mL ethanol, distilled water, quercetin as the standard flavonoid compound, and Tinospora crispa stem extract. Sostaric et al. (2024) validated the effectiveness of $AlCl_3$ as a derivatizing reagent for UV-Vis quantification of flavonoids.

To prepare the standard curve, 25 mg of quercetin was weighed and dissolved in 10 mL of ethanol to make a 1000 ppm stock solution. Serial dilutions were then performed to obtain standard solutions with concentrations of 0, 6, 8, 10, 12, and 14 ppm. Each standard solution was mixed with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, 1.5 mL of ethanol, and 2.8 mL of distilled water. The mixtures were homogenized and incubated at room temperature for 30 minutes. Absorbance was measured at 435 nm using the UV-Vis spectrophotometer, and the absorbance values were plotted against the concentrations to generate the standard calibration curve. Measurement at 435 nm was chosen to match the maximum absorbance of the flavonoid-$AlCl_3$ complex and to ensure consistency between standard and sample analysis.

For sample preparation, 100 grams of Tinospora crispa stems were decocted in 1000 mL of distilled water until the volume was reduced to approximately 500 mL. The extract was centrifuged at 3000 rpm for 10 minutes to separate solids from the supernatant. A 5 mL portion of the supernatant was then dried, and the residue was weighed to determine the total solids content. This step was intended to estimate the dry extract concentration and to calculate flavonoid content relative to dry weight. Bansal et al. (2015) confirmed the accuracy of quercetin calibration curves for measuring total flavonoid content.

Flavonoid analysis was performed by taking 0.5 mL of the supernatant and mixing it with the same reagents used in the standard preparation: 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, 1.5 mL of ethanol, and 2.8 mL of distilled water. The solution was homogenized and incubated for 30 minutes at room temperature. Absorbance was then measured at 435 nm using the UV-Vis spectrophotometer. Sharma et al. (2015) also used UV- Vis spectrophotometry effectively in evaluating flavonoids in herbal extracts.

The flavonoid concentration in the samples was determined by comparing the absorbance values to the standard curve. The final results were expressed as milligrams of quercetin equivalents (QE) per 100 grams of extract. Hasanah et al. (2021) highlighted Tinospora crispa as a traditional remedy containing bioactive flavonoid compounds.

Result and Discussion

The extraction of brotowali stems (Tinospora crispa) was carried out using the decoction method, which involves boiling 100 grams of plant stems in 1000 mL of distilled water until the volume is reduced to approximately 500 mL. The resulting extract is cloudy yellow in color, as shown in Figure 1, indicating the presence of water-soluble phytochemical compounds, including flavonoids. Before boiling, the brotowali stems are cut into pieces approximately 2– 3 cm in length and 1–2 cm in diameter (Figure 2) to increase the surface area and maximize extraction efficiency. Purnamasari et al. (2022) used similar spectrophotometric analysis on other Indonesian herbal species with comparable results.

Figure 1. Brotowali extract

The brotowali plants used were obtained from Depok, Sleman Regency, Special Region of Yogyakarta. Although this study did not compare flavonoid levels between geographical locations, it is important to note that environmental factors such as soil, rainfall, temperature, and light intensity can affect the content of secondary metabolites such as flavonoids. Therefore, information on the location of sample collection is provided as background information to support the repeatability of the study. Afriana et al. (2016) demonstrated the benefit of integrating chemical experiments to strengthen conceptual understanding.

Figure 2. Small pieces of Brotowali stems

Widyastuti (2010) found a positive correlation between total flavonoid content and antioxidant capacity in several local plants.

The determination of total flavonoid content was performed using the aluminum chloride ($AlCl_3$) colorimetric method combined with UV-Vis spectrophotometry. This method was chosen because it is specific to flavonoids that have aromatic hydroxyl groups. The reaction between flavonoids and $AlCl_3$ forms a yellow complex that shows maximum absorption at a wavelength of 435 nm. The principle of operation of the UV-Vis spectrophotometer is based on measuring the intensity of light before (I_0) and after (I) passing through the sample. Transmittance is calculated using the formula $T = I/I_0$, and absorbance is obtained through $A = -\log_{10}(T)$.

Flavonoid content was measured by comparing the absorbance value of the sample to a quercetin standard solution. The total flavonoid content in two brotowali extract samples is shown in Table 1.

Table 1. Total Flavonoid Content of Brotowali Stem

Sample	Absorbance	Concentration (mg/L)	Weight of extract (g)	Vad (L)	FP	mg QE	g QE/g	mg QE/ 100 g	% Flavonoid
Brotowali	0.206	4.066	0.005	0.005	1	0.020	0.00407	4.066	0.407
Extract	0.205	4.043	0.005	0.005	1	0.020	0.00404	4.043	0.404
	0.205	4.043	0.005	0.005	1	0.020	0.00404	4.043	0.404

The absorbance values for the two samples were 0.206 and 0.205. Based on the quercetin standard curve, the flavonoid concentrations obtained were 4.066 mg/L and 4.043 mg/L. The calculations were performed using the linear regression equation from the calibration:

$x = (A - 0.034) / 0.0423$

Where A is the absorbance of the sample. The concentration results (in mg/L) were then multiplied by the volume of the solution (0.005 L), divided by the dry weight of the extract (0.005 g), and multiplied by 100 to obtain the final unit of mg QE/100 g of extract.

For comparison, standard quercetin solutions were prepared at concentrations of 0, 6, 8, 10, 12, and 14 ppm. The absorbance of each concentration was measured after reaction with $AlCl_3$ and potassium acetate. The values are presented in Table 2.

Table 2. Absorbance Values of Quercetin Standard Solutions

Concentration (ppm)	Absorbanc
0	0.0
6	0.27
8	0.37
10	0.44
12	0.55
14	0.62

From the data, a calibration curve for quercetin was created (Figure 3), showing a linear relationship between concentration and absorbance. The regression equation obtained is:

$y = 0.0423x + 0.034$ with $R^2 = 0.9974$

The R^2 value is close to 1, indicating that the data fits the regression line very well. However, the intercept on the Y-axis of 0.034 (not 0) requires attention. This suggests the possibility of initial absorbance from the blank or the influence of other compounds in the solvent that absorb at the same wavelength. Nevertheless, this value is still within the acceptable range for spectrophotometric measurements.

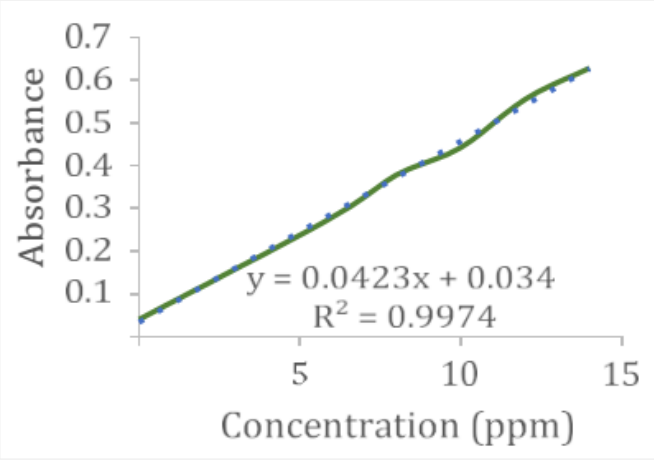

Figure 3. Standard curve graph of quercetin as a comparison standard solution

The results of the study indicate that brotowali extract has relatively stable and consistent flavonoid levels across samples. The range of levels from 404.255 to 406.619 mg QE/100 g of extract shows that the extraction and analysis methods used produce reproducible data. These results also support previous literature stating that Tinospora crispa contains active flavonoid compounds with potential as natural antioxidants. Additionally, the UV-Vis spectrophotometry method has proven to be effective, sensitive, and economical, making it highly suitable for phytochemical analysis in educational laboratories or advanced research settings.

Conclusion

Based on the results obtained, it can be concluded that the brotowali (Tinospora crispa) stem extract contains total flavonoid levels ranging from 0.404% to 0.407%, equivalent to 4.043 mg/L to 4.066 mg/L of quercetin equivalent (QE), as measured using the $AlCl_3$ colorimetric method and UV-Vis spectrophotometry at 435 nm. While this indicates relatively consistent measurement values, the levels observed can be categorized as moderate when compared to other herbal plants reported in previous studies. However, this consistency primarily reflects the precision of the measurement method rather than confirming the extract's stability across different batches or storage conditions. This study also highlights the applicability of the $AlCl_3$ colorimetric method as a practical technique for estimating total flavonoid content. Nevertheless, potential limitations include the inability to distinguish individual flavonoid compounds and possible interference from other constituents in the extract. Future research is recommended to identify the specific flavonoids present in Tinospora crispa, explore their biological activity, and investigate factors influencing flavonoid stability under various storage or processing conditions. Such studies would contribute to a more comprehensive evaluation of brotowali's potential in the development of herbal or functional food products.

Acknowledgments
I would like to thank all parties involved in this research, in particular I would like to thank Suparno M.App.Sc., Ph.D. as my supervisor in the Physics Education study program at Yogyakarta State University who has provided opportunities and encouragement to his students to have a big heart in conducting research.

Author Contributions
Conceptualization, formal analysis by Yunike Kurnia Unda' (Y.K.U), Elisabet Sa Wulo (E.S.W) and Suparno (S); Yunike Kurnia Unda' (Y.K.U), writing- early drafts, results and discussion; Elisabet Sa Wulo (E.S.W), methodology, supervision and review; Suparno (S), supervision, conclusions.

Funding
This research received no external funding and funded by personal funding.

Conflicts of Interest

The authors declare no conflict of interest in the decision to publish the results of this study.

References

Afriana, J., Permanasari, A., & Fitriani, A. (2016). Project based learning integrated to STEM to enhance elementary school's students' scientific literacy. *Jurnal Pendidikan IPA Indonesia*, *5*(2), 261–267. Retrieved from https://journal.unnes.ac.id/nju/jpii/article/view/5493

Bansal, V., Sharma, A., Ghanshyam, C., & Singla, M. L. (2015). Rapid HPLC Method for Determination of Vitamin C, Phenolic Acids, Hydroxycinnamic Acid, and Flavonoids in Seasonal Samples of Emblica officinalis Juice. *Journal of Liquid Chromatography & Related Technologies*, *38*(5), 619–624. https://doi.org/10.1080/10826076.2014.936608

Bhandari, S., & Kwak, J.-H. (2015). Chemical Composition and Antioxidant Activity in Different Tissues of Brassica Vegetables. *Molecules*, *20*(1), 1228–1243. https://doi.org/10.3390/molecules20011228

Chang, C.-N., Ma, Y.-S., Fang, G.-C., Chao, A. C., Tsai, M.-C., & Sung, H.-F. (2004). Decolorizing of lignin wastewater using the photochemical UV/TiO2 process. *Chemosphere*, *56*(10), 1011–1017. https://doi.org/10.1016/j.chemosphere.2004.04.021

Cong-Hau, N., Anh-Dao, L. T., Nhon-Duc, L., & Thanh-Nho, N. (2021). Spectrophotometric determination of total flavonoid contents in tea products and their liquors under various brewing conditions. *Malaysian Journal of Analytical Sciences*, *25*(5), 740–750. Retrieved from https://mjas.analis.com.my/mjas/v25_n5/pdf/Nguyen_25_5_4.pdf

De Araújo, E. P., Maschio, L. J., Vieira, R., Gouvêa, L. H., & Ferroni Pereira, L. G. (2022). Laboratory scale method for preparation of mixture modeled composite fuels for hybrid propulsion. *Journal of Energetic Materials*, *40*(4), 486–503. https://doi.org/10.1080/07370652.2021.1898491

Harborne, J. B. (1989). *Phytochemical methods: A guide to modern techniques of plant analysis*. Springer.

Harjono, A., Andani, T. G., Gunada, I. W., & Susilawati, S. (2022). Implementation of Blended-Flipped Classroom Model Assisted by Video to Improve Students' Creative Thinking Skills. *Jurnal Penelitian Pendidikan IPA*, *8*(6), 3180–3186. https://doi.org/10.29303/jppipa.v8i6.2255

Hasanah, A., Khoerunnisa, A., Aisyah, S., Aeni, F. N., & Yuniarsih, N. (2022). Review artikel: Kajian Tanaman Herbal yang Digunakan untuk Penyakit Saraf melalui Sistem Penghantaran Obat Sistem Saraf Pusat. *Jurnal Pendidikan Dan Konseling*, *4*(6), 12441–12447. Retrieved from https://journal.universitaspahlawan.ac.id/index.php/jpdk/article/view/10497/8027

Mansour, F. R., Abdallah, I. A., Bedair, A., & Hamed, M. (2025). Analytical Methods for the Determination of Quercetin and Quercetin Glycosides in Pharmaceuticals and Biological Samples. *Critical Reviews in Analytical Chemistry*, *55*(1), 187–212. https://doi.org/10.1080/10408347.2023.2269421

Nabi, N. G., & Shrivastava, M. (2017). Phytochemical Screening and Antioxidant Activity of Ethanol Extract of Psoralea corylifolia seeds. *Pharmaceutical and Biosciences Journal*, *8*(2), 01–07. https://doi.org/10.20510/ukjpb/5/i2/147015

Neldawati, R., & Gusnedi. (2013). Absorbance value analysis to determine flavonoid levels of medicinal plant leaves. *Pillar of Physics*, *2*, 76–83. https://doi.org/10.24036/756171074

Pawar, A. (2024). Recent Innovations in High-Performance Liquid Chromatography (HPLC): Method Development and Validation Strategies. *Ournal of Drug Delivery and Biotherapeutics*, *1*(1), 55–61. https://doi.org/10.61920/jddb.v1i01.140

Purnamasari, A., Zelviani, S., Sahara, S., & Fuadi, N. (2022). Absorbance analysis of flavonoid content in herbal plants using UV-Vis spectrophotometer. *Teknosains*, *16*(1), 57–64. https://doi.org/10.24252/teknosains.v16i1.241

Rani, P., Nanda, B. P., Paul, P., Chawla, R., & Bhatia, R. (2024). Exploring advanced strategies in SPME-HPLC-DAD: Enhancing analytical precision and diverse applications in modern era. *Journal of Liquid Chromatography & Related Technologies*, *47*(6–10), 181–200. https://doi.org/10.1080/10826076.2024.2349146

Sharma, R., & Kumar, R. (2025). Chemometric analysis of UV–visible spectral data for the differentiation of Dalbergia latifolia and Dalbergia sissoo woods. *Chemometrics and Intelligent Laboratory Systems*, *264*, 105448. https://doi.org/10.1016/j.chemolab.2025.105448

Silalahi, J., Situmorang, P., Patilaya, P., & Silalahi, Y. C. E. (2016). Antibacterial activity of chitosan and hydrolyzed coconut oil and their combination against bacillus cereus and escherichia coli. *Asian Journal of Pharmaceutical and Clinical Research*, *9*(5), 69–73. https://doi.org/10.22159/ajpcr.2016.v9i5.11768

Wang, J., & Chu, L. (2016). Irradiation treatment of pharmaceutical and personal care products (PPCPs) in water and wastewater: An overview.

Radiation Physics and Chemistry, *125*, 56–64. https://doi.org/10.1016/j.radphyschem.2016.03.012

Widiastuti, D., Isya, F. K., & Setiyani, E. (2019). Efek Antibakteri Sodium Hypochlorite terhadap Staphylococcus aureus Antibacterial Effect of Sodium Hypochlorite to Staphylococcus aureus. *Jurnal Ilmiah Kesehatan Masyarakat*, *11*(4), 302–307. https://doi.org/10.52022/jikm.v11i4.34

Widyastuti, N. (2010). *Pengukuran aktivitas antioksidan dengan metode CUPRAC, DPPH, dan FRAP serta korelasinya dengan fenol dan flavonoid pada enam tanaman*. Retrieved from https://repository.ipb.ac.id/handle/123456789/26745