

Original Article

e-ISSN: 2581-0545 - <https://journal.itera.ac.id/index.php/jsat/>

Received 12th May 2025
Accepted 19th May 2025
Published 19th May 2025

Open Access

DOI: 10.35472/jsat.v9i1.2190

Quantitative Profiling of Total Flavonoid Content in Cassava Leaf (*Manihot esculenta* Crantz) Varieties and Isolation of Flavonoid Compounds from a High-Flavonoid Variety

Syaikhul Aziz^a, Vinta Marito Siringoringo^a, Isna Mulyani^a, Ngatinem^b^a Program Studi Farmasi, Fakultas Sains, Institut Teknologi Sumatera, Lampung Selatan, Lampung, Indonesia.^b Pusat Riset Bahan Baku Obat dan Obat Tradisional, Badan Riset dan Inovasi Nasional, Lampung Tengah, Lampung, Indonesia.* Corresponding E-mail: syaikhul.aziz@fa.itera.ac.id

Abstract: *Cassava* (*Manihot esculenta* Crantz) is widely utilized across Indonesia as both a staple food and a traditional medicinal resource, and its leaves are known to contain bioactive secondary metabolites such as flavonoids. This study aimed to quantify the total flavonoid content in several cassava leaf varieties and to isolate a flavonoid compound from the variety with the highest flavonoid content. Total flavonoid content was determined using UV-Visible spectrophotometry. The isolation of flavonoid compounds was carried out using vacuum liquid chromatography (VLC) and column chromatography (CC), with thin-layer chromatography (TLC) employed to monitor the separation results. The total flavonoid content of the Gajah, UJ-3 (Thailand), UJ-5 (Katesart), Cimanggu and Kaspro varieties were found to be 1.029 ± 0.003 mgQE/g, 0.746 ± 0.006 mgQE/g, 0.970 ± 0.009 mgQE/g, 0.936 ± 0.004 mgQE/g and 1.220 ± 0.003 mgQE/g, respectively. The Kaspro variety exhibited the highest flavonoid content. Isolation and characterization of the flavonoid from Kaspro leaves yielded a yellowish powder with absorption maxima at 269 nm (Band II) and 334 nm (Band I). Further analysis using shift reagents identified the isolated compound as apigenin, a member of the flavone group.

Keywords: *Manihot esculenta*, cassava leaves, flavonoids, apigenin, isolation.

Introduction

Cassava (*Manihot esculenta* Crantz) is a staple food crop extensively cultivated in tropical regions, including Indonesia, where its production between 2018 and 2022 was concentrated in seven key provinces. Among them, Lampung emerged as the leading contributor, accounting for 39.74% of national production with approximately 5.95 million tons recorded in 2022 [1]. While cassava is predominantly cultivated for its starchy roots, its leaves also hold substantial value due to their rich phytochemical composition and traditional medicinal uses.

Recent pharmacological studies have highlighted the therapeutic potential of cassava leaf extracts. These include significant antioxidant activity through free radical scavenging and the reduction of oxidative stress, which are crucial in mitigating cellular damage and chronic disease development [2].

Furthermore, anti-inflammatory properties have been reported, notably the inhibition of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) in activated macrophages [3]. Cassava leaf extracts have

also shown cytotoxic and anti-proliferative effects against human colon adenocarcinoma (HT-29) cells [4], and neuroprotective effects, as demonstrated by their ability to attenuate amyloid-beta-induced memory impairment via oxidative stress reduction in the hippocampus [5]. Additionally, flavonoids and polyphenols from cassava leaves have demonstrated benefits for skin health, including anti-melanogenesis effects and collagen synthesis stimulation, supporting their potential in cosmetic applications[6].

Flavonoids, as a major class of phenolic compounds in cassava leaves, are secondary metabolites with well-documented antioxidant, anti-inflammatory, and antimicrobial properties [7]. Given the increasing global demand for natural antioxidants in functional foods and therapeutic formulations, the exploration and profiling of flavonoid compounds from cassava leaves represent a promising area of research.

Despite their known ethnomedicinal applications, systematic scientific investigations into the flavonoid content of cassava leaves remain limited. In Indonesia, cassava is cultivated in numerous varieties, including Mentega, Sembung, Adira, Gajah, CNM0300512,



Malang-4, Kaspro, UJ-5, UJ-3, Butoijo, and Cimanggu [8]. However, previous studies have focused on a limited number of common cultivars, neglecting the broader genetic diversity that could harbor higher levels of bioactive compounds. This is a notable limitation, as flavonoid concentration is influenced by genetic, environmental and agronomic factors. A comprehensive comparative study across diverse cassava varieties is thus essential to identify those with superior bioactive potential. The objective of this study was to quantify the total flavonoid content of five cassava leaf varieties: Gajah, Thailand (UJ-3), Kasetsart (UJ-5), Cimanggu and Kaspro. The variety with the highest flavonoid level was then selected for further isolation and characterization of its bioactive compounds.

Method

Preparation of Plant Material

Fresh cassava leaves from five different varieties (Gajah, Thailand, Kasetsart, Cimanggu and Kaspro) were collected from the Research Center for Raw Materials and Traditional Medicines, Central Lampung. The leaves were separated from their stems and extraneous materials, thoroughly washed under running water, and oven-dried at 45°C. The dried material was ground into a fine powder and stored in airtight containers for further analysis [9].

Determination of Total Flavonoid Content [10]

a) Preparation of Test Solution

Approximately 1 gram of cassava leaf powder was weighed and placed in an Erlenmeyer flask. A total of 25 mL of 96% ethanol was added, and the mixture was extracted by magnetic stirring for 1 hour at room temperature. The extract was filtered through filter paper into a volumetric flask, rinsed with 96% ethanol, and brought to the final volume.

b) Quercetin Standard Curve and Total Flavonoid Determination

A 10 mg sample of quercetin standard was dissolved in 25 mL of 96% ethanol to produce a 400 µg/mL solution. Serial dilutions were prepared to obtain concentrations of 50, 70, 90, 110 and 130 µg/mL. From each standard solution and test solution, 1 mL was pipetted into a test tube, followed by the addition of 3 mL ethanol, 0.2 mL of 5% AlCl₃, 0.2 mL of 1 M sodium acetate, and 5.6 mL of distilled water. The mixtures were incubated at room temperature for 30 minutes before being analyzed using

UV-Vis spectrophotometry [10]. The absorbance values were measured and plotted to generate a standard curve with a linear regression equation.

Statistical Analysis

All data were obtained in triplicate and expressed as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS version 23 (IBM Corp., USA). One-way ANOVA was used to assess differences in total flavonoid content among cassava leaf varieties, following confirmation of data normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). Significant results ($p < 0.05$) were further analyzed using Tukey's HSD post hoc test.

Isolation of Flavonoid

a) Extraction

A total of 290 grams of Kaspro variety cassava leaf powder was subjected to Ultrasound-Assisted Extraction (UAE) using 96% ethanol at a temperature below 50°C for 30 minutes [11]. The extract was filtered and then concentrated using a rotary evaporator.

b) Extract Monitoring (TLC Analysis)

The presence of compounds in the extract was monitored using Thin-layer chromatography (TLC) with silica gel F₂₅₄ as the stationary phase and the mobile phase consisted of chloroform:methanol:formic acid (10:2:1). Spots were visualized under UV light at 254 and 366 nm and with FeCl₃ spray reagent.

c) Fractionation and Fraction Monitoring

Liquid-Liquid Partitioning

The extract was partitioned using solvents of increasing polarity: n-hexane, ethyl acetate and water. TLC monitoring was performed using the same mobile phase and visualization methods as described above.

Vacuum Liquid Chromatography

Fractions containing flavonoids were further separated by VLC using silica gel 60H as the stationary phase. A solvent gradient of n-hexane : ethyl acetate : ethanol was used with a sample:silica:solvent ratio of 8:80:200. The fractions were collected and monitored using TLC as described above.

Column Chromatography

Flavonoid-rich fractions were purified using column chromatography using silica gel 60 with a sample:silica:solvent ratio of 1.7:15:40. A gradient elution system of n-hexane : ethyl acetate : ethanol was

used. Fraction monitoring was conducted using TLC using n-hexane:ethyl acetate (4:6) and visualized under UV light and with FeCl_3 spray reagent.

d) Purity Test

Two-dimensional TLC was used to assess the purity of isolated compounds, using two mobile phases with different polarities: (i) n-hexane : ethyl acetate (4:6) and (ii) chloroform : methanol (8:2). A single spot observed under UV light and H_2SO_4 visualization indicated that the isolated compound was pure.

e) Characterization and Identification

UV-Vis spectrophotometry was employed for characterization using shift reagents. Isolates dissolved in ethanol were scanned from 200–800 nm. Shift reagents such as 2M NaOH, 5% AlCl_3 , HCl, sodium acetate, and boric acid were sequentially added, and spectral changes were recorded [12].

Results And Discussion

In this study, five cassava leaf varieties: Gajah, Uj3 (Thailand), Uj5 (Kasetsart), Cimanggu, and Kaspro were evaluated. All varieties were cultivated under standardized agronomic conditions, maintaining identical planting ages and uniform soil characteristics across each variety. Visual comparisons of the morphological appearance of each cassava variety are shown in **Figure 1**.

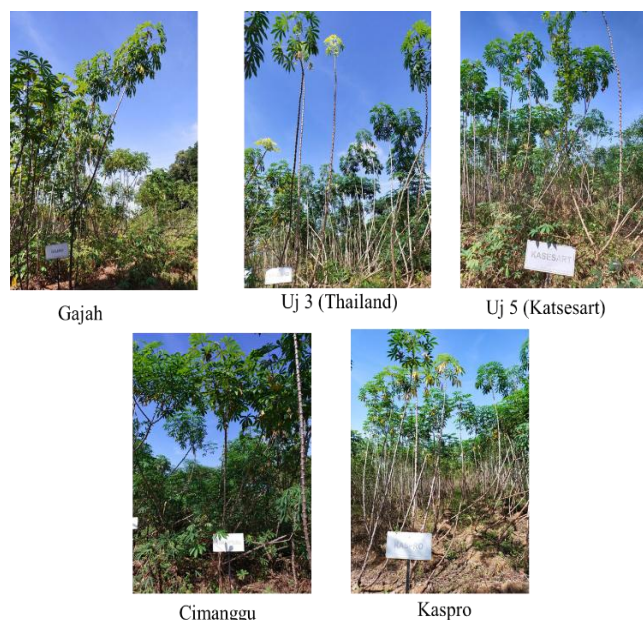


Figure 1. Variety of Cassava

The maximum absorption wavelength of quercetin was determined to be 439 nm, consistent with the typical range of 415–440 nm reported in the literature [13]. This wavelength corresponds to the optimum absorbance of the quercetin– AlCl_3 complex, which is essential for accurate quantification of flavonoid content using UV-Vis spectrophotometry [14]. The identified wavelength was subsequently employed to construct the quercetin standard curve and to quantify the total flavonoid content in the five tested cassava leaf varieties. The UV-Vis absorption spectrum of quercetin is presented in **Figure 2**.

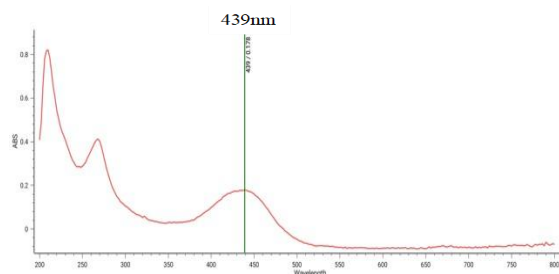


Figure 2. UV-Visible Spectrum of the Quercetin Standard

The linear regression equation derived from the calibration curve is expressed as $y = 0.0052x + 0.0088$, with a coefficient of determination (r^2) of 0.998. The r^2 value, which approaches 1, indicates a strong linear relationship between concentration and absorbance values [15]. The linear regression is depicted in **Figure 3**.

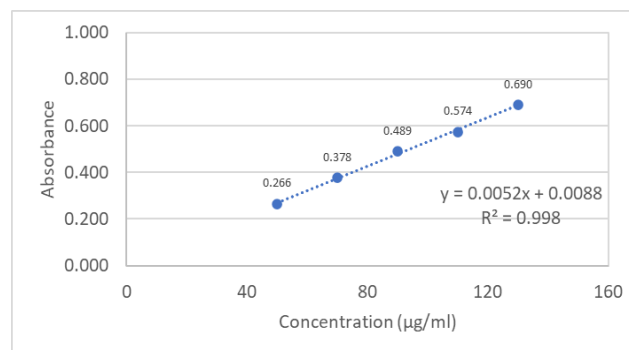


Figure 3. Quercetin Standard Curve

The determination of total flavonoid content in the five cassava leaf varieties was performed in triplicate to ensure data accuracy. The total flavonoid content is presented in **Figure 4**.

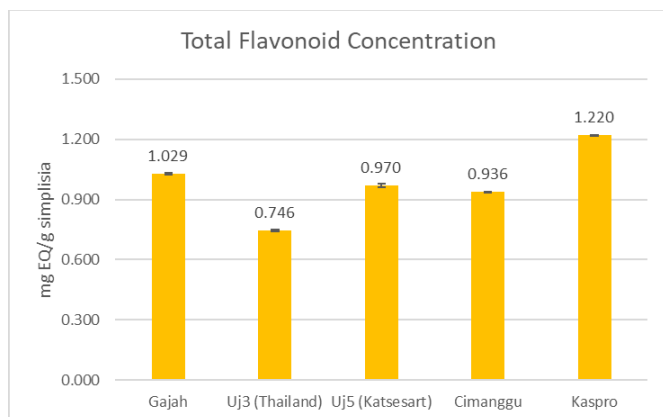


Figure 4. Total Flavonoid Content in Five Cassava Leaf Varieties

The analysis of total flavonoid content in five cassava leaf varieties showed statistically significant variation. A one-way ANOVA test yielded an F-value of 3262.38 with a p-value < 0.0001, indicating a highly significant difference in total flavonoid content among the varieties. To identify which pairs of varieties differed significantly, a Tukey HSD post hoc test was conducted (Figure 5). The results showed that every pairwise comparison between varieties exhibited statistically significant differences ($p < 0.05$), both among the varieties.

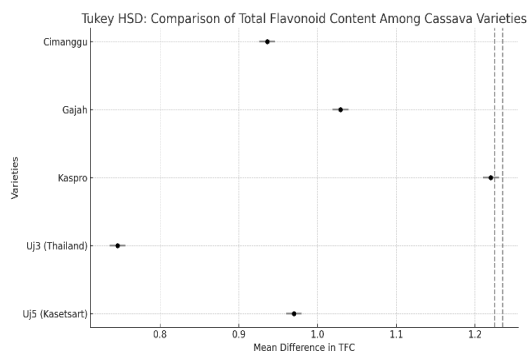


Figure 5. Tukey HSD: Comparison of Total Flavonoid Content among Cassava Varieties

The Kaspro variety had the highest average total flavonoid content at 1.220 mgQE/g, which was significantly higher than that of the other varieties. In contrast, the Uj3 (Thailand) variety showed the lowest flavonoid content (0.746 mg QE/g). These differences may be attributed to genetic variability between cultivars as well as their secondary metabolite responses to environmental and agronomic conditions [16]. This finding is supported by the Tukey HSD plot, where each

variety forms a statistically distinct group with non-overlapping confidence intervals of the mean.

This is consistent with findings from a comprehensive study on 15 cassava genotypes across two growing seasons and multiple locations with varying climatic conditions. The study reported significant variation in flavonoid levels, total phenolic content, and antioxidant capacity between the two seasons, with higher concentrations observed in the first season likely due to differences in temperature and rainfall. Strong correlations were found between total phenolic content, antioxidant capacity, and temperature, suggesting environmental sensitivity of these phytochemicals. Additionally, genotypes with higher antioxidant capacity exhibited reduced leaf damage caused by *Bemisia tabaci*, indicating a potential defensive role of antioxidants against pest attacks. Among the tested genotypes, UG 120257, UG 120291, and UG 120124 consistently showed high antioxidant capacity and stable performance across environments, making them promising candidates for future cassava improvement programs targeting resilience and phytochemical enrichment [17].

The Kaspro variety, which showed the highest total flavonoid content at 1.220 ± 0.003 mgQE/g of crude drug, was selected for further isolation studies. The Kaspro variety was extracted and yielding 24.48% (w/w). Extract monitoring was conducted using quercetin as a reference compound using TLC as shown in Figure 6.

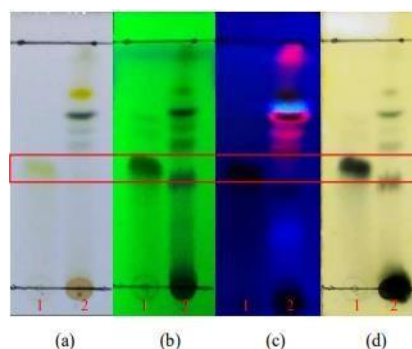


Figure 6. Thin-layer chromatogram of the ethanol extract of Kaspro variety. Stationary phase: silica gel F₂₅₄; mobile phase: chloroform: methanol: formic acid (10:2:1). (a) under visible light, (b) under UV light λ 254 nm, (c) under UV light λ 366 and (d) detection with FeCl₃ reagent. (1) Quercetin, (2) Ethanol extract.

The extract was fractionated by stepwise liquid–liquid partitioning using solvents of increasing polarity (n-hexane, ethyl acetate, and distilled water) at 1:5 ratio. TLC analysis revealed flavonoid-related spots in all fractions, with the ethyl acetate fraction showing the most prominent spot ($R_f=0.5$) and a positive FeCl_3 reaction. This fraction was selected for further isolation.

The ethyl acetate fraction was purified by VLC using a gradient elution, which produces 26 fractions. TLC analysis identified fractions number 11-16 with spot profiles similar to quercetin under UV 254 nm. These fractions were pooled and confirmed as flavonoid-rich based on FeCl_3 spray results, which produced coloration consistent with quercetin. The combined fraction was then subjected to further purification via column chromatography. This process yielded 9 pooled sub-fractions. Among these, sub-fraction number 5 produced a prominent yellow spot with an R_f value similar to that of quercetin and tested positive with FeCl_3 spray reagent, indicating the presence of flavonoid compounds. Two-dimensional TLC analysis of sub-fraction 5 showed a single, well-defined spot, confirming that the flavonoid compound was successfully isolated in a pure form (**Figure 7**).

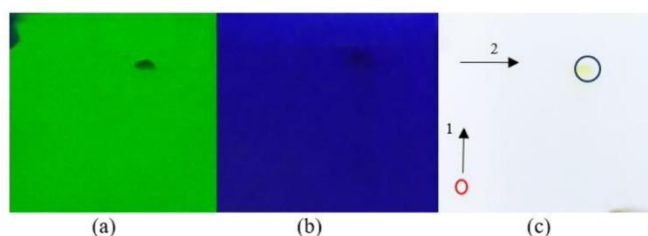


Figure 7. Two-dimensional thin-layer chromatogram of isolate (sub-fraction 5). (a) under UV light λ 254 nm, (b) under UV light λ 366 nm, and (c) after spraying with H_2SO_4 reagent.

The isolated compound was identified using UV-Visible spectrophotometry combined with the shift reagent method. The isolate was dissolved in 96% ethanol, and the spectrum was measured within a wavelength range of 200–800 nm. An initial blank measurement using 96% ethanol revealed absorption bands at λ 334 nm (Band I) and λ 269 nm (Band II). Based on the characteristic absorption ranges of flavonoids, these bands indicate that the isolated compound belongs to the flavone group [12][18]. The spectral shifts resulting from shift reagents are presented in **Table 1**.

Table 1. UV-Vis spectral data of flavonoid isolates before and after the addition of shift reagents

Treatment	λ maks (nm)		Shift (nm)		Interpretation
	Band II	Band I	Band II	Band I	
Isolate	269	334	–	–	Flavon
Isolate + NaOH	274	383	+5	+49	4'-OH
Isolate + NaOH (5 min)	274	383	+5	+49	
Isolate + AlCl_3	279	343	+10	+8	5-OH
Isolate + AlCl_3 / HCl	280	343	+11	+8	
		384		+49	7-OH
Isolate + NaOAc	275	369	+6	+37	/ H_3BO_3
Isolate + NaOAc	271	–	+2		

Hydroxyl groups were detected using sodium hydroxide (NaOH), a strong base [19]. The addition of NaOH to the isolate resulted in a bathochromic shift of 49 nm in Band I and 5 nm in Band II, indicating the presence of a hydroxyl group at position 4' of ring B. No spectral decomposition was observed within 5 minutes of measurement, as indicated by the stability of Band I and II positions (**Figure 8**).

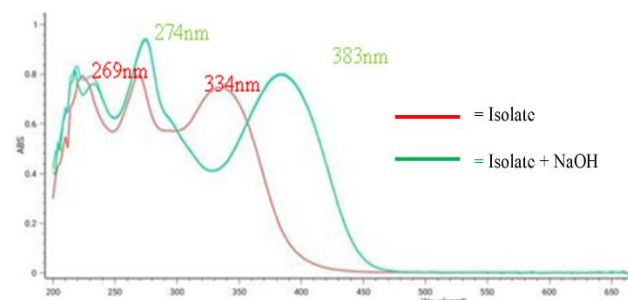


Figure 8. The Spectrum of Isolate with Shift Reagent NaOH

Aluminum chloride (AlCl_3) was used to identify ortho-dihydroxy and keto-dihydroxy substitutions by forming complexes with these groups [20][21]. The subsequent addition of hydrochloric acid (HCl) breaks down these complexes, especially those involving ortho-dihydroxy groups, due to the instability introduced by aluminum complexation. The addition of AlCl_3 induced a bathochromic shift of 10 nm in Band II and 8 nm in Band I. The subsequent addition of HCl resulted in an additional bathochromic shift of 11 nm in Band II and 8 nm in Band I. The appearance of a

new band upon AlCl_3 addition, with a shift of 49 nm, supports the presence of a hydroxyl group at position 5 (Figure 9).

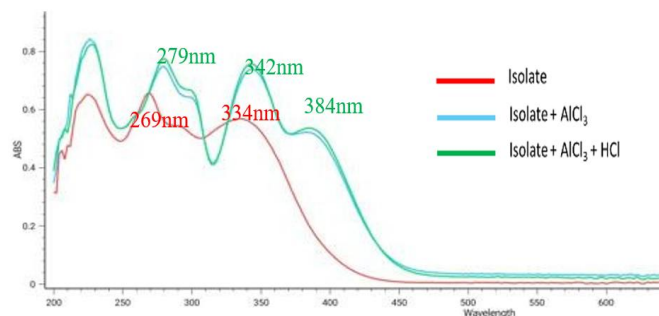


Figure 8. The Spectrum of Isolate with Shift Reagent AlCl_3 and $\text{AlCl}_3 + \text{HCl}$

Sodium acetate (NaOAc) was added to ionize the most acid-resistant hydroxyl group at position 7 (7-OH), typically causing a bathochromic shift in Band II. The addition of boric acid (H_3BO_3) helps bridge ortho-dihydroxy groups, forming a borate complex [22]. Upon NaOAc addition, a bathochromic shift of 6 nm in Band II and 37 nm in Band I was observed. The combination of NaOAc and H_3BO_3 produced a 2 nm shift in Band I (Figure 10).

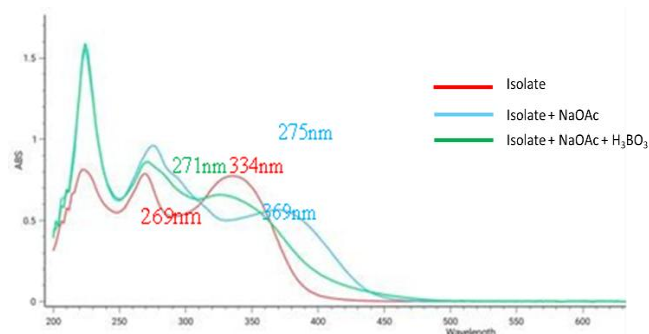


Figure 9. The Spectrum of Isolate with Shift Reagent NaOAc and $\text{NaOAc} + \text{H}_3\text{BO}_3$

Based on spectral interpretation, the flavonoid isolated from sub-fraction 5 was identified as 5,7,4'-trihydroxyflavone, commonly known as apigenin. The chemical structure of apigenin is presented in Figure 11. Apigenin has been previously reported in *Manihot esculenta* leaves, where it contributes significantly to their antioxidant and anti-inflammatory activities [23][24][25]. The identification of apigenin in this study further supports the potential of cassava leaves, particularly the Kaspro variety, as a valuable source of bioactive compounds for pharmaceutical and nutraceutical applications [23][26][27].

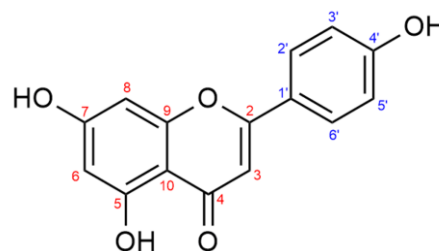


Figure 10. Structure of apigenin

Conclusions

The total flavonoid content of various cassava leaf (*Manihot esculenta* Crantz) varieties was quantitatively ranging from 0.746 ± 0.006 to 1.220 ± 0.003 mgQE/g. Among of them, the Kaspro variety demonstrated the highest flavonoid concentration. Further phytochemical investigation of this variety led to the successful isolation and identification of apigenin, a flavonoid compound belonging to the flavone group. These findings suggest that the Kaspro cassava leaf variety is a promising source of bioactive flavonoids with potential health and functional food applications.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank for Research Center for Raw Materials and Traditional Medicines, Central Lampung and the Institut Teknologi Sumatera for the facilities provided.

References

- [1] Kementerian Pertanian, "Analisis Kinerja Perdagangan Ubi Kayu," Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal, Kementerian Pertanian, vol. 12 Nomor 2A, 2023.
- [2] S. R. N. S. P. Mohidin, S. Moshawih, A. Hermansyah, M. I. Asmuni, N. Shafqat, and L. C. Ming, "Cassava (*Manihot esculenta* Crantz): A Systematic Review for the Pharmacological Activities, Traditional Uses, Nutritional Values, and Phytochemistry," Jan. 01, 2023, SAGE Publications Ltd. doi: 10.1177/2515690X231206227.
- [3] Z. Meilawaty and B. Kusumawardani, "Effect of Cassava leaf flavonoid extract on TNF- α expressions in rat models suffering from periodontitis," *Dent J*, vol. 49, no. 3, pp. 137–142, Sep. 2016, doi: 10.20473/j.djmk.v49.i3.p137-142.
- [4] V. Chinnadurai, P. Viswanathan, K. Kalimuthu, A. Vanitha, V. Ranjitha, and A. Pugazhendhi, "Comparative studies of phytochemical analysis and pharmacological activities of wild and micropropagated plant ethanol extracts of *Manihot esculenta*,"

- Biocatal Agric Biotechnol*, vol. 19, p. 101166, May 2019, doi: 10.1016/J.BCAB.2019.101166.
- [5] G. S. Carrazoni, N. B. Garces, C. R. Cadore, P. M. Sosa, R. Cattaneo, and P. B. Mello-Carpes, "Supplementation with Manihot esculenta Crantz (Cassava) leaves' extract prevents recognition memory deficits and hippocampal antioxidant dysfunction induced by Amyloid- β ," *Nutr Neurosci*, vol. 27, no. 9, pp. 942–950, Sep. 2024, doi: 10.1080/1028415X.2023.2280815;SUBPAGE:STRING:ACCESS.
- [6] M. Jampa, K. Sutthanut, N. Weerapreeyakul, W. Tukummee, J. Wattanathorn, and S. Muchimapura, "Multiple Bioactivities of Manihot esculenta Leaves: UV Filter, Anti-Oxidation, Anti-Melanogenesis, Collagen Synthesis Enhancement, and Anti-Adipogenesis," *Molecules* 2022, Vol. 27, Page 1556, vol. 27, no. 5, p. 1556, Feb. 2022, doi: 10.3390/MOLECULES27051556.
- [7] E. Fachriyah, I. B. Haryanto, D. Kusrini, P. R. Sarjono, and N. Ngadiwiyan, "Antioxidant Activity of Flavonoids from Cassava Leaves (Manihot esculenta Crantz)," *Jurnal Kimia Sains dan Aplikasi*, vol. 26, no. 1, pp. 10–18, Jan. 2022, doi: 10.14710/JKSA.26.1.10-18.
- [8] F. N. Amanu and W. H. Susanto, "Pembuatan Tepung Mocaf di Madura (Kajian Varietas dan Lokasi Penanaman) terhadap Mutu dan Rendemen Mocaf," vol. 2, pp. 161–169, Jul. 2014.
- [9] M. R. Ghozaly, S. Aziz, N. S. G. Gunarti, and L. Fikayuniar, *Metode Fitokimia untuk Farmasi*, 1st ed. Jejak Pustaka, 2023.
- [10] Kementerian Kesehatan RI, "Farmakope Herbal Indonesia Edisi II", 2017.
- [11] S. Aziz, "Teknologi Ekstraksi Bahan Alam," in *Bunga Rampai Senyawa Bahan Alam: Uji Aktivitas dan Teknologinya*, Eds 1, ITERA Press, 2025.
- [12] K. R. Markham, *Techniques of Flavonoid Identification*. London: Academic press, 1982.
- [13] M. Taniguchi, C. A. LaRocca, J. D. Bernat, and J. S. Lindsey, "Digital Database of Absorption Spectra of Diverse Flavonoids Enables Structural Comparisons and Quantitative Evaluations," *J Nat Prod*, vol. 86, no. 4, pp. 1087–1119, Apr. 2023, doi: 10.1021/ACS.JNATPROD.2C00720/SUPPL_FILE/NP2C00720_SI_001.PDF.
- [14] S. S. Shanko, T. S. Badessa, and A. M. Tura, "Method development and validation for the quantitative determination of total flavonoids through the complexation of iron (III) and its application in real sample," *Anal Chim Acta*, vol. 1301, p. 342443, May 2024, doi: 10.1016/J.ACA.2024.342443.
- [15] Q. Hong, Y. Shen, S. Liu, and Y. Zhang, "Re-Examination of Plotting Analytical Response against Different Forms of Concentration," *Anal Chem*, vol. 93, no. 35, pp. 11910–11914, Sep. 2021, doi: 10.1021/ACS.ANALCHEM.1C02683/ASSET/IMAGES/MEDIUM/AC1C02683_0006.GIF.
- [16] H. W. Gitonga, S. Kyamanywa, P. Arusei, M. M. Lukanda, R. Edema, and I. O. Dramadri, "Genotype $\times$ Environment Interaction Influence Secondary Metabolite in Cowpea Infested by Flower Bud Thrips," *Agronomy* 2022, Vol. 12, Page 3210, vol. 12, no. 12, p. 3210, Dec. 2022, doi: 10.3390/AGRONOMY12123210.
- [17] N. Mwila, E. Nuwamanya, T. L. Odong, A. Badji, S. Agbahoungba, P. A. Ibanda, M. Mwala, P. Sohati, S. Kyamanywa & P. R. Rubaihayo, "Genotype by Environment Interaction Unravels Influence on Secondary Metabolite Quality in Cassava Infested by Bemisia tabaci," *Journal of Agricultural Science*, vol. 10, no. 8, p. p192, Jul. 2018, doi: 10.5539/JAS.V10N8P192.
- [18] N. A. Tyukavkina and N. N. Pogodaeva, "Ultraviolet absorption of flavonoids II. Ionization constants of 7- and 4'-hydroxy derivatives of flavone and flavonol," *Chem Nat Compd*, vol. 7, no. 1, pp. 8–11, 1971, doi: 10.1007/BF01032014/METRICS.
- [19] T. Okselni and M. Efdi, "Isolation, identification and α -glucosidase inhibition evaluation of a phenolic compound from Elaeocarpus mastersii King leaves," *E3S Web of Conferences*, vol. 503, Mar. 2024, doi: 10.1051/E3SCONF/202450307004.
- [20] L. Jurd, "Aluminum complexes of phenolic flavones. Spectral and structural correlations," *Phytochemistry*, vol. 8, no. 2, pp. 445–462, Feb. 1969, doi: 10.1016/S0031-9422(00)85447-3.
- [21] T. Asada and H. Tamura, "Isolation of bilberry anthocyanidin 3-glycosides bearing ortho -dihydroxyl groups on the B ring by forming an aluminum complex and their antioxidant activity," *J Agric Food Chem*, vol. 60, no. 42, pp. 10634–10640, Oct. 2012, doi: 10.1021/JF302476N/ASSET/IMAGES/MEDIUM/JF-2012-02476N_0006.GIF.
- [22] L. Jurd, "A spectrophotometric method for the detection of o-dihydroxyl groups in flavonoid compounds," *Arch Biochem Biophys*, vol. 63, no. 2, pp. 376–381, 1956, doi: 10.1016/0003-9861(56)90052-2.
- [23] M. Jampa, K. Sutthanut, N. Weerapreeyakul, W. Tukummee, J. Wattanathorn, and S. Muchimapura, "Multiple Bioactivities of Manihot esculenta Leaves: UV Filter, Anti-Oxidation, Anti-Melanogenesis, Collagen Synthesis Enhancement, and Anti-Adipogenesis," *Molecules*, vol. 27, no. 5, Mar. 2022, doi: 10.3390/MOLECULES27051556.
- [24] C. N. Tsumbu, G. D. Dupont, M. Tits, L. Angenot, T. Franck, D. Sertheyn and A. M. Mickalad, "Antioxidant and Antiradical Activities of Manihot esculenta Crantz (Euphorbiaceae) Leaves and Other Selected Tropical Green Vegetables Investigated on Lipoperoxidation and Phorbol-12-myristate-13-acetate (PMA) Activated Monocytes," *Nutrients*, vol. 3, no. 9, pp. 818–838, 2011, doi: 10.3390/NU3090818.
- [25] R. H. Patil, R. L. Babu, M. N. Kumar, K. M. K. Kumar, S. M. Hegde, R. Nagesh, G. T. Ramesh & S. C. Sharma, "Anti-Inflammatory Effect of Apigenin on LPS-Induced Pro-Inflammatory Mediators and AP-1 Factors in Human Lung Epithelial Cells," *Inflammation*, vol. 39, no. 1, pp. 138–147, Feb. 2016, doi: 10.1007/S10753-015-0232-Z.
- [26] B. Salehi, A. Venditti, M. S. Rad, D. Kregiel, J. S. Rad, A. Durazzo, M. Lucarini, A. Santini, E. B. Souto, E. Novellino, H. Antolak, E. Azzini, W. N. Setzer & N. Martins, "The Therapeutic Potential of Apigenin," *Int J Mol Sci*, vol. 20, no. 6, Mar. 2019, doi: 10.3390/IJMS20061305.
- [27] A. Singh, J. Singh, G. Parween, R. Khator, and V. Monga, "A comprehensive review of apigenin a dietary flavonoid: biological sources, nutraceutical prospects, chemistry and pharmacological insights and health benefits.," *Crit Rev Food Sci Nutr*, 2024, doi: 10.1080/10408398.2024.2390550.