ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 26 (1) (2023): 10–18

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Antioxidant Activity of Flavonoids from Cassava Leaves (*Manihot esculenta* Crantz)

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https://doi.org/10.14710/jksa.26.1.10-18

Article Info

Article history:

Received: 7th January 2022
Revised: 15th December 2022
Accepted: 23rd December 2022
Online: 31st January 2023

Keywords:
Manihot esculenta Crantz; flavonoids; total flavonoids; antioxidants

Abstract

The community uses cassava leaves for cooking because of their high protein content, β-carotene, Fe, Mg, Zn, S, Ca, Zn, Ni, and K. Cassava leaves contain various secondary metabolites, including flavonoids, saponins, tannins, phenolics, steroids, anthocyanins, and anthraquinones. The antioxidant activity of flavonoid compounds in cassava leaves (*Manihot esculenta* Crantz) has been successfully investigated. This study consisted of six stages: the preparation of ethanol extract of cassava leaves, phytochemical screening, determination of total flavonoid content in the extract, isolation of flavonoids, and purity testing of flavonoid isolates using the chromatographic method, identification of the structure of pure flavonoid isolates using a UV-Vis spectrophotometer with the addition of a shear reagent, FTIR, and LC-MS/MS. As well as test the antioxidant activity using the DPPH method. The yield of ethanol extract from cassava leaf in this study was 14.67%. The results of the phytochemical screening showed that the cassava leaves and the ethanol extract of cassava leaves contained alkaloids, flavonoids, saponins, tannins, quinones, steroids, phenolics, and triterpenoids. The total content of flavonoids in the ethanol extract was 35.71 mg EQ/g extract. UV-Vis, FTIR, and LC-MS/MS analysis revealed that the flavonoid isolate was quercetin. The results of the antioxidant activity of the ethanol extract and BC isolate showed IC_{50} of 81.76 ± 0.505 mg/L and 77.85 ± 4.708 mg/L.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a member of the Euphorbiaceae family, often found in the Asian continent, including Indonesia. The component of cassava that is most frequently used by the community is the tuber. At the same time, the lower leaves are utilized as animal feed. Only a few leaves are used as vegetables, particularly the apex [1, 2]. People use cassava leaves for cooking as a vegetable because they contain high protein [3], β-carotene, Fe, Mg, Zn, S, Ca, and Ni [4, 5]. Cassava leaves contain various secondary metabolites: flavonoids, saponins, tannins, phenolics, steroids, anthocyanins, and anthraquinones [6]. Cassava leaves are known to have a high content of flavonoids [7]. The most abundant flavonoid found in cassava leaves is rutin [8]. Flavonoids are secondary metabolites produced by plants and have many functions, one of which is as an antioxidant [7]. Antioxidant compounds inhibit the activity of free radicals in the body by giving electrons to free radical molecules so that these molecules become stable.

Several studies on the activity of cassava leaves have been carried out. Research by Rikomah *et al.* [9] reported that 70% ethanol extract had an antirheumatic effect on pain in mice (*Mus musculus*), with the best effect at a dose of 0.65 mg. Ethanol extract has activity as an antibacterial [10, 11, 12], analgesic [13], antidiarrheal [14], lowers blood sugar [15], anthelmintic [16], antioxidant [10, 17, 18, 19, 20, 21, 22, 23]. Research on cassava leaf flavonoids and antioxidant activity has not been widely reported. Therefore, isolation of flavonoids and determination of antioxidant activity from cassava leaves was carried out in this study. An antioxidant

activity test was performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

2. Experiment

2.1. Materials and Tools

The materials used were cassava leaves, 2 N HCl (Merck), ammonia (Merck), Mg powder, concentrated HCl, 1% $FeCl_3$, H_2SO_4 (Merck), Mayer's reagent, Dragendroff's reagent, $AlCl_3$ 10% (Merck), CH_3COOK 1 M, 100% glacial acetic acid (Merck), NaOH 2 M (Merck), $AlCl_3$ 5% (Merck), NaOAc (Merck), H_3BO_3 (Merck), DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich), 60 F254 silica gel plate (Merck), GF254 silica plate (Merck), 60 G silica (Merck), and quercetin standard (Sigma-Aldrich). In addition, technical grade and analytical grade (Merck) of ethanol, n-hexane, ethyl acetate were also used. Amyl alcohol (Merck), petroleum ether (Merck), acetic acid anhydride, methanol, butanol (Merck), and chloroform (Merck) were of analytical grade.

The tools used were standard research glassware, bunsen, a set of round bottom reflux flasks, Buchi B480 rotary evaporator, TLC chamber, magnetic stirrer, vials, spatula, capillary tube, 254 and 365 nm UV lamps, gravity column chromatography, vials, and aluminum foil.

2.2. Research Procedures

The research started with plant determination, sample preparation, phytochemical screening test on powder and ethanol extract of cassava leaves, isolation of flavonoid compounds, separation of flavonoids, testing of flavonoid purity, and identification of flavonoid compounds. Testing of antioxidant activity of flavonoid isolates using the DPPH method. Isolation of flavonoids was carried out by maceration with 96% ethanol and removal of chlorophyll by adding distilled water and incubating for 1 × 24 hours. The chlorophyll-free ethanol extract was extracted with n-hexane to obtain non-polar compounds such as fat. The fraction which was insoluble in n-hexane was hydrolyzed with 2 N HCl, partitioned using ethyl acetate, then fractionated with amyl alcohol. Flavonoids Amyl alcohol fraction was separated by gravity column chromatography and preparative TLC and tested for purity using TLC of various eluents and two-dimensional TLC. The structure of flavonoid isolates was identified using UV-Vis spectrophotometer (Shimadzu UV-1280). The shear reagents used were sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$), hydrochloric acid (HCl), sodium acetate (NaOAc), and boric acid (H_3BO_3).

2.2.1. Sample Preparation

Cassava leaf samples were obtained from the Bulusan area, Semarang, Indonesia, at coordinates -7.0589640, 110.4417452. Cassava leaves were cleaned and aerated at room temperature. Dried cassava leaves were mashed to become simplicia powder. Simplicia powder was macerated with 96% ethanol, evaporated, and ethanol extract was obtained.

2.2.2. Production of Ethanol Extract

As much as 1600 g of cassava leaf simplicia was macerated with 96% technical ethanol. Maceration was carried out by replacing the solvent regularly every 24 hours and repeated until the macerated filtrate turned clear and then filtered. The filtrate was evaporated using a rotary evaporator at 60°C. The concentrated ethanol extract was dissolved in ethanol. Then the chlorophyll was removed by adding distilled water (1:1), allowed to stand for 1 × 24 hours, and filtered. The ethanol-water extract was further extracted with n-hexane. The n-hexane layer and the ethanol-water layer were separated. The ethanol-water extract obtained was concentrated and weighed.

2.2.3. Phytochemical Screening

Simplicia and ethanol extract from cassava leaves were tested for phytochemical screening. The tests conducted were alkaloid test (Dragendorff and Mayer reagent), saponin test (HCl), flavonoid test (magnesium metal powder), phenolic test ($FeCl_3$), quinone test (NaOH), tannin test ($FeCl_3$), and steroid/triterpenoid test (Liebermann-Burchard reagent) [24, 25].

2.2.4. Determination of Total Flavonoid Content

Preparation of quercetin standard curve. 1000 ppm stock solution was prepared by dissolving 10 mg of quercetin with methanol in a 10 mL volumetric flask until the end limit. Concentrations were varied to 0, 2, 4, 6, 8, and 10 ppm. 1 mL of the extract solution was added with 3 mL of methanol, 0.2 mL of $AlCl_3$, 0.2 mL of 1 M CH_3COONa, and distilled water to 10 mL. The solution was homogenized and incubated again in the dark for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at 432.5 nm.

Determination of the total content of flavonoid compounds. 0.1 g of ethanol extract was dissolved with methanol in a 10 mL volumetric flask up to the mark. 1 mL of ethanol extract was added with 3 mL of methanol, 0.2 mL $AlCl_3$, 0.2 mL CH_3COONa 1 M, and distilled water up to 10 mL. The solution was homogenized and incubated in the dark for 30 minutes. The absorbance of the solution was measured using a UV-Vis spectrophotometer at 432.5 nm [26].

2.2.5. Isolation of Flavonoid Compounds

The ethanol-water extract was hydrolyzed using 2 N HCl for 1 hour at 85°C [27]. The hydrolysis results were fractionated using ethyl acetate so that two layers were formed, resulting in two layers that were subsequently separated: the lower layer was the ethyl acetate fraction, while the upper layer was the ethanol-water fraction. The ethyl acetate fraction was tested for sugar content using Benedict's reagent. This was done to ensure that the flavonoids in the fraction were free of sugar.

The ethyl acetate fraction was concentrated with a rotary evaporator to obtain the ethyl acetate fraction. The ethyl acetate fraction was dissolved in ethyl acetate, then fractionated with amyl alcohol. Two fractions were formed and separated between the upper layer (amyl alcohol fraction) and the lower layer (ethyl acetate

fraction). The amyl alcohol fraction obtained was concentrated with a rotatory evaporator to obtain amyl alcohol extract and weighed [28].

2.2.6. Separation of Flavonoid Compounds

The amyl alcohol fraction was then analyzed using TLC to determine a suitable eluent for gravitational column chromatography (GCC). The TLC method used the stationary phase of silica gel GF254 and various single eluents (methanol, ethyl acetate, chloroform, ethanol, dichloromethane, and n-hexane) and mixtures. The best eluent was used to separate compound components using the gravitational column chromatography method.

In the GCC method, the eluate produced from the column was collected every 15 mL in vials and analyzed for each vial using the TLC method. The eluate that produced the same stain pattern was combined into one large fraction. These fractions were analyzed for flavonoids by the TLC method by adding $AlCl_3$ spotting.

The positive fraction containing flavonoids was analyzed using the TLC method to determine the eluent composition that could provide the best separation. The flavonoid's positive fraction was then separated using the preparative TLC method. The most striking color intensity results were scraped and dissolved in methanol to obtain flavonoid isolates.

2.2.7. Purity Test

The purity of the flavonoid isolates was tested using TLC with a single or mixed eluent. The single eluents used were dichloromethane, chloroform, ethyl acetate, and methanol. Meanwhile, the mixed eluents were chloroform: ethyl acetate: ethanol (7:3:1). In the two-dimensional TLC, the eluent mixture of chloroform: ethyl acetate: ethanol (7:3:1) was used in the first elution, after being rotated 90° it was eluted with methanol: ethyl acetate (5:3).

2.2.8. Identification of Flavonoid Structures

Structural identification of flavonoid isolates was performed by UV-Vis spectrophotometry, shear reagent, FTIR (PerkinElmer Frontier), and LC-MS/MS (Xevo G2-XS QTof). LC-MS/MS operational conditions were 1.7-micrometer ACQUITY UPLC®BEH C8 column 2.1 x 100 mm, solvent system A (0.1%FA/WA) and B (Acetonitrile+0.1FA), flow rate of 0.3 mL/min, and eluent gradient A: B of 0.95: 0.05 to 0.00: 1.00 for 16 minutes [27, 28].

2.2.9. Antioxidant Activity Test

Preparation of test solutions. 0.1 mM DPPH solution was prepared by dissolving 3.94 mg of DPPH powder in 100 mL of methanol (analytical grade). The 1000 mg/L ethanol extract test solution was made by dissolving 25 mg ethanol extract in methanol into a 25 mL volumetric flask. On the other hand, 2.5 mg of the isolate was dissolved in methanol in a 25 mL volumetric flask to prepare 100 mg/L of the flavonoid isolate test solution.

Antioxidant activity test. Antioxidant activity test was performed in several stages. The first step was to determine the optimum wavelength of the DPPH solution. 3 mL of 0.1 mM DPPH was added with 1 mL of methanol and shaken. The absorbance of the solution was measured at a wavelength of 400–600 nm, and a maximum wavelength of 516 nm was obtained. Before testing the antioxidant activity, the concentration of the ethanol extract was varied to 25, 50, 75, 125, and 150 ppm. In addition, isolates were prepared by varying concentrations to 6, 8, 20, 30, and 40 ppm. Likewise, quercetin concentration was also varied to 2, 4, 6, 8, and 10 ppm. Antioxidant activity was tested by adding 3 mL of 0.1 mM DPPH solution to 1 mL of the sample (ethanol extract, isolate, and quercetin standard) at various concentrations. The mixture was homogenized and incubated for 30 minutes in a dark room. The absorbance was measured using a UV-Vis spectrophotometer at 516 nm in the control solution [29].

3. Results and Discussion

A total of 1600 g of cassava leaf powder was macerated with 96% ethanol solvent to obtain 234.83 g of ethanol extract with a yield of 14.67%. This result is higher than the study by Rikomah *et al.* [9], which produced a yield of 10.48% using 70% ethanol, contrary to this study using 96% ethanol. The simplicia and ethanol extract of cassava leaves were then subjected to phytochemical screening. The results of the phytochemical screening are shown in Table 1.

Table 1. Results of phytochemical screening of simplicia and ethanol extract of cassava leaves

Test	Simplicia	Ethanol extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Quinone	+	+
Tannins	+	+
Steroids	+	+
Triterpenes	+	+
Phenolic	+	+

(+): contains a group of secondary metabolite compounds

It can be seen in Table 1 that cassava leaves contain secondary metabolites of alkaloids, including flavonoids, phenolics, tannins, quinones, triterpenoids, and steroids. These results are in accordance with a study conducted by Chinnadurai *et al.* [30]. In determining the total levels of flavonoids, quercetin was used as the standard solution. The standard quercetin calibration curve obtained, namely concentration versus absorbance, is shown in Figure 1.

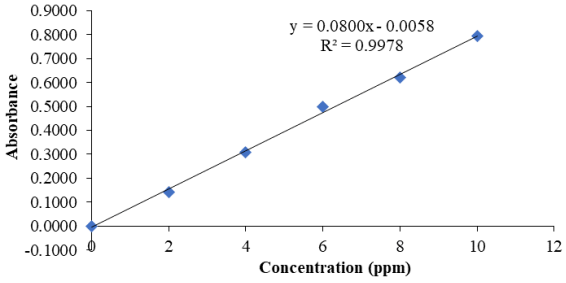


Figure 1. Calibration curve of quercetin

The result was a graph of concentration versus absorbance which produced a linear regression equation y = 0.0800x – 0.0058 with a value of R^2 = 0.9978. From this equation, the total flavonoid content was 35.71 ± 0 mg QE/g extract for 2000 ppm ethanol extract.

The ethanol fraction obtained from removing chlorophyll and fat was then subjected to acid hydrolysis, which aimed to break the O-glycoside bonds in the flavonoid framework to produce sugar-free flavonoids [27]. Free flavonoids can be taken by fractionation using ethyl acetate. The ethyl acetate fraction obtained was then tested for sugar with Benedict's reagent, which produced a brown solution, indicating that the flavonoids were sugar-free. The ethyl acetate fraction was fractionated with amyl alcohol to extract more polar flavonoid compounds.

The amyl alcohol fraction obtained was then identified for its flavonoid content using the TLC method. The amyl alcohol fraction was eluted with chloroform: ethyl acetate: butanol with a ratio of 6: 1: 0.5, as shown in Figure 2.

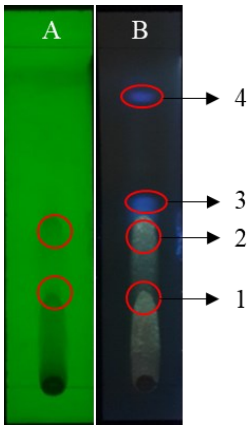


Figure 2. TLC profile of amyl alcohol fraction with chloroform as eluent: ethyl acetate: butanol (6: 1: 0.5) (A) at UV 254 nm (B) UV 365 nm after being sprayed with $AlCl_3$

TLC results of the amyl alcohol fraction with chloroform: ethyl acetate: butanol (6:1:0.5) eluent were still not completely separated, even though the target stain had been separated from the other stains. These stains were seen in 254 nm and 365 nm UV light to produce four spots with different Rf. The Rf value of each stain can be seen in Table 2.

Table 2. Rf value and color on TLC results of amyl alcohol fraction (UV 365 nm)

Spot	Rf	Color before spraying $AlCl_3$	Color after spraying $AlCl_3$
1	0.27	Yellow	Bright yellow
2	0.54	Yellow	Bright yellow
3	0.60	Light blue	Purplish blue
4	0.87	Purple	Purple

The TLC results of the amyl alcohol fraction with the eluent chloroform: ethyl acetate: butanol (6: 1: 0.5) were still not completely separated, then separation was conducted using GCC. The eluent was chloroform: ethyl acetate: butanol (6: 1: 0.5) and 120 g of 60 G silica gel as the stationary phase. The resulting eluates were 90 vials @ 15 mL. Each vial of the eluate was analyzed using TLC with chloroform: ethyl acetate: butanol (6: 1: 0.5) as the eluent. TLC results with the same spot pattern were combined into six large fractions. The fractions from the column chromatography results are shown in Table 3. The TLC results for fractions A, B, C, D, E, and F can be seen in Figure 3.

Table 3. Fractions from column chromatography results

	Number of vials	Fraction color
A	1-11	Blackish green
B	12-26	Yellowish green
C	27-49	Yellow
D	50-71	Yellow
E	72-83	Chocolate
F	84-90	Chocolate

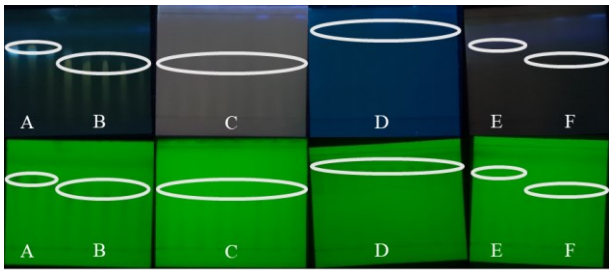


Figure 3. TLC profiles of column chromatography on six fractions (A, B, C, D, E, and F) with chloroform: ethyl acetate: butanol (6:1:0.5) as eluent at (Y) UV 254 nm and (X) 365nm

Fractions A, B, C, D, E, and F were re-identified using TLC with the same eluent (chloroform: ethyl acetate: butanol (6:1:0.5)) and accompanied by a specific spot of flavonoid $AlCl_3$ 5%. The results of separation with TLC are presented in Figure 4.

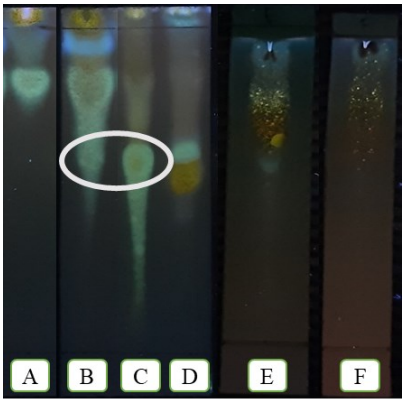


Figure 4. TLC profile of gravity column chromatography results on fractions A, B, C, D, E, and F with chloroform: acetic acid: water (90:45:6) as eluent sprayed with $AlCl_3$ spotting agent at UV 365 nm

The TLC results in Figure 4 of the B and C fractions produced the same target spot pattern so that the B and C fractions were combined. The combination of fractions B and C was called the BC fraction. The BC fraction was suspected of containing quercetin compounds and

strengthening this suspicion. TLC was carried out on the BC fraction with quercetin standards. The TLC results of the BC fraction with quercetin standards can be seen in Figure 5.

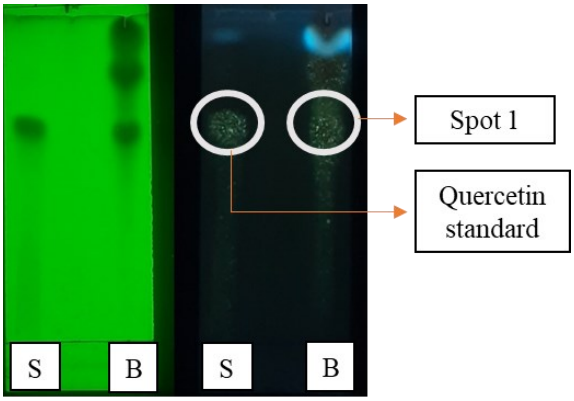


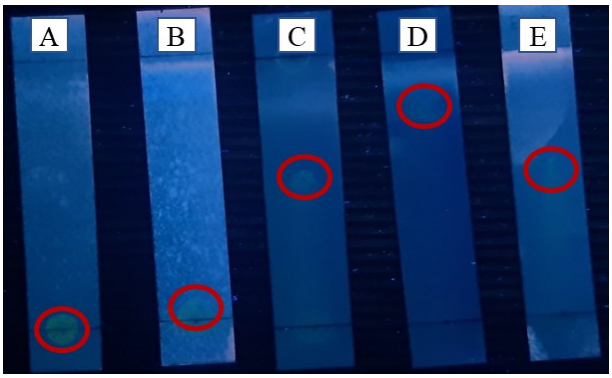
Figure 5. TLC results on quercetin standard and BC fraction with chloroform: acetic acid: water (90:45:6) at UV 254 and 365 nm (S: quercetin standard; B: BC fraction) sprayed with $AlCl_3$ spotting

The TLC results in Figure 5 show that spot 1 and the quercetin standard have the same Rf of 0.66. The target spot to be taken is spot 1. The BC fraction was separated by flavonoid compounds using the preparative TLC method (silica gel plate 60 F254) with a 10 × 20 cm size. Separation of flavonoids in the BC fraction used chloroform: acetic acid: water (90:45:6) as the eluent. The results of preparative TLC on the BC fraction can be seen in Figure 6.

Figure 6. Results of preparative TLC of the BC fraction with chloroform: acetic acid: water (90:45:6) as eluent at UV 365 nm

The results of the preparative TLC of the BC fraction showed three well-separated bands. The isolated target compound was band 1 because it was yellow under UV 365. It was identified as a flavonoid compound and suspected to be quercetin.

The next step was band 1 scraped, dissolved in methanol, then filtered. The filtrate obtained was referred to as BC isolate, then tested for purity using TLC. The eluent used was dichloromethane, chloroform, ethyl acetate, and methanol. Another eluent was a mixture of chloroform: ethyl acetate: ethanol. The results of the BC isolate purity test can be seen in Figure 7.



A: chloroform B: dichloromethane C: ethyl acetate
D: methanol E: ethyl acetate: chloroform: ethanol (7:3:1)

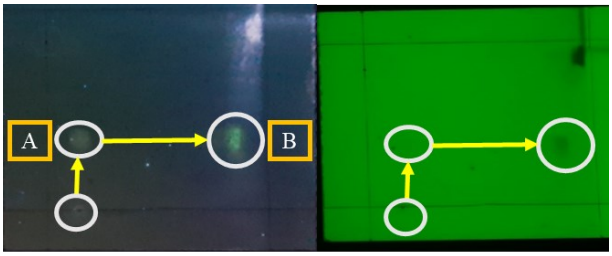
Figure 7. TLC results of BC isolate with various eluents at UV light 365 nm accompanied by the appearance of H_2SO_4 spots

Figure 7 shows that the BC isolate is pure, because only one spot is visible. The TLC Rf values of each eluent are presented in Table 4.

Table 4. Rf value of BC isolate in single and mixed eluents

Code	Eluent	Rf
A	Chloroform	0
B	Dichloromethane	0.11
C	Ethyl acetate	0.6
D	Methanol	0.85
E	Ethyl acetate: chloroform: ethanol	0.61

BC isolate was identified for its purity by two-dimensional TLC. The first elution was tested using chloroform: ethyl acetate: ethanol (7:3:1) as a mixed eluent and using methanol: ethyl acetate (5:3) as eluent after rotating the TLC plate by 90°. Two-dimensional TLC results of BC isolate at UV 365 nm and 254 nm can be seen in Figure 8.



A: chloroform: ethyl acetate: ethanol (7:3:1)
B: methanol: ethyl acetate (5:3)

Figure 8. The two-dimensional TLC of BC isolate at UV 365 nm and 254 nm with H_2SO_4 spotting

The two-dimensional TLC results of the BC isolate showed only one spot, revealing that the BC isolate was pure. TLC was performed using a quercetin standard to confirm that the BC isolate was a quercetin compound. The results can be seen in Figure 9.

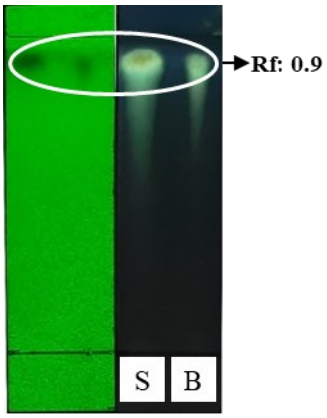


Figure 9. TLC results of quercetin standard (S) and BC isolate (B) using butanol: methanol: acetic acid (1:8:1) as eluent with $AlCl_3$ spots appearing

The TLC results on standard quercetin and BC isolate had the same Rf value of 0.9. This strengthens the notion that the BC isolate is a quercetin compound. Evaporation of the BC isolate produced 34.6 mg of yellowish-green solid isolate. Furthermore, structural analysis of BC isolates was identified using UV-Vis spectrophotometry, shear reagents, FTIR, and LC-MS/MS.

BC isolate was dissolved in methanol and analyzed using a UV-Vis spectrophotometer. Standard quercetin spectra were analyzed to confirm that the BC isolate was a quercetin compound. The results of the UV-Vis spectrophotometer analysis of BC isolates and quercetin standards can be seen in Figure 10.

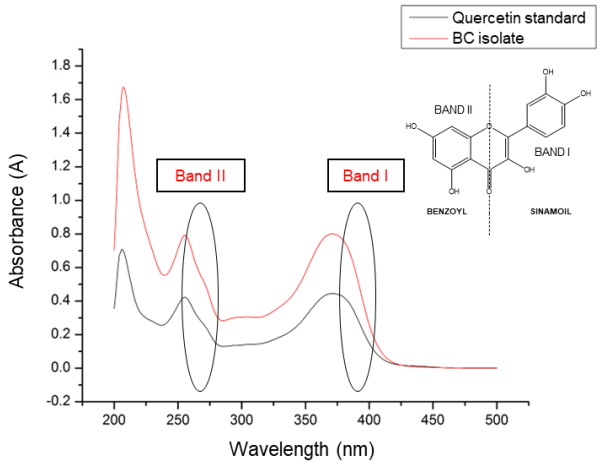


Figure 10. UV-Vis spectra of BC isolate and quercetin standard in methanol

A UV-Vis spectrophotometer analysis showed that BC isolate and standard quercetin exhibited two absorption wavelengths at 371 nm (band I) and 255 nm (band II). The analysis results using a UV-Vis spectrophotometer absorption band I and II bands on BC isolates have similarities with standard quercetin. Furthermore, the BC isolate was analyzed for the position of free hydroxyphenol groups in the flavonoid core, which can be determined by adding a shear reagent to the sample solution and observing the shift in the absorption peaks that occur. The shear reagents used were sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$), hydrochloric acid (HCl), sodium acetate (NaOAc), and boric acid (H_3BO_3). The results of the shear reagent from BC isolate can be analyzed for the location of the free hydroxy groups in the flavonoid compound by observing the shift in the absorption peaks. Interpretation of the position of the hydroxyl group in BC isolate can be seen in Table 5.

Table 5. Interpretation of the position of the hydroxyl groups in BC isolate with the addition of shear reagent

Shear Reactant	λ			λ shift		Interpretation
	Band I (nm)	New band (nm)	Band II (nm)	Band I (nm)	Band II (nm)	
Methanol	371	-	255	-	-	Flavonol
Methanol + NaOH	442	324	285	+71	+30	7-OH
Methanol + NaOH (5 minutes)	444	325	286	+73	+31	7-OH
Methanol + $AlCl_3$	431	-	266	+60	+11	3-OH (with or without 5-OH)
Methanol + $AlCl_3$ + HCl	428	-	266	+57	+11	3-OH (with or without 5-OH)
Methanol + NaOAc	373	-	256	+2	+1	Possibly 7-OH
Methanol + NaOAc + H_3BO_3	387	-	260	+16	+5	*o*-di OH in B ring

Based on Table 5, it is known that the flavonoid compound from BC isolate is quercetin. Furthermore, an analysis was carried out using an FTIR spectrophotometer to strengthen the prediction of the compound by indicating the presence of several functional groups. The results of FTIR analysis on BC isolates are shown in Figure 11.

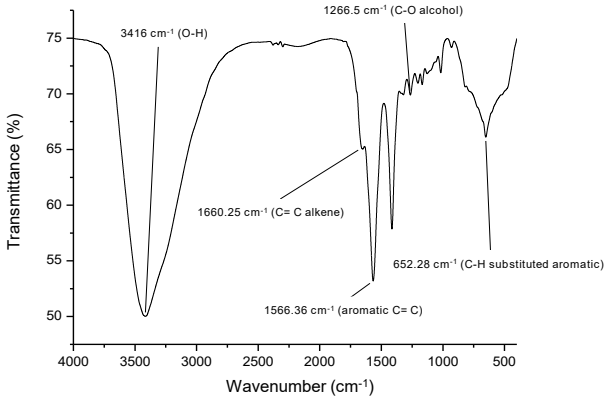


Figure 11. FTIR spectrum of BC isolate

The FTIR spectrum of BC isolate shows an absorption band in the 3550–3200 cm^{-1} region resulting from O-H stretching vibrations with an absorption peak at 3416 cm^{-1}, indicating the O-H groups. The absorption band in the 1680–1600 cm^{-1} corresponds to a C=C alkene stretching vibration with an absorption peak at 1660.25 cm^{-1}. An aromatic C=C stretching vibration appears at 1566.36 cm^{-1}. An absorption peak assigned at 1266 cm^{-1} is the C-O stretching vibrations of alcohol. The band at 652.28 cm^{-1} indicates the presence of out-of-

plane aromatic C-H vibrations (aromatic substitution) [7, 8]. The deconvolution results of IR spectra of BC isolate in the range of 4000–1500 cm^{-1} shows the presence of C-H aromatic and C=O vibrations at 3102.75 cm^{-1} and 1759.5 cm^{-1}, respectively.

BC isolates were then analyzed using LC-MS/MS to determine the purity, structure, and molecular weight of the compound. The results of the analysis of BC isolate using LC-MS are presented in Figure 12. The LC analysis showed that the isolate BC was pure because only one peak appeared at a retention time of 6.01 minutes. The spectrogram on the BC isolate can be seen in Figure 13.

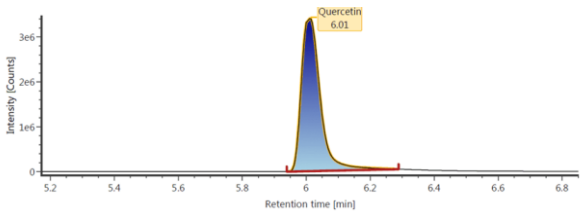


Figure 12. LC Chromatogram of BC isolate

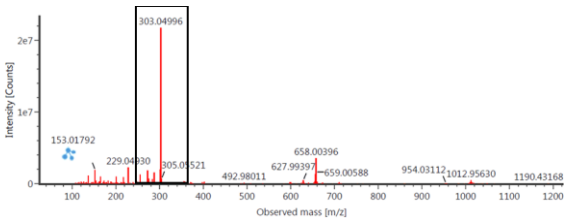


Figure 13. Mass spectrogram of BC isolate

The results of the BC isolate spectrogram showed [M+H]+ = 303.0499 (Figure 13), meaning that the compound's molecular weight is 302.04265 g/mol. This strengthens the results of UV-Vis spectrophotometer analysis using shear reagents. The results of FTIR analysis showed that the compound present in BC isolate was quercetin. These results are in accordance with Tao *et al.* [8], which reported that the highest flavonoid content was rutin. Hydrolysis was carried out in this research to hydrolyze the existing rutin to produce quercetin. The structure of quercetin can be seen in Figure 14. The pattern of quercetin fragmentation based on LCMS/MS results is shown in Figure 15.

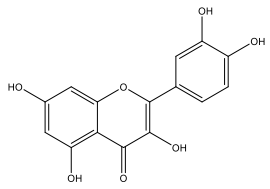


Figure 14. The structure of quercetin

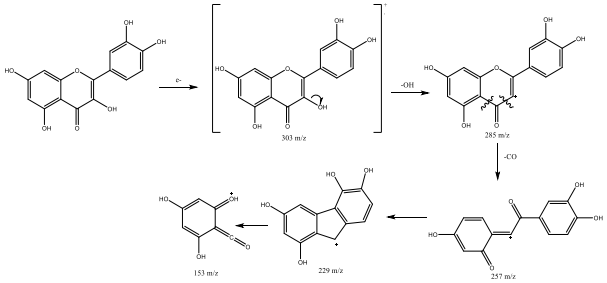


Figure 15. The pattern of quercetin fragmentation (BC isolate) based on LCMS/MS results

An antioxidant activity test was carried out on ethanol extract, BC isolate, and quercetin standard as a comparison to determine the IC_{50} value. The results of the antioxidant activity test are shown in Table 6.

Table 6. The antioxidant activities (IC_{50}) of quercetin standard, BC isolate, and ethanol extract of cassava leaves

Sample	IC_{50} (mg/L)
Quercetin standard	7.36 ± 0.039 mg/L
BC isolate	77.85 ± 4.708 mg/L
Ethanol extract from cassava leaves	81.76 ± 0.505 mg/L

Based on Table 6, BC isolate and ethanol extract had IC_{50} values of 77.85 ± 4.708 mg/L and 81.76 ± 0.505 mg/L, respectively. The IC_{50} value of BC isolate differed from that of quercetin because quercetin is a pure compound with very strong antioxidant activity. As for the BC isolate, there may still be antagonistic fabric/dirt compounds that result in lower antioxidant activity. According to Jun *et al.* [31], a strong antioxidant activity has an IC_{50} value of 50-100 mg/L.

This showed that BC isolate and ethanol extract had strong antioxidant activities. BC isolates had a strong antioxidant because it was a quercetin compound with five hydroxy groups. According to Cai *et al.* [32] and Lin *et al.* [33], a flavonoid compound with more hydroxy groups (one to six hydroxy groups) has increased antioxidant activity because more substituted hydroxy groups allow for more hydrogen atoms to be donated, which strengthens a molecule's potential to capture free radicals.

4. Conclusion

Simplicia and ethanol extract of cassava leaves contained alkaloids, flavonoids, saponins, tannins, quinones, steroids, phenolics, and triterpenoids. The total content of flavonoids in the ethanol extract was 35.71 mg of quercetin equivalent per gram of extract. Identification of BC isolates using a UV-Vis spectrophotometer showed that BC isolates had maximum absorption at a wavelength of 371 nm (band I) and 255 nm (band II). Identification of the structure using the shear reagent showed that BC isolate belonged to the flavonol group, with the possibility that there are hydroxy groups at C-3, C-5, C-7, C-3', and C-4'. Analysis using FTIR showed that BC isolate had the functional groups of O-H (3416 cm^{-1}), C-H aromatic (3102.75 cm^{-1}), C=O (1759.5 cm^{-1}), C=C aromatic (1566.36 cm^{-1}), C=C alkene (1660.25 cm^{-1}), C-O alcohol (1266.5 cm^{-1}), and C-H aromatic substitution (652.28 cm^{-1}). LC-MS/MS analysis revealed that the structure of the BC isolate was a quercetin compound. Ethanol extract and BC isolate had IC_{50} of 81.76 ± 0.505 mg/L and 77.85 ± 4.708 mg/L, respectively. BC isolate and ethanol extract had strong antioxidant activities.

Acknowledgment

This research was funded based on a Surat Penugasan Pelaksanaan Kegiatan Penelitian Sumber Dana Selain APBN Faculty of Science and Mathematics Diponegoro University 2022 No: 1263H/UN7.5.8/PP/2022.

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