DETERMINATION OF GLUCOSE CONTENT IN CELLULOSE DERIVED FROM BANANA STEM (*MUSA SPP.*) USING UV-VIS SPECTROPHOTOMETRY AND HPLC METHODS

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This study investigates the glucose content in hydrolyzed microcrystalline cellulose (MCC) derived from banana stem (*Musa* spp.) in a comparative analysis by two techniques: UV-Vis spectrophotometry and high-performance liquid chromatography (HPLC). MCC was isolated by delignification, bleaching, hydrothermal re-alkalization, and HCl hydrolysis, then characterized using physicochemical tests, FTIR, and XRD. Glucose levels were analyzed using two spectrophotometric methods (phenol-sulfuric acid and sulfuric acid–UV) and two HPLC systems (UV detector and refractive index detector). Results showed that banana stem MCC had properties similar to Avicel PH 101, but contained higher moisture and ash. The sulfuric acid–UV method provided accurate glucose quantification and is suitable for routine analysis, while HPLC-UV offered the highest sensitivity for detecting low glucose concentrations. Thus, UV-Vis spectrophotometry is more practical, whereas HPLC-UV is ideal for highly sensitive analyses.

Keywords: banana stem, glucose, microcrystalline cellulose, UV-Vis spectrophotometry, HPLC

INTRODUCTION

The banana plant, which belongs to the Musaceae family, is native to the Malaysia-Indonesia region in Southeast Asia. Bananas are widely cultivated and represent an abundant natural resource in tropical and subtropical countries.[1–3] The banana stem is a potential lignocellulosic material due to its high cellulose content, making it a promising source of glucose through the hydrolysis process.[4–6] The glucose obtained from the hydrolysis can be used as a raw material in various industries, such as bioethanol, pharmaceutical, and food industries.[7–9]

However, the utilization of lignocellulosic waste, such as banana stem, requires several steps, including cellulose isolation, purification, and characterization to ensure its quality.[10,11] One of the forms of cellulose obtained from isolation is microcrystalline cellulose (MCC), which possesses high functional properties and broad applicability.[12–16] To assess the efficiency of the conversion process, accurate and precise analytical methods are required to determine the glucose content resulting from cellulose hydrolysis. In addition, accurate determination of glucose levels in plant matrices is essential for evaluating their potential applications in industrial or biotechnological fields.

UV-Vis spectrophotometry and High Performance Liquid Chromatography (HPLC) are two commonly used analytical methods for glucose determination.[17–20] UV-Vis spectrophotometry offers advantages in terms of simplicity and low cost, as well as being fast and providing good precision and accuracy.[21,22] Meanwhile, HPLC is known as a highly sensitive and accurate method for compound separation and quantification, while also being relatively simple, fast, and economical.[21,23,24]

Although UV–Vis spectrophotometry and HPLC have been widely used in carbohydrate analysis, studies specifically comparing these methods for glucose determination in banana stems are still limited. Understanding the strengths and limitations of each method is crucial, particularly

in selecting the most appropriate technique based on the available resources in each laboratory. Therefore, this study was conducted to evaluate and compare the performance of UV–Vis spectrophotometry and HPLC methods in analyzing glucose from banana stem extract obtained through hydrolysis.

EXPERIMENTAL

Materials and equipment

The materials used in this study include 5% sodium hydroxide (NaOH) solution, 37% hydrochloric acid (HCl), 30% (w/w) hydrogen peroxide (H_2O_2) in water, 95–97% sulfuric acid (H_2SO_4), 0.1 N potassium permanganate solution, 10% potassium iodide solution, 0.1 N sodium thiosulfate solution, 0.2% starch solution, 0.1 N potassium dichromate solution, glucose solutions (1%, 2%, 4%, and 5%), distilled water, and Avicel PH 101 as the standard microcrystalline cellulose.

The equipment included a Shimadzu LC 2030 High Performance Liquid Chromatography system, a Nicolet Avatar 360 IR Fourier Transform Infrared Spectrophotometer, a Bruker D2 Phaser X-Ray Diffractometer, a pH meter (Mettler Toledo), and an analytical balance (Ohaus).

Sample preparation

A 100 g sample of banana stem was soaked in deionized water at 80 °C for 3 hours with continuous stirring, then rinsed with running water to remove soluble glucoses and impurities. The banana stem fibers were dried in an oven at 60 °C for 3 hours, cut into small pieces (~5 mm), and ground using a blender until a fine powder was obtained. The powder was then weighed until a constant weight was achieved.

Alkalization process

A total of 40 g of sample powder was mixed with 800 mL of 5% (w/v) sodium hydroxide (NaOH) solution and stirred for 2 hours at 90 °C. The ratio of the sample to sodium hydroxide was maintained at 1:20 (w/v). The mixture was then filtered and thoroughly washed with deionized water until a neutral pH (pH 7) was reached. Afterward, the material was dried and weighed to determine the yield.

Bleaching process

The second stage is known as the bleaching process, in which the remaining hemicelluloses and lignin are removed. This is done by adding 14 g of alkali-treated sample powder to 400 mL of 3% (v/v) hydrogen peroxide (H_2O_2) solution and 160 mL of 5% sodium hydroxide (NaOH) solution, maintaining a solid-to-liquid ratio of 1:40 (w/v). The mixture was stirred at 55 °C for 90 minutes using a hotplate stirrer, then cooled to a temperature of 27–30 °C and filtered using vacuum filtration. The bleached sample powder was washed with deionized water until the pH reached 6, then dried and weighed.

Hydrothermal re-alkalization

To remove residual lignin from the sample, a re-alkalization process was carried out using the same method as in the initial alkalization, with a modification from conventional thermal treatment to a hydrothermal process. The sample was mixed with 5% NaOH solution at a ratio of 1:20 (w/v), and the mixture was placed in an autoclave and heated at 90 °C for 1 hour. The resulting solid was then filtered and washed with deionized water until the pH reached 7, followed by drying at 60 °C.

Preparation of microcrystalline cellulose

The sample prepared in the previous process was hydrolyzed using hydrochloric acid (HCl) to produce microcrystalline cellulose (MCC). The sample powder was added to 3.5 N HCl solution at a ratio of 1:20 (w/v), and the mixture was heated at 70 °C for 90 minutes. The resulting solid was filtered and washed with distilled water until the supernatant reached a neutral pH. The solid residue was then separated by filtration to ensure that no unreacted cellulose remained in the microcrystalline cellulose powder. Finally, the powder was dried at 60 °C for 24 hours and stored for further analysis. The yield of microcrystalline cellulose was calculated using Equation 1:

$$\text{MCC Yield (\%)} = \frac{W1}{W2} \times 100\% \quad (1)$$

where W1 is the weight of the microcrystalline cellulose, and W2 is the weight of the sample fiber used during the isolation process.

Characterization of microcrystalline cellulose

The characterization of microcrystalline cellulose was carried out by measuring moisture content, ash content, pH, and swelling index, performed on both the isolated microcrystalline cellulose and commercial Avicel PH 101.

Sample hydrolysis

The microcrystalline cellulose sample was hydrolyzed using a sonicator at room temperature for 60 minutes.

Glucose determination by spectrophotometric method

Glucose determination was carried out using UV-Vis spectrophotometry with the phenol-sulfuric acid and sulfuric acid–UV methods, as developed by Albalasmeh.[25]

Glucose determination by HPLC method

Glucose separation was performed using an HPLC system equipped with two different detectors: a UV detector and a Refractive Index Detector (RID). The operational conditions of the HPLC-UV and HPLC-RID

systems shared several similarities as well as key differences. Both systems utilized an LC-2030 Controller with a low-pressure gradient mode and employed a mobile phase consisting of a mixture of water and acetonitrile.

The flow rate for the HPLC-UV system was 0.5 mL/min, slightly higher than that of the HPLC-RID system, which was 0.45 mL/min. The rising speed and sampling rate were the same for both systems, at 35 µL/s and 15 µL/s, respectively. The purging time was also consistent across both systems, at 10 minutes. However, only the HPLC-RID system featured an oven temperature control set at 30 °C, with an operational capacity up to a maximum temperature of 90 °C.

Another major difference was in the type of detector used: the HPLC-UV system employed an LC-2030 UV Detector with a detection wavelength of 195 nm,[26] while the HPLC-RID system used a RID-20A refractive index detector, which does not require wavelength settings.

RESULTS AND DISCUSSION

Physicochemical characteristics of microcrystalline cellulose

The microcrystalline cellulose (MCC) extracted from banana stem exhibited physicochemical characteristics that closely resemble those of the standard Avicel PH 101 (Table 1), although some notable differences were observed. In terms of moisture content, banana stem MCC showed a value of 2.65%, which is higher than that of Avicel PH 101 (0.915%). This higher moisture content may affect product stability, as lower moisture levels are preferable to prevent microbial growth and extend shelf life. Additionally, the ash content of banana stem MCC was also higher (1.248%) compared to Avicel PH 101 (0.249%), indicating the possible presence of inorganic residues from the raw material or incomplete purification during processing. Regarding pH, both materials had the same value of 5, suggesting compatibility in terms of acidity and supporting their potential use in pharmaceutical and food formulations, without risk of chemical incompatibility.

For the swelling index, banana stem MCC had a value of 1.2, slightly lower than Avicel PH 101 (1.25). This indicates that the water absorption and swelling capability of banana stem MCC are nearly comparable to the standard, making it suitable for applications requiring such properties, such as pharmaceutical tablets.

However, the permanganate number of banana stem MCC was higher (0.65) than that of Avicel PH 101 (0.42), suggesting the possible presence of impurities or partial cellulose degradation during the extraction process.

Table 1
Comparison of test results between banana stem microcrystalline cellulose and Avicel PH 101

Testing parameters	Microcrystalline cellulose	Avicel PH 101
Moisture content (%)	2.65	0.915
Ash content (%)	1.248	0.249
pH	5	5
Swelling index	1.2	1.25
Permanganate number	0.65	0.42

Overall, microcrystalline cellulose from banana stem demonstrates characteristics similar to those of Avicel PH 101 in aspects such as pH and swelling index. Nevertheless, the higher moisture and ash content suggest that further optimization of the purification process is needed to meet standard specifications. Despite these differences, banana stem-derived MCC shows promise as a sustainable alternative MCC source.

Characterization of microcrystalline cellulose by FTIR

The FTIR spectra (Fig. 1) compare the standard Avicel PH 101 (black) with the microcrystalline cellulose (MCC) sample isolated from banana stem (red). In general, both spectra exhibit similar patterns, indicating that the isolated MCC has a primary structure comparable to that of Avicel PH 101. Several characteristic absorption peaks can be observed, including the region around 3400–3500 cm^{-1}, which corresponds to O–H stretching vibrations of hydroxyl groups; around 2900 cm^{-1}, corresponding to C–H stretching vibrations; and peaks in the region of 1100–1000 cm^{-1}, which signify the presence of glycosidic bonds characteristic of the cellulose structure.

Nevertheless, there are a few minor differences between the spectra of banana-stem-derived MCC and Avicel PH 101. One example is a possible shift or change in intensity in the 3400–3500 cm^{-1} region, which may indicate differences in crystallinity or water content in the isolated MCC.

Additionally, variations in the region around 1500–1200 cm^{-1} may be due to the presence of residual hemicelluloses or lignin that were not completely removed during the isolation process. If the banana-stem MCC spectrum shows a high degree of similarity to that of Avicel PH 101, it can be concluded that the isolation process has successfully produced MCC with high purity.

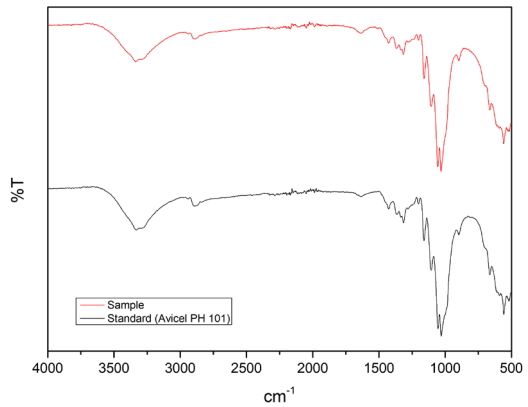

Figure 1: FTIR characterization of microcrystalline cellulose compared to Avicel PH 101

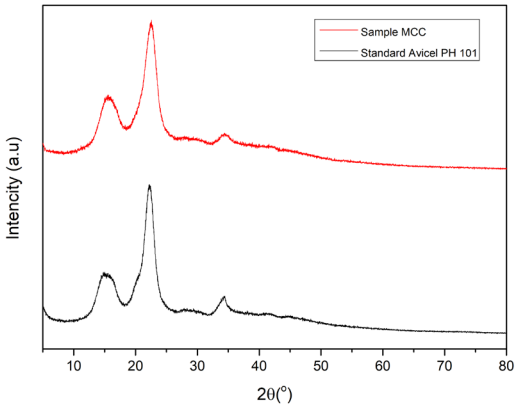

Figure 2: XRD patterns of (a) synthesized MCC and (b) Avicel PH 101 MCC

Characterization of microcrystalline cellulose using XRD

The XRD diffractogram (Fig. 2) compares the standard Avicel PH 101 (black) with the microcrystalline cellulose (MCC) sample isolated from banana stem (red). This diffraction pattern provides information on the degree of crystallinity and the structural arrangement of the produced MCC compared to the standard.

In general, both spectra exhibit similar crystalline peaks, particularly around 2θ = 15°, 22°, and 34°, which are characteristic of cellulose type I. The sharp peak observed at around 22° indicates the presence of crystalline regions, while the flatter parts of the spectrum suggest the presence of amorphous phases within the MCC structure. The similarity in the diffraction patterns between banana stem-derived MCC and Avicel PH 101 indicates that the isolation process successfully preserved the crystalline structure of cellulose.

However, there are several notable differences between the two diffractograms. The peak intensities of the banana stem MCC (red) appear lower than those of Avicel PH 101 (black), suggesting that the isolated MCC has a slightly lower degree of crystallinity. This may be attributed to factors such as the isolation method used, the possible presence of residual hemicelluloses or lignin, or degradation during the extraction process.

Additionally, if peak broadening is observed in the banana stem MCC compared to Avicel PH 101, it indicates an increase in the amorphous fraction within the material. A higher amorphous fraction is typically associated with reduced crystallinity, which can affect the physical properties of MCC, such as solubility and water-binding capacity.

Overall, the XRD results indicate that MCC derived from banana stem retains a crystalline structure comparable to that of Avicel PH 101, although with a slightly lower degree of crystallinity.

Glucose characterization using FTIR

The FTIR spectra (Fig. 3) compare the glucose standard (black) with the hydrolyzed product of microcrystalline cellulose (MCC) derived from banana stem (red). This analysis aims to confirm whether the hydrolysis product contains functional groups consistent with glucose as the final product.

In the FTIR spectrum of the glucose standard, several characteristic peaks indicate the presence of specific functional groups, such as: a broad absorption band around 3200–3500 cm^{-1}, corresponding to the stretching vibrations of hydroxyl (-OH) groups, which is typical for carbohydrate compounds; an absorption band around 2900 cm^{-1}, associated with the stretching vibrations of C-H bonds; absorption bands in the 1500–1000 cm^{-1} region, indicating the stretching vibrations of C-O bonds from hydroxyl and ether groups, which are key characteristics of glucose.

When compared with the sample spectrum (red), the absorption pattern shows a clear similarity to the glucose standard spectrum,

particularly in the regions around 3200–3500 cm^{-1} and 1000–1200 cm^{-1}. These similarities indicate the presence of hydroxyl groups and C–O bonds characteristic of glucose. This suggests that the hydrolysis product of MCC from banana stem contains compounds with spectral features closely resembling those of glucose.

However, differences in intensity or slight peak shifts may occur due to factors such as the degree of purity, the presence of residual cellulose oligomers or other compounds from the hydrolysis process, and potential structural changes caused by the extraction method used.

Overall, the similarity between the hydrolyzed MCC spectrum and the glucose standard spectrum confirms that the hydrolysis process successfully converted MCC derived from banana stem into glucose.

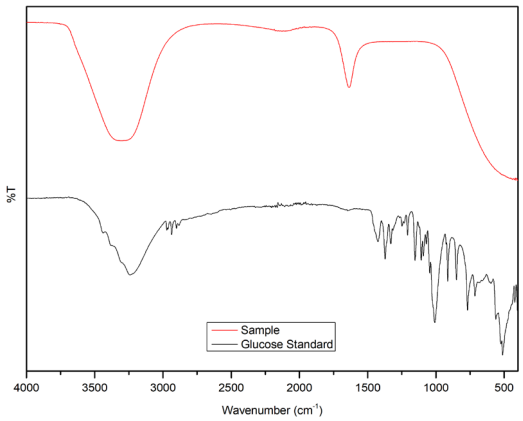

Figure 3: FTIR spectra of sample and glucose standard

Determination of glucose using the UV-Vis spectrophotometric method

The validation parameters evaluated in this study include linearity, limit of detection (LOD), limit of quantification (LOQ), and precision. Table 2 presents the results of the validation tests along with the acceptance criteria for the determination of glucose concentration in banana stem hydrolysate samples, using a comparative analysis between the phenol-sulfuric acid method and the sulfuric acid method.

Linearity

Linearity is one of the parameters used in the validation of an analytical method. The result is a linear curve, typically expressed as the coefficient of determination (R^2). Glucose measurement using the phenol-sulfuric acid method was performed at a wavelength of 490 nm, whereas glucose measurement using the sulfuric acid-UV method was conducted at a wavelength of 315 nm.[25]

In the phenol-sulfuric acid method, the regression equation obtained was $y = 11.566x + 0.0032$, with an R^2 value of 0.9999, indicating that nearly all variations in absorbance can be explained by the glucose concentration. An R^2 value approaching 1 suggests that the method exhibits excellent linearity.[27] Thus, it is capable of providing highly accurate predictions of glucose concentration based on the measured absorbance.

Meanwhile, the sulfuric acid-UV method yielded a regression equation of $y = 10.789x + 0.0163$, with an R^2 value of 0.9965. Although it still demonstrates good linearity, the R^2 value is slightly lower than that of the phenol-sulfuric acid method. This indicates that the method remains suitable for accurate glucose quantification, albeit with slightly greater variability in the measurement data compared to the phenol-sulfuric acid method.

Based on the regression equations, the sensitivity of the methods can be compared using the slope values (regression line gradients). The phenol-sulfuric acid method has a slope of 11.566, which is higher than that of the sulfuric acid-UV method (10.789). A greater slope value indicates higher sensitivity, as each increase in glucose concentration results in a larger change in absorbance. This higher sensitivity is advantageous for detecting low concentrations of glucose with better response performance.

However, in terms of precision, the sulfuric acid-UV method shows a higher intercept (0.0163) compared to the phenol-sulfuric acid method (0.0032). A higher intercept may indicate a greater influence of interfering factors in the sulfuric acid-

UV method, potentially arising from matrix effects or variability in measurement technique.

The phenol-sulfuric acid method is more recommended for analyses requiring high sensitivity, especially for detecting trace levels of glucose. With near-perfect linearity and a higher slope, this method offers more accurate results in low concentration ranges.

On the other hand, the sulfuric acid-UV method remains a suitable alternative, particularly when other factors such as ease of use, equipment availability, or robustness against matrix interferences are primary considerations. Although it exhibits slightly lower sensitivity, the method still demonstrates a strong linear relationship and can be reliably applied in various glucose analysis applications.

Overall, both methods exhibit excellent linearity, as indicated by their high R^2 values (>0.995). The phenol-sulfuric acid method outperforms in terms of sensitivity, whereas the sulfuric acid-UV method maintains good accuracy despite slightly higher variability in data. The choice of method should be guided by the specific analytical requirements, such as required detection limits, precision, and potential matrix interferences.[28]

Table 2
Glucose content test results determined using the spectrophotometric method

Parameter	Unit	Testing method	
		Phenol-sulfuric acid	Sulfuric acid-UV
Linearity		0.9999	0.9965
Regression equation		y = 11.566x + 0.0032	y = 10.789x + 0.0163
LOD	mg/mL	0.011	0.004
LOQ	mg/mL	0.036	0.012
Precision	%	3.352	0.677
Sample concentration	mg/mL	0.102	0.597

Limit of detection (LOD) and limit of quantification (LOQ)

The determination of the LOD (limit of detection) value is conducted to detect the lowest concentration of an analyte in a sample that can be identified by the instrument, though not necessarily quantifiable. On the other hand, the LOQ (limit of quantification), also known as the limit of reporting, is the lowest amount of analyte in a sample that can be quantified with appropriate precision and accuracy. In this study, the detection limit was calculated by dividing three times the standard deviation by the slope of the calibration curve for each method. The quantification limit was determined by dividing ten times the standard deviation by the slope value.[29–31] The LOD and LOQ values obtained for glucose concentration testing using the phenol-sulfuric acid method and the sulfuric acid method are presented in Table 2.

Based on Table 2, the sulfuric acid-UV method exhibits a lower LOD value (0.004 mg/mL) compared to the phenol-sulfuric acid method (0.011 mg/mL). This indicates that the sulfuric acid-UV method is more sensitive, as it can detect lower concentrations than the phenol-sulfuric acid method. The same trend is observed for the LOQ, where the sulfuric acid-UV method has a smaller LOQ value (0.012 mg/mL) compared to the phenol-sulfuric acid method (0.036 mg/mL). This suggests that the sulfuric acid-UV method can measure glucose concentrations with better accuracy and precision at lower levels compared to the phenol-sulfuric acid method.

Overall, these results demonstrate that the sulfuric acid-UV method outperforms the phenol-sulfuric acid method in terms of sensitivity and quantification limits. Therefore, for analyses requiring the detection of very low glucose concentrations, the sulfuric acid-UV method is recommended.

Determination of precision

Precision is an important parameter in the method validation process, expressed as repeatability, to assess random errors originating from sample preparation, solution preparation, filtration, and instrument conditions.[32] Precision testing in this study was conducted to assess the repeatability of the methods when performed multiple times by the same analyst, under identical conditions, laboratory settings, equipment, and reagents, within the same time frame. The first round of testing for both the phenol-sulfuric acid

method and the sulfuric acid-UV method was carried out for repeatability, as shown in Table 3.

Table 3
Glucose testing using the phenol-sulfuric acid method and sulfuric acid-UV method

Parameter	Phenol-sulfuric acid	Sulfuric acid-UV
Glucose concentration	0.102	0.597
SD	0.003	0.004
%RSD	3.352	0.677
2/3 CV Horwitz	6.771	5.193

Table 3 presents the total glucose content results using two methods: the phenol-sulfuric acid method and the sulfuric acid-UV method. The glucose concentration obtained from the phenol-sulfuric acid method is 0.102 g/L, while the sulfuric acid-UV method shows a higher value of 0.597 g/L. This difference may be attributed to the sensitivity of each method in detecting reducing and non-reducing glucose. In terms of precision, the sulfuric acid-UV method has a lower %RSD value (0.677) compared to the phenol-sulfuric acid method (3.352), indicating more consistent results. However, the standard deviation (SD) of the phenol-sulfuric acid method is smaller (0.003) than that of the sulfuric acid-UV method (0.004), suggesting that the former method has lower error in repeated measurements. Overall, the sulfuric acid-UV method excels in precision, whereas the phenol-sulfuric acid method is more stable in repeated measurements. Therefore, the selection of the more suitable method depends on the analysis objectives and the type of sample used.

Glucose determination using HPLC

Glucose determination in banana stem samples was performed using HPLC with both a refractive index (RI) detector and an ultraviolet (UV) detector at a maximum wavelength of 319 nm. The results of glucose determination in the samples using the chromatographic method are presented in Table 4.

Glucose analysis from banana stem using the HPLC-UV method showed consistent results with good precision. The retention times for the three samples were within the range of 0.268 to 0.269 minutes, reflecting the reproducibility of the method. The peak areas obtained showed slight variation, with values of 2756, 2068, and 2156, all remaining within reasonable limits. The glucose concentrations calculated from the calibration curve also showed uniformity, with values of 0.0202 mg/L, 0.0199 mg/L, and 0.01996 mg/L. The small differences in the repeatability results indicate that the HPLC-UV method is reliable for glucose quantification in banana stems.

Glucose analysis from banana stem using the HPLC-RI method showed stable and consistent results. The retention times for the three samples were within the range of 7.027 to 7.048 minutes, reflecting good method reproducibility. The peak areas obtained showed slight variation, with values of 23682905, 24909671, and 24779178, all within reasonable limits for repeat analysis. The glucose concentrations calculated from the calibration curve also showed uniform results, with values of 0.632 mg/L, 0.663 mg/L, and 0.660 mg/L. The small differences in these results suggest that the HPLC-RI method is reliable for glucose quantification in banana stems.

Table 4
Sample analysis results using HPLC-UV and HPLC-RI

Sample	Retention time (min)		Peak area		Sample concentration (mg/L)	
	HPLC-UV	HPLC-RI	HPLC- UV	HPLC-RI	HPLC-UV	HPLC-RI
1	0.268	7.048	2756	23682905	0.020201571	0.632
2	0.269	7.027	2068	24909671	0.019922856	0.663
3	0.269	7.032	2156	24779178	0.019958505	0.66

Table 5
Determination of linearity using the HPLC method

Testing method	Regression equation	Slope	Intercept	R^2	r
HPLC-UV	y =2468471.4286x + 47111	2468471.4286	47111	0.9947	0.9974
HPLC-RI	y =39386720.5116279x +1199160	39386720.5116	1199160	0.9945	0.9972

Determination of linearity

Linearity was determined by plotting a line between the concentration of the standard solution and the peak area. The results of the linearity determination are presented in Table 5.

From the linearity data (Table 5) for the glucose standard solutions obtained using HPLC-UV and HPLC-RI, it is evident that both methods exhibit a very good linear relationship between the standard solution concentration and the instrument response.

In the HPLC-UV method, the regression equation obtained is y = 2,468,471.4286x + 47.111, with a coefficient of determination (R^2) of 0.9947 and a correlation coefficient (r) of 0.9974. Meanwhile, the HPLC-RI method yields the regression equation y = 39,386,720.5116x + 1,199,160, with an R^2 of 0.9945 and r of 0.9972.

The R^2 values approaching 1 for both methods indicate that the measurement data exhibit a very high degree of linearity, meaning that changes in glucose concentration produce consistent detection responses from the instrument. The correlation coefficient (r) values, also close to 1, further reinforce that the relationship between concentration and detection signal is linear and strong.

From the slope values, it can be observed that the HPLC-RI method has a larger slope compared to HPLC-UV, indicating that this method produces a higher signal response to changes in glucose concentration. However, the relatively large intercept in HPLC-RI suggests the possibility of some bias in detection at low concentrations.

Overall, both methods demonstrate excellent linearity in glucose measurement from banana stem samples, making them highly reliable for accurate and precise quantitative glucose analysis.

Determination of LOD and LOQ

Table 6 presents the limit of detection (LOD) and limit of quantification (LOQ) data for glucose determination in banana stem samples using two High-Performance Liquid Chromatography (HPLC) methods: HPLC-UV and HPLC-RI.

In the HPLC-UV method, the LOD value is 0.0021 mg/L, while the LOQ is 0.0070 mg/L. The detected sample concentration with this method is 0.0200 mg/L, which is above the LOQ. This indicates that the HPLC-UV method exhibits good sensitivity, as the detected glucose concentration falls within the measurable range with good accuracy (above the LOQ).

For the HPLC-RI method, the LOD is 0.134 mg/L, and the LOQ is 0.447 mg/L. The detected sample concentration using this method is 0.6514 mg/L, which is also above the LOQ. This means that the HPLC-RI method can also measure glucose concentration in banana stems with good accuracy.

Overall, since the LOD and LOQ values for both methods are lower than the measured sample concentrations, the test results are reliable and valid. This indicates that the glucose concentration in banana stems detected by both methods is within the range that allows for accurate quantification. However, the HPLC-UV method has significantly lower LOD and LOQ values compared to HPLC-RI, suggesting that it is more sensitive in detecting glucose at low concentrations.

Table 6
Determination of LOD and LOQ using the HPLC method

Testing method	Sample concentration (mg/L)	LOD (mg/L)	LOQ (mg/L)
HPLC-UV	0.0200	0.0021	0.0070
HPLC-RI	0.6514	0.134	0.447

Table 7
Determination of precision using the HPLC method

Testing method	Sample concentration (mg/L)	%RSD	CV-Horwitz	2/3 CVHorwitz
HPLC-UV	0.0200	0.7573	28.8237	19.2158
HPLC-RI	0.6514	2.63	17.0663	11.3775

Determination of precision

Table 7 presents the results of glucose determination in banana stem samples using two High-Performance Liquid Chromatography (HPLC) methods: HPLC-UV and HPLC-RI. The data presented include the sample concentration, relative standard deviation (%RSD), CV-Horwitz value, and the 2/3 CV-Horwitz limit as acceptance criteria.

In the HPLC-UV method, the detected glucose concentration is 0.0200 mg/L, with an %RSD value of 0.7573%. This value is compared to the 2/3 CV-Horwitz limit, which is 19.2158, and the %RSD is within the acceptable range. Therefore, the HPLC-UV method is considered valid and acceptable for glucose determination in banana stems.

Meanwhile, the HPLC-RI method shows a higher glucose concentration of 0.6514 mg/L, with an %RSD of 2.63%. This value also remains below the 2/3 CV-Horwitz limit of 11.3775, thus meeting the acceptance criteria.

Overall, both HPLC methods used for glucose determination in banana stems yield acceptable results based on the CV-Horwitz criteria. Although the HPLC-RI method has a higher %RSD compared to HPLC-UV, both methods are within the allowed limits. Therefore, both methods can be used for glucose analysis in banana stems, with HPLC-UV showing better precision due to its lower %RSD.

Comparative study of UV-Vis Spectrophotometry and HPLC methods for glucose determination in banana stem samples

Table 8 shows that HPLC-UV is the best method for glucose determination in banana stems. This method exhibits good linearity; although UV-Vis Spectrophotometry with the phenol-sulfuric acid reagent demonstrates a higher R^2, the difference is relatively small and still within the acceptance limits. Moreover, HPLC-UV shows the highest sensitivity, with the lowest LOD and LOQ values compared to other methods. This enables the detection and quantification of glucose at very low concentrations, making it the superior method for analyzing samples with low glucose content.

In terms of precision, HPLC-UV also performs best, with the lowest RSD value (0.7573%) and the smallest standard deviation (SD) of 0.0002. This very low variability in results indicates that the HPLC-UV method provides more stable and reliable measurements compared to other methods. Therefore, HPLC-UV is recommended as the primary method for glucose analysis in banana stems, particularly if the necessary equipment is available.

Table 8
Comparison of UV-Vis Spectrophotometry and HPLC methods for glucose determination

Parameters	Unit	UV-Vis Spectrophotometry		HPLC	
		Phenol-sulfuric acid	Sulfuric acid-UV	UV	RI
1	mg/mL	0.1037	0.5994	0.0202	0.6317
2	mg/mL	0.0985	0.5920	0.0199	0.6629
3	mg/mL	0.1050	0.5985	0.0200	0.6596
Linearity		0.9999	0.9965	0.9947	0.9945
LOD	mg/mL	0.011	0.004	0.002	0.134
LOQ	mg/mL	0.036	0.012	0.007	0.447
Sample concentration	mg/mL	0.102	0.597	0.020	0.651
SD		0.003	0.004	0.0002	0.0171
RSD	%	3.352	0.677	0.7573	2.6261
2/3 CV Horwitz		6.771	5.193	19.2158	11.3775

Overall, when considering aspects such as equipment availability, cost, and result accuracy, the sulfuric acid-UV spectrophotometric method is the most balanced choice, as it offers high

precision, good sensitivity, and near-maximum quantification results. On the other hand, HPLC-UV is more suitable for applications requiring very high sensitivity, especially for samples with low glucose concentrations. The phenol-sulfuric acid method, while having the highest linearity, is less recommended due to its high RSD (>3%), indicating poorer precision. As for the HPLC-RI method, despite being able to detect the highest glucose levels, it has drawbacks in terms of sensitivity and precision, making it less ideal as the primary method.

CONCLUSION

Based on the evaluation and comparison of performance between UV–Vis spectrophotometry and HPLC methods for glucose analysis from hydrolyzed banana stem extract, it was found that both techniques have their own advantages. The UV–Vis spectrophotometric method, particularly using sulfuric acid-UV reagent, demonstrated excellent precision with a low RSD value (0.677%) and high glucose quantification result (0.597 mg/mL), making it a reliable, cost-effective, and accessible method for routine analysis. Meanwhile, the HPLC method with a UV detector showed the highest sensitivity, with the lowest LOD and LOQ values, making it suitable for detecting very low concentrations of glucose, although it produced lower quantification results and had a higher Horwitz value. On the other hand, the HPLC method with an RI detector and the spectrophotometric method using phenol-sulfuric acid reagents were found to have limitations in terms of sensitivity and precision. Therefore, the UV–Vis spectrophotometric method (sulfuric acid-UV) can be recommended as an efficient alternative for glucose analysis in banana stem extract samples, while HPLC-UV is more suitable for high-sensitivity analytical needs.

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