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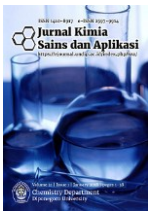

Total Phenolic Content Analysis, Antioxidant Activity, and Isolation of Phenolic Acid Compounds from Methanol Extract of Celery (*Apium graveolens* L.)

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Abstract

Celery (*Apium graveolens* L.), a member of the Apiaceae family, is rich in bioactive compounds and exhibits various health benefits, including antihypertensive, antibacterial, anti-inflammatory, and antioxidant activities. The presence of diverse phenolic acids, as identified in previous studies, prompted this research to analyze the total phenolic content, evaluate antioxidant activity, and isolate phenolic acids from the methanol extract of celery. The study involved phytochemical screening, maceration extraction, determination of total phenolics using the Folin–Ciocalteu method, antioxidant testing via the DPPH method, and phenolic acid isolation using three approaches: base hydrolysis (HB), acid hydrolysis (HA), and non-hydrolysis (TH). The isolates were analyzed using UV–Vis spectrophotometry, CMS, and LC/MS-MS QTOF. The methanol extract of celery yielded 16.59% and was found to contain flavonoids, phenolic acids, saponins, tannins, and steroids. The total phenolic content was 17.268 ± 0.057 mg GAE/g extract, while the IC_{50} value was 1712.782 ± 0.115 mg/L, indicating very weak antioxidant activity. Isolation of phenolic acids revealed that the HA fraction contained pyrogallol and caffeic acid. This was supported by the A3 band (λ_{max} 230 nm and 269 nm) with a molecular weight of 126 m/z, characteristic of pyrogallol. The A4 band (λ_{max} 218, 242, 298, and 325 nm) with a molecular weight of 180.0387 m/z was characteristic of caffeic acid.

1. Introduction

Celery (*Apium graveolens* L.) is a plant from the Apiaceae family known for its various health benefits, primarily attributed to its secondary metabolites, including flavonoids, phenolic acids, tannins, saponins, steroids, and essential oils [1]. Traditionally, it has been used in the treatment of several conditions, such as hypertension [2], hyperuricemia, bacterial infections [3], oxidative stress [4], diarrhea [5], inflammation, and fungal infections [6].

Celery has been reported to contain various types of phenolic acids [7, 8]. Sorour *et al.* [7] identified 15 phenolic acids in celery, with the four most abundant being ellagic acid, pyrogallol, chlorogenic acid, and gallic acid. Ashoush *et al.* [8], using the HPLC method, identified 22 phenolic acids, with pyrogallol, vanillic acid, ellagic acid, and chlorogenic acid as the predominant components. The novelty of the present study lies in the isolation of phenolic acids from celery, which, to the best of our knowledge, has not been previously reported. According to Zadernowski *et al.* [9], phenolic acids in plants may exist in free form, bound to sugars, or as esters. These compounds, belonging to the phenolic group, are known for their antioxidant properties. The antioxidant activity of a plant or extract is closely related to its total phenolic content [10]; the higher the phenolic content, the stronger the antioxidant activity.

Based on the diversity of phenolic acid compounds found in celery, this study aimed to isolate phenolic acids from the methanol extract of celery using three methods: alkaline hydrolysis, acid hydrolysis, and non-hydrolysis.

In addition, the total phenolic content and antioxidant activity were evaluated. Total phenolic content is a key parameter in assessing the antioxidant potential of plant materials, as phenolic compounds play a significant role in preventing oxidative damage [11]. The antioxidant activity of the methanol extract was measured to evaluate its capacity to scavenge free radicals, providing insight into celery's potential as a natural source of antioxidants [12].

2. Experimental

2.1. Materials and Tools

The materials used in this study included celery plants obtained from Bandungan, Semarang, and various analytical-grade chemicals such as methanol, chloroform, ethyl acetate, and diethyl ether (Smart-Lab); sodium hydroxide, sulfuric acid, hydrochloric acid, sodium carbonate, anhydrous sodium sulfate, ferric chloride, and the Folin–Ciocalteu reagent (Merck); n- hexane, sodium bicarbonate, acetic acid, amyl alcohol, acetic anhydride, and magnesium powder; and distilled water. Additional reagents included Dragendorff and Mayer reagents, and standards such as gallic acid, caffeic acid, and pyrogallol (Sigma-Aldrich); salicylic acid (Merck); DPPH (Sigma-Aldrich); and TLC plates coated with silica gel 60 GF254.

The equipment used included standard glassware, an analytical balance, a rotary evaporator (Büchi B-480), drop plates, micropipettes, a chromatographic chamber, a UV detector lamp (254 nm and 365 nm), a Genesys 10S UV-Vis spectrophotometer, an Advion Expression CMS, and an LC-MS/MS system (UPLC H-Class with a Xevo G2-S QToF mass spectrometer).

2.2. Preparation of Methanol Extract

A total of 12 kg of fresh celery plants was washed thoroughly, chopped, air-dried, and ground into a fine powder. Approximately 900 grams of the powdered material were subjected to maceration using methanol as the extraction solvent. The maceration process was carried out for six consecutive 24-hour periods, replacing the solvent each day until it became clear. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 50°C to obtain a viscous methanol extract [13].

2.3. Phytochemical Screening

Phytochemical screening was performed to detect the presence of major secondary metabolites in the dried celery plant powder (simplicia) and the methanol extract. The simplicia was tested using standard qualitative chemical assays as described by Ngelu *et al.* [14], including tests for flavonoids, phenolics, tannins, saponins, alkaloids, and steroids. The methanol extract was analyzed by thin-layer chromatography (TLC) following Pratiwi *et al.* [15], using silica gel GF254 plates and appropriate solvent systems. Visualization was done under UV light (254 nm and 365 nm) and by spraying with specific reagents to identify compound classes.

2.4. Determination of Total Phenolic Content

2.4.1. Preparation of Gallic Acid Standard Curve

A 1,000 ppm stock solution was prepared by dissolving 25 mg of gallic acid in methanol in a 25 mL volumetric flask. The stock solution was then diluted to obtain standard solutions with concentrations of 20, 30, 40, 50, and 60 ppm. For each concentration, 0.5 mL of the standard solution was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3, shaken, and incubated in the dark for 30 minutes. The absorbance of each mixture was measured at a wavelength range of 400–800 nm using a UV-Vis spectrophotometer. The resulting absorbance values were used to construct a standard calibration curve [16].

2.4.2. Determination of Total Phenolic Content

A 3,000 ppm extract solution was prepared by dissolving 75 mg of the methanol extract in methanol in a 25 mL volumetric flask, then diluted to obtain a 1,000 ppm solution. A total of 0.5 mL of the 1,000 ppm extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3, then shaken and incubated in the dark for 30 minutes. The absorbance was measured at 740 nm using a UV-Vis spectrophotometer, following the method reported in [17] with slight modifications. The total phenolic content was determined by applying the absorbance value to the linear equation of the gallic acid standard curve.

2.5. Isolation of Phenolic Acid Compounds

Phenolic acid compounds in plants may exist in three forms: free, bound to sugars, or as esters [17]. In this study, the isolation of phenolic acids from celery was conducted using three methods: acid hydrolysis, base hydrolysis, and non-hydrolysis [18].

2.5.1. Base Hydrolysis

The base hydrolysis method used in this study refers to the procedure reported by Fachriyah *et al.* [19] with slight modifications. A total of 10 grams of methanol extract was dissolved in methanol to a final volume of 100 mL, followed by defatting and dechlorophyllation. The resulting fat- and chlorophyll-free extract was treated with an excess of 2 N NaOH solution and hydrolyzed for 24 hours at room temperature in the dark. The hydrolysate was then acidified to pH 3 using 10% H_2SO_4 and extracted with 40 mL of ether thrice. The upper (non-polar) layer was collected and evaporated until approximately 50 mL of solution remained, then re-extracted with 32 mL of $NaHCO_3$. The resulting lower (polar) layer was acidified again to pH 3 with 10% H_2SO_4 and re-extracted with 40 mL of ether, forming two layers. The upper layer was collected and evaporated to dryness, yielding the base hydrolysis (HB) fraction.

2.5.2. Acid Hydrolysis

The acid hydrolysis procedure used in this study refers to the method described by Fachriyah *et al.* [19] with slight modifications. A total of 10 grams of methanol extract was dissolved in methanol to a final volume of 100 mL, followed by defatting and dechlorophyllation. The

resulting fat- and chlorophyll-free extract was then mixed with 40 mL of 4 N H_2SO_4 and hydrolyzed in a water bath at 60°C for 2 hours. The hydrolysate was extracted three times with 40 mL of ether. The upper (non-polar) layer was collected and evaporated until approximately 50 mL of solution remained, then re-extracted with 32 mL of 20% $NaHCO_3$. The lower (polar) layer was collected, acidified to pH 3 using 10% H_2SO_4, and extracted again with 40 mL of ether. The resulting upper layer was evaporated to dryness, yielding the acid hydrolysis (HA) fraction.

2.5.3. Non-Hydrolysis

The procedure of non-hydrolysis was carried out following the method reported by Fachriyah *et al.* [19] with slight modifications. A total of 10 grams of methanol extract was dissolved in methanol to a final volume of 100 mL, followed by defatting and dechlorophyllation. The resulting fat- and chlorophyll-free solution was extracted thrice with 40 mL of ether. The upper (non-polar) layer was collected and evaporated until approximately 50 mL of solution remained, then re-extracted with 32 mL of 20% $NaHCO_3$, yielding two layers. The lower (polar) layer was collected, acidified to pH 3 using 10% H_2SO_4, and re-extracted with 40 mL of ether, forming two layers. The upper layer was collected and evaporated to dryness to obtain the non-hydrolysis (TH) fraction.

2.6. Separation of Phenolic Acid Compounds

The HA, HB, and TH fractions were dissolved in methanol and analyzed by TLC, using standard phenolic acids (gallic acid, caffeic acid, salicylic acid, and pyrogallol) for comparison based on their Rf values. The stationary phase was silica gel 60 GF254, and the mobile phase consisted of chloroform: ethyl acetate: acetic acid (3:5:0.4). TLC plates were observed under UV light at 254 nm and 365 nm. The spots from each fraction were compared with those of the standards to identify similarities in Rf values [19].

The fraction displaying a spot with an Rf value matching one of the phenolic acid standards was further separated using preparative TLC with the same eluent. The dominant band was scraped off, dissolved in methanol, and filtered for further analysis.

2.7. Purity Test

The purity of the phenolic acid isolates obtained from preparative TLC was assessed using TLC with various eluents and two-dimensional TLC. The isolates were considered pure, as indicated by the presence of a single spot in each TLC run and in the two-dimensional TLC analysis [19].

2.8. Identification of Phenolic Acid Structure

The structure of the isolated phenolic acid was analyzed by measuring its λ_{max} using a UV-Vis spectrophotometer. The molecular weight of the compound was further determined using LC-MS/MS analysis.

2.9. Antioxidant Test

The antioxidant activity of the methanol extract of celery was evaluated using the DPPH method, as described by Prinanda *et al.* [20]. The procedure consisted of four main steps: solution preparation, maximum wavelength measurement, preparation of the gallic acid standard curve, and antioxidant testing of the extract.

2.9.1. Solution Preparation

A 40 ppm DPPH solution was prepared by dissolving 4 mg of DPPH in methanol in a 100 mL volumetric flask. A 100 ppm gallic acid stock solution was prepared by dissolving 2.5 mg of gallic acid in methanol in a 25 mL volumetric flask. Additionally, a 4,000 ppm extract stock solution was prepared by dissolving 100 mg of the methanol extract in methanol in a 25 mL volumetric flask.

2.9.2. Maximum Wavelength Measurement

To determine the λ_{max} value of DPPH, 3 mL of a 40 ppm DPPH solution was mixed with 1 mL of methanol. The solution was shaken and incubated in the dark for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer over the wavelength range of 400–600 nm. The resulting value was used as the blank absorbance.

2.9.3. Preparation of Gallic Acid Standard Curve

Gallic acid standards were prepared at concentrations of 2, 4, 6, 8, and 10 ppm in methanol. Each standard (1 mL) was reacted with 3 mL of 40 ppm DPPH solution, shaken, and incubated in the dark for 30 minutes. The absorbance was then measured at the previously determined λ_{max} using a UV-Vis spectrophotometer.

2.9.4. Antioxidant Test of Celery Methanol Extract

The 4,000 ppm extract stock solution was diluted to concentrations of 500, 1,000, 2,000, and 2,500 ppm. For each concentration, 1 mL of the extract was mixed with 3 mL of 40 ppm DPPH solution, shaken, and incubated in the dark for 30 minutes. The absorbance was measured at λ_{max} using a UV-Vis spectrophotometer. Antioxidant activity was evaluated by calculating the IC_{50} value, derived from the linear regression of the % inhibition versus concentration curve [19]. The percentage of DPPH radical scavenging activity (%inhibition) was calculated using Equation (1).

$$\%Inhibition = \frac{A_{control\,(DPPH)} - A_{sample}}{A_{control\,(DPPH)}} \times 100\% \quad (1)$$

3. Results and Discussion

3.1. Extraction Yield and Phytochemical Screening

Methanol extraction of celery produced a blackish-green paste weighing 149.31 grams, yielding 16.59%. Phytochemical screening was conducted on the simplicia and its methanol extract to identify the presence of secondary metabolites. The results are summarized in Table 1. These findings are consistent with the results reported by Shalsyabillah and Sari [21], who also observed the presence of flavonoids, tannins/phenolics, saponins, and steroids in celery plants.

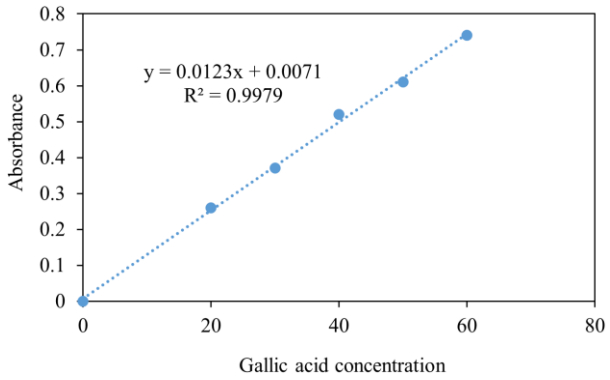

Figure 1. Gallic acid standard curve

Table 1. Phytochemical screening results of the simplicia and methanol extracts of celery plants

Secondary metabolites	Simplicia	Methanol extract
Alkaloid		
a) Dragendorff	(-)	(-)
b) Mayer	(-)	(-)
Flavonoid	(+)	(+)
Tannin/Phenolic	(+)	(+)
Saponin	(+)	(+)
Quinone	(-)	(-)
Steroid	(+)	(+)
Triterpenoid	(-)	(-)

(+) contains secondary metabolite compounds
(-) does not contain secondary metabolite compounds

3.2. Total Phenolic Content Determination

The total phenolic content was determined using a colorimetric method with the Folin–Ciocalteu reagent. A calibration curve was constructed using gallic acid as a standard, showing a linear relationship between phenolic concentration and absorbance. The absorbance measurements were conducted at a λ_{max} of 740 nm. The linear regression curve of gallic acid absorbance is shown in Figure 1.

Based on the total phenolic content determination, the standard curve for gallic acid was generated with a linear regression equation of y = 0.0123x + 0.0071 and a coefficient of determination (R^2) of 0.9979, as shown in Figure 1. Using this equation, the total phenolic content of the methanol extract of celery plants was calculated to be 17.268 ± 0.057 mg GAE/g extract. This result is consistent with the findings of Derouich *et al.* [22], but lower than those reported by Ashoush *et al.* [8] and Jung *et al.* [23].

The observed differences in phenolic content are likely influenced by several factors, including varietal differences and environmental conditions. Variations in environmental conditions, such as geographical location, climatic factors, and planting season, can significantly affect the biosynthesis of secondary metabolites. Meanwhile, genetic differences among plant varieties may influence both the composition and distribution of secondary metabolites, including phenolic compounds, as well as their antioxidant activity [24].

3.3. Phenolic Acid Isolation Results

3.3.1. Base Hydrolysis Method

The base hydrolysis method was employed to isolate phenolic acids in their esterified form. In this process, esters are hydrolyzed using an excess base to produce carboxylate salts, as illustrated in Figure 2. The resulting carboxylate salts were then acidified to regenerate free carboxylic acids, which are more soluble in organic solvents such as diethyl ether. Diethyl ether was used to extract phenolic acids by separating them from water-soluble impurities in the aqueous phase [25]. Using this method, a dry HB fraction weighing 0.175 grams was obtained. The fraction appeared greenish brown in color, with a yield of 1.75%.

3.3.2. Acid Hydrolysis Method

The isolation of phenolic acids by acid hydrolysis aims to release phenolic acids bound to sugar moieties as glycosides. This method uses strong acids to protonate the glycosidic bonds, resulting in the cleavage of the sugar-phenolic acid linkage. The reaction mechanism of this hydrolysis process is illustrated in Figure 3, which shows how acid treatment breaks down glycosidic bonds to free phenolic acids. The hydrolysis process disrupts the sugar-phenolic acid linkage by breaking down the glycosidic bond, allowing the formerly bound phenolic acids to become extractable. This is particularly important in plant-based samples, where a substantial proportion of phenolic acids may exist in conjugated forms with carbohydrates.

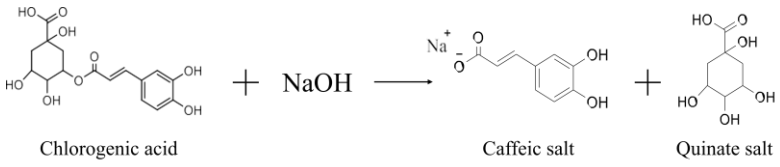

Figure 2. Hydrolysis reaction of esters by bases [26]

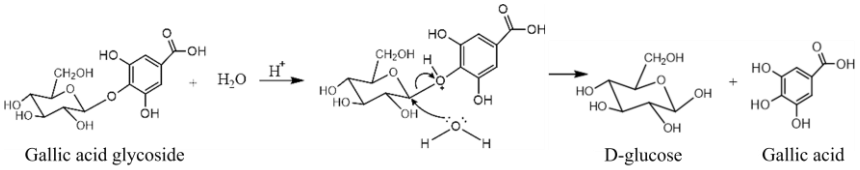

Figure 3. The hydrolysis reaction of sugar-bound phenolic acid using acid [27]

Following hydrolysis and extraction, a dry HA fraction was obtained with a weight of 0.105 grams. The isolated fraction appeared yellowish-brown in color and had a yield of 1.05%. The color of the fraction may indicate the presence of specific phenolic compounds or degradation products that can arise under acidic conditions. This result highlights the role of acid hydrolysis as an effective approach to isolate sugar-bound phenolic acids, complementing base hydrolysis, which targets ester-bound forms.

3.3.3. Non-Hydrolysis Method

The isolation of phenolic acids using the non-hydrolysis method targets the extraction of free phenolic acids naturally present in the sample without breaking any chemical bonds. This method yielded a dry TH fraction weighing 0.110 grams, with a blackish-brown coloration and a yield of 1.10%.

When comparing the amounts of phenolic acid isolated by different methods, the yield from base hydrolysis was the highest, followed by the non-hydrolysis method, and lastly, the acid hydrolysis method. This trend can be attributed to the fact that the excess base used in base hydrolysis is more effective at breaking both ester and glycosidic bonds, thereby releasing more bound phenolic acids.

The phenolic acid yields obtained from the non-hydrolysis and acid hydrolysis methods were very similar, differing by only 0.05%. The lower yield from acid hydrolysis compared to base hydrolysis may result from the milder hydrolytic effect of the acid and the shorter hydrolysis time employed. In contrast, the longer hydrolysis duration and stronger basic conditions likely enhanced the cleavage of bound phenolic compounds in the base hydrolysis method.

3.4. Separation of Phenolic Acid Compounds

The HA, HB, and TH fractions obtained from the hydrolysis and non-hydrolysis methods were analyzed by TLC to separate and identify the phenolic acid components. The TLC analysis was performed using silica gel GF254 as the stationary phase and a mobile phase consisting of a mixture of chloroform: ethyl acetate: acetic acid (3:5:0.4). The chromatograms of the three fractions were compared to standard phenolic acids, namely gallic acid (AG), caffeic acid (KF), salicylic acid (AS), and pyrogallol (PG), as shown in Figure 4.

After development, the TLC plates were observed under UV light at 254 nm and 365 nm to detect the presence of phenolic compounds. The Rf values of each spot from the HA, HB, and TH fractions, along with the phenolic acid standards, are summarized in Table 2. Based on the data, spot 3 from each fraction exhibited an Rf value of 0.754, closely matching that of standard pyrogallol. This identification was further confirmed by post-chromatographic visualization using 2% $FeCl_3$ spray reagent, as presented in Figure 5, where the $FeCl_3$ reacted with the phenolic hydroxyl groups to give characteristic colored spots.

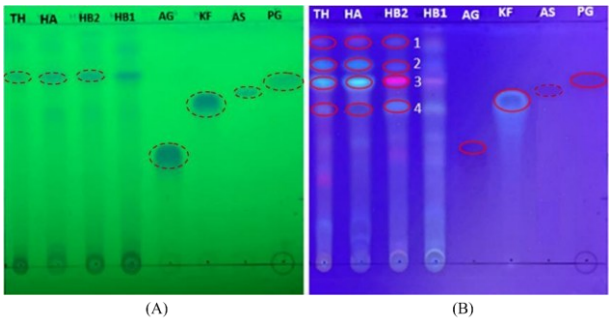

Figure 4. TLC profiles of HA, HB, TH fractions, and standard phenolic acids. (A) Observed under UV light at 254 nm; (B) observed under UV light at 365 nm

Table 2. Rf values of phenolic compounds in HB, HA, and TH fractions compared to standard phenolic acids

Compound	Number of spots	Rf value			
		Spot 1	Spot 2	Spot 3	Spot 4
HB	4	0.923	0.812	0.747	0.669
HA	4	0.907	0.815	0.754	0.662
TH	4	0.907	0.815	0.754	0.646
AG	1	0.461	-	-	-
KF	1	0.662	-	-	-
AS	1	0.707	-	-	-
PG	1	0.754	-	-	-

Among the three fractions, the HA fraction showed the most intense and dominant staining in Figure 5, indicating a higher concentration of phenolic content. Therefore, the HA fraction was selected for further separation using preparative TLC. Notably, spot 4 in the HA fraction exhibited an Rf value of 0.662, corresponding to standard caffeic acid. These findings suggest that the methanol extract of celery in the HA fraction contains pyrogallol and caffeic acid. This result is consistent with previous studies [7, 8], which also reported the presence of pyrogallol and caffeic acid in celery extracts.

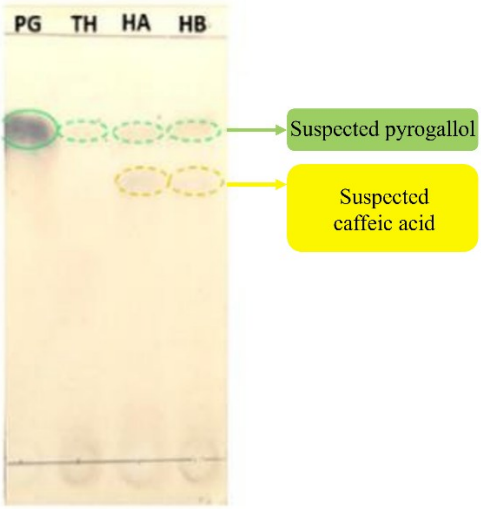

Figure 5. TLC results of HB, HA, TH fractions, and pyrogallol standards with chloroform: ethyl acetate: acetic acid (3:5:0.4) eluent sprayed with 2% $FeCl_3$

Table 3. Rf values of isolates A3 and A4 using various eluents, compared to standard phenolic acids

Eluent system	Rf value			
	A3	A4	Pyrogallol	Caffeic acid
C: EA: AA (3:5:0.4)	0.754	0.662	0.754	0.662
EA: M (1:1)	0.785	0.738	-	-
M: C (3:2)	0.646	-	-	-
M: C (1:2)	-	0.769	-	-
Chloroform	-	-	-	-

C = chloroform, EA = ethyl acetate, M = methanol, AA = acetic acid; " –" indicates no spot observed

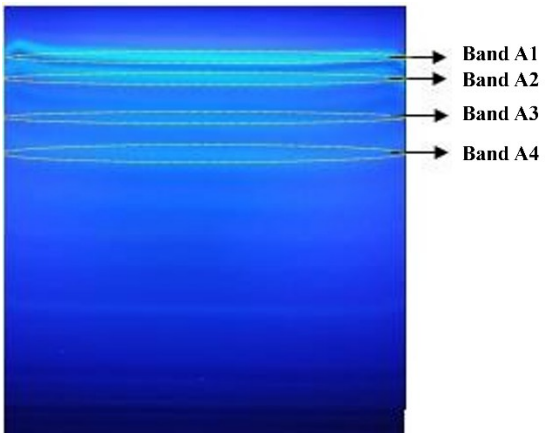

Figure 6. Preparative TLC chromatogram of the HA fraction under UV light at 365 nm

3.4.1. Separation of HA Fractions by Preparative TLC

The HA fraction was further purified using preparative TLC to isolate individual phenolic acid compounds. The separation was performed on a silica gel GF254-coated glass plate as the stationary phase, using a mobile phase consisting of chloroform: ethyl acetate: acetic acid (3:5:0.4). The preparative TLC chromatogram of the HA fraction is presented in Figure 6.

Based on the Rf values obtained from the earlier analytical TLC results, isolate A3 is suspected to correspond to pyrogallol, while isolate A4 is presumed to represent caffeic acid. These identifications were based on the similarity of Rf values between the isolates and their respective standards. To confirm the identity of the isolated compounds, both A3 and A4 were subjected to UV-Vis spectrophotometric analysis, and their spectra were compared with those of authentic phenolic acid standards.

3.5. Purity Test Results

3.5.1. Purity Test of Isolates A3 and A4

The purity of isolates A3 and A4 was evaluated using TLC with various eluent systems and two-dimensional (2D) TLC. The goal was to ensure that each isolate contained only a single compound, with no co-eluting impurities. The TLC results using different solvent systems are summarized in Table 3.

Table 3 shows that both isolates A3 and A4 consistently produced a single spot across multiple solvent systems, indicating high purity. In particular, their Rf values in the chloroform: ethyl acetate: acetic acid (3:5:0.4) system matched precisely with the standard compounds pyrogallol (for A3) and caffeic acid (for A4).

Further confirmation was obtained by conducting two-dimensional TLC. In this technique, the plate was first developed in one solvent system, then rotated 90° and developed in a second solvent. Both A3 and A4 exhibited a single spot in both directions, with no evidence of additional components. This confirms that both isolates are chemically pure and suitable for further characterization or bioactivity testing.

3.6. Phenolic Acid Structure Identification

3.6.1. Structural Identification of Isolate A3

The chemical structure of isolate A3 was identified using UV-Vis spectroscopy and mass spectrometry (MS). The UV-Vis absorption spectra of isolate A3 and the standard compound pyrogallol are presented in Figure 7. As shown in Figure 7, isolate A3 exhibits two major absorption peaks (λ_{max}) at 230 nm and 268 nm, which closely match the λ_{max} values of the pyrogallol standard at 229 nm and 267 nm, respectively. These absorption bands are characteristic of aromatic phenolic compounds.

This observation is further supported by previous findings from S. *et al.* [28], who reported pyrogallol absorption peaks at 228 nm and 265 nm. The slight variation in wavelength may be due to solvent effects or instrument calibration differences. To further confirm the molecular identity, isolate A3 was subjected to mass spectrometric analysis, and the resulting spectrum is shown in Figure 8.

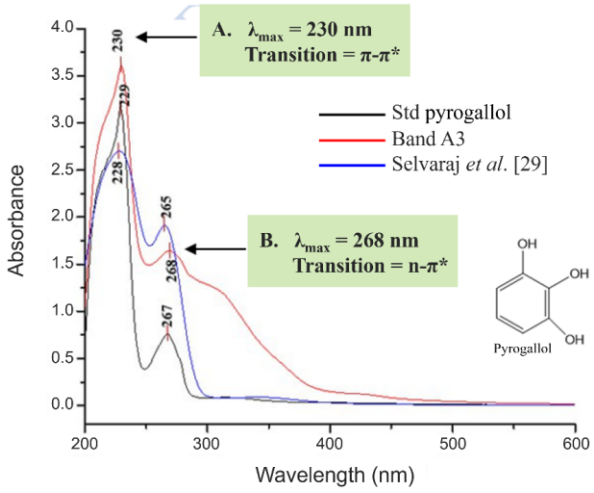

Figure 7. UV-Vis spectra of isolate A3 and pyrogallol standard

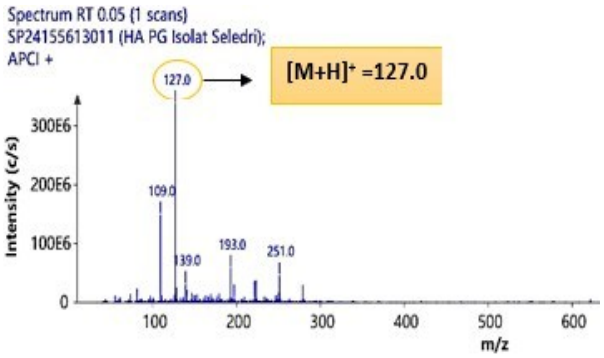

Figure 8. Mass spectrum of isolate A3

The mass spectrogram reveals a prominent peak at m/z 127.0, corresponding to the $[M+H]^+$ ion. This indicates that the molecular ion [M] has a mass of 126.0 m/z, which matches the molecular weight of pyrogallol ($C_6H_6O_3$). The high-intensity peak at this value suggests that pyrogallol is the major compound present in isolate A3. The UV-Vis spectral data and mass spectrometry results confirm that isolate A3 is identified as pyrogallol.

3.6.2. Structural Identification of Isolate A4

The chemical structure of isolate A4 was analyzed using UV-Vis spectroscopy and LC-MS/MS. The UV-Vis absorption spectra of isolate A4 and the standard compound caffeic acid are shown in Figure 9. The isolate A4 displays four absorption peaks at 218, 242, 298, and 325 nm, which closely correspond to the standard caffeic acid absorption peaks at 219, 241, 297, and 322 nm, respectively. These results are in agreement with previous findings by Catauro *et al.* [29], who reported caffeic acid absorption maxima at 216, 242, 296, and 324 nm. The spectral similarity strongly suggests that isolate A4 contains caffeic acid. Further confirmation was carried out through LC-MS/MS analysis. The LC chromatogram of isolate A4 is presented in Figure 10.

As shown in Figure 10, although several peaks are present—indicating the presence of impurities—the major peak at a retention time of 4.98 minutes accounts for 74.94% of the total peak area, suggesting that caffeic acid is the dominant component in isolate A4. To determine the molecular identity of this dominant compound, the peak at 4.98 minutes was further analyzed using a QToF (Quadrupole Time-of-Flight) mass spectrometer. The mass spectrum is shown in Figure 11.

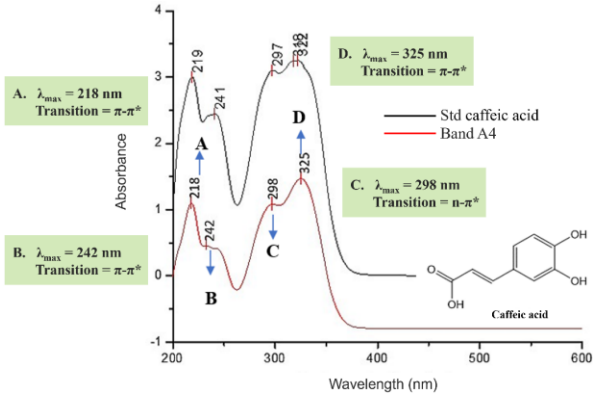

Figure 9. UV-Vis spectra of isolate A4 and caffeic acid standard

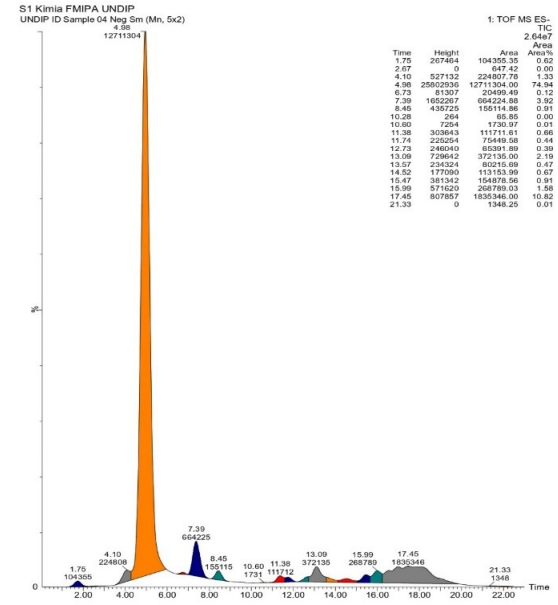

Figure 10. LC chromatogram of isolate A4

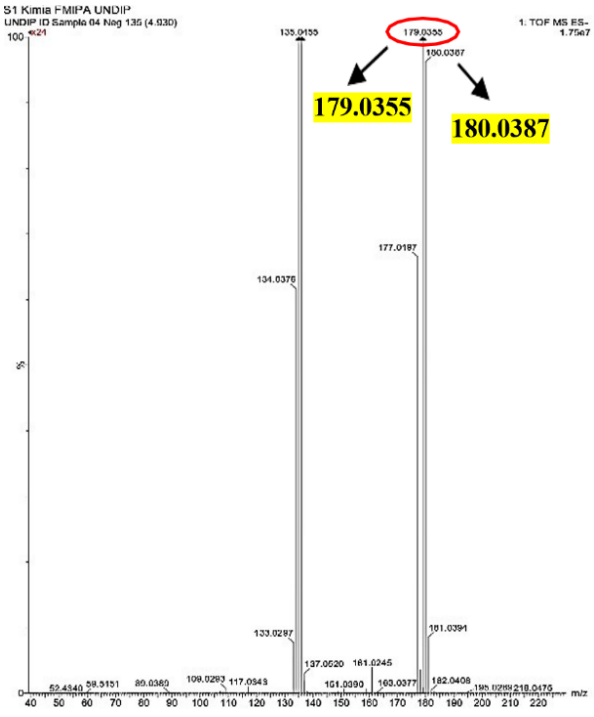

Figure 11. Mass spectrum of isolate A4

The mass spectrometric analysis revealed a major peak at [M+H]+ = 179.0355 m/z, corresponding to a neutral molecular mass [M] = 180.0387 m/z, which matches the molecular weight of caffeic acid ($C_9H_8O_4$). Based on the combined evidence from UV-Vis spectroscopy, LC retention time, and high-resolution mass spectrometry, isolate A4 is identified as caffeic acid, despite minor impurities. The molecular structure of caffeic acid is shown in Figure 12.

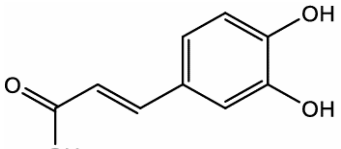

Figure 12. Structure of caffeic acid

Table 4. Inhibition values and absorbance of methanol extract solutions from the celery plant

Concentration (ppm)	Absorbance		Sample	Sample	% Inhibition
	1	2	1	2	
2500	0.217	0.215	0.495	0.498	69.684
2000	0.310	0.313	0.402	0.400	56.281
1500	0.391	0.389	0.321	0.324	45.263
1000	0.480	0.485	0.232	0.228	32.281
500	0.571	0.572	0.141	0.141	19.789
Positive control	0.712	0.713	-	-	-

3.7. Antioxidant Activity

The absorbance values and inhibition percentages of celery methanol extract solutions at various concentrations are shown in Table 4. As shown in Table 4 and illustrated in Figure 3, the percentage of DPPH inhibition increases as the concentration of the methanol extract increases. This indicates that the higher the extract concentration, the stronger the DPPH radical scavenging activity, which correlates with a lower absorbance value due to reduced purple color intensity. This trend suggests a dose-dependent antioxidant effect.

The antioxidant strength of a sample is commonly expressed by its IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals. A lower IC_{50} value reflects stronger antioxidant activity [30]. The results of the quantitative antioxidant test are presented in Table 5. The IC_{50} value of gallic acid was 2.86 mg/L, whereas that of celery methanol extract was 1712.782 ± 0.115 mg/L. This result is consistent with the study conducted by Sukweenadhi *et al.* [31], which reported an IC_{50} of 2221.00 mg/L. However, it significantly differs from the findings of Jung *et al.* [23], likely due to species variation and differing growing environments, as their samples were obtained from Korea.

According to the classification by Blois [32], antioxidant activity is categorized as follows: <50 mg/L (very strong), 50–100 mg/L (strong), 100–150 mg/L (moderate), 150–200 mg/L (weak), and >500 mg/L (very weak). The celery methanol extract in this study, with an IC_{50} above 1700 mg/L, falls into the very weak category.

The low antioxidant activity of the celery methanol extract may be attributed to several factors, including the sample's origin, species differences, and the nature of the extract, which contains a complex mixture of compounds unlike pure gallic acid. Additionally, the type of solvent used plays a crucial role. For instance, a study by Nurmiati *et al.* [33] on the celery water extract from North Sulawesi reported an IC_{50} of 23.71 mg/L, indicating very strong antioxidant activity, highlighting the influence of the extraction method on antioxidant potential.

Table 5. Results of IC_{50} value calculations in the antioxidant activity test

Sample	IC_{50} (mg/L)	Information
Gallic acid standard	2.86	Very strong
Celery methanol extract	1.712,782 ± 0.115	Very weak

4. Conclusion

The celery plant was found to contain various secondary metabolites, including flavonoids, phenolics, tannins, saponins, and steroids. Phenolic acid isolates were successfully obtained through three different methods: base hydrolysis (1.75%), acid hydrolysis (1.05%), and extraction without hydrolysis (1.10%). Structural analysis identified isolate A3 as pyrogallol and isolate A4 as caffeic acid. In addition, the methanol extract of celery exhibited a total phenolic content (TPC) of 17.268 ± 0.057 mg GAE/g extract. However, its antioxidant capacity was relatively low, with an IC_{50} value of 1.712,782 ± 0.115 mg/L, categorizing it as having "very weak" antioxidant activity. These results indicate that while celery contains phenolic compounds, their antioxidant potency is limited.

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