Ripeness-Dependent Variation in Phenolic, Flavonoid Content, and Antioxidant Activity of Plantain (Musa paradisiaca 'ABB') Peel Extracts Using Single and Mixed Solvent Systems

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Ripeness-Dependent Variation in Phenolic, Flavonoid Content, and Antioxidant Activity of Plantain (*Musa paradisiaca* 'ABB') Peel Extracts Using Single and Mixed Solvent Systems

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Abstract: *This study investigates the impact of ripeness and solvent system composition on the extraction efficiency of phenolic and flavonoid compounds from plantain (Musa paradisiaca 'ABB') peels and their associated antioxidant activities. Peels were collected at two ripeness stages (unripe and ripe), and extractions were performed using single, binary, ternary, and quaternary solvent combinations of water, methanol, ethanol, and acetone. Total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity were assessed spectrophotometrically. Results demonstrated that ripe peels consistently yielded higher TPC, TFC, and DPPH scavenging activity compared to unripe ones. Among solvent systems, ternary combinations—particularly water:ethanol:acetone (1:1:1)—exhibited the highest bioactive recovery and antioxidant potential. Correlation analysis revealed strong positive interdependence between TPC, TFC, and antioxidant activity ($r > 0.87$), highlighting the contributory role of polyphenols in free radical scavenging. Principal component analysis (PCA) and two-way ANOVA further supported the significant effects of both ripeness and solvent system on extract quality. This work provides a scientific basis for optimizing plantain peel utilization in functional food or green chemistry applications.*

Keywords: *Plantain peel; ripeness; total phenolic content; total flavonoid content; antioxidant activity; green solvent extraction;*

1. INTRODUCTION

Plantain, (*Musa acuminata* × *balbisiana*, ABB genome group) or commonly known as *Pisang kepok* in Indonesia, is a triploid hybrid cultivar extensively cultivated across Southeast Asia, including Indonesia, the Philippines, Malaysia, and Thailand [1][2]. Characterized by its large pseudostem and angular-shaped fruits, plantain is predominantly consumed after cooking due to its starchy texture, and it holds significant agronomic and economic importance in the region [3]. Indonesia, for instance, reports a monthly production exceeding 900 tons in regions such as East Kutai Regency, highlighting its relevance as a staple crop [4].

The *plantain* peel, which constitutes approximately 40% of the total fruit biomass, is often discarded as agricultural waste despite its rich composition of bioactive secondary metabolites including polyphenols, flavonoids, alkaloids, steroids, tannins, saponins, and triterpenoids [5]. Among these, phenolic compounds—particularly catechins—are recognized for their potent antioxidant properties and ability to act as electron donors, thereby neutralizing free radicals and reducing oxidative stress [6], [7].

The biochemical composition and antioxidant potential of banana peel are known to be influenced by the fruit's maturation and ripening processes, which modulate the synthesis, degradation, and polymerization of phenolic compounds and flavonoids. Ripening induces physiological and metabolic changes that can either enhance or diminish the concentration of bioactive constituents, subsequently affecting the antioxidant capacity as measured by free radical scavenging assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) [8]. Despite the established importance of ripeness on phenolic profiles in various fruits, comprehensive investigations specific to plantain peel remain scarce.

Moreover, the extraction of phenolic compounds from plant matrices is highly dependent on the choice of solvent system. Conventional extraction approaches commonly employ single or binary solvent mixtures, which may be suboptimal due to the complex chemical nature and polarity diversity of phenolic compounds [9]. This study proposes in exploring the effect of single, binary, ternary, and quaternary solvent extraction system, to enhance the solubilization and recovery efficiency of total phenolic and flavonoid compounds from plantain peel. Optimizing extraction protocols is critical for maximizing yield and preserving bioactivity, which are essential for subsequent applications in food, pharmaceutical, and environmental sectors [9].

The objectives of this study are twofold: (1) to elucidate the influence of ripeness stages on the total phenolic content, total flavonoid content, and antioxidant activity (DPPH radical scavenging) of plantain peel extracts; and (2) to evaluate the efficacy of a quaternary solvent system for phenolic compound extraction compared to conventional solvent systems.

This research holds significance for the valorization of banana peel waste, contributing to the sustainable utilization of agro-industrial by-products by identifying optimal harvesting conditions and advanced extraction methodologies. Enhanced recovery of antioxidant compounds can facilitate the development of natural antioxidant formulations, functional ingredients, and bioactive agents, supporting health-related applications and environmental sustainability initiatives such as green synthesis of nanomaterials and waste-to-resource strategies.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Plantain peels from *Musa paradisiaca* 'ABB' were sourced from a plantation located at coordinates –6.2302917, 107.0529099. The samples were harvested and sorted byt the ripening stages, as determined by standardized peel color indices [10]. Post-harvest, the

peels were manually separated, washed thoroughly with distilled water to remove surface contaminants, and cut into uniform sections. The samples were then oven-dried at 50 ± 2°C until a constant weight was achieved, to prevent enzymatic degradation. The dried material was subsequently milled into a fine powder using a laboratory-grade grinder and stored in airtight containers in -20^{O}C freezer until use.

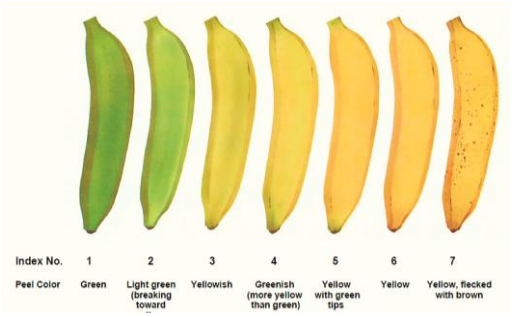

Fig. 1. Banana ripening guide[10]

2.2 Extraction Procedure

Phenolic and flavonoid compounds were extracted from plantain peel powder using various solvent systems, including single solvent, binary, ternary, and quaternary combinations of distilled water, ethanol, and acetone in different volumetric ratios. A solid-to-solvent ratio of 1:5 (w/v) was maintained for all treatments, wherein 1 g of peel powder was mixed with 5 mL of the respective solvent system. The extraction process was conducted at 75 ± 1°C for 120 minutes under continuous agitation using an orbital shaker to enhance solvent penetration and mass transfer. Following extraction, the mixtures were allowed to cool to room temperature and filtered using Whatman No. 1 filter paper. The filtrates were subsequently collected, aliquoted into amber vials to prevent light-induced degradation, and stored at -4°C until further analyses of phytochemical contents, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity via DPPH radical scavenging assay.

2.3 Determination of Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu method stated in Dahanayake *et al.*, 2019 with slight modifications [11]. In this assay, 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated for 5 minutes at room temperature. Then, 2 mL of 7.5% sodium carbonate (Na_2CO_3) was added to the mixture. The reaction was incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Gallic acid was used as a standard, and results were expressed as mg gallic acid equivalent (GAE) per gram of dry weight of sample.

2.4 Determination of Total Flavonoid Content (TFC)

The total flavonoid content was measured using the aluminum chloride colorimetric method [11]. In this assay, 0.5 mL of the extract was mixed with 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. The mixture was incubated at room temperature for 30 minutes, and absorbance was recorded at 415 nm. Quercetin was used as the calibration standard, and results were expressed as mg quercetin equivalent (QE) per gram of dry weight of sample.

2.5 DPPH Radical Scavenging Activity

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [11]. A 0.1 mM solution of DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 1.0 mL of the extract at various concentrations. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm. The scavenging activity was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \left(\frac{\text{Ac} - \text{As}}{\text{Ac}}\right) \times 100\%$$

With Ac as the absorbance of control and As as the absorbance of sample.

3. RESULTS AND DISCUSSION

Plantain peels were obtained from *Musa paradisiaca* 'ABB', harvested at a plantation located at coordinates – 6.2302917, 107.0529099. The fruits were categorized into two groups based on the USDA plantain ripening scale [10]. The first group, coded as U (Unripe), consisted of plantains at ripening stages 1 and 2 with predominantly green peels. The second group, R (Ripe), comprised plantains at stages 3 and 4, with greenish yellow peel coloration. The average peel-to-fruit mass ratio was 48.27% for the unripe group and 44.23% for the ripe group.

Table 1. Peel to fruit ratio and water content of plantain peel

Plantain peel	Peel to Fruit Ratio (%)	Water content (%)	Water content of dried peel powder (%)
Unripe (U)	48,2727	84,8071	6,0145
Ripe (R)	44,2241	83,8365	6,2106

Fresh peels were thoroughly washed and oven-dried at 50°C until constant weight was achieved. Moisture content was determined in triplicate for both fresh peels and powdered samples. Unripe plantain peels exhibited a higher average of initial moisture content (84.81%) than ripe peels (83.84%), which is consistent with findings by Pham et al. (2022), indicating that greener plantain peels retain more water [12]. This higher moisture content may hinder solvent penetration during extraction, potentially reducing the extraction yield and efficiency. Hence, ripeness-based sorting of plantain peels is recommended to ensure homogeneity and reproducibility in extraction processes.

Drying was conducted not only to reduce moisture content but also to inhibit microbial metabolism that may alter the concentration and integrity of secondary metabolites, including phenolic compounds.

Additionally, drying suppresses unwanted activity of polyphenol oxidase (PPO) [13], which is naturally present in plantain peels and associated with enzymatic browning, which is phenolic degradation during fruit ripening.

Following drying, the peels were ground and sieved to obtain a 200-mesh powder (~76 µm particle size). The powder was stored in dark containers at –4°C to protect phenolic constituents from thermal degradation, enzymatic oxidation, and photolytic damage. This step also enhances the surface area-to-volume ratio, facilitating optimal solvent–solute interaction during extraction.

Table 2. Content of TPC, TFC, and DPPH scavenging Activity

*Ripeness	Water (%)	Methanol (%)	Ethanol (%)	Acetone (%)	Means of TPC (mg GAE/g)	Means of TFC (mg QE/g)	Means of DPPH (%)
0	100	0	0	0	28.796 ± 0.576	11.017 ± 0.22	28.797 ± 0.576
0	0	100	0	0	30.69 ± 0.614	13.043 ± 0.261	31.216 ± 0.624
0	0	0	100	0	31.11 ± 0.622	13.163 ± 0.263	30.735 ± 0.615
0	0	0	0	100	27.133 ± 0.543	8.946 ± 0.179	27.542 ± 0.551
0	50	50	0	0	29.938 ± 0.599	12.1 ± 0.242	30.108 ± 0.602
0	50	0	50	0	30.283 ± 0.606	14.732 ± 0.295	29.917 ± 0.598
0	50	0	0	50	27.986 ± 0.56	10.022 ± 0.2	28.23 ± 0.565
0	0	50	50	0	31.168 ± 0.623	12.832 ± 0.257	31.21 ± 0.624
0	0	50	0	50	28.869 ± 0.577	10.894 ± 0.218	29.353 ± 0.587
0	0	0	50	50	28.724 ± 0.574	11.005 ± 0.22	29.508 ± 0.59
0	33.3	33.3	33.3	0	30.239 ± 0.605	12.65 ± 0.253	29.933 ± 0.599
0	33.3	33.3	0	33.3	29.084 ± 0.582	10.944 ± 0.219	29.299 ± 0.586
0	0	33.3	33.3	33.3	29.505 ± 0.59	11.76 ± 0.235	30.011 ± 0.6
0	33.3	0	33.3	33.3	34.959 ± 0.699	14.765 ± 0.295	29.514 ± 0.59
0	25	25	25	25	29.306 ± 0.586	11.797 ± 0.236	30.323 ± 0.606
1	100	0	0	0	32.861 ± 0.657	12.867 ± 0.257	33.906 ± 0.678
1	0	100	0	0	35.054 ± 0.701	14.856 ± 0.297	36.249 ± 0.725
1	0	0	100	0	35.107 ± 0.702	15.022 ± 0.3	36.197 ± 0.724
1	0	0	0	100	31.149 ± 0.623	11.106 ± 0.222	32.548 ± 0.651
1	50	50	0	0	33.848 ± 0.677	14.197 ± 0.284	35.168 ± 0.703
1	50	0	50	0	34.083 ± 0.682	14.059 ± 0.281	34.855 ± 0.697
1	50	0	0	50	31.998 ± 0.64	12.053 ± 0.241	33.738 ± 0.675
1	0	50	50	0	34.674 ± 0.693	15.061 ± 0.301	35.639 ± 0.713
1	0	50	0	50	32.773 ± 0.655	13.114 ± 0.262	34.347 ± 0.687
1	0	0	50	50	32.925 ± 0.658	13.067 ± 0.261	34.491 ± 0.69
1	33.3	33.3	33.3	0	34.683 ± 0.694	14.416 ± 0.288	35.286 ± 0.706
1	33.3	33.3	0	33.3	33.017 ± 0.66	12.776 ± 0.256	34.838 ± 0.697
1	0	33.3	33.3	33.3	33.647 ± 0.673	13.703 ± 0.274	35.054 ± 0.701
1	33.3	0	33.3	33.3	35.332 ± 0.707	14.001 ± 0.28	34.4 ± 0.688
1	25	25	25	25	33.661 ± 0.673	13.462 ± 0.269	34.638 ± 0.693

*Unripe plantain, Ripeness = 0
Ripe plantain, Ripeness = 1

3.1 Determination of Total Phenolic Content (TPC)

Phenolic compounds are recognized as key secondary metabolites in plants, with important roles in plant defense and as bioactive ingredients [14]. The total phenolic content (TPC) of plantain peel extracts was determined using the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g).

Among the single solvents, ethanol and methanol showed higher TPC values compared to distilled water and acetone. This is consistent with previous studies indicating that alcoholic solvents, especially methanol and ethanol, are effective in extracting phenolic compounds due to their polarity and ability to disrupt plant cell walls [15]. However, water as a polar solvent showed moderate extraction capability, mainly for highly polar phenolic compounds. Acetone, while moderately polar, exhibited relatively lower TPC values when used alone, likely due to limited interaction with the broad spectrum of phenolic structures in plantain peel [16].

The binary solvent systems demonstrated enhanced extraction efficiency, particularly when water was mixed with ethanol or methanol in a 1:1 volumetric ratio. The water:ethanol (1:1) combination yielded significantly

higher TPC than any single solvent, likely due to the synergistic interaction of water's hydrogen-bonding capacity and ethanol's ability to solubilize semi-polar compounds [16]. Similarly, water:methanol (1:1) showed comparable TPC, reinforcing the importance of mixed polarity in solvent selection. In contrast, binary mixtures of two organic solvents (e.g., methanol:acetone or ethanol:acetone) resulted in moderate TPC values, likely because of reduced ability to extract highly polar phenolics.

Among all tested solvent combinations, the ternary system of water:ethanol:acetone (1:1:1) yielded the highest TPC values, indicating a synergistic extraction effect. This can be attributed to the combined polarity range of the solvents that facilitates the solubilization of both polar and semi-polar phenolic compounds. Water forms hydrogen bonds with hydroxyl groups on phenolics, ethanol enhances membrane permeability and diffusion, and acetone helps dissolve less polar phenolic structures. The three-way interaction appears to create an optimal environment for maximum phenolic extraction.

The quaternary solvent mixture of water:methanol:ethanol:acetone (1:1:1:1) showed slightly lower TPC values compared to the best ternary system. This may be due to dilution of each solvent's functional role or competing interactions that reduce overall extraction efficiency. Although the quaternary system still showed high TPC content, it doesn't show any significancies.

Across all solvent systems, plantain peels at ripening stages 3 and 4 consistently produced higher TPC values than those at stages 1 and 2. This trend supports the findings of Pham et al. (2022), who reported that higher moisture content in more mature samples enhances solute-solvent interactions, thus improving phenolic extraction [12]. Moisture facilitates cell wall swelling and loosening, promoting solvent penetration and compound diffusion.

Plantain peel is known to contain high levels of phenolic compounds that possess reductive capability towards Fe^{2+} and Fe^{3+} ions, rendering them promising for green synthesis of nanomaterials such as nZVI. For example, phenolic concentration in plantain peel extract (158 mg gallic acid equivalent/100 g dry mass) surpasses that of plantain pulp (29.6 mg/100 g dry mass) [17]. Several specific phenolic constituents identified in Musa paradisiaca 'Kepok' peels include 2-methoxy-4-vinylphenol, 4-methoxy-2-vinylphenol, and 2-methoxy-5-vinylphenol [18]. Additionally, plantain peel polyphenols are reported to include catecholamines, flavanones, flavonols, tocopherols [19], as well as p-hydroxybenzoic acid, ferulic acid, caffeic acid, and vanillic acid
[12].

3.2 Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of plantain peel extracts was determined using the aluminum chloride colorimetric method and expressed as milligrams of quercetin equivalent per gram of dry sample (mg QE/g).

Among single solvents, ethanol and methanol exhibited higher TFC values than distilled water and acetone, which aligns with previous findings on the efficiency of alcohols in solubilizing flavonoids due to their intermediate polarity and ability to form hydrogen bonds. The binary solvent systems—particularly water:ethanol (1:1) and water:methanol (1:1)—showed an even greater ability to extract flavonoids, likely due to enhanced solubility from combined polar and semi-polar interactions [15].

Interestingly, the ternary system of water:ethanol:acetone (1:1:1) yielded one of the highest TFC values, suggesting a synergistic solvent effect that promotes the extraction of a broader range of flavonoid compounds. This can be attributed to the combined solvent polarity range, which enhances mass transfer and solubilization of both glycosylated and aglycone flavonoids.

The quaternary mixture of water:methanol:ethanol:acetone (1:1:1:1) produced slightly lower TFC than the best ternary combination, possibly due to dilution of the most effective polarity window or unfavorable solvent-solvent interactions.

These findings reinforce that solvent polarity, hydrogen bonding capacity, and miscibility play critical roles in flavonoid extraction efficiency. Comparable trends were reported by Pham et al. (2022), where mixed solvent systems enhanced the recovery of phenolic and flavonoid compounds from plant materials [12].

Similar to total phenolic content (TPC), ripeness significantly influenced TFC values. Flavonoid content was generally higher in ripe samples (ripening stages 3 and 4) than in unripe samples (stages 1 and 2). This trend supports the hypothesis that ripening increases the biosynthesis of secondary metabolites, including flavonoids, as a response to physiological and oxidative changes in plant tissues. Increased enzymatic activity, cell wall softening, and membrane permeability during ripening likely contribute to greater extractability of flavonoids from the plantain peel matrix. These findings mirror the behavior of TPC under similar ripening conditions and are consistent with the report by Pham et al. (2022), which observed enhanced bioactive compound release in ripe fruit peels [12].

3.3 Determination of DPPH Scavenging Activity

The antioxidant activity of plantain (Musa paradisiaca 'ABB') peel extracts was evaluated using the DPPH radical scavenging assay and expressed as percentage inhibition (%). The values varied across different solvent systems and ripeness stages, highlighting the influence of both variables on antioxidant potential.

There is a clear distinction between the antioxidant activities of unripe and ripe plantain peels. On average, ripe samples exhibit higher DPPH scavenging percentages than unripe ones. Ripe peels extracted with methanol and ethanol individually showed DPPH inhibition values of $35.054 \pm 0.701\%$ and $35.107 \pm 0.702\%$, respectively.In contrast, unripe peels extracted with the same solvents only yielded $30.690 \pm 0.614\%$ (methanol) and $31.110 \pm 0.622\%$ (ethanol).

This trend suggests that ripening enhances the antioxidant activity of plantain peel, likely due to increased synthesis or availability of phenolic compounds and flavonoids during the maturation process [20], [21]. This is consistent with the strong positive correlation observed between ripeness and DPPH activity ($r = 0.94$), indicating that maturity is a significant factor contributing to the antioxidant potency.

Single solvent systems show variable effectiveness. Methanol and ethanol outperformed water and acetone, consistent with their intermediate polarity and ability to extract both polar and moderately nonpolar antioxidant compounds. Acetone alone resulted in the lowest scavenging value in unripe samples ($27.133 \pm 0.543\%$) and relatively moderate activity in ripe ones ($31.149 \pm 0.623\%$), likely due to limited solubilization of polar bioactives.

Binary and ternary mixtures generally enhanced DPPH activity. Water-ethanol and water-methanol 1:1 combinations offered higher DPPH values compared to single solvents, benefiting from synergistic solvent interactions. The ternary mixture of water:ethanol:acetone (1:1:1) showed notable improvement, especially in ripe samples ($34.683 \pm 0.694\%$), supporting the hypothesis that a balanced polarity range facilitates the extraction of a broader spectrum of antioxidants.

Interestingly, a unique spike is seen in the unripe extract with methanol-ethanol-acetone (33.3% each) showing a DPPH value of $34.959 \pm 0.699\%$, suggesting that specific solvent synergies can enhance antioxidant extraction even in immature peels.

Overall, the combination of ripeness and solvent polarity balance plays a critical role in maximizing antioxidant activity. Mixed solvents amplify compound solubility, while ripening enriches the concentration and diversity of antioxidant constituents such as flavonoids, quinones, tannins, and polyphenols [20], [21].

This synergy is especially evident in ripe samples extracted with ternary or quaternary mixtures, which consistently demonstrated DPPH values above 33%, indicating moderate to high antioxidant capacity.

3.4 Correlation Matrix

The correlation matrix provides insights into the relationships between extraction parameters (ripeness and solvent composition) and bioactive compound content (total phenolic content, TPC; total flavonoid content, TFC) as well as antioxidant activity (DPPH radical scavenging).

Ripeness shows a strong positive correlation with TPC ($r = 0.79$) and DPPH ($r = 0.94$), and a moderate positive correlation with TFC ($r = 0.51$). This suggests that as plantain peel matures (from unripe to ripe), its phenolic and antioxidant content increases significantly, with flavonoid content also improving but to a lesser extent. These results are consistent with previous findings [12], which attribute the rise in bioactives to enhanced cell wall permeability and metabolic shifts during ripening.

Among the solvents ethanol shows moderate positive correlation with TFC ($r = 0.46$) and a weaker correlation with TPC and DPPH. This supports its known role as an effective solvent for flavonoid extraction due to its semi-polar nature [20], [21].

Table 3. Correlation Matrix

	Means of TPC (mg GAE/g)	Means of TFC (mg QE/g)	Means of DPPH (%)
Ripeness	0.788	0.514	0.936
Water (%)	-0.045	-0.042	-0.104
Methanol (%)	0.115	0.115	0.203
Ethanol (%)	0.285	0.460	0.172
Acetone (%)	-0.356	-0.634	-0.271
Means of TPC (mg GAE/g)	1.000	0.875	0.878
Means of TFC (mg QE/g)	0.875	1.000	0.714
Means of DPPH (%)	0.878	0.714	1.000

Methanol presents weak positive correlations with all bioactive metrics, suggesting a modest contribution to solubilization, especially for phenolics. Acetone shows a negative correlation with all variables, most notably with TFC ($r = –0.63$) and TPC ($r = –0.36$). This may be due to its inability to efficiently solubilize more polar compounds like glycosylated flavonoids [20], [21].

Water demonstrates near-zero or negative correlations, implying limited extraction efficiency, likely due to reduced solubility of less polar compounds in purely aqueous systems.

There are strong positive correlations among TPC, TFC, and DPPH, particularly TPC and DPPH ($r = 0.88$), and TPC and TFC ($r = 0.88$).

This implies that higher total phenolic content is associated with both greater antioxidant potential and higher flavonoid concentration. It supports the hypothesis that phenolics and flavonoids compounds—are key contributors to the antioxidant capacity of plantain peel extracts.

The correlation coefficient of 0.88 between TPC and DPPH suggests that phenolic compounds are the primary contributors to the antioxidant activity of the plantain peel extracts. Phenolics are known to possess multiple hydroxyl groups capable of donating hydrogen atoms or electrons to neutralize free radicals such as DPPH•. The strong linear relationship implies that an increase in TPC almost proportionally enhances the radical scavenging capacity of the extract. This finding is consistent with prior studies indicating that phenolics act as chain-breaking antioxidants that interrupt lipid peroxidation and stabilize free radicals [7].

TFC also shows a strong positive correlation with DPPH activity ($r = 0.71$), although slightly lower than that of TPC. This supports the understanding that flavonoids—being a subclass of phenolic compounds—play a significant role in antioxidant defense, but may not be the sole contributors. The lower correlation compared to TPC-DPPH may also reflect the presence of other phenolic subgroups (e.g., phenolic acids, tannins, or quinones) in the extract that also influence antioxidant activity.

The high correlation between TPC and TFC ($r = 0.88$) indicates that a significant proportion of total phenolics extracted are flavonoid compounds. This suggests that

both compound classes share similar extraction behaviors and affinities for the solvent systems used, especially in mixed-polarity combinations (e.g., water:ethanol or ternary systems). Since both are polyphenolic structures with conjugated double bonds and hydroxyl substitutions, they likely respond similarly to variations in polarity, temperature, and solvent ratios.

These strong correlations collectively suggest that DPPH scavenging activity in plantain peel extracts is not driven by a single compound class, but rather the cumulative effect of various phenolics, including flavonoids. The interplay between hydroxyl-rich structures and conjugated aromatic rings across different polyphenols may produce synergistic antioxidant behavior, where the combination of compounds offers greater activity than any single one alone.

Additionally, these results support the validity of using TPC and TFC as predictive indicators of antioxidant potential in phytochemical screening and solvent optimization studies. However, the slightly lower TFC-DPPH correlation also implies that non-flavonoid antioxidants (e.g., betacyanins, quinones, tocopherols) present in the matrix may contribute to the total observed activity, reinforcing the need for comprehensive phytochemical profiling.

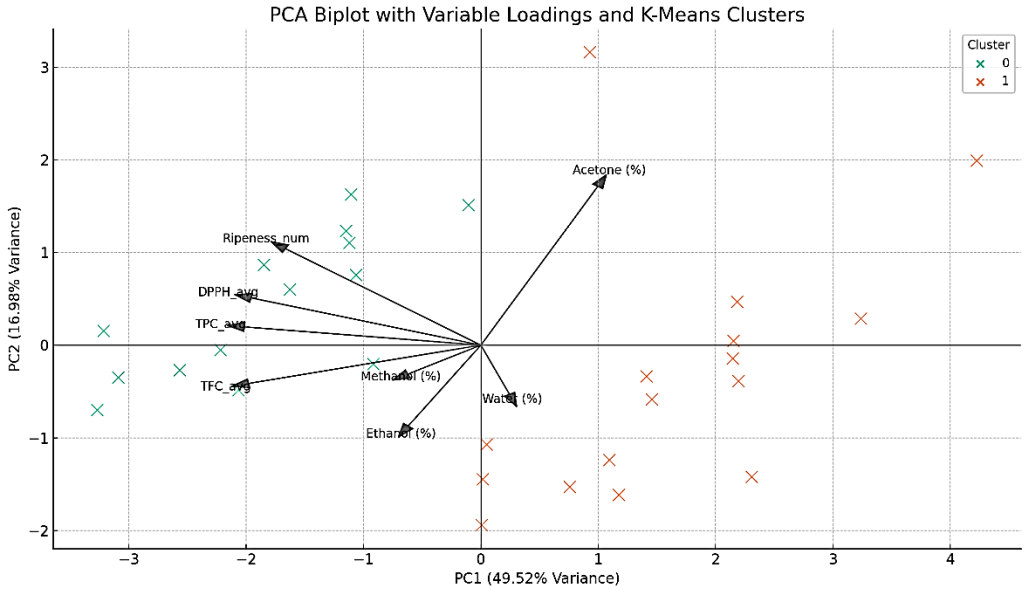

Fig. 2. Graph of Principal Component Analysis

3.5 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was conducted to identify patterns and reduce dimensionality in the dataset composed of ripeness level, solvent system composition, and measured phytochemical responses (TPC, TFC, DPPH). The first two principal components (PC1 and PC2) explained a substantial proportion of the total variance in the data, making them suitable for graphical interpretation.

Principal Component 1 (PC1) accounted for the largest percentage of the data variance. The loadings indicate that TPC, TFC, and DPPH all have strong positive contributions to PC1, suggesting that this axis primarily reflects bioactive content and antioxidant capacity of the extracts. Samples positioned positively along PC1 are characterized by high phenolic and flavonoid content, and strong DPPH scavenging activity. These are mainly extracts from ripe plantain peel, particularly those obtained using mixed-polarity solvent systems (e.g., water:ethanol, or ternary mixtures).

Principal Component 2 (PC2) captured a secondary variance component and was primarily influenced by ripeness and solvent composition—especially acetone and water content, which had opposite contributions. PC2 appears to differentiate samples based on ripeness and extraction polarity extremes, with unripe samples and high-acetone conditions clustering separately from ripe and water-inclusive systems. This suggests that PC2 reflects a polarity-driven extraction efficiency gradient modulated by sample maturity.

Projection of the samples onto the PC1–PC2 space, combined with k-means clustering, revealed two distinct groups. Cluster 1 comprised mainly of unripe samples and single/pure solvent extractions, particularly those with high acetone or water ratios. These samples are characterized by lower phenolic and flavonoid content and reduced antioxidant activity. Cluster 2 is encompassing ripe samples and multi-solvent systems (e.g., binary, ternary, or quaternary) that yielded higher levels of TPC, TFC, and DPPH activity. These extracts are chemically richer and potentially more bioactive.

The PCA biplot showed that TPC, TFC, and DPPH are closely grouped with long arrows in the same direction, indicating strong collinearity (supported by correlation matrix), Similar response to ripeness and optimal solvent conditions, Co-contribution to overall antioxidant potential. On the other hand, acetone loading is oriented in the opposite direction, reinforcing its inverse correlation with polyphenol extraction efficiency and antioxidant performance in this matrix.

From PCA, we can conclude that ripeness is a dominant driver of bioactive compound abundance. Mixed-polarity solvents (especially water-alcohol combinations) enhance the extraction results of bioactive compounds while acetone alone or in excess, negatively affects recovery of polar phytochemicals.

These findings provide a multivariate confirmation of trends observed in univariate correlation and ANOVA analyses and support the use of PCA in optimizing extraction protocols and assessing sample clustering

based on chemical and functional characteristics.

4. CONCLUSION

Ripeness and solvent system significantly influence the extraction of bioactive compounds from plantain peel. Ripe samples extracted with ternary solvents—particularly water:ethanol:acetone—yielded the highest levels of TPC, TFC, and antioxidant activity. Ethanol and methanol emerged as effective solvents for recovering flavonoids, while acetone and water alone were less efficient. Strong positive correlations among TPC, TFC, and DPPH activity reinforce the critical role of phenolic compounds, especially flavonoids, in the antioxidant capacity of the extract. These findings support the valorization of plantain peel as a sustainable source of natural antioxidants and provide insights for green extraction strategies in functional product development.

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