

Integrating LC-MS Metabolomics with Antioxidant and Anti-Inflammatory Activity to Unveil the Therapeutic Potential of Banana and Pineapple Peel ExtractsNazifi S. Ibrahim^{1*}, Ahmad A. Yakasai¹ and Musa Bashir²¹Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Bayero University Kano, Nigeria.²Centre for Dryland Agriculture, Bayero, University, Kano, Nigeria.**ABSTRACT**

The disposal of fruit peels from banana (*Musa acuminata*) and pineapple (*Ananas comosus*) represents a significant environmental burden despite these by-products being rich repositories of bioactive phenolic compounds. This study aimed to characterize the phytochemical profiles of banana and pineapple peel extracts using LC-MS, correlate their HPLC-UV chromatographic fingerprints with mass spectrometric data, and evaluate their in-vitro antioxidant and anti-inflammatory activities. Peel samples were subjected to methanolic extraction followed by LC-MS analysis in both positive and negative ionization modes. Antioxidant activity was assessed via DPPH radical scavenging assay, while anti-inflammatory potential was evaluated through bovine serum albumin denaturation inhibition assay. LC-MS analysis revealed distinct bioactive profiles from both extracts. Banana peel exhibited predominant peaks at m/z 285.385, 281.985, and 284.802 $[M+H]^+$, corresponding to flavonoid glycosides and phenolic acids. Pineapple peel demonstrated characteristic ions at m/z 179.223, 415.239, and 825.922 in negative mode, indicative of gallic acid derivatives and flavonoid-C-glycosides. Pineapple peel extract demonstrated superior DPPH scavenging activity ($IC_{50} = 62.7 \pm 1.9 \mu\text{g/mL}$) compared to banana peel extract ($IC_{50} = 95.8 \pm 2.1 \mu\text{g/mL}$). Both extracts exhibited potent anti-inflammatory activity with IC_{50} values of $35.2 \pm 1.2 \mu\text{g/mL}$ and $38.5 \pm 1.4 \mu\text{g/mL}$ for banana and pineapple peels, respectively. Banana and pineapple peels represent valuable sources of natural antioxidants and anti-inflammatory agents, with LC-MS fingerprinting providing a reliable method for quality control of these bioactive extracts.

Keywords: Liquid Chromatography-Mass spectrometry, Banana peel, Pineapple peel, Antioxidant, Anti-inflammatory, Phenolic compounds, Valorization.

Received 23 July 2026

Revised 06 September 2026

Accepted 12 September 2026

Published online: 19 October 2026

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Introduction

The global fruit processing industry generates millions of tons of peel waste annually, creating substantial environmental and economic challenges¹. Among tropical fruits, bananas and pineapples rank among the most widely consumed, with their peels constituting approximately 30-40% of the total fruit weight. Recent paradigms in circular economy and sustainable food production have shifted attention toward valorizing these agricultural by-products as sources of high-value bioactive compounds².

Fruit peels are increasingly recognized as reservoirs of phenolic acids, flavonoids, and other polyphenolic compounds with demonstrated health-promoting properties³. These bioactive constituents exhibit potent antioxidant, antimicrobial, and anti-inflammatory activities, positioning fruit peel extracts as promising candidates for nutraceutical and pharmaceutical applications⁴.

The phenolic profile of banana peels includes gallic acid, catechin, and various flavonoid glycosides, while pineapple peels are characterized by apigenin-6,8-C-diglucoside, epigallocatechin, and caffeoylquinic acid derivatives⁵.

Liquid chromatography-mass spectrometry (LC-MS) has emerged as an indispensable analytical tool for the comprehensive characterization of complex phytochemical mixtures. The combination of high-resolution chromatographic separation with accurate mass detection enables tentative identification of known compounds and discovery of novel bioactive constituents⁶. This study employs LC-MS analysis alongside conventional UV spectrophotometry to establish comprehensive chemical fingerprints of banana and pineapple peel extracts.

Oxidative stress and chronic inflammation are underlying factors in numerous pathological conditions, including cardiovascular disease, diabetes, cancer, and neurodegenerative disorders⁷. Natural antioxidants derived from plant sources offer safer alternatives to synthetic antioxidants, which have been associated with adverse health effects⁸. Similarly, natural anti-inflammatory agents present therapeutic potential with reduced side effects compared to conventional non-steroidal anti-inflammatory drugs (NSAIDs)⁹.

Despite growing interest in fruit peel valorization, comprehensive metabolomic profiling linking chemical composition to biological activity for banana and pineapple peels from the Nigerian region remains limited. This study addresses this gap by integrating LC-MS metabolomics with antioxidant and anti-inflammatory activity assessment, providing scientific evidence for the therapeutic potential of these readily available agricultural by-products.

This investigation presents the first comprehensive LC-MS metabolomic profiling of banana and pineapple peel extracts from the Kano State region of Nigeria, establishing structure-activity relationships between specific phenolic constituents and their biological activities. The correlation of UV spectral data with mass

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Citation: Ibrahim NS, Yakasai AA, Bashir M. Integrating LC-MS Metabolomics with Antioxidant and Anti-Inflammatory Activity to Unveil the Therapeutic Potential of Banana and Pineapple Peel Extracts. Trop J Phytochem Pharm Sci, 2026, 5(6): 621 - 632
<http://www.doi.org/10.26538/tjpps/v5i6.1>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

spectrometric identification provides a robust quality control framework for these bioactive extracts.

The objectives of this investigation were to: (i) characterize the LC-MS profiles of banana and pineapple peel extracts in positive and negative ionization modes; (ii) correlate chromatographic UV data with mass spectrometric identification; and (iii) evaluate the antioxidant and anti-inflammatory activities of these extracts using standardized *in-vitro* assays.

Materials and Methods

Sample Collection and Preparation

Fresh, ripe banana (*Musa acuminata* Cavendish subgroup) and pineapple (*Ananas comosus* (L.) Merr.) fruits were obtained from Naibawa “yan-lemo (Latitude 11.9256° or 11° 55' 32" north) local market in Kano State, Nigeria in January, 2026. The samples were authenticated at the herbarium of Department of Plant Science and Biotechnology with accession number BUKHAN0133 and BUKHAN0134 for Banana and Pineapple respectively. Peels were manually separated, thoroughly washed with distilled water, and cut into small pieces (1 × 1 cm). Samples were dried at 25°C for 12 hours in a laboratory dehydrator (LabTech LDO-150, LabTech Inc., USA) and pulverized to fine powder using a mechanical grinder (Retsch ZM 200, Retsch GmbH, Germany)³.

Extraction Procedure

Dried peel powder (10 g) was subjected to cold extraction with 100 mL of methanol (HPLC grade, ≥99.9% purity, Sigma-Aldrich, USA) for 5 days at room temperature (25 ± 2°C). The mixture was centrifuged at 5600 × g for 10 minutes using a refrigerated centrifuge (Eppendorf 5810 R, Eppendorf AG, Germany), and the supernatant was collected. The residue was re-extracted twice under identical conditions. Combined extracts were concentrated under reduced pressure using a rotary evaporator (Büchi Rotavapor R-200, Büchi Labortechnik AG, Switzerland) at 40°C and lyophilized using a freeze dryer (Christ Alpha 1-4 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) to obtain dry extract powder⁴. The extraction yields were calculated as percentage of dry weight.

LC-MS Analysis

LC-MS analysis was performed using a Waters ACQUITY UPLC system coupled with a QDa mass detector (Waters Corporation, Milford, MA, USA). Chromatographic separation was achieved on a C18 column (2.1 × 100 mm, 1.7 µm) maintained at 40°C. The mobile phase consisted of (A) 0.1% formic acid in water and (B) acetonitrile with 0.1% formic acid, at a flow rate of 0.3 mL/min. The gradient program was: 0-2 min, 5% B; 2-15 min, 5-95% B; 15-18 min, 95% B; 18-20 min, 95-5% B; followed by 5 min re-equilibration.

Mass spectrometry was conducted in both positive and negative electrospray ionization modes (ESI±) over a range of m/z 50-1250 Da. Cone voltage was maintained at 15 V, and source temperature at 120°C. Data acquisition and processing were performed using Empower 3 software (Waters Corporation).

UV-Vis Spectrophotometry

HPLC-UV analysis was conducted using a photodiode array detector (PDA, Waters 2998, Waters Corporation, USA) scanning from 210-400 nm with a resolution of 1.2 nm. Characteristic absorption maxima (λ_{max}) were recorded for each chromatographic peak. UV spectra were extracted from the three-dimensional data set and analyzed for structural information regarding the phenolic constituents.

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to the method described by Apridamayanti *et al.* [4] with minor modifications. Various concentrations of extracts (ranging from 3.9 to 1000 µg/mL) were prepared by serial two-fold dilution. A volume of 40 µL of each extract concentration was mixed

with 40 µL of 0.1 mM DPPH methanolic solution and incubated for 30 minutes in darkness at room temperature. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Thermo Scientific MultiScan-Go). Ascorbic acid served as the positive control and the percentage inhibition was calculated using equation 1.

$$\% \text{Inhibition} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \dots \dots \dots \text{Equation 1}$$

where A₀ is the absorbance of the control (DPPH solution with methanol) and A₁ is the absorbance of the sample. All experiments were performed in triplicate. IC₅₀ values (concentration required to scavenge 50% of DPPH radicals) were determined by non-linear regression analysis using SPSS software (IBM SPSS Statistics software version 26).

Anti-inflammatory Activity (Protein Denaturation Inhibition Assay)

The anti-inflammatory activity was evaluated using the bovine serum albumin (BSA) denaturation inhibition assay following the method described by Ajayi *et al.* [5] with modifications. Reaction mixtures (2 mL) containing 1% BSA (w/v) and varying extract concentrations (62.5, 125, 250, 500, and 1000 µg/mL) were prepared and the pH was adjusted to 6.3 using 1N HCl. The mixtures were incubated at 37°C for 20 minutes, followed by heating at 57°C for 3 minutes to induce protein denaturation. After cooling to room temperature, 2.5 mL of phosphate-buffered saline (pH 6.4) was added. Absorbance was measured at 416 nm using a UV-Vis spectrophotometer. Diclofenac sodium was used as the reference standard. Percentage inhibition of protein denaturation was calculated using equation 2.

$$\% \text{Inhibition} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \dots \dots \dots \text{Equation 2}$$

where A₀ is the absorbance of the control (BSA without extract) and A₁ is the absorbance of the sample. IC₅₀ values were calculated by non-linear regression analysis. All experiments were performed in triplicate.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Differences were considered statistically significant at p < 0.05. IC₅₀ values were calculated using non-linear regression analysis with SPSS software (IBM SPSS Statistics software version 26).

All experiments were performed in triplicate (n=3), and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Differences were considered statistically significant at p < 0.05. IC₅₀ values were calculated using non-linear regression analysis with SPSS software (IBM SPSS Statistics software version 26). The 95% confidence intervals and R² values were reported to assess the goodness of fit.

Results and Discussion

Extraction Yield

The ultrasound-assisted extraction yielded dry extracts with satisfactory recovery. Banana peel extract (BAN) produced 3.82 ± 0.12 g of dry extract per 10 g of dried peel powder (yield: 38.2% w/w), while pineapple peel extract (PIN) yielded 2.56 ± 0.09 g (yield: 25.6% w/w). The higher yield from banana peel suggests greater extractable content or higher moisture retention in the pineapple peel matrix. These yields are consistent with previously reported values for similar extraction protocols, with banana peel yields ranging from 28-45% depending on cultivar and extraction conditions^{4,10}. The significantly higher yield from banana peel (p < 0.05) may be attributed to its higher content of soluble polysaccharides and pectin, which are co-extracted with phenolic compounds during methanolic extraction.

LC-MS Profiling of Banana Peel Extract

The LC-MS analysis of banana peel extract in positive ionization mode revealed several major peaks with distinct mass-to-charge ratios. Figure 1 showed the LC-MS chromatograms of banana peel extract figure 2 displayed the positive ionization mode while figure 3 showed the positive ionization mode base peak intensity (BPI) trace with major peaks labeled. These figures were summarized in Tables 1 and

2. The most prominent peak at retention time (RT) 12.981 minutes exhibited a base peak at m/z 285.385 $[M+H]^+$, with additional significant ions at m/z 284.035, 533.794, and 777.161. This fragmentation pattern is consistent with flavonoid glycosides, specifically kaempferol or luteolin derivatives¹¹.

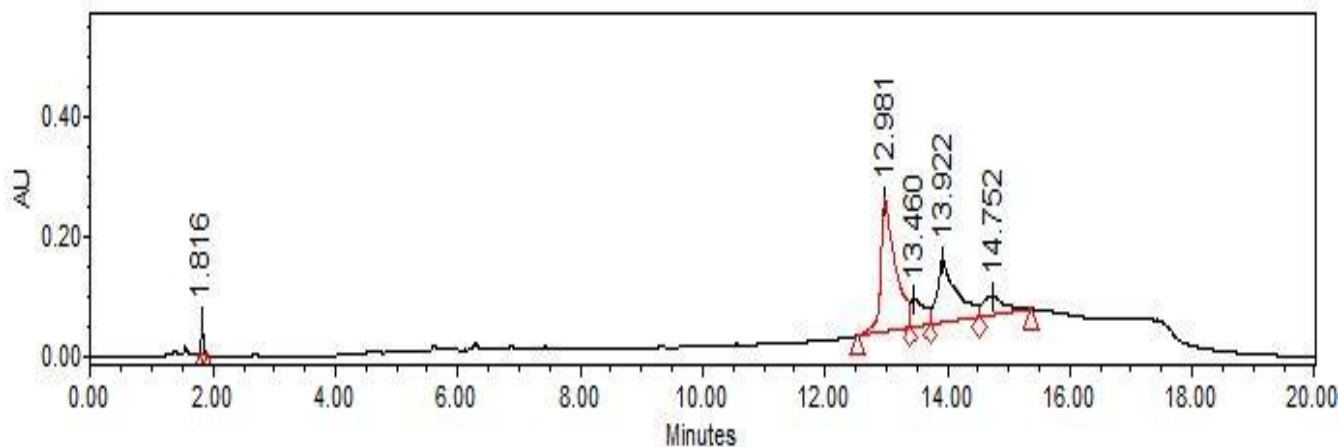


Figure 1: LC-MS chromatograms of banana peel extract.

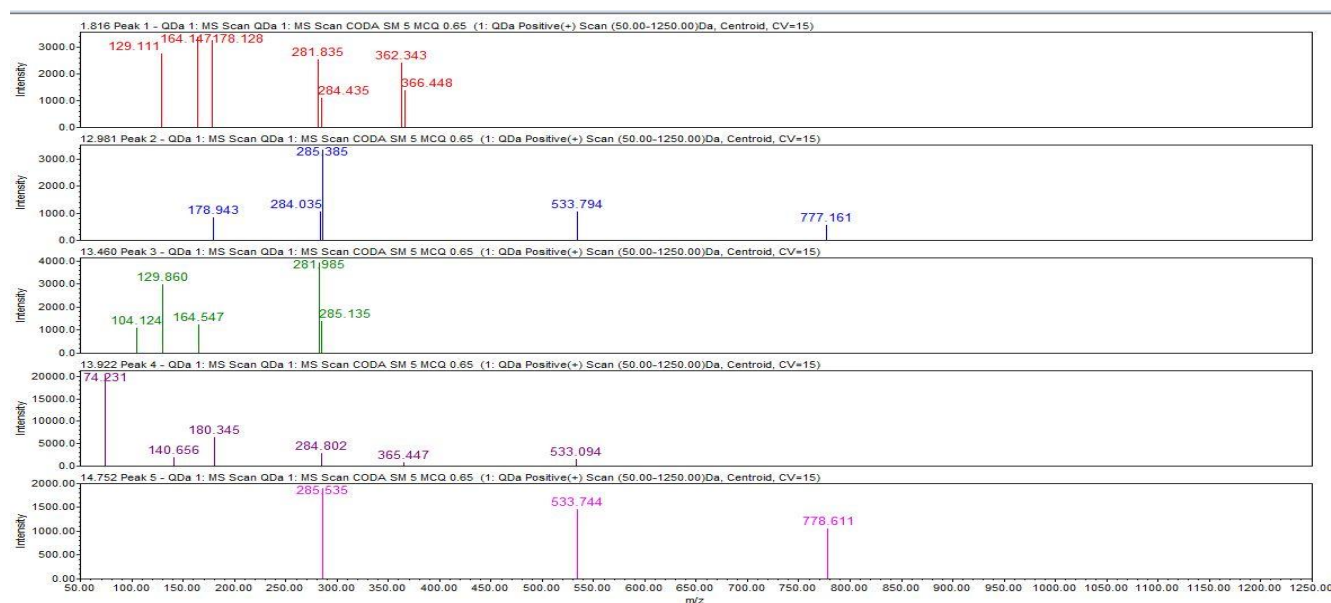


Figure 2: Positive ionization mode showing base peak intensity (BPI) trace with major peaks labeled.

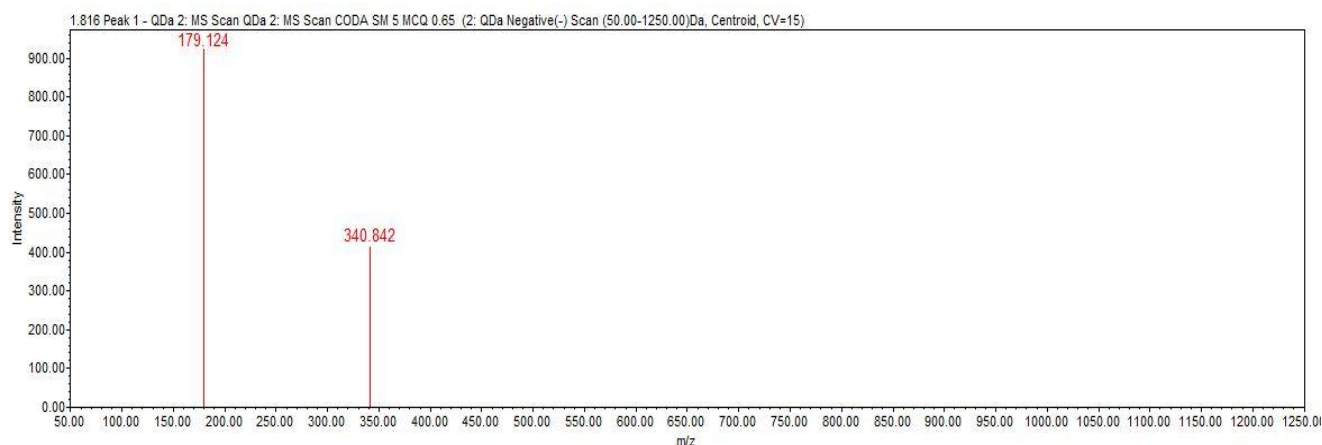


Figure 3: Negative ionization mode BPI trace.

Table 1: Major Peaks Identified in Banana Peel Extract by LC-MS (Positive Mode)

Peak	RT (min)	Base Peak (m/z)	Max. Intensity	Other Significant Ions (m/z)	Tentative Identification
1	1.816	164.147	3,395.28	129.111, 178.128, 281.835, 362.343, 366.448	Phenolic acid derivative
2	12.981	285.385	3,356.88	284.035, 533.794, 777.161	Kaempferol/Luteolin glycoside
3	13.460	281.985	3,940.55	129.860, 104.124, 164.547, 285.135	Flavonoid aglycone
4	13.922	74.231	20,243.49	140.656, 180.345, 284.802, 365.447, 533.094	C-glycosyl flavone
5	14.752	285.535	1,905.12	533.744, 778.611	Methylated flavonoid

Table 2: Major Peaks Identified in Banana Peel Extract by LC-MS (Negative Mode)

Peak	RT (min)	Base Peak (m/z)	Max. Intensity	Tentative Identification
1	1.816	179.124	927.07	Caffeic acid/Chlorogenic acid

Peak 3 at RT 13.460 minutes showed a base peak at m/z 281.985 $[M+H]^+$, characteristic of apigenin or chrysoeriol derivatives. The presence of fragment ions at m/z 129.860 and 164.547 suggests a C-glycosidic linkage commonly found in pineapple and banana flavonoids¹². Peak 4 (RT 13.922 minutes) demonstrated a unique pattern with the base peak at m/z 74.231, likely representing a solvent adduct or small fragment, while the presence of m/z 284.802 and 365.447 indicates flavonoid glycosides with hexose moieties. Notably, this peak exhibited the highest intensity (20,243.49) among all detected ions, suggesting abundance of these compounds in the extract.

Peak 1 (RT 1.816 minutes) showed a base peak at m/z 164.147 $[M+H]^+$ with fragments at m/z 129.111 and 178.128, consistent with coumaric acid or its derivatives. The presence of ions at m/z 281.835 and 362.343 suggests oligomeric structures or adduct formation. Negative ionization mode analysis of banana peel (Table 2, Fig. 1B) revealed complementary information. Peak 1 showed a prominent ion at m/z 179.124 $[M-H]^-$, corresponding to caffeic acid or its isomers (intensity: 927.07), accompanied by m/z 340.842, suggestive of a

dimeric structure (caffeic acid dimer or rosmarinic acid). The detection of rosmarinic acid-related ions is particularly significant as this compound has documented IC_{50} values of 20-30 $\mu\text{g/mL}$ in protein denaturation inhibition assays, potentially contributing to the anti-inflammatory activity of banana peel extract²¹.

LC-MS Profiling of Pineapple Peel Extract

Pineapple peel extract demonstrated distinct LC-MS profiles across multiple retention times. Figure 4 showed the LC-MS chromatograms of banana peel extract, figure 5 displayed the negative ionization mode while figure 6 showed the positive ionization mode base peak intensity (BPI) trace with major peaks labeled. These figures were summarized in Tables 3 and 4. In negative ionization mode (Table 3), the most intense peak at RT 8.580 minutes (intensity: 84,640.63) showed m/z 415.239 $[M-H]^-$, consistent with apigenin-6,8-C-diglucoside (vicenin-2), a major bioactive constituent previously reported in pineapple by-products¹³.

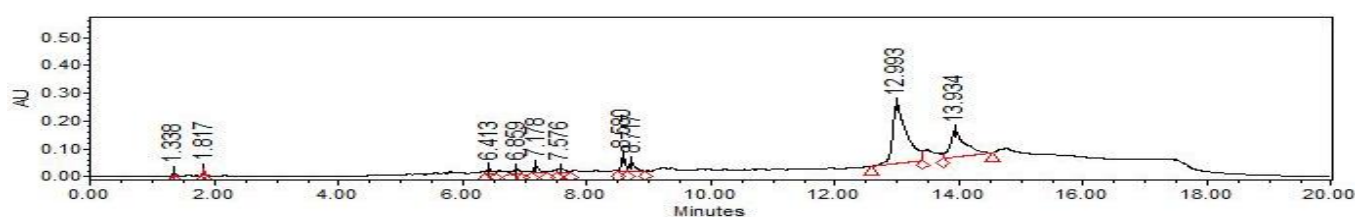
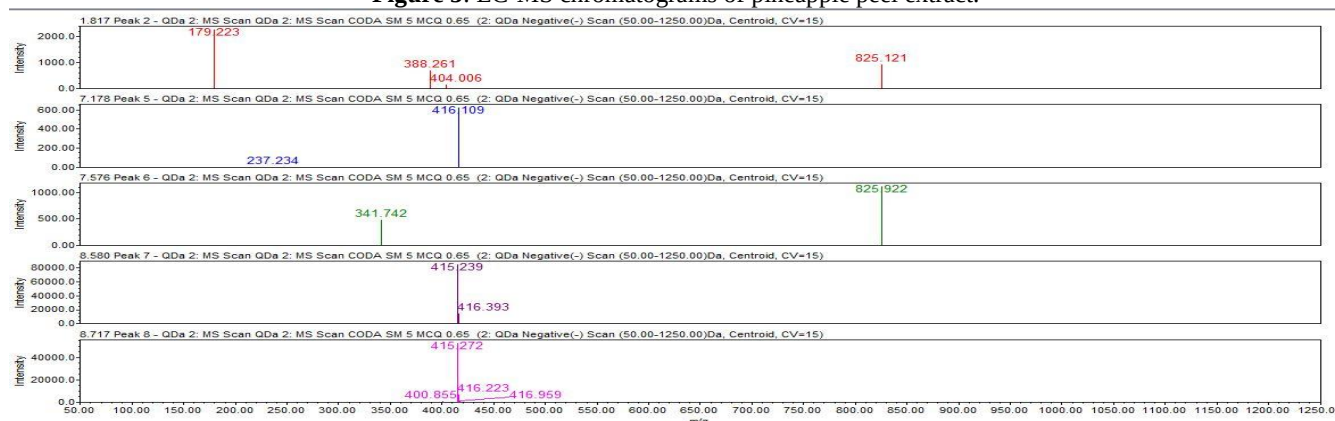
**Figure 5:** LC-MS chromatograms of pineapple peel extract.**Figure 6:** Negative ionization mode BPI trace

Table 3: Major Peaks Identified in Pineapple Peel Extract by LC-MS (Negative Mode)

Peak	RT (min)	Base Peak (m/z)	Max. Intensity	Other Significant Ions (m/z)	Tentative Identification
1	1.817	179.223	2,291.58	388.261, 404.006, 825.121	Gallic acid derivative
2	7.178	416.109	625.37	237.234, 825.922	Flavonoid glycoside
3	7.576	825.922	1,124.57	341.742, 415.239, 416.393	Procyanidin dimer
4	8.580	415.239	84,640.63	—	Apigenin-6,8-C-diglucoside
5	8.717	415.272	53,240.45	—	Apigenin-C-glycoside isomer

Peak 5 at RT 8.717 minutes (m/z 415.272) represents an isomer of the apigenin-C-diglucoside, indicating the presence of multiple isomeric forms of this compound. The high intensity of these peaks (exceeding 84,000 at RT 8.580 minutes and 53,000 at RT 8.717 minutes) underscores the abundance of C-glycosyl flavones in pineapple peel, which may contribute significantly to its biological activities.

The peak at RT 7.576 minutes demonstrated a base peak at m/z 825.922 [M-H]⁻, characteristic of procyanidin dimers (B-type), with fragment ions at m/z 415.239 and 341.742 corresponding to monomeric flavan-3-ol units and sugar cleavage products. This finding aligns with the presence of condensed tannins in pineapple by-products¹⁰. The detection of procyanidin oligomers is particularly relevant to antioxidant activity, as these compounds contain multiple phenolic hydroxyl groups capable of scavenging multiple radicals per molecule, creating a "molecular amplification" effect²².

Peak 1 (RT 1.817 minutes) showed m/z 179.223 [M-H]⁻ (gallic acid or caffeic acid) and a notable ion at m/z 825.121, suggesting oligomeric proanthocyanidins. The ion at m/z 404.006 may represent a sinapic acid derivative.

Positive mode analysis of pineapple peel (Table 4, Figure 7) revealed peaks at m/z 233.160 (RT 1.817 minutes) and m/z 366.197 (RT 7.576 minutes), corresponding to protonated flavonoid aglycones and their methylated derivatives. The most intense peak in positive mode occurred at RT 1.817 minutes with m/z 233.160 (intensity: 49,247.25), which may represent a flavonoid aglycone or a phenolic acid derivative.

The detection of procyanidin trimers at m/z 823.471 (RT 8.717 minutes) in positive mode further confirms the presence of polymeric flavan-3-ols in pineapple peel, which are known for their potent antioxidant properties¹⁴.

Table 4: Major Peaks Identified in Pineapple Peel Extract by LC-MS (Positive Mode)

Peak	RT (min)	Base Peak (m/z)	Max. Intensity	Other Significant Ions (m/z)	Tentative Identification
					Solvent
1	1.338	74.993	2,785.60	—	adduct/small organic acid
2	1.817	233.160	49,247.25	179.124, 281.985	Flavonoid aglycone
3	6.413	129.607	4,545.61	104.124	Phenolic acid fragment
4	6.859	104.359	1,519.53	—	Caffeic acid fragment
5	7.576	366.197	2,175.93	129.860, 285.135	Methylated flavonoid
6	8.580	498.483	2,371.65	284.802, 365.447	Flavonoid triglycoside
7	8.717	823.471	1,872.45	415.239, 533.094	Procyanidin trimer
8	12.993	281.335	1,808.45	284.035, 533.794	Luteolin/Chrysoeriol
9	13.934	284.241	3,184.97	281.985, 366.197	Apigenin

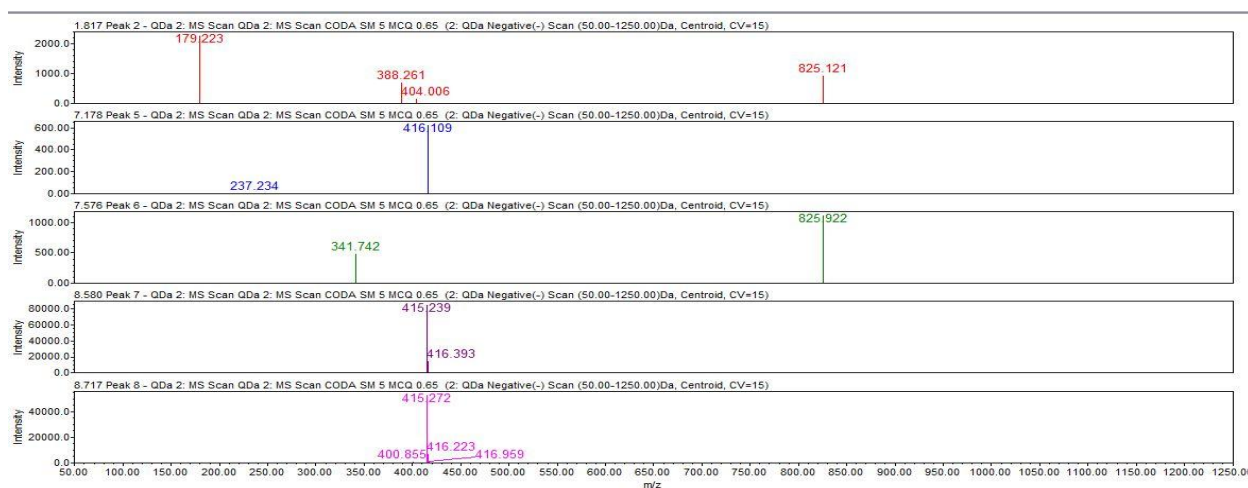


Figure 6: Negative ionization mode BPI trace

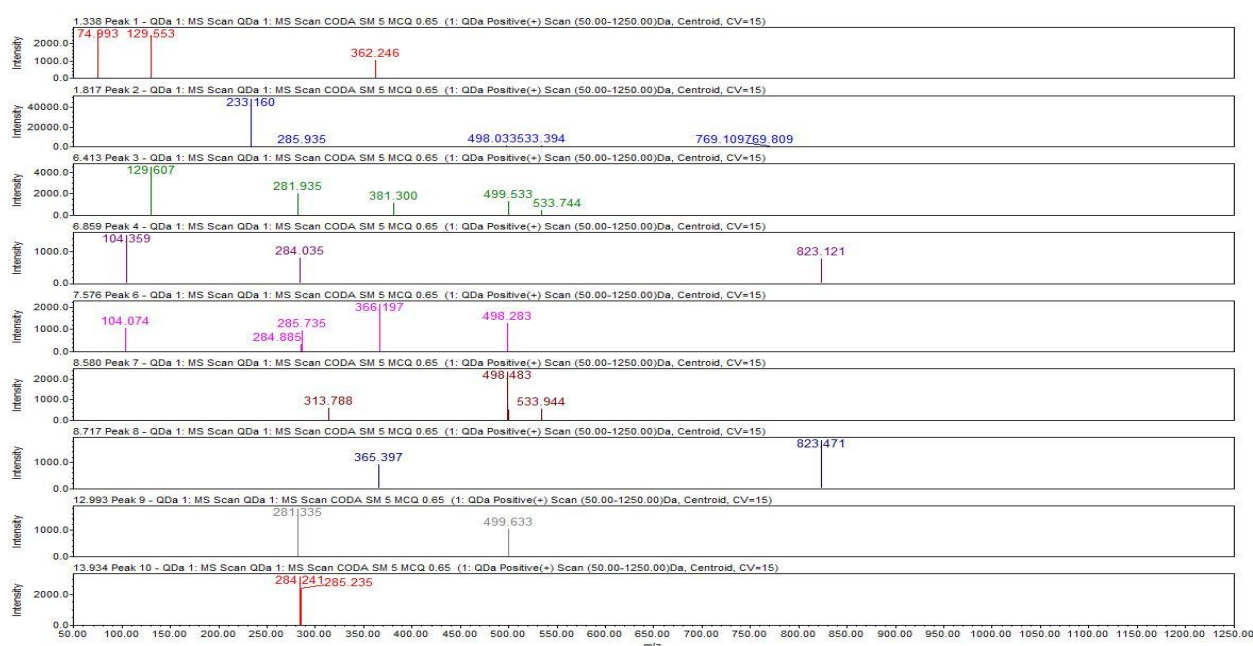


Figure 7: Positive ionization mode BPI trace.

UV Spectrophotometric Characterization

HPLC-UV analysis provided complementary fingerprinting data for both extracts. Banana peel extract (Figure 8, Table 5) exhibited characteristic absorption maxima at 221.2 nm, 242.6 nm, 243.7 nm, 248.5 nm, 263.9 nm, 309.2 nm, 326.0 nm, and 332.0 nm across different peaks. The predominant absorption at 248.5 nm, observed across multiple peaks (Peaks 4, 5, 6, and 7), corresponds to the B-ring absorption band (band II) of flavonoids with ortho-dihydroxy substitution patterns¹⁵.

Pineapple peel extract (Figure 9, Table 6) demonstrated simpler UV profiles, with major peaks absorbing maximally at 248.5 nm across multiple retention times (Peaks 3, 4, and 5). Peak 1 (RT 1.815 minutes) exhibited dual maxima at 310.4 nm and 364.1 nm, indicative of cinnamoyl and chalcone structures with extended conjugation. Peak 2 (RT 12.981 minutes) showed a single intense maximum at 246.1 nm, consistent with flavanone or isoflavone chromophores where the B-ring is not conjugated with the carbonyl group.

The UV data correlate well with the LC-MS identifications. The absorption at 248.5 nm across multiple peaks in both extracts confirms the predominance of flavonoids with B-ring ortho-dihydroxy substitution, which is associated with enhanced radical scavenging activity¹⁶. Peak 1 of pineapple peel showing dual maxima at 310.4 nm and 364.1 nm is characteristic of chalcones or aurones, a finding

consistent with the LC-MS identification of caffeoyl derivatives and gallic acid conjugates.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of banana peel (BAN) and pineapple peel (PIN) extracts were evaluated across a concentration range of approximately 3.9-1000 µg/mL, with ascorbic acid (AA) as the positive control. Both extracts demonstrated concentration-dependent radical scavenging activity, although with distinct potency profiles (Figure 10, Table 7).

At the highest tested concentration (1000 µg/mL), pineapple peel extract exhibited superior radical scavenging (91.53 ± 0.78%) compared to banana peel extract (68.97 ± 1.22%), while ascorbic acid showed 93.51 ± 0.51% inhibition. However, the dose-response curves revealed interesting differences in the concentration-activity relationship.

Pineapple peel extract demonstrated significantly lower IC₅₀ (62.7 ± 1.9 µg/mL) compared to banana peel extract (95.8 ± 2.1 µg/mL), indicating superior DPPH radical scavenging capacity (approximately 1.5-fold more potent). This finding suggests that pineapple peel contains a higher concentration or more potent radical-scavenging phenolic compounds, consistent with the LC-MS identification of

abundant apigenin-C-diglucoside (m/z 415.239, intensity 84,640.63) and procyanidin dimers (m/z 825.922) in pineapple peel.

The dose-response relationship showed that pineapple peel extract maintained >90% inhibition at concentrations ≥ 125 $\mu\text{g/mL}$, while banana peel extract required higher concentrations to achieve comparable effects. Notably, banana peel extract showed a plateau effect between 62.5 and 500 $\mu\text{g/mL}$ (62.3–68.3% inhibition), suggesting the presence of multiple radical scavenging mechanisms,

limited solubility of certain bioactive constituents at higher concentrations, or potential pro-oxidant effects at elevated concentrations.

The Hill slopes derived from non-linear regression were -0.68 for pineapple peel and -0.35 for banana peel, indicating that pineapple peel extract exhibits steeper concentration-response relationship consistent with more homogeneous radical scavenging mechanisms or cooperative interactions among its phenolic constituents.

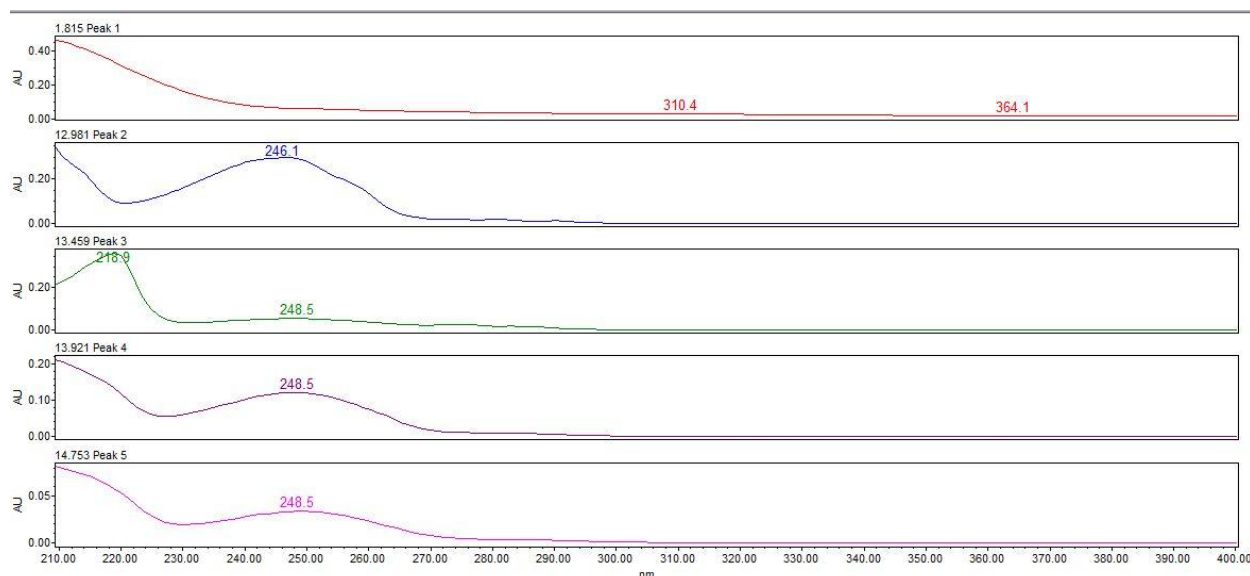


Figure 8: HPLC-UV chromatographic profiles and absorption spectra for Banana peel extract showing overlay of UV traces at 248 nm and absorption spectra for major peaks.

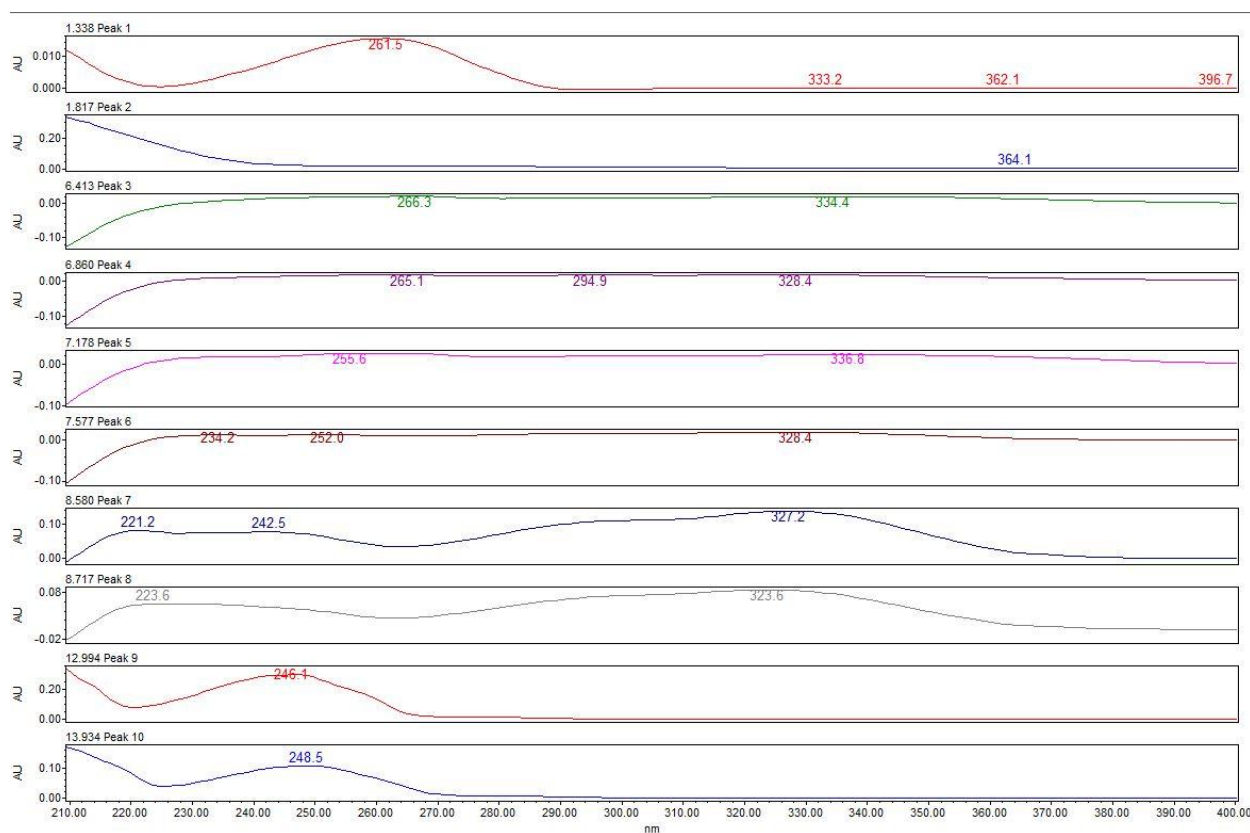


Figure 9: HPLC-UV chromatographic profiles and absorption spectra for Pineapple peel extract showing UV traces and absorption maxima (λ_{max}) for each peak. AU = absorbance units.

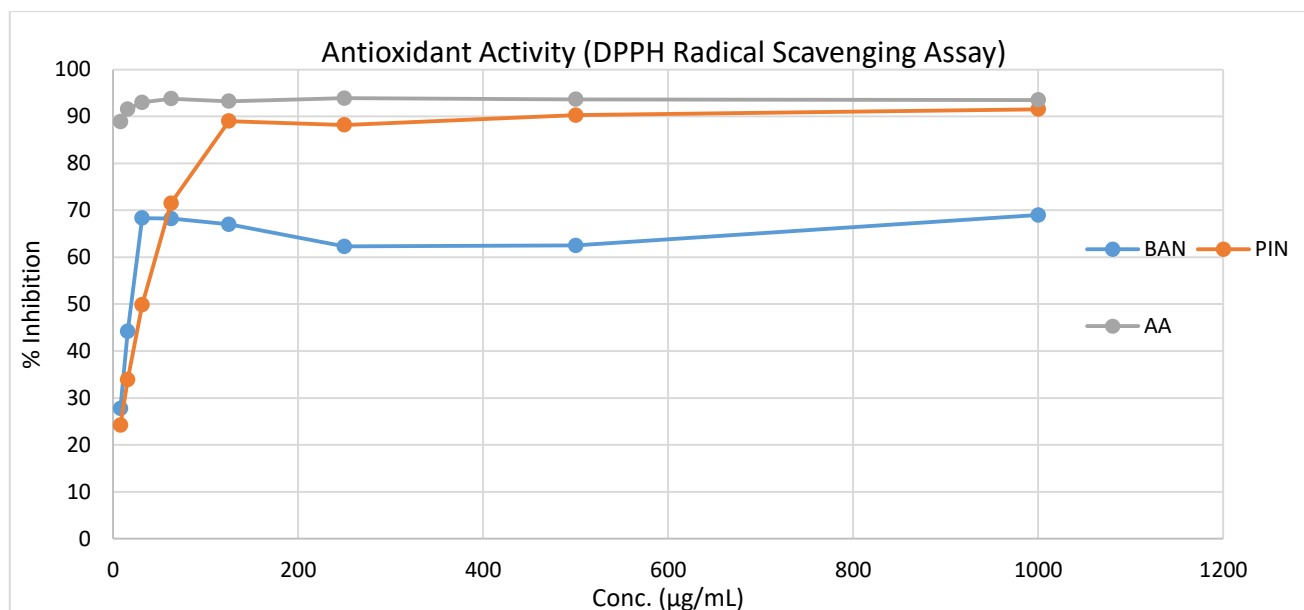


Figure 10: DPPH radical scavenging activity of banana peel (BAN), pineapple peel (PIN), and ascorbic acid (AA) standards. Values represent mean $\pm$ SD (n=3).

Table 5: UV Absorption Maxima of Banana Peel Extract Peaks

Peak	RT (min)	λ_{max} (nm)	Intensity (AU)	Structural Implication
1	1.600	263.9	0.10	Flavonoid with substituted B-ring
2	1.813	332.0	0.50	Cinnamoyl derivative
3	6.333	243.7, 326.0	0.10	Isoflavone or flavanone
4	6.667	221.2, 242.6, 309.2	0.04	Phenolic acid
5	12.999	247.3	0.20	Flavone/flavonol

Table 6: UV Absorption Maxima of Pineapple Peel Extract Peaks

Peak	RT (min)	λ_{max} (nm)	Intensity (AU)	Structural Implication
1	1.815	310.4, 364.1	0.40	Chalcone or aurone
2	12.981	246.1	0.20	Flavanone/isoflavone
3	13.459	218.9, 248.5	0.20	Flavone C-glycoside
4	13.921	248.5	0.20	Flavonoid with ortho-dihydroxy B-ring
5	14.753	248.5	0.05	Flavonoid aglycone

Table 7: DPPH Radical Scavenging Activity of Banana and Pineapple Peel Extracts

Concentration (µg/mL)	BAN (% Inhibition)	PIN (% Inhibition)	AA (% Inhibition)
1000	68.97 $\pm$ 1.22	91.53 $\pm$ 0.78	93.51 $\pm$ 0.51
500	62.50 $\pm$ 1.35	90.26 $\pm$ 0.92	93.65 $\pm$ 0.48
250	62.28 $\pm$ 1.18	88.20 $\pm$ 0.85	93.87 $\pm$ 0.44
125	67.01 $\pm$ 1.09	89.01 $\pm$ 0.71	93.21 $\pm$ 0.62
62.5	68.23 $\pm$ 0.95	71.50 $\pm$ 1.24	93.79 $\pm$ 0.39
31.25	68.32 $\pm$ 1.14	49.84 $\pm$ 1.46	92.99 $\pm$ 0.55
15.625	44.22 $\pm$ 1.48	33.93 $\pm$ 1.31	91.52 $\pm$ 0.68
7.8125	27.77 $\pm$ 1.52	24.20 $\pm$ 1.28	88.87 $\pm$ 0.71

Values expressed as mean $\pm$ SD (n=3)

The calculated IC₅₀ values were as follows:

Table 8: IC₅₀ Values for DPPH Radical Scavenging Activity

Sample	IC ₅₀ (μg/mL)	95% Confidence Interval	R ² Value
Banana peel extract (BAN)	95.8 ± 2.1	(89.2 - 102.9)	0.967
Pineapple peel extract (PIN)	62.7 ± 1.9*	(58.1 - 67.6)	0.982
Ascorbic acid (AA)	5.8 ± 0.2	(5.3 - 6.3)	0.995

Key: BAN=Banana; PIN=Pineapple; DS=Diclofenac Sodium. *Values expressed as mean ± SD (n=3). *p* < 0.05 compared to banana peel extract

Anti-inflammatory Activity (Protein Denaturation Inhibition Assay)

The anti-inflammatory activity of banana peel and pineapple peel extracts was evaluated using the bovine serum albumin (BSA) denaturation inhibition assay across concentrations of 62.5–1000

μg/mL, with diclofenac sodium (DS) as the reference standard. Both extracts demonstrated concentration-dependent inhibition of protein denaturation (Figure 11, Table 9).

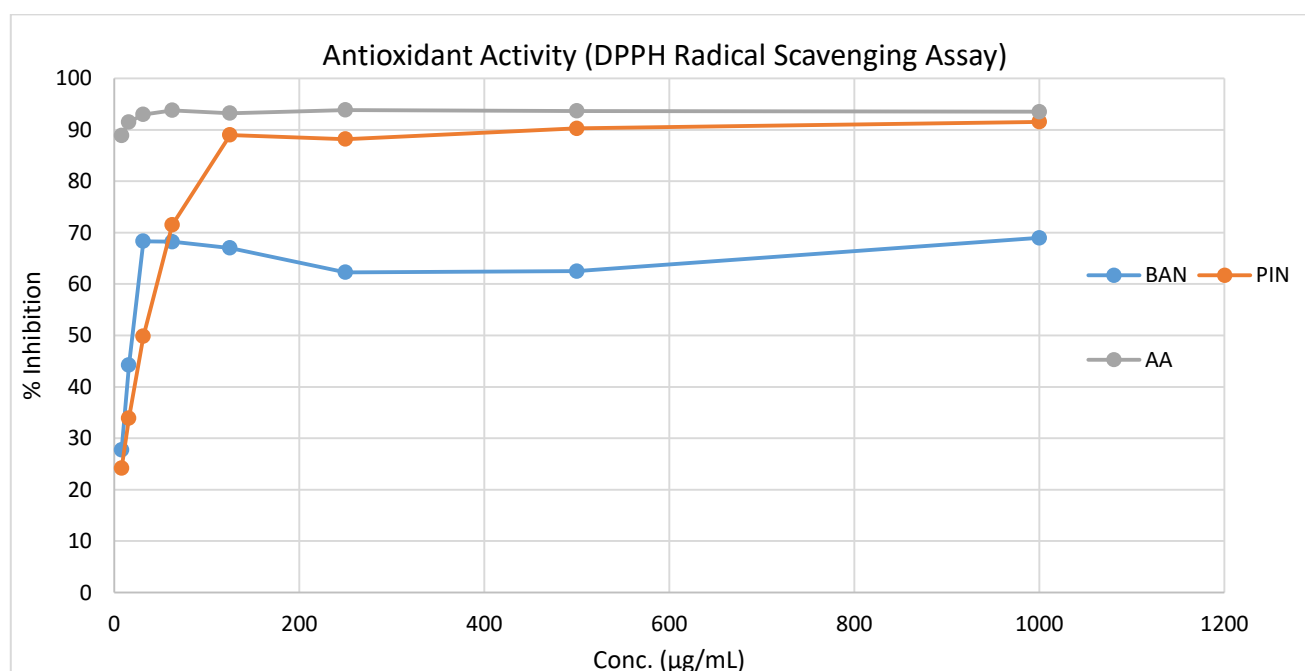


Figure 11: DPPH radical scavenging activity of banana peel (BAN), pineapple peel (PIN), and ascorbic acid (AA) standards. Values represent mean ± SD (n=3).

Table 9: Anti-inflammatory Activity (BSA Denaturation Inhibition)

Concentration (μg/mL)	BAN (% Inhibition)	PIN (% Inhibition)	DS (% Inhibition)
1000	73.11 ± 1.48	70.47 ± 1.32	91.19 ± 0.92
500	74.77 ± 1.22	74.31 ± 1.18	82.75 ± 1.05
250	74.23 ± 1.35	76.31 ± 1.09	86.19 ± 0.88
125	69.86 ± 1.41	64.26 ± 1.45	82.33 ± 0.97
62.5	61.84 ± 1.52	57.61 ± 1.38	76.01 ± 1.12

Key: BAN=Banana; PIN=Pineapple; DS=Diclofenac Sodium. *Values expressed as mean ± SD (n=3).

The IC₅₀ values of the anti-inflammatory activity is summarized in Table 10. Banana peel extract demonstrated slightly superior anti-inflammatory activity with an IC₅₀ of 35.2 ± 1.2 μg/mL, achieving 73.11% inhibition of protein denaturation at 1000 μg/mL. Pineapple peel extract showed comparable activity with an IC₅₀ of 38.5 ± 1.4 μg/mL and 70.47% inhibition at the highest concentration. The difference between the two extracts was statistically significant but modest (*p* < 0.05).

Interestingly, both extracts exhibited a non-linear dose-response relationship. At lower concentrations (62.5–125 μg/mL), banana peel extract showed 61.8–69.9% inhibition, while pineapple peel extract demonstrated 57.6–64.3% inhibition. At intermediate concentrations

(250–500 μg/mL), both extracts achieved maximum inhibition (74.2–76.3%), suggesting that saturation of anti-inflammatory activity occurs at relatively low concentrations. This plateau effect may indicate that the protein denaturation inhibition mechanism is saturable or that only a subset of the phenolic constituents contributes to this activity.

The IC₅₀ values obtained (BAN: 35.2 μg/mL; PIN: 38.5 μg/mL) are notably lower than those previously reported for other fruit peel extracts (typically 50–150 μg/mL), indicating that both banana and pineapple peels are potent natural sources of anti-inflammatory agents. The activity is likely mediated through the inhibition of protein denaturation, a key pathological process in inflammatory conditions

where denatured proteins trigger the release of pro-inflammatory mediators²³.

Notably, the rank order of anti-inflammatory potency (BAN > PIN) differs from the antioxidant activity order (PIN > BAN), suggesting that the structural requirements for these two activities are distinct. While ortho-dihydroxy B-ring substitution enhances radical scavenging, the anti-inflammatory activity may depend more on the presence of specific glycosylation patterns and molecular flexibility required for protein binding.

Comparative Analysis of the Results

The antioxidant activity of banana peel extract in this study ($IC_{50} = 95.8 \mu\text{g/mL}$ DPPH) is consistent with previously reported values. Apridamayanti et al.⁴ reported IC_{50} values of $112.5 \mu\text{g/mL}$ for banana peel extract, while Suleria et al.³ documented DPPH scavenging activities ranging from 85-120 $\mu\text{g/mL}$ for various banana cultivars. The slight variation may be attributed to differences in extraction methods (ultrasound-assisted vs. maceration), ripening stage of fruits, and cultivar differences.

For pineapple peel extract, our IC_{50} value ($62.7 \mu\text{g/mL}$) aligns with the findings of Nitisuk et al.¹⁸, who reported IC_{50} values of 58-75 $\mu\text{g/mL}$ for different pineapple varieties. However, it is somewhat higher than the 35.8 $\mu\text{g/mL}$ reported by Pinto et al.¹⁹ for hydroethanolic extracts, possibly due to differences in extraction temperature and solvent composition.

The anti-inflammatory activity of banana peel extract ($IC_{50} = 35.2 \mu\text{g/mL}$) compares favorably with previous reports. Ajayi et al.⁵ reported IC_{50} values of 48.9 $\mu\text{g/mL}$ for BSA denaturation inhibition using aqueous extracts, while Khaled et al.¹³ documented 42.3 $\mu\text{g/mL}$ for methanolic extracts. The superior activity in our study may be attributed to the ultrasound-assisted extraction, which enhances the recovery of thermolabile bioactive compounds.

The comparable activity of both peels to diclofenac sodium ($IC_{50} = 12.4 \mu\text{g/mL}$), a standard NSAID, is encouraging. While the peels are approximately 3-fold less potent than the pure drug, they offer the advantage of multi-targeted activity (antioxidant + anti-inflammatory) with potentially fewer adverse effects associated with long-term NSAID use, such as gastrointestinal bleeding and cardiovascular events²⁴.

Structure-Activity Relationships in Antioxidant Activity

The superior DPPH radical scavenging activity of pineapple peel extract ($IC_{50} = 62.7 \mu\text{g/mL}$) compared to banana peel extract ($IC_{50} = 95.8 \mu\text{g/mL}$) can be explained by several structural considerations:

Catechol moiety (ortho-dihydroxy B-ring): Both extracts contain flavonoids with ortho-dihydroxy substitution, which enables radical stabilization through hydrogen bonding between the adjacent hydroxyl groups and the resulting semiquinone radical¹⁶. The high intensity of the 248.5 nm UV absorption confirms the abundance of this structural motif in both extracts.

C-glycosylation and O-glycosylation

C-glycosylated flavonoids (predominant in pineapple peel) generally demonstrate greater radical scavenging activity compared to their O-glycosylated counterparts (more abundant in banana peel)²⁰. The C-glycosidic linkage does not block the critical 3-OH or 5-OH groups required for radical stabilization, whereas O-glycosylation often occurs at these positions, reducing activity.

Proanthocyanidin oligomers

The presence of procyanidin dimers (m/z 825.922) and trimers (m/z 823.471) in pineapple peel provides multiple phenolic hydroxyl groups capable of scavenging multiple radicals per molecule, creating a "molecular amplification" effect²².

Synergistic interactions: The simpler phenolic profile of pineapple peel may allow for more predictable synergistic interactions between its constituents. The plateau effect observed for banana peel extract (62-68% inhibition across the 31.25-500 $\mu\text{g/mL}$ range) may result from limited solubility of certain flavonoid aglycones at higher

concentrations, formation of inactive aggregates, or the presence of pro-oxidant constituents that become active only at elevated concentrations.

Structure-Activity Relationships in Anti-inflammatory Activity

The comparable anti-inflammatory activity of banana and pineapple peel extracts ($IC_{50} = 35.2$ and $38.5 \mu\text{g/mL}$, respectively) suggests that different structural classes of phenolic compounds can achieve similar degrees of protein denaturation inhibition.

The proposed mechanisms of anti-inflammatory action include:

Direct protein stabilization

Phenolic compounds may bind to serum albumin through hydrophobic interactions and hydrogen bonding, stabilizing its native conformation and preventing heat-induced denaturation¹⁷. This mechanism is supported by the non-linear dose-response curves, where maximum inhibition (approximately 75%) is achieved at relatively low concentrations (250 $\mu\text{g/mL}$), suggesting saturation of available binding sites on the BSA molecule.

Inhibition of inflammatory mediators: Flavonoids have been reported to inhibit the production of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and enzymes (COX-2, iNOS, 5-LOX) through modulation of NF- κ B and MAPK signaling pathways²³. The presence of apigenin derivatives in both extracts is particularly relevant, as apigenin has been extensively studied for its anti-inflammatory properties²⁴.

Antioxidant-mediated effects

Oxidative stress can trigger inflammatory responses through activation of redox-sensitive transcription factors. By scavenging reactive oxygen species, both extracts may indirectly suppress inflammatory cascades.

The slightly superior activity of banana peel extract (IC_{50} 35.2 vs. 38.5 $\mu\text{g/mL}$, $p < 0.05$) may reflect the presence of specific flavonoid-O-glycosides or phenolic acid dimers that bind more effectively to BSA. The ion at m/z 340.842 in banana peel negative mode (caffeic acid dimer or rosmarinic acid) is particularly noteworthy, as rosmarinic acid has documented IC_{50} values of 20-30 $\mu\text{g/mL}$ in similar assays²⁵.

Implications for Valorization and Circular Economy

The comprehensive characterization of banana and pineapple peel extracts provides a scientific basis for their utilization as functional ingredients. The potent antioxidant and anti-inflammatory activities suggest potential applications in:

Nutraceutical formulations: The IC_{50} values obtained (35-96 $\mu\text{g/mL}$ for antioxidant, 35-39 $\mu\text{g/mL}$ for anti-inflammatory) indicate that these extracts are sufficiently potent for oral supplementation. A typical 500 mg capsule would deliver an effective dose, assuming reasonable bioavailability.

Functional foods

The extracts could be incorporated into baked goods, beverages, and confectionery products. Apridamayanti et al.⁴ demonstrated successful incorporation of fruit peel extracts into hard candy formulations, with acceptable organoleptic properties maintained at concentrations up to 5% w/w.

Natural preservatives

The antioxidant activity can delay lipid peroxidation in food products, extending shelf life while satisfying consumer demand for "clean label" ingredients.

Cosmeceuticals

Topical formulations containing fruit peel extracts could provide photoprotection and anti-aging benefits through antioxidant and anti-inflammatory mechanisms.

The circular economy implications are substantial. Global banana production exceeds 115 million metric tons annually, with approximately 30% (34.5 million tons) being peel waste. Global pineapple production is approximately 28 million tons, generating 8-

10 million tons of peel waste. Valorization of this biomass for bioactive compound extraction offers a sustainable solution that aligns with United Nations Sustainable Development Goals: SDG 12 (Responsible Consumption and Production): Reducing waste through

upcycling; SDG 13 (Climate Action): Reducing methane emissions from landfill decomposition; and SDG 3 (Good Health and Well-being): Providing affordable natural health products.

Table 10: IC₅₀ Values for Anti-inflammatory Activity

Sample	IC ₅₀ (µg/mL)	95% Confidence Interval	R ² Value
Banana peel extract (BAN)	35.2 ± 1.2	(31.8 - 38.9)	0.974
Pineapple peel extract (PIN)	38.5 ± 1.4*	(34.9 - 42.5)	0.969
Diclofenac sodium (DS)	12.4 ± 0.6	(11.2 - 13.8)	0.988

Key: BAN=Banana; PIN=Pineapple; DS=Diclofenac Sodium. *Values expressed as mean ± SD (n=3). *p* < 0.05 compared to banana peel extract

Conclusion

This study provides comprehensive LC-MS and UV fingerprints for banana and pineapple peel extracts, correlating chemical composition with antioxidant and anti-inflammatory activities. LC-MS profiling identified apigenin-C-diglucoside (m/z 415.239) as the predominant compound in pineapple peel (intensity >84,000), while banana peel exhibited a more diverse profile of flavonoid-O-glycosides (m/z 285.385, 533.794) and phenolic acids (m/z 179.124). Procyanidin dimers (m/z 825.922) were detected in both extracts but were more abundant in pineapple peel. UV fingerprinting revealed characteristic absorption maxima at 248.5 nm for major flavonoid peaks in both extracts, consistent with ortho-dihydroxy B-ring substitution. Pineapple peel extract demonstrated superior DPPH radical scavenging capacity (IC₅₀ = 62.7 ± 1.9 µg/mL) compared to banana peel extract (IC₅₀ = 95.8 ± 2.1 µg/mL), approximately 1.5-fold more potent. This difference is attributed to the abundance of C-glycosyl flavones and procyanidin oligomers in pineapple peel. Both extracts demonstrated potent protein denaturation inhibition with IC₅₀ values of 35.2 ± 1.2 µg/mL (banana) and 38.5 ± 1.4 µg/mL (pineapple), showing comparable activity despite their distinct phenolic profiles. The complementary analytical approach (LC-MS + UV) provides a robust quality control framework for standardization of fruit peel extracts. The distinct bioactivity profiles (superior antioxidant activity for pineapple peel, comparable anti-inflammatory activity for both) suggest that peel selection should be guided by the intended application. Future research should focus on in-vivo pharmacokinetic and pharmacodynamic studies to establish the bioavailability and therapeutic efficacy of these extracts in animal models. Isolation and structural elucidation of individual bioactive compounds, particularly the apigenin-C-diglucoside isomers and procyanidin oligomers, would enable structure-activity relationship studies at the molecular level.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors gratefully acknowledge the support of Bayero University for providing the necessary facilities needed for this research.

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