


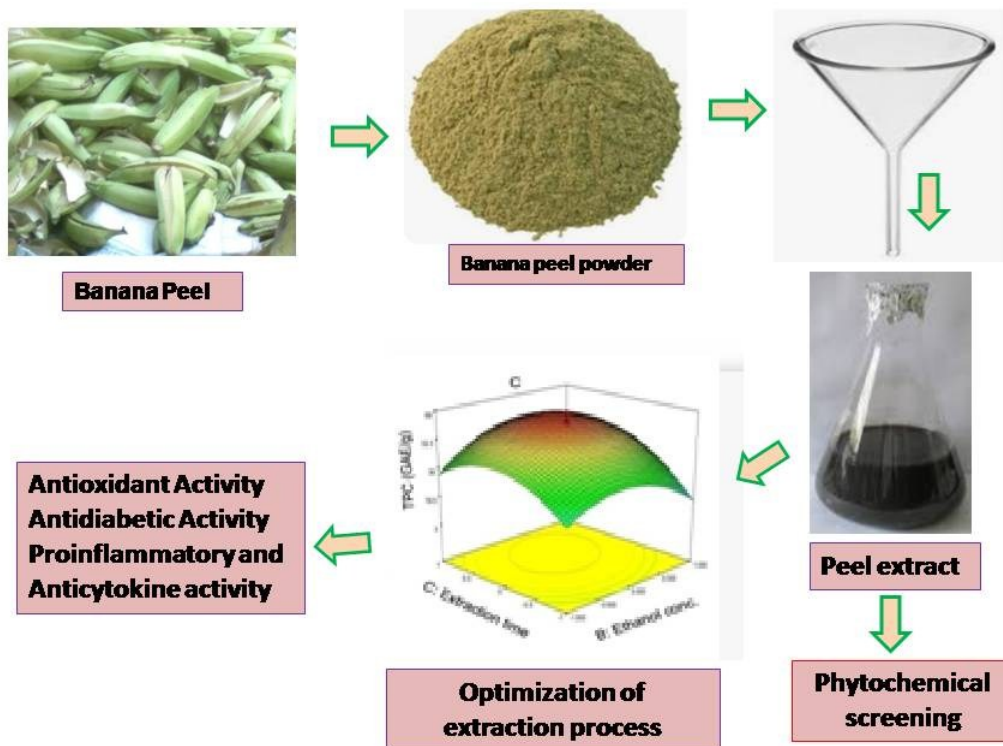
Extraction of Polyphenols from Unripe Banana Peels via Response Surface Methodology and Analysis of *In Vitro* Bioactive Properties

Mansour K. Gatasheh  *

*Corresponding author: mgatasheh@ksu.edu.sa

DOI: 10.15376/biores.21.2.4678-4700

GRAPHICAL ABSTRACT



Extraction of Polyphenols from Unripe Banana Peels via Response Surface Methodology and Analysis of *In Vitro* Bioactive Properties

Mansour K. Gatasheh  *

Banana peels are rich in phytochemicals, including tannins, phenolic compounds, flavonoids, glycosides, saponins, and terpenoids. This study focused on the use of unripe banana peels of *Musa × paradisiaca* var. Monthan for the extraction of total phenolic content (TPC) and total flavonoid content (TFC). Three independent variables (solid–solvent ratio, ethanol concentration, and extraction time) were selected for optimizing the ultrasound-assisted extraction of the TPC and TFC via response surface methodology. The banana peel extract presented a maximum TPC and TFC of 68.2 mg GAE/g and 15.4 QE/g, respectively, when extracted under the following extraction conditions: a 6% solid–solvent ratio, 50% ethanol, and 40 min extraction time. Banana peels showed 75.4±2.1% inhibition in the DPPH assay and 59.9±1.1% inhibition in the ABTS assay at a concentration of 100 µg/mL. Banana peels presented significant enzyme inhibitor activity. The IC₅₀ values of the α-amylase and α-glucosidase inhibitory activities of the peel extract were 7.4±0.2 mg/mL and 9.5±0.09 mg/mL, respectively. Banana peel extract suppressed the synthesis of proinflammatory cytokines such as IFN-γ, IL-1α, TNF-α, and IL-6 and upregulated the synthesis of anti-inflammatory cytokines (IL-13 and IL-10). Banana peel can be utilized for its anti-inflammatory, antioxidant, and antidiabetic activities.

DOI: 10.15376/biores.21.2.4678-4700

Keywords: *Banana peels; Polyphenols; Flavonoids; Bioactive*

Contact information: Department of Biochemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; *Corresponding author: mgatasheh@ksu.edu.sa

INTRODUCTION

Banana peel is a major source of functional compounds that can be utilized because of its anti-inflammatory, antioxidant, antimicrobial, and cytotoxic properties (Manzoor and Ahmad 2021). The functional properties of banana peels vary on the basis of the availability of phytochemical compounds. The utilization of banana peels can improve the circular economy of farmers. Climatic changes result in an increase in UVB radiation and cause oxidative stress in keratinocytes (Jin *et al.* 2007). The available polyphenols in banana peels reduce the risk of oxidative stress and reduce the risk of secondary risks upon UVB radiation (Sánchez-Marzo *et al.* 2017). Agricultural waste can be extracted to obtain phytochemical compounds with potential in cosmetics and biopharmaceuticals. The selection of waste can be based on the presence of specific bioactive functional molecules. Banana peels are rich in valuable phytochemicals, such as pectin. They can be extracted via several methods, including microwave-assisted extraction. Thus, pectin extracted from banana peels can be utilized as a stabilizing agent and plays a critical role in regulating

digestive processes (Rivadeneira *et al.* 2020). Several previous reports have revealed that unripe banana peels contain significant amounts of natural antioxidants, including norepinephrine and dopamine (Kanazawa and Sakakibara 2000). The total phenolic content varied with topographic condition and cultivar. For example, in Brazil, unripe banana peels contain 29.0 to 61.0 mg GAE/100 g of total phenolic content (Hikal *et al.* 2022). Banana peels also contain significant amounts of phenolic compounds (gallic acid and (+)-catechin) and flavonoids (Khamsaw *et al.* 2024). Unripe banana peels contain 49.1% quercetin, whereas ripe peels contain 41.8% quercetin (Oboh *et al.* 2024). Unripe banana products have been used for the preparation of baby food and banana chips. Hence, a substantial number of peels are generated and wasted without any industrial processing (Chitra 2015). *Musa paradisiacal* var Monthan is a rare endangered banana species cultivated in India. The unripe banana of the Monthan variety is used for the preparation of natural fiber sources to feed babies (Sasipriya *et al.* 2014). *M. paradisiaca* can withstand severe oxidative stress caused by temperature (Kanazawa and Sakakibara 2000). Owing to its ability to tolerate oxidative stress, the present study attempted to utilize this rare banana variety to determine its bioactive properties. The biotechnological applications of banana peels include bioethanol production, protein isolation for farm animals, and enzyme extraction (Jamal *et al.* 2012). The bioactive compounds identified in banana peels promote probiotic development and significantly reduce the risk of cardiovascular diseases (CVDs) and cancer (Kris-Etherton *et al.* 2002). Banana peel and pulp are rich in carotenoids, phenolic compounds, biogenic amines, flavonoids, *etc.* (Pereira and Maraschin 2015).

Bananas have greater antioxidant capacity than herbs, berries, and vegetables do, which is attributed to the presence of several bioactive compounds (Moongngarm *et al.* 2014). The available pigments in banana, including β -carotene, α -carotene, and β -cryptoxanthin, exhibit provitamin A activity; similarly, other compounds (lycopene and lutein) exhibit antioxidant potential (Erdman *et al.* 1993). The provitamin A capacity of banana peel phytochemicals varies with cultivar, and the iron and zinc contents do not vary significantly among *Musa* spp. (Davey *et al.* 2009). Major phenolic compounds, such as sinapic, ferulic, salicylic, *p*-hydroxybenzoic, gallic, vanillic, gentisic, syringic, and *p*-coumaric acids, are present in banana. Moreover, the ferulic acid composition is high among these phenolic compounds (Russell *et al.* 2009). Bananas are rich in flavonoids such as myricetin, cyanidin, quercetin, and kaempferol, which have several health benefits, including antioxidant activity (Kevers *et al.* 2007). Most of these reported phenolic compounds exhibit antiviral, anti-inflammatory, antiallergenic, antithrombotic, antibacterial, and vasodilator functions (Ali *et al.* 2021). The anti-inflammatory and antidiabetic properties of unripe banana peels have been reported recently. Moreover, these activities may vary with cultivar, topographical conditions and season (Raveena *et al.* 2024).

The extraction of phytochemical compounds is an important step in determining the amount of phenols. The extraction conditions, such as the solvent concentration, type of solvent, extraction time, temperature, and solid–solvent ratio, are the major factors influencing the phytochemical yield. The extraction parameters varied with the type of plant material and the amount of phytochemical compounds. Therefore, the present study aimed to establish optimum extraction conditions by analyzing the influence of extraction parameters and the bioactive potential of phenolic and flavonoid compounds from unripe banana peels.

EXPERIMENTAL

Phytochemical Screening

Banana peels were collected from unripe banana *Musa × paradisiaca* var. Monthan. The peels were separated manually from the whole banana and washed two times with water. The peels were chopped and dried for three days under sunlight or until completely dry. The peels were blended mechanically, and a powder was obtained. The phytochemical contents of the banana peels were determined as described previously (Sadasivam 1996). Briefly, 1.0 g of banana peel powder was mixed with 20 mL of double distilled water at a 1:20 (w/v) ratio, 75% ethanol, and 75% methanol for 24 h with constant shaking at ambient temperature. The extract was subsequently filtered through Whatman No. 1 filter paper, and the filtrate was used for phytochemical (alkaloid, flavonoid, glycoside, saponin, terpenoid, tannin, and steroid) screening.

Ultrasound-Assisted Extraction

Banana peels (0.5 g) were mixed with 5 mL of ethanol for five minutes. The mixture was further homogenized for 60 s and then placed in an ultrasonic bath. After 60 s, the extract was centrifuged at 5000 ×g for 10 min at 4 °C. The upper layer was separated and stored at −20 °C for the determination of TFC and TPC (Klangpetch *et al.* 2016; Mostafa *et al.* 2018).

Optimization of the Ultrasound-assisted Extraction of Total Flavonoids and Total Phenolic Compounds

Total flavonoids and phenolic compounds from banana peels were extracted via response surface methodology with a central-composite design. Three independent variables were selected for optimized ultrasound-assisted extraction *via* an ultrasonic bath. These parameters included the solid–solvent ratio (2% to 10%), ethanol concentration (25% to 75%), and extraction time (20 min to 60 min). This experimental design comprised six axial points, totaling 20 experiments. The experimental design and analysis were carried out *via* Design Expert software (version 8.0.1; State-Ease, Inc., Minneapolis, USA). The extraction procedure was carried out with 2 g of dried banana peels, and an appropriate amount of ethanol was added to a 100 mL Erlenmeyer flask and incubated at predetermined times. Ultrasound-assisted extraction was performed, and the clear extract was filtered through Whatman No. 1 filter paper. The TFC and TPC of the peel extracts were tested. This experiment was performed in triplicate, and the results were analyzed *via* Design Expert software. The effects of the selected independent parameters on phytochemical yield were analyzed by developing a second-order polynomial model, and the interactions were analyzed via Eq.1,

$$Y_k = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (1)$$

where Y_k represents the predicted response; β_0 denotes the intercept; X_i and X_j are independent variables; β_i represents the linear regression coefficient; β_{ii} represents the quadratic regression coefficient; and β_{ij} represents the interaction effect.

A minimum threshold of 95% using analysis of variance (ANOVA) and fitness of the model was used to test the overall model significance along with the model coefficient values. All experiments were performed in triplicate, and the average mean values were used for analysis.

Analysis of Phytochemical Compounds

Determination of total phenolic contents

The total phenolic content (TPC) of the banana peel powder was assayed *via* the Folin–Ciocalteu method. Briefly, banana peel extract was prepared at a 1.0 mg/mL concentration in Millipore water. Approximately 0.2 mL of extract was mixed with 1.98 mL of 10% Folin–Ciocalteu reagent. To this mixture, 0.5 mL of 10% sodium carbonate was added, and the mixture was incubated in the dark for 30 min. Gallic acid was prepared at various concentrations and used as a reference standard. The absorbance of the sample was read at 765 nm, and the result was expressed as mg of gallic acid equivalent/g of extract. The results are expressed as the mean \pm standard deviation (SD) (Salar *et al.* 2012).

Determination of total flavonoids

The total flavonoid content (TFC) of the banana peels was assayed *via* a colorimetric test. The banana peel extract was prepared at a concentration of 1.0 mg/mL and used for analysis. Catechin was used as the reference standard, and the results are expressed as mg of catechin equivalents/g sample. Approximately 0.2 mL of extract was mixed with 0.8 mL of 5% NaNO₂ and incubated for 5 min in the dark. Then, 0.8 mL of 10% AlCl₃·6H₂O was added and further incubated for 5 min. To the test tubes, 1 N NaOH (1.5 mL) was added, and the sample was neutralized and incubated for 15 min. The absorbance of the sample was read at 510 nm against the reagent blank, and the result was expressed as the mean \pm standard deviation (SD) (Lamaison and Carnat 1991).

Antioxidant Assay

DPPH method

The unripe banana peel extract was diluted in methanol to a concentration of 10 mg/mL. The mixture was further diluted to 200, 400, 600, 800, and 1000 μ g/mL. Then, 0.1 mL of extract was mixed with 1.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and incubated in the dark for 30 min at 520 nm (Brand-Williams *et al.* 1995). The percentage inhibition was finally calculated using Eq. 2:

$$\text{Inhibition (\%)} = \left(A_{\text{Control}} - (A_{\text{Sample}} - A_{\text{Blank}}) \right) 100 / A_{\text{Control}} \quad (2)$$

ABTS method

The Trolox equivalent antioxidant capacity of banana peels was determined as described previously by Re *et al.* (1999). Unripe banana peels were prepared at a 10 mg/mL concentration in methanol. Then, it was further diluted with methanol at concentrations of 200, 400, 600, 800, and 1000 μ g/mL and used as the working concentration. To 0.1 mL of extract, 1.9 mL of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was added, and the mixture was incubated for 5 min at room temperature. After 5 min, the absorbance of the sample was read at 734 nm with a colorimeter. The percentage inhibition was calculated via Eq. 2.

FRAP assay method

The unripe banana peel extract was combined with methanol and diluted to a concentration of 10 mg/mL. Then, it was further diluted with methanol to concentrations ranging from 200 to 1000 μ g/mL. Then, 0.1 mL of extract was combined with 1.9 mL of ferric reducing/antioxidant power (FRAP) reagent and incubated for 10 min at 37 °C. The absorbance of each sample was subsequently read at 600 nm. Ferrous sulfate was prepared

at five different concentrations, and the results were used as a reference standard. The results are expressed as μmol of FeSO_4 equivalent/ μg of peel extract (Wu *et al.* 2019). The results are expressed as the means \pm standard deviations (SDs).

Antidiabetic Activity

α -Amylase inhibition activity

The α -amylase inhibitory activity of banana peel extract was tested as described previously by Oboh *et al.* (2015), with slight modifications. To analyze the α -amylase inhibitory activity, 0.2 mL of banana peel extract (2 to 10 mg/mL) was incubated with 0.2 mL of porcine pancreatic α -amylase (10 U/mL). Acarbose (25 to 250 $\mu\text{g}/\text{mL}$) was used as the standard. The tubes were incubated for 10 min at 37 °C, 0.2 mL of 1% soluble starch solution was added, and the mixture was further incubated for 10 min. Then, 0.5 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added, and the mixture was placed in a boiling water bath for 5 min and cooled. The color intensity was read at 540 nm via a spectrophotometer. The percentage of α -amylase inhibition was calculated via Eq. 3:

$$\text{Inhibition}(\%) = \left[1 - \left(\frac{A_{\text{Sample}} - A_{\text{Control}}}{A_{\text{test}} - A_{\text{Blank}}} \right) \right] \times 100 \quad (3)$$

where A_{sample} represents the absorption of the mixture of banana peel extract, substrate and DNS reagent; A_{control} represents the mixture of buffer, substrate and DNS reagent without amylase; A_{test} represents the mixture of substrate, buffer, enzyme and DNS reagent; and A_{blank} represents the mixture of banana peel extract, substrate, and DNS reagent without amylase.

α -Glucosidase Inhibition Activity

The α -glucosidase inhibitory activity of the banana peel extract was determined as described previously by Oboh *et al.* (2015), with slight modifications. The banana peel extract was prepared in 0.1 M phosphate buffer (pH 7.2) at various concentrations (2 to 10 mg/mL), and the polyphenol standard was prepared at 25 to 250 $\mu\text{g}/\text{mL}$ concentrations. α -Glucosidase was prepared in phosphate buffer (pH 7.2, 0.1 M). The banana extract (0.2 mL) was mixed with 0.2 mL of the enzyme mixture and incubated for 10 min at 37 °C. Then, 0.1 mL of 5 mM *p*-nitrophenyl- α -D-glucopyranoside was added, the mixture was incubated for 10 min, and the color intensity was read at 405 nm. The percentage α -glucosidase inhibitory effect was calculated as previously described (Eq. 2).

Determination of Proinflammatory and Anti-Inflammatory Cytokines

The macrophage cell line (RAW 264.7) was prepared at a density of 3×10^5 cells per well and seeded in a microtiter plate. The RAW 264.7 cell line was cultivated in Roswell Park Memorial Institute medium supplemented with 1% antibiotics and 10% fetal bovine serum. The cells were further incubated at 37 °C in a humidified incubator under 5% CO_2 and were frequently subcultured before the experiments were performed. The plates were subsequently incubated with ethanolic extracts of banana peels at two different concentrations (100 and 200 $\mu\text{g}/\text{mL}$) for six hours. After 6 h of incubation, lipopolysaccharide (1.0 $\mu\text{g}/\text{mL}$) was added, and the mixture was incubated in a 5% CO_2 atmosphere for 24 h at 37 °C. The culture medium was subsequently centrifuged at $4000 \times g$ at 4 °C for 10 min, after which the supernatant was retained and stored at -80 °C. Then, 0.2 mL of phosphate-buffered saline was added, and 0.2 mL of RIPA lysis buffer (containing 50 mM Tris pH 7.4 to 8.0, 150 mM NaCl, 1% NP-40 or Triton X-100, 0.5% sodium

deoxycholate, 0.1% SDS) containing phosphatase inhibitor cocktails (PICs) was added, after which the mixture was further incubated for 3 min. The cells were treated with lysis buffer, agitated for 30 min at 4 °C and sonicated for 60 s at 30 kHz. Then, the lysate was stored on ice and centrifuged at 10000×g for 10 min. The microtiter plates were coated with centrifuged medium (0.1 mL) and further incubated for 12 h at 4 °C. The mixture was subsequently discarded, and the samples were further rinsed with PBS containing 0.05% Tween 20. BSA was used as a blocking agent, and the samples were treated with PBS containing 0.05% Tween 20. Then, primary antibodies against IL-1 α , TNF- α , TNF- α , IL-6, IL-1 α , INF- γ , IL-13, and IL-10 were added, and the mixture was incubated for 4 h. The plates were subsequently incubated for 24 h, and 0.1 mL of secondary antibody was added and incubated for 4 h at 4 °C with constant agitation. The plates were subsequently washed with PBS buffer, and newly prepared TMB substrate was added to all the wells and incubated for 30 min at room temperature. Then, 1 N sulfuric acid was added to the well, and the absorbance of the plates was measured at 490 nm. The result was expressed as mg of cell protein. The total protein content of the lysate was determined *via* the bicinchoninic acid (BCA) protein assay method with bovine serum albumin (BSA) as a standard.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical analysis of banana peel extract revealed a characteristic color indicating the presence of tannins, saponins, flavonoids, and polyphenols, and a visible color intensity was observed (Table 1).

Table 1. Phytochemical Contents of Banana Peel Extracts

Phytochemicals	Results		
	Ethanol	Methanol	Water
Alkaloids	+++	++	+
Flavonoids	++++	+++	++
Glycosides	+	++	+
Saponins	++	++	++
Terpenoids	+++	++	+
Tannins	++++	++	++
Steroids	-	-	-

Moreover, the steroid test did not reveal any characteristic color intensity and was considered negative. Ethanol presented a significant amount of phytochemical compounds in the extract, followed by methanol and water. In phytochemical extraction, suitable solvent selection is very important since each solvent has its own polarity (Kauffmann and Castro 2023). Ethanol and methanol are polar solvents, and these two solvents were able to dissolve significant amounts of phytochemicals compared with water, which is consistent with previous reports. Flavonoids and polyphenol derivatives are polar compounds that dissolve better in polar solvents than in nonpolar solvents (Al-Khayri *et al.* 2022). In phytochemical extraction, methanol and ethanol (aliphatic alcohols) and ethyl acetate and acetone (polar solvents) are the general choices for saponin and polyphenol

extraction from plant material (Yang *et al.* 2010). Thus, the results of the phytochemical screening of banana peel extracts in various solvents indicate that ethanol resulted in higher phytochemical extraction efficiency than did methanol or water.

Optimization of the Extraction Process

The selection of suitable extraction conditions that improve the recovery of phytochemical compounds has gained interest because of the current trend of recycling and valorizing agricultural wastes (Mateos-Aparicio 2021; Alarjani *et al.* 2024; Alodaini *et al.* 2025). The extraction parameters were fitted to a quadratic polynomial regression model for the TPC and TFC. The initial screening experiments revealed the influence of three independent factors on the improvement in the TPC and TFC yield. The three independent variables (X1, X2, and X3) selected were the solid–solvent ratio (%), ethanol concentration (%), and extraction time (min), respectively, and were tested at five different levels: $-\alpha$, -1 , 0 , $+1$, and $+\alpha$. The extraction potential was tested by constructing the RSM and analyzing the impact of independent factors *via* Design Expert software. The model values (TPC and TFC yield) are depicted in Table 2. The significance of the designed model was evaluated through ANOVA, where a small p value and large F value of each term in the models revealed a significant impact on the product yield (Yuan *et al.* 2008). The present findings revealed that the designed model was statistically significant for the TPC ($P < 0.0001$) and TFC ($P = 0.0007$). Statistical analysis of independent variables revealed that the solid–solvent ratio and extraction time significantly affected the TPC of the extract ($P < 0.01$). The model F value of 53.13 implies that the designed RSM model was significant. In this model, the variables A (solid–solvent ratio), C (extraction time), AB , AC , BC , A^2 , B^2 , and C^2 were significant model terms. The insignificant lack of fit of response Y1 showed that the designed model was well fit. The R-squared value for the TPC was 0.979, which was in good agreement with the adjusted R-squared value (0.961). The analysis of the model revealed that the quadratic model was a good fit, and the results are presented in Table 3. The coefficient estimate was positive for extraction time (8.71), solid–solvent ratio (5.03), and ethanol concentration (1.18). The final polynomial equation in terms of the coded factor was as follows:

$$\begin{aligned} \text{TPC} = & 61.93 + 5.03A + 1.18B + 8.71C + 4.70AB + 13.83AC \\ & + 5.85BC - 6.00A^2 - 14.77B^2 - 15.94C^2 \end{aligned} \quad (4)$$

The banana peel extract presented a maximum TPC and TFC of 68.2 mg GAE/g and 15.4 mg QE/g, respectively, when extracted under the following extraction conditions: a 6% solid–solvent ratio, 50% ethanol, and 40 min extraction time. The experimental results of the TFC analysis constitute the basis of fitting the second-order polynomial regression model and are illustrated by the following equation.

$$\begin{aligned} \text{TFC} = & 12.24597 + 0.307197B + 1.672474C + 0.8375AB + 2.6875AC \\ & + 0.4125BC + 0.232943A^2 - 2.5071B^2 - 3.28491C^2 \end{aligned} \quad (5)$$

where A , B , and C are the coded values of the selected independent variables (solid-solvent ratio, ethanol concentration, and extraction time).

The regression coefficients of the designed model were analyzed, and P values were determined for the TFC. The designed extraction model was significant for flavonoid extraction, and the model P value was < 0.0007 . The second-order polynomial model was best fitted, and the lack of fitness was insignificant. In this model, extraction time

significantly influenced the flavonoid yield compared with the other two individual variables (solid–solvent ratio and ethanol concentration). All three selected independent factors had a positive effect, indicating that the variable range was within the optimum limit for improving the TFC of the extract. The interaction effect of the solid–solvent ratio and ethanol concentration was not significant. Analysis of variance has been widely used to identify the effects of tested factors on the response and test whether these effects should be considered (Hefied *et al.* 2023). Figure 1 shows the 3D surface plots of the TPC and TFCs from banana peel extract. The findings indicate a maximum of TPCs and TFCs with middle values. TPC recovery initially increased with increasing banana peel-to-ethanol ratio, ethanol concentration, and extraction time. After the midpoint, there was a sharp reduction in the TPC yield, and the results are depicted in Figs. 1a to 1c. Moreover, the shape of the 3D surface plot of the TFC was different from that of the TPC, revealing variation in the distributions of phenolic and flavonoid compounds in the extract (Figs. 1d–1f).

In ultrasound-assisted extraction, sound waves or frequencies from 20 kHz to 100 kHz affect pressure changes in the solvent and generate microbubbles. This method causes the rupture of the plant cell wall, thus increasing the penetration of the solvent and improving the mass transfer kinetics. This method results in higher yields with shorter extraction times. This method is eco-friendly and an alternative to traditional solvent extraction methods (Das and Arora 2021).

Compared with absolute ethanol, ethanol concentrations between 40% and 50% were found to be suitable for extracting phenolic compounds (Özbek *et al.* 2020). In other studies, ethanol and water mixtures at a 50:50 ratio were reported to be suitable for the extraction of phenolic compounds from solvent mixtures (Kaur and Ubeyitogullari 2023). The polarity of the solvent is one of the critical factors in phytochemical extraction from the solvent, and the solubilities of the phenolic compounds vary on the basis of the polarity of the solvent used for extraction (Arruda *et al.* 2017). Binary solvent systems have also been used for the extraction of phytochemical compounds. The binary solvent systems provided intermediate polarity, which was similar to that of phenolic compounds, thus improving the solubility and yield of phytochemicals (Barba *et al.* 2020; Osorio-Tobón 2020). The available water in the solvent system causes the plant material to swell, improving the contact surface area between the solvent and solid, whereas ethanol breaks the hydrogen bonds. An increase in ethanol concentration can improve damage to the plant cell membrane of the plant biomass (Drevelegka and Goula 2020).

In the present study, the solid–solvent ratio and solvent concentration were not statistically significant, and only the extraction time was significant; however, the former two variables presented optimum values at the “0” level. Figure 1 shows that the TFC significantly increased with increasing extraction time, as corroborated by the results in Table 4. The lack-of-fit test was used to determine the fitness of the model, and an insignificant lack of fit ($p > 0.05$) observed in this study indicated the suitability of the designed model to predict the variations significantly (Quanhong and Caili 2005). In CCD experiments, coefficient of variation was used to measure the relative variability from the average value, which showed that the model was highly reliable and indicates good precision and reproducibility. The coefficient of variation values obtained for TFC (23.54%) and TPC (12.24%) were highly accurate and highly reliable (Mohamed Mahzir *et al.* 2018).

Haas *et al.* (2018) reported that a high solvent volume can yield more phenolic compounds due to mass transfer. In addition, the determination of the effective solid–

solvent ratio is important, which allows the extraction of more TPCs and TFCs with maximum antioxidant activity. The presence of hydrophilic and lipophilic phenolic compounds in plants strongly correlates with their antioxidant properties (Lang *et al.* 2024). In the present study, extraction time was identified as the prominent independent factor that had a major impact on the extraction of TPC and TFC. Similarly, the extraction time, type of solvent, and solid–solvent ratio have major effects on the extraction process (Selahvarzi *et al.* 2022). Thus, optimizing ideal extraction parameters is a significant factor for reducing organic solvent consumption, extraction time, costs and energy expenditure. It is important to determine the minimum extraction time without compromising the TPC or TFC yields. However, the optimum extraction time allows the solvent to penetrate the plant tissue, dissolving the phytochemical compounds. It has been reported that a longer contact time of solvents with plant materials can significantly improve the release of phytochemicals from the plant biomass to the solvent (Arruda *et al.* 2017). The results of the present investigation and those of previous reports were in good agreement with the use of response surface methodology as an important tool to analyze the impact of extraction conditions for optimizing the yield of polyphenols from various byproducts (Moutinho *et al.* 2023).

Table 2. Experimental Design and Response of the Three Independent Variables Selected for the Extraction of TPC and TFC from Banana Peels

Runs	A:Solid-Solvent Ratio (%)	B:Ethanol Conc (%)	C:Extraction Time (min)	TPC (mg GAE/g)	TFC (mg QE/g)
1	6	50	40	62.4	12.9
2	10	25	60	43	9.2
3	10	25	20	11.4	3.4
4	6	50	40	62	10.2
5	6	50	40	68.2	15.4
6	6	50	40	55.8	12.2
7	2	25	60	14	3.2
8	10	75	60	66	12.5
9	10	75	20	5.7	2.1
10	2	75	60	12.9	4.2
11	6	50	40	60	11.8
12	-0.72	50	40	41	14.9
13	2	25	20	32.4	9.2
14	6	50	73.6	31	7.6
15	12.72	50	40	50	13.1
16	2	75	20	13.2	3.5
17	6	7.95	40	15	4.2
18	6	92.04	40	26.4	8.3
19	6	50	40	63	14.2
20	6	50	6.34	3.8	0.5

Table 3. Results of ANOVA of the Fitted Quadratic Model for TPC Extraction from Banana Peels

Source	Sum of Squares	df	Mean Square	F-Value	P value Prob> F
Model	9727.267	9	1080.807	53.12645	< 0.0001
A-Solid-solvent ratio	345.9549	1	345.9549	17.00521	0.0021
B-Ethanol conc	19.15139	1	19.15139	0.941375	0.3548
C-Extraction time	1035.953	1	1035.953	50.92165	< 0.0001
AB	176.72	1	176.72	8.686567	0.0146
AC	1529.045	1	1529.045	75.1593	< 0.0001
BC	273.78	1	273.78	13.45749	0.0043
A ²	519.5829	1	519.5829	25.53979	0.0005
B ²	3144.977	1	3144.977	154.5895	< 0.0001
C ²	3661.369	1	3661.369	179.9724	< 0.0001
Residual	203.4406	10	20.34406		
Lack of Fit	121.4606	5	24.29211	1.481588	0.3384
Pure Error	81.98	5	16.396		
Cor Total	9930.708	19			

Table 4. Results of ANOVA of the Fitted Quadratic Model for TFC Extraction from Banana Peels

Source	Sum of Squares	df	Mean Square	F-Value	P value Prob> F
Model	353.1831	9	39.24256	9.918021	0.0007
A-Solid-solvent ratio	1.21459	1	1.21459	0.306971	0.5917
B-Ethanol conc	1.288801	1	1.288801	0.325727	0.5808
C-Extraction time	38.20052	1	38.20052	9.654658	0.0111
AB	5.61125	1	5.61125	1.418167	0.2612
AC	57.78125	1	57.78125	14.60342	0.0034
BC	15.96125	1	15.96125	4.033987	0.0724
A ²	0.781994	1	0.781994	0.197638	0.6661
B ²	90.58257	1	90.58257	22.8935	0.0007
C ²	155.5072	1	155.5072	39.30233	< 0.0001
Residual	39.56693	10	3.956693		
Lack of Fit	29.1586	5	5.831719	2.801467	0.1414
Pure Error	10.40833	5	2.081667		
Cor Total	392.75	19			

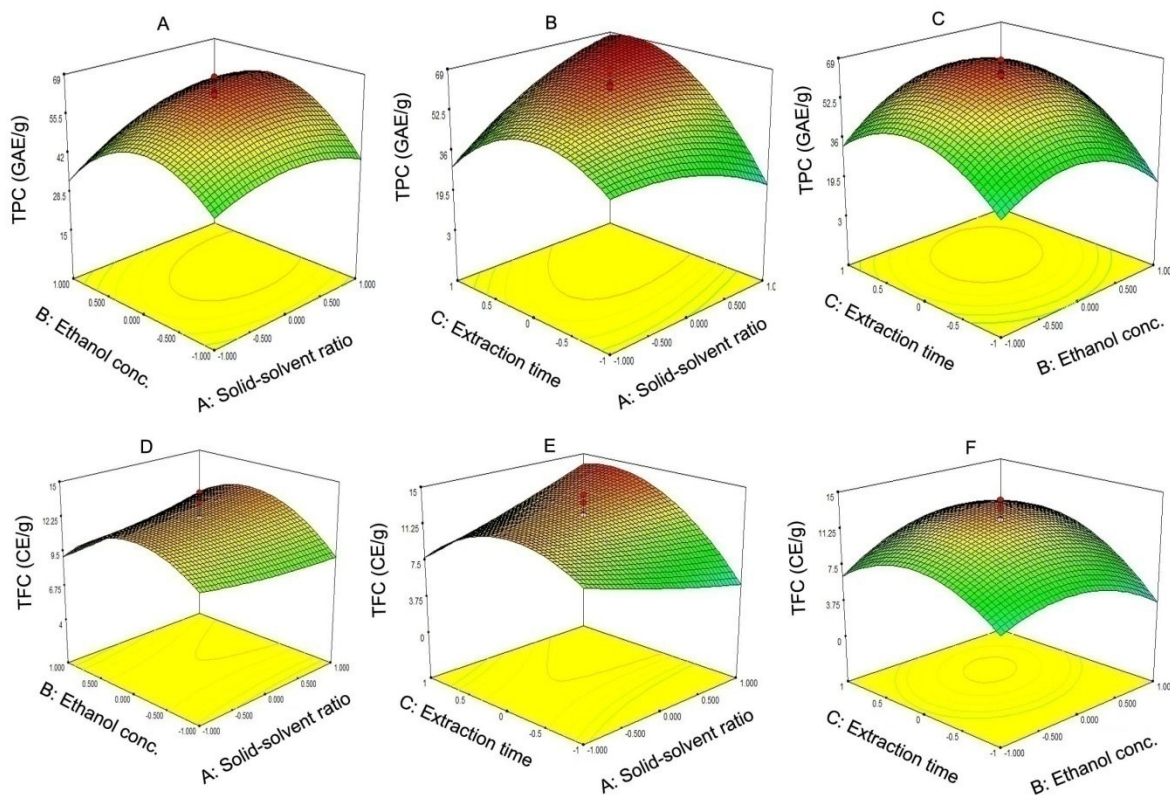


Fig. 1. 3D-Response surface plot illustrating the effects of independent variables on the TPC and TFC (a to c: interaction of ethanol concentration (%), solid–solvent ratio (%), and extraction time (min) for phenolic compounds; d to f: interaction of ethanol concentration (%), solid–solvent ratio (%), and extraction time for flavonoids (min))

Total Phenolic and Flavonoid Contents

The TPC and TFC of the banana peel extract are described in Table 5. The ethanol extract of the banana peel extract had a significant amount of TPC (33.2 ± 1.3 mg GAE/g dry extract), followed by the methanolic extract (26.4 ± 2.2 mg GAE/g dry extract) and water (1.8 ± 0.4 mg GAE/g dry extract). The ethanol extract of the banana peels had the highest TFC (3.8 ± 0.4 mg QE/g dry extract), followed by methanol (1.7 ± 0.2 mg QE/g dry extract) and water (1.42 ± 0.3 mg QE/g dry extract). Ethanol is considered an effective solvent for the extraction of phenolic compounds and TFCs from banana peels. Similar results were obtained by Safdar *et al.* (2017) for mango peels and Ranjha *et al.* (2020) for apple and pomegranate peels. The TPC contents are higher in banana peels than in other fruit peels, such as those of avocado, pine apple, passion fruit, water melon and papaya fruits (Morais *et al.* 2015). Athanasiadis *et al.* (2023) used overripe banana peels for the extraction of polyphenols, and an antibacterial effect was reported by Chen and Cai (2024) due to the presence of polyphenols. Granella *et al.* (2023) recovered phenolic compounds from pretreated banana peels, and the bioactive properties of banana peels were established recently by Wani and Dhanya (2025). Banana peel can be considered a natural source of antioxidants because of the presence of significant polyphenols (Islam *et al.* 2023; Bhavani *et al.* 2023). Islam *et al.* (2023) used enzyme treatment to improve the TPC of banana peel extract. The TPC of the banana peels was found to be 25.4 mg GAE/g DM under the optimized extraction conditions. The TPC yield obtained in this study was greater than that reported by Islam *et al.* (2023). Moreover, the TFC observed in this study (3.8 ± 0.4 mg

QE/g dry extract) was lower than that of enzyme-mediated extraction of banana peels (Islam *et al.* 2023). An optimum extraction temperature and solvent percentage are required to assist TFC extraction from the raw material (Görgüç *et al.* 2019). In this study, the TPC and TFC varied widely with respect to the solvent system used for phytochemical extraction. The effectiveness of the extraction step is based on the solvents used, the extraction conditions selected, and the type of plant material used for extraction (Adefegha *et al.* 2015).

Table 5. Total Phenolic Content and Total Flavonoid Content of Unripe Banana Peels

Extractions	Total Phenolic Content (mg GAE/g dry extract)	Total Flavonoid content (mg QE/g dry extract)
Ethanol	33.2±1.3	3.8±0.4
Methanol	26.4±2.2	1.7±0.2
Water	1.8 ±0.4	1.42±0.3

Antioxidant Activity

To determine the antioxidant activity of the fruit peels, various antioxidant assays were selected on the basis of different mechanisms. ABTS and DPPH activities were used to analyze the radical scavenging activity, whereas the FRAP assay was used to analyze the reducing power of peels. The results are presented in Fig. 2 as % radical scavenging activity for ABTS and DPPH and μM FeSO_4 equivalents for the FRAP assay. The DPPH assay has been widely used to analyze free radical scavenging power, which is due mainly to the presence of polyphenol compounds (Yang *et al.* 2020). Banana peels presented higher DPPH activity (75.4±2.1% inhibition), followed by ABTS activity (59.9±1.1% inhibition), at a concentration of 100 $\mu\text{g}/\text{mL}$. The antioxidant activity was dose dependent, which was similar to reports of dose-dependent DPPH activity (Ajila *et al.* 2007). In this study, freeze-dried banana peels were used for the determination of antioxidant activity, and the freeze-drying process neutralized free radicals and generated redox-active metabolites, as reported by Castro-Vazquez *et al.* (2016). The DPPH assay is largely considered a nonspecific free radical scavenging assay because of its ability to detect the antioxidant power of both nonphenolic compounds and phenolic compounds. Therefore, alternative methods have been recommended for testing the *in vitro* antioxidant power of phytochemicals.

The ABTS assay method has been recommended for evaluating antiradical scavenging potential on the basis of the ability of phenolic compounds to donate hydrogen atoms (Yang *et al.* 2020). The antioxidant activity observed *via* the ABTS assay method was similar to that reported in studies of freeze-dried grape peel extract (Castro-Vazquez *et al.* 2016), kiwi fruit extract (Pal *et al.* 2015), and Hass and Fuerte peel samples (Tremocoldi *et al.* 2018).

A FRAP assay was used in this study to analyze the potential of banana peels to donate electrons to reduce the Fe^{+3} -TPTZ complex, and the peel extract presented significant FRAP reducing power, with a value of $1.3\pm 0.009 \mu\text{MFeSO}_4$. The present findings were comparable to the results reported by Agunloye *et al.* (2019) in orange and apple peels, who detected the presence of bound flavonoids and phenolics. Similarly, the FRAP activities of lime, banana, kiwifruit, and mango have been reported previously (Guo *et al.* 2003), which was consistent with the results of this study.

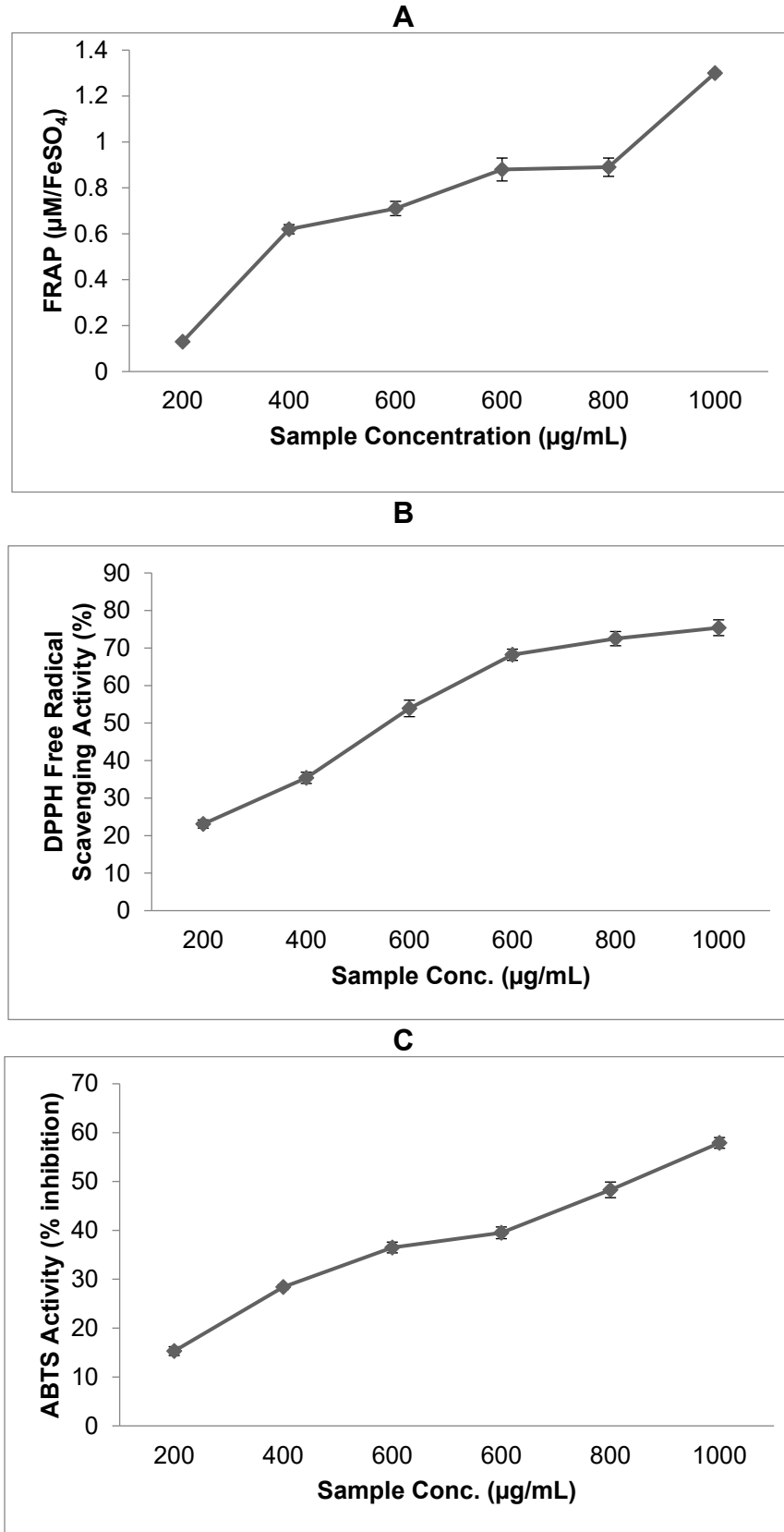


Fig. 2. Antioxidant activities of banana peel extracts at various concentrations: (A) FRAP antioxidant activity, (B) DPPH free radical scavenging activity (%), and (C) ABTS radical scavenging activity (%)

Banana peel is considered an important source of antioxidant phytochemicals that alleviate the generation of ROS associated with oxidative stress. The recovery of antioxidant phytochemicals from fruit waste supports their application in the preparation of cosmetics with antioxidant activity by reducing the generation of free radicals in the dermis (Arraibi *et al.* 2021). Similarly, the antioxidant activity of banana peels was established by Islam *et al.* (2023), and the TPC was extracted via enzyme treatment. The increased level of antioxidant properties in the banana peel extract may be the result of increased TFC and TPC (Islam *et al.* 2021). The banana peel extract presented significant amounts of flavonoids and phenolic compounds, which potentially had antioxidant activity in terms of ABTS and DPPH radical scavenging activities, and the present findings were comparable to those of earlier reports on other fruit peels (Suleria *et al.* 2020). The phenolic content detected in fruit peels and seeds, including watermelon byproducts and avocado peels, was directly associated with antioxidant activity. The TPC and TFC contents of fruit peels have been reported, and the amounts of these phytochemicals vary on the basis of agricultural byproducts/residues used for extraction (Shi *et al.* 2021; Neglo *et al.* 2021; Gaber *et al.* 2021).

Antidiabetic Activity of Banana Peel Extract

The digestive enzyme inhibitory effect of banana peel extract was determined, and the enzyme inhibitory effect increased in a dose-dependent manner, while the enzyme activity decreased. The IC_{50} value of the α -amylase inhibitory activity of the banana peel extract was 7.4 ± 0.2 mg/mL, which was 14 times greater than that of the positive control (0.51 ± 0.2 mg/mL). Similarly, the IC_{50} value of the α -glucosidase inhibitory effect on banana peels was 9.5 ± 0.09 mg/mL, which was more than 15 times greater than that of the positive control (0.62 ± 0.1 mg/mL) (Fig. 3). A chitinase-like protein was identified previously from banana varieties that exhibited α -amylase inhibitory activity, and the IC_{50} values ranged from 7.22 ± 0.41 to 15.5 ± 0.02 mg/mL (Karnchanatat and Sangvanich 2012), which was similar to the results of this study. The phytochemicals from the pseudostem, flower, and peels of banana inhibited α -glucosidase activity and insulin levels (Ramu *et al.* 2015).

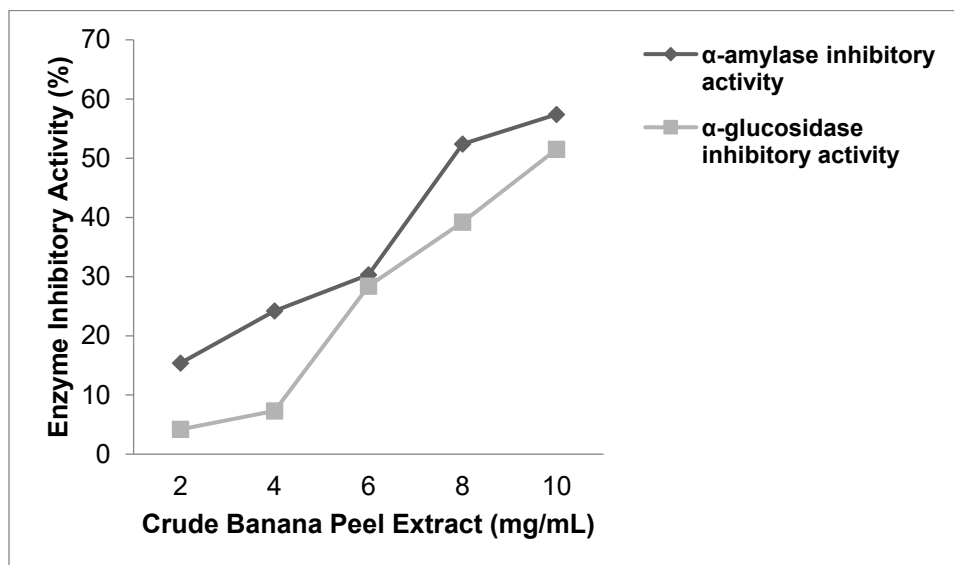


Fig. 3. Antidiabetic activity of banana peel extract at various concentrations

Phytochemical compounds such as (+)-catechin and epicatechin have been reported to have α -glucosidase inhibitory activity (Tadera *et al.* 2006; Alothman *et al.* 2009). Similarly, Wang *et al.* (2015) reported the α -glucosidase inhibitor activity of gallic acid from the extract. The present study and previous reports revealed that banana peels can be used as natural antidiabetic agents to manage diabetes-associated complications (Wang *et al.* 2022; Vijay *et al.* 2022). The screening of antidiabetic agents from natural sources is becoming more popular because of the side effects of commercial antidiabetic agents (Rahman *et al.* 2022). At 10 mg/mL concentration, the α -amylase inhibitory effect was $57.4 \pm 2.9\%$, which was $51.5 \pm 2.1\%$ on α -glucosidase inhibitory activity at the same concentration. The present finding was consistent with the previous result reported by Islam *et al.* (2023). *In vitro* studies previously reported that phenolic compounds potentially affect α -glucosidase activity (Papoutsis *et al.* 2021).

***In Vitro* Effects of Banana Peel Extract on Proinflammatory and Anti-inflammatory Activity**

To determine the impact of banana peel extract on cytokine synthesis in inflamed patients, an ELISA-based method was used. At 200 $\mu\text{g/mL}$, banana peel metabolites suppressed the synthesis of proinflammatory cytokines such as IFN- γ , IL-1 α , TNF- α , and IL-6 and upregulated the synthesis of anti-inflammatory cytokines (IL-13 and IL-10). In addition, the level of the anti-inflammatory cytokine IL-10 was increased (Table 6). LPS activates macrophages to produce proinflammatory cytokines, and these anti-inflammatory cytokines control the response of proinflammatory cytokines. IL-10 is considered to have potent anti-inflammatory properties, and the upregulation of IL-10 is implicated in the anti-inflammatory response mediated by banana peel phytochemicals through the downregulation of proinflammatory cytokine receptors. Anti-inflammatory molecules regulate the production of cytokines at various levels (Martinez-Espinosa *et al.* 2021). ELISA revealed that the increased level of the anti-inflammatory cytokine IL-10 was due to the presence of phytochemicals in the banana peel extracts. The *in vitro* anti-inflammatory activity of the banana peel extract observed in this study was similar to that reported by Hong *et al.* (2023). They reported the anti-inflammatory response of banana peel extracts tested *in vitro* and *in vivo*. Oral administration of the sample reduced the inflammatory response, lowered the serum TNF- α and IL-6 levels, and regulated the T-cell population (Hong *et al.* 2023).

Table 6. *In Vitro* Suppression of Proinflammatory Cytokines and Upregulation of the Anti-inflammatory Response by Banana Peel Extract

Experiment	Protein ($\mu\text{g/mL}$)					
	IL-1 α	TNF- α	IL-6	INF- γ	IL-13	IL-10
LPS	0.52 ± 0.04	0.82 ± 0.03	0.48 ± 0.02	0.57 ± 0.03	0.28 ± 0.05	0.37 ± 0.02
Control	0.35 ± 0.01	0.53 ± 0.02	0.42 ± 0.03	0.51 ± 0.04	0.37 ± 0.03	0.63 ± 0.05
100 μmL EX	0.31 ± 0.04	0.48 ± 0.05	0.35 ± 0.04	0.42 ± 0.02	0.39 ± 0.05	0.66 ± 0.02
200 μmL EX	0.19 ± 0.02	0.37 ± 0.02	0.27 ± 0.03	0.29 ± 0.05	0.52 ± 0.04	0.72 ± 0.03
Positive control	0.11 ± 0.01	0.28 ± 0.04	0.22 ± 0.04	0.22 ± 0.02	0.64 ± 0.02	0.84 ± 0.05

CONCLUSIONS

1. Unripe banana peels are sources of bioactive secondary metabolites with bioactive properties.
2. Response surface methodology was used to optimize the total phenolic and flavonoid compounds in the extracts. The optimized concentrations of the independent variables were as follows: 6% solid–solvent ratio, 50% ethanol, and 40 min of extraction.
3. Banana peels presented higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity, followed by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) activity, and ferric reducing/antioxidant power (FRAP) reducing power. The ethanol extract presented α -amylase and α -glucosidase inhibitory activity.
4. Banana peel extract suppressed the synthesis of proinflammatory cytokines and upregulated the synthesis of anti-inflammatory cytokines. At higher concentrations, the anti-inflammatory response increased in a dose-dependent manner.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Ongoing Research Funding Program (ORF-2026-393), King Saud University, Riyadh, Saudi Arabia.

Conflict of Interest

The authors do not have any conflicts of interest related to the publication of this research article.

Use of Generative AI

The authors did not use any AI tools in the preparation of text, data analysis, or collation of references.

REFERENCES CITED

- Adefegha, S. A., Oyeleye, S. I., and Oboh, G. (2015). "Distribution of phenolic contents, antidiabetic potentials, antihypertensive properties, and antioxidative effects of soursop (*Annona muricata* L.) fruit parts *in vitro*," *Biochemistry Research International* 2015(1), article 347673. <https://doi.org/10.1155/2015/347673>
- Agunloye, O. M., Oboh, G., Ademiluyi, A. O., Ademosun, A. O., Akindahunsi, A. A., Oyagbemi, A. A., Omobowale, T. O., Ajibade, T. O., and Adedapo, A. A. (2019). "Cardio-protective and antioxidant properties of caffeic acid and chlorogenic acid: Mechanistic role of angiotensin converting enzyme, cholinesterase and arginase activities in cyclosporine induced hypertensive rats," *Biomedicine and Pharmacotherapy* 109, 450-458. <https://doi.org/10.1016/j.biopha.2018.10.044>
- Ajila, C. M., Naidu, K. A., Bhat, S. G., and Rao, U. P. (2007). "Bioactive compounds and antioxidant potential of mango peel extract," *Food Chemistry* 105(3), 982-988. <https://doi.org/10.1016/j.foodchem.2007.04.052>

- Alarjani, K. M., Elshikh, M. S., Alghmdi, M. A., Arokiyaraj, S., and Ponnuswamy, V. (2024). "Valorization of date molasses and municipal solid waste for the production of cellulases by *Trichoderma reesei* Al-K1 149 in a tray reactor," *Waste and Biomass Valorization* 15(10), 5833-5842. <https://doi.org/10.1007/s12649-024-02665-3>
- Ali, A., Ponnampalam, E. N., Pushpakumara, G., Cottrell, J. J., Suleria, H. A., and Dunshea, F. R. (2021). "Cinnamon: A natural feed additive for poultry health and production—A review," *Animals* 11(7), article 2026. <https://doi.org/10.3390/ani11072026>
- Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., and Al-Mssallem, M. Q. (2022). "Flavonoids as potential anti-inflammatory molecules: A review," *Molecules* 27(9), article 2901. <https://doi.org/10.3390/molecules27092901>
- Alodaini, H. A., Sheeja, R. R., Joselin, J., Hatamleh, A. A., Al-Dosary, M. A., Rani, S. A., Vijayaraghavan, P., and Arokiyaraj, S. (2025). "Agro-industrial residues: Low-cost biomass for the production of bioactive extracellular polysaccharides by *Lactobacillus plantarum* in solid state fermentation," *BioResources* 20(3), 7358-7377. <https://doi.org/10.15376/biores.20.3.7358-7377>
- Alothman, M., Bhat, R., and Karim, A. A. (2009). "Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents," *Food Chemistry* 115(3), 785-788. <https://doi.org/10.1016/j.foodchem.2008.12.005>
- Arraibi, A. A., Liberal, Â., Dias, M. I., Alves, M. J., Ferreira, I. C., Barros, L., and Barreira, J. C. (2021). "Chemical and bioactive characterization of Spanish and Belgian apple pomace for its potential use as a novel dermocosmetic formulation," *Foods* 10(8), article 1949. <https://doi.org/10.3390/foods10081949>
- Arruda, H. S., Pereira, G. A., and Pastore, G. M. (2017). "Optimization of extraction parameters of total phenolics from *Annona crassiflora* Mart. (Araticum) fruits using response surface methodology," *Food Analytical Methods* 10(1), 100-110. <https://doi.org/10.1007/s12161-016-0554-y>
- Athanasiadis, V., Chatzimitakos, T., Mantiniotou, M., Kalompatsios, D., Bozinou, E., and Lalas, S. I. (2023). "Investigation of the polyphenol recovery of overripe banana peel extract utilizing cloud point extraction," *Eng.* 4(4), 3026-3038. <https://doi.org/10.3390/eng4040170>
- Barba, F. J., Alcántara, C., Abdelkebir, R., Bäuerl, C., Pérez-Martínez, G., Lorenzo, J. M., Carmen Collado, M., and García-Pérez, J. V. (2020). "Ultrasonically-assisted and conventional extraction from *Erodium glaucophyllum* roots using ethanol: Water mixtures: Phenolic characterization, antioxidant, and anti-inflammatory activities," *Molecules* 25(7), article 1759. <https://doi.org/10.3390/molecules25071759>
- Bhavani, M., Morya, S., Saxena, D., and Awuchi, C. G. (2023). "Bioactive, antioxidant, industrial, and nutraceutical applications of banana peel," *International Journal of Food Properties* 26(1), 1277-1289.
- Brand-Williams, W., Cuvelier, M. E., and Berset, C. L. W. T. (1995). "Use of a free radical method to evaluate antioxidant activity," *LWT-Food Science and Technology* 28(1), 25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Castro-Vazquez, L., Alañón, M. E., Rodríguez-Robledo, V., Pérez-Coello, M. S., Hermosín-Gutierrez, I., Díaz-Maroto, M. C., Jordán, J., Galindo, M. F., and Arroyo-Jimenez, M. D. M. (2016). "Bioactive flavonoids, antioxidant behaviour, and cytoprotective effects of dried grapefruit peels (*Citrus paradisi* Macf.)," *Oxidative Medicine and Cellular Longevity* 2016(1), article 8915729. <https://doi.org/10.1155/2016/8915729>

- Chen, J., and Cai, F. (2024). "Analysis of the antibacterial activity of polyphenols in banana peel," *Academic Journal of Materials & Chemistry* 5(1), 43-48.
<https://doi.org/10.25236/AJMC.2024.050108>
- Chitra, P. (2015). "Development of banana-based weaning food mixes for infants and its nutritional quality evaluation," *Reviews on Environmental Health* 30(2), 125-130.
<https://doi.org/10.1515/reveh-2015-0002>
- Das, I., and Arora, A. (2021). "Kinetics and mechanistic models of solid-liquid extraction of pectin using advance green techniques – A review," *Food Hydrocolloids* 120, article 106931. <https://doi.org/10.1016/j.foodhyd.2021.106931>
- Davey, M. W., Van den Bergh, I., Markham, R., Swennen, R., and Keulemans, J. (2009). "Genetic variability in *Musa* fruit provitamin A carotenoids, lutein and mineral micronutrient contents," *Food Chemistry* 115(3), 806-813.
<https://doi.org/10.1016/j.foodchem.2008.12.088>
- Drevelegka, I., and Goula, A. M. (2020). "Recovery of grape pomace phenolic compounds through optimized extraction and adsorption processes," *Chemical Engineering and Processing-Process Intensification* 149, article 107845.
<https://doi.org/10.1016/j.cep.2020.107845>
- Erdman Jr, J. W., Bierer, T. L., and Gugger, E. T. (1993). "Absorption and transport of carotenoids," *Annals of the New York Academy of Sciences* 691(1), 76-85.
<https://doi.org/10.1111/j.1749-6632.1993.tb26159.x>
- Gaber, N. B., El-Dahy, S. I., and Shalaby, E. A. (2021). "Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues," *Biomass Conversion and Biorefinery* 13(5), 4011-4020. <https://doi.org/10.1007/s13399-021-01386-0>
- Görgüç, A., Bircan, C., and Yılmaz, F. M. (2019). "Sesame bran as an unexploited by-product: Effect of enzyme and ultrasound-assisted extraction on the recovery of protein and antioxidant compounds," *Food Chemistry* 283, 637-645.
<https://doi.org/10.1016/j.foodchem.2019.01.077>
- Granella, S. J., Bechlin, T. R., Christ, D., Machado, S. R. C., Triques, C. C., and da Silva, E. A. (2023). "Pretreated banana peels as a source for the recovery of phenolic compounds: Extraction kinetics, ultrasound optimization, and conventional extraction methods," *Journal of Applied Research on Medicinal and Aromatic Plants* 34, article 100484. <https://doi.org/10.1016/j.jarmap.2023.100484>
- Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., and Jiang, Y. (2003). "Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay," *Nutrition Research* 23(12), 1719-1726. <https://doi.org/10.1016/j.nutres.2003.08.005>
- Haas, I. C. D. S., Toaldo, I. M., Burin, V. M., and Bordignon-Luiz, M. T. (2018). "Extraction optimization for polyphenolic profiling and bioactive enrichment of extractives of nonpomace residue from grape processing," *Industrial Crops and Products* 112, 593-601. <https://doi.org/10.1016/j.indcrop.2017.12.058>
- Hefied, F., Ahmed, Z. B., and Yousfi, M. (2023). "Optimization of ultrasonic-assisted extraction of phenolic compounds and antioxidant activities from *Pistacia atlantica* Desf. galls using response surface methodology," *Journal of Applied Research on Medicinal and Aromatic Plants* 32, article 100449.
<https://doi.org/10.1016/j.jarmap.2022.100449>
- Hikal, W. M., Said-Al Ahl, H. A., Bratovcic, A., Tkachenko, K. G., Sharifi-Rad, J., Kačaniová, M., Elhourri, M., and Atanassova, M. (2022). "Banana peels: A waste

- treasure for human being,” *Evidence-Based Complementary and Alternative Medicine* 2022(1), article 7616452. <https://doi.org/10.1155/2022/7616452>
- Islam, M. R., Haque, A. R., Kabir, M. R., Hasan, M. M., Khushe, K. J., and Hasan, S. K. (2021). “Fruit by-products: The potential natural sources of antioxidants and α -glucosidase inhibitors,” *Journal of Food Science and Technology* 58(5), 1715-1726. <https://doi.org/10.1007/s13197-020-04681-2>
- Islam, M. R., Kamal, M. M., Kabir, M. R., Hasan, M. M., Haque, A. R., and Hasan, S. K. (2023). “Phenolic compounds and antioxidants activity of banana peel extracts: Testing and optimization of enzyme-assisted conditions,” *Measurement: Food* 10, article 100085. <https://doi.org/https://doi.org/10.1016/j.meafoo.2023.100085>
- heed, O. K., and Alam, Z. (2012). “Bio-valorization potential of banana peels (*Musa sapientum*): An overview,” *Asian Journal of Biotechnology* 4, 1-14. <https://doi.org/10.3923/ajbkr.2012.1.14>
- Jin, G. H., Liu, Y., Jin, S. Z., Liu, X. D., and Liu, S. Z. (2007). “UVB induced oxidative stress in human keratinocytes and protective effect of antioxidant agents,” *Radiation and Environmental Biophysics* 46(1), 61-68. <https://doi.org/10.1007/s00411-007-0096-1>
- Kanazawa, K., and Sakakibara, H. (2000). “High content of dopamine, a strong antioxidant, in cavendish banana,” *Journal of Agricultural and Food Chemistry* 48(3), 844-848. <https://doi.org/10.1021/jf9909860>
- Karnchanatat, A., and Sangvanich, P. (2012). “A chitinase-like protein with α -amylase inhibitory activity from KluaiHom thong banana fruit: *Musa* (AAA group),” *Food Biotechnology* 26(3), 218-238. <https://doi.org/10.1080/08905436.2012.698769>
- Kauffmann, A. C., and Castro, V. S. (2023). “Phenolic compounds in bacterial inactivation: A perspective from Brazil,” *Antibiotics* 12(4), article 645. <https://doi.org/10.3390/antibiotics12040645>
- Kaur, S., and Ubeyitogullari, A. (2023). “Extraction of phenolic compounds from rice husk via ethanol-water-modified supercritical carbon dioxide,” *Heliyon* 9(3), article e14196.
- Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommès, J., and Pincemail, J. (2007). “Evolution of antioxidant capacity during storage of selected fruits and vegetables,” *Journal of Agricultural and Food Chemistry* 55(21), 8596-8603. <https://doi.org/10.1021/jf071736j>
- Khamsaw, P., Sommano, S. R., Wongkaew, M., Willats, W. G., Bakshani, C. R., Sirilun, S., and Sunanta, P. (2024). “Banana peel (*Musa* ABB cv. Nam Wa Mali-Ong) as a source of value-adding components and the functional properties of its bioactive ingredients,” *Plants* 13(5), article 593. <https://doi.org/10.3390/plants13050593>
- Klangpetch, W., Phromsurin, K., Hannarong, K., Wichaphon, J., and Rungchang, S. (2016). “Antibacterial and antioxidant effects of tropical citrus peel extracts to improve the shelf life of raw chicken drumettes,” *International Food Research Journal* 23(2), article 700.
- Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E., and Etherton, T. D. (2002). “Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer,” *The American Journal of Medicine* 113(9), 71-88. [https://doi.org/10.1016/S0002-9343\(01\)00995-0](https://doi.org/10.1016/S0002-9343(01)00995-0)
- Lamaison, J. L., and Carnat, A. (1991). “Teneursen principaux flavonoides des fleurset des feuilles de *Crataegus monogyna* Jacq. et de *Crataegus laevigata* (Poiret) DC. en

- fonction de la periode de vegetation,” *Plantes Médicinales et Phytothérapie* 25, 12-16.
- Lang, Y., Gao, N., Zang, Z., Meng, X., Lin, Y., Yang, S., Yang, Y., Jin, Z., and Li, B. (2024). “Classification and antioxidant assays of polyphenols: A review,” *Journal of Future Foods* 4(3), 193-204. <https://doi.org/10.1016/j.jfutfo.2023.07.002>
- Manzoor, A., and Ahmad, S. (2021). “Banana peel: Characteristics and consideration of its extract for use in meat products preservation: A review,” *ACS Food Science and Technology* 1(9), 1492-1506. <https://doi.org/10.1021/acsfoodscitech.1c00235>
- Martinez-Espinosa, I., Serrato, J. A., and Ortiz-Quintero, B. (2021). “Role of IL-10-producing natural killer cells in the regulatory mechanisms of inflammation during systemic infection,” *Biomolecules* 12(1), article 4. <https://doi.org/10.3390/biom12010004>
- Mateos-Aparicio, I. (2021). “Plant-based by-products,” *Food Waste Recovery* 367-397. <https://doi.org/10.1016/B978-0-12-820563-1.00022-6>
- Mohamed Mahzir, K. A., AbdGani, S. S., Hasanah Zaidan, U., and Halmi, M. I. E. (2018). “Development of *Phaleria macrocarpa* (Scheff.) boerl fruits using response surface methodology focused on phenolics, flavonoids and antioxidant properties,” *Molecules* 23(4), article 724. <https://doi.org/10.3390/molecules23040724>
- Moongngarm, A., Tiboobun, W., Sanpong, M., Sriwong, P., Phiewtong, L., Prakitrum, R., and Huychan, N. (2014). “Resistant starch and bioactive contents of unripe banana flour as influenced by harvesting periods and its application,” *American Journal of Agricultural and Biological Sciences* 9(3), 457-465. <https://doi.org/10.3844/ajabssp.2014.457.465>
- Morais, D. R., Rotta, E. M., Sargi, S. C., Schmidt, E. M., Bonafe, E. G., Eberlin, M. N., Sawaya, A.C., and Visentainer, J. V. (2015). “Antioxidant activity, phenolics and UPLC–ESI (–)–MS of extracts from different tropical fruits parts and processed peels,” *Food Research International* 77, 392-399. <https://doi.org/10.1016/j.foodres.2015.08.036>
- Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., and Bakri, M. M. (2018). “Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases,” *Saudi Journal of Biological Sciences* 25(2), 361-366. <https://doi.org/10.1016/j.sjbs.2017.02.004>
- Moutinho, J., Gouvinhas, I., Domínguez-Perles, R., and Barros, A. (2023). “Optimization of the extraction methodology of grape pomace polyphenols for food applications,” *Molecules* 28(9), article 3885. <https://doi.org/10.3390/molecules28093885>
- Neglo, D., Tettey, C. O., Essuman, E. K., Kortei, N. K., Boakye, A. A., Hunkpe, G., Amarh, F., Kwashie, P., and Devi, W. S. (2021). “Comparative antioxidant and antimicrobial activities of the peels, rind, pulp and seeds of watermelon (*Citrullus lanatus*) fruit,” *Scientific African* 11, article e00582. <https://doi.org/10.1016/j.sciaf.2020.e00582>
- Oboh, F. O., Ugheighele, S. E., Davidson, D. D., and Ugbeva, N. E. (2024). “Nutritional, phytochemical composition and in vitro functional properties of ripe and unripe plantain (*Musa paradisiaca*) fruit peels,” *Systematic Reviews in Pharmacy* 15(9), 302-310. <https://doi.org/10.31858/0975-8453.15.9.302-310>
- Oboh, G., Ademosun, A. O., Ayeni, P. O., Omojokun, O. S., and Bello, F. (2015). “Comparative effect of quercetin and rutin on α -amylase, α -glucosidase, and some pro-oxidant-induced lipid peroxidation in rat pancreas,” *Comparative Clinical Pathology* 24(5), 1103-1110. <https://doi.org/10.1007/s00580-014-2040-5>

- Osorio-Tobón, J. F. (2020). “Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds,” *Journal of Food Science and Technology* 57(12), 4299-4315. <https://doi.org/10.1007/s13197-020-04433-2>
- Özbek, H. N., Halahlıh, F., Göğüş, F., KoçakYanık, D., and Azaizeh, H. (2020). “Pistachio (*Pistacia vera* L.) Hull as a potential source of phenolic compounds: Evaluation of ethanol–water binary solvent extraction on antioxidant activity and phenolic content of pistachio hull extracts,” *Waste and Biomass Valorization* 11(5), 2101-2110. <https://doi.org/10.1007/s12649-018-0512-6>
- Pal, R. S., Kumar, V. A., Arora, S., Sharma, A. K., Kumar, V., and Agrawal, S. (2015). “Physicochemical and antioxidant properties of kiwifruit as a function of cultivar and fruit harvested month,” *Brazilian Archives of Biology and Technology* 58, 262-271. <https://doi.org/10.1590/s1516-8913201500371>
- Papoutsis, K., Zhang, J., Bowyer, M. C., Brunton, N., Gibney, E. R., and Lyng, J. (2021). “Fruit, vegetables, and mushrooms for the preparation of extracts with α -amylase and α -glucosidase inhibition properties: A review,” *Food Chemistry* 338, article 128119. <https://doi.org/10.1016/j.foodchem.2020.128119>
- Pereira, A., and Maraschin, M. (2015). “Banana (*Musa* spp) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance for human health,” *Journal of Ethnopharmacology* 160, 149-163. <https://doi.org/10.1016/j.jep.2014.11.008>
- Quanhong, L., and Caili, F. (2005). “Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein,” *Food Chemistry* 92(4), 701-706. <https://doi.org/10.1016/j.foodchem.2004.08.042>
- Rahman, M. M., Dhar, P. S., Anika, F., Ahmed, L., Islam, M. R., Sultana, N. A., Cavalu, S., Pop, O., and Rauf, A. (2022). “Exploring the plant-derived bioactive substances as antidiabetic agent: An extensive review,” *Biomedicine & Pharmacotherapy* 152, article 113217.
- Ramu, R., Shirahatti, P. S., Zameer, F., and Nagendra Prasad, M. N. (2015). “Investigation of antihyperglycaemic activity of banana (*Musa* sp. var. Nanjangud rasa bale) pseudostem in normal and diabetic rats,” *Journal of the Science of Food and Agriculture* 95(1), 165-173. <https://doi.org/10.1002/jsfa.6698>
- Ranjha, M. M. A. N., Amjad, S., Ashraf, S., Khawar, L., Safdar, M. N., Jabbar, S., Nadeem, M., Mahmood, S., and Murtaza, M. A. (2020). “Extraction of polyphenols from apple and pomegranate peels employing different extraction techniques for the development of functional date bars,” *International Journal of Fruit Science* 20(sup3), S1201-S1221. <https://doi.org/10.1080/15538362.2020.1782804>
- Raveena, N. K., Rajan, S., Priya, S., Lankalapalli, R. S., and Reshma, M. V. (2024). “First report on glucocerebrosides from unripe banana peel: Its anti-inflammatory and α -glucosidase inhibition properties,” *Food Chemistry Advances* 4, article 100700. <https://doi.org/10.1016/j.focha.2024.100700>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. (1999). “Antioxidant activity applying an improved ABTS radical cation decolorization assay,” *Free Radical Biology and Medicine* 26(9-10), 1231-1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Rivadeneira, J. P., Wu, T., Ybanez, Q., Dorado, A. A., Migo, V. P., Nayve Jr, F. R. P., and Castillo-Israel, K. A. T. (2020). “Microwave-assisted extraction of pectin from “Saba” banana peel waste: Optimization, characterization, and rheology study,”

- International Journal of Food Science* 2020(1), article 8879425.
<https://doi.org/10.1155/2020/8879425>
- Russell, W. R., Labat, A., Scobbie, L., Duncan, G. J., and Duthie, G. G. (2009). "Phenolic acid content of fruits commonly consumed and locally produced in Scotland," *Food Chemistry* 115(1), 100-104.
<https://doi.org/10.1016/j.foodchem.2008.11.086>
- Sadasivam, S. (1996). *Biochemical Methods*, New Age International, New Delhi, India, 1996; ISBN 978-81-224-0976-5.
- Safdar, M. N., Kausar, T., and Nadeem, M. (2017). "Comparison of ultrasound and maceration techniques for the extraction of polyphenols from the mango peel," *Journal of Food Processing and Preservation* 41(4), article e13028.
<https://doi.org/10.1111/jfpp.13028>
- Salar, R. K., Certik, M., and Brezova, V. (2012). "Modulation of phenolic content and antioxidant activity of maize by solid state fermentation with *Thamnidium elegans* CCF 1456," *Biotechnology and Bioprocess Engineering* 17(1), 109-116.
<https://doi.org/10.1007/s12257-011-0455-2>
- Sánchez-Marzo, N., Pérez-Sánchez, A., Castillo, J., Herranz-López, M., Barrajón-Catalán, E., and Micol, V. (2017). "Prevention of UVB-induced oxidative stress and DNA damage in human keratinocytes by citrus and olive formulations," *Free Radical Biology and Medicine* 108, article S82.
<https://doi.org/10.1016/j.freeradbiomed.2017.04.272>
- Sasipriya, G., Maria, C. L., and Siddhuraju, P. (2014). "Influence of pressure cooking on antioxidant activity of wild (*Ensete superbum*) and commercial banana (*Musa paradisiaca* var. Monthan) unripe fruit and flower," *Journal of Food Science and Technology* 51(10), 2517-2525. <https://doi.org/10.1007/s13197-012-0791-z>
- Selahvarzi, A., Ramezan, Y., Sanjabi, M. R., Namdar, B., Akbarmivehie, M., Mirsaedghazi, H., and Azarikia, F. (2022). "Optimization of ultrasonic-assisted extraction of phenolic compounds from pomegranate and orange peels and their antioxidant activity in a functional drink," *Food Bioscience* 49, article 101918.
<https://doi.org/10.1016/j.fbio.2022.101918>
- Shi, D., Xu, W., Balan, P., Wong, M., Chen, W., and Popovich, D. G. (2021). "In vitro antioxidant properties of New Zealand hass avocado byproduct (peel and seed) fractions," *ACS Food Science & Technology* 1(4), 579-587.
<https://doi.org/10.1021/acsfoodscitech.0c00018>
- Suleria, H. A., Barrow, C. J., and Dunshea, F. R. (2020). "Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels," *Foods* 9(9), article 1206. <https://doi.org/10.3390/foods9091206>
- Tadera, K., Minami, Y., Takamatsu, K., and Matsuoka, T. (2006). "Inhibition of α -glucosidase and α -amylase by flavonoids," *Journal of Nutritional Science and Vitaminology* 52(2), 149-153. <https://doi.org/10.3177/jnsv.52.149>
- Tremocoldi, M. A., Rosalen, P. L., Franchin, M., Massarioli, A. P., Denny, C., Daiuto, É. R., Paschoal, J. A. R., Melo, P. S., and Alencar, S. M. D. (2018). "Exploration of avocado byproducts as natural sources of bioactive compounds," *PloS One* 13(2), article e0192577. <https://doi.org/10.1371/journal.pone.0192577>
- Vijay, N., Shashikant, D., and Mohini, P. (2022). "Assessment of antidiabetic potential of *Musa acuminata* peel extract and its fractions in experimental animals and characterisation of its bioactive compounds by HPTLC," *Archives of Physiology and Biochemistry* 128(2), 360-372. <https://doi.org/10.1080/13813455.2019.1683585>

- Wang, M., Yang, F., Yan, X., Chao, X., Zhang, W., Yuan, C., and Zeng, Q. (2022). “Anti-diabetic effect of banana peel dietary fibers on type 2 diabetic mellitus mice induced by streptozotocin and high-sugar and high fat diet,” *Journal of Food Biochemistry* 46(10), article e14275. <https://doi.org/10.1111/jfbc.14275>
- Wang, T., Li, X., Zhou, B., Li, H., Zeng, J., and Gao, W. (2015). “Anti-diabetic activity in type 2 diabetic mice and α -glucosidase inhibitory, antioxidant and anti-inflammatory potential of chemically profiled pear peel and pulp extracts (*Pyrus* spp.),” *Journal of Functional Foods* 13, 276-288. <https://doi.org/10.1016/j.jff.2014.12.049>
- Wani, K. M., and Dhanya, M. (2025). “Unlocking the potential of banana peel bioactives: extraction methods, benefits, and industrial applications,” *Discover Food* 5(1), article 8. <https://doi.org/10.1007/s44187-025-00276-y>
- Wu, Z., Tan, B., Liu, Y., Dunn, J., MartorellGuerola, P., Tortajada, M., Cao, Z., and Ji, P. (2019). “Chemical composition and antioxidant properties of essential oils from peppermint, native spearmint and scotch spearmint,” *Molecules* 24(15), article 2825. <https://doi.org/10.3390/molecules24152825>
- Yang, D., Dunshea, F. R., and Suleria, H. A. (2020). “LC-ESI-QTOF/MS characterization of Australian herb and spices (garlic, ginger, and onion) and potential antioxidant activity,” *Journal of Food Processing and Preservation* 44(7), article e14497. <https://doi.org/10.1111/jfpp.14497>
- Yang, L., Cao, Y. L., Jiang, J. G., Lin, Q. S., Chen, J., and Zhu, L. (2010). “Response surface optimization of ultrasound-assisted flavonoids extraction from the flower of *Citrus aurantium* L. var. *amara* Engl,” *Journal of Separation Science* 33(9), 1349-1355. <https://doi.org/10.1002/jssc.200900776>
- Yuan, Y., Gao, Y., Mao, L., and Zhao, J. (2008). “Optimisation of conditions for the preparation of β -carotene nanoemulsions using response surface methodology,” *Food Chemistry* 107(3), 1300-1306. <https://doi.org/10.1016/j.foodchem.2007.09.015>

Article submitted: February 10, 2026; Peer review completed: March 15, 2026; Revised version received and accepted: April 2, 2026; Published: April 13, 2026.

DOI: 10.15376/biores.21.2.4678-4700