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RESEARCH ARTICLE

Optimization of Extraction Parameters of Phenolic Compounds from Cau Banana (*Musa paradisiaca*) Peel and Evaluation for Antioxidant and Antibacterial Potential

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ABSTRACT

Objective: This study aims to demonstrate the potential reuse of Cau banana peel (CBP), a fruit by-product in the Mekong Delta, Vietnam, based on its antioxidant and antibacterial activity.

Materials and Methods: The determination of optimal extraction conditions for phenolics using response surface methodology from CBP powder. Then, the CBP extract was evaluated for total phenolic content (TPC), flavonoid content (TFC), phytochemical constituents, and antioxidant activity by the DPPH free radical scavenging assay, whereas antibacterial effects against the pathogens *Staphylococcus aureus* and *Escherichia coli* were determined by the agar diffusion and broth dilution methods.

Results: The highest TPC (38.42 mgGAE/g) and TFC (9.75 mgQE/g) were achieved with 1 g of the CBP powder, 58 mL of 60% ethanol solution, and 76 min of processing time at 56°C. The CBP extract contained tannins, flavonoids, alkaloids, saponins, and glycosides, with TPC and TFC of 543.48 mgGAE/g and 158.96 mgQE/g, respectively. The IC_{50} was 105.85 µg/mL, and the MBC/MIC ratios were ≤2.0 mg/mL. Therefore, it could be a strong antioxidant and bactericidal agent.

Conclusion: These results show that the peel waste of CBP could be helpful in the medical or health food industry for various applications instead of dumping them in landfills.

Keywords: Cau banana peel, Response surface methodology, Antioxidant, Antibacterial activity.

INTRODUCTION

Cau banana (*Musa paradisiaca*) belongs to the *Musaceae* family and is a native crop and a rich source of nutritional fruit in Vietnam. It is classified as a plantain banana and is typically eaten when fully ripened although it can also be cooked. In the production of frozen bananas, the peel is often discarded and comprises 18%-30% of the whole fruit weight,[1] leading to serious waste management challenges. Several recent studies have explored using banana peel waste, including composting,[2] protein,[3] ethanol,[4] alpha-amylase,[5] and dietary fibre production[6] through biotechnological processes.

In addition, there is growing interest in the medicinal properties of banana peels among researchers worldwide.[7,8] In Vietnamese ethnomedicine, banana peels have been traditionally used for various health purposes, such as treating gastric ulceration, persistent diarrhoea, dysentery, acne, and warts.[9] Recent analyses have shown that ripe banana peels contain a variety of phytonutrients and phytochemicals, including high levels of carbohydrates, dietary fibre, crude protein, crude fat, ash, starch, and resistant starch, respectively.[10] They also contain pigments (xanthophyll, beta-carotenoid) and vitamins (vitamins A and C) with antioxidant activity,[11-13] as well as essential minerals, such as potassium, calcium, sodium, manganese, and iron.[14] Banana peels are also rich in phenolics and have been linked to numerous health benefits, including the prevention of cardiovascular diseases, cancer, diabetes, and obesity.[15] They consist mainly of gallocatechin, catechin, and epicatechin.[16] Pereira et al.[17] demonstrated that the phenolic content was high in banana peel, accounting for 244 mgGAE/g. Toxic substances in banana peels, hydrogen cyanide, and oxalates were present at low levels of 1.3 mg/g and 0.5 mg/g, respectively, and within the safety limits Anhwange et al.[18] suggested that they are safe for human consumption.

In light of the growing problem of drug-resistant bacterial pathogens, which render antibiotics ineffective in treatment, there is a pressing need for new antibacterial agents,[19] such as using plant extracts as an effective therapy against resistant

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bacterial strains.[20] Banana peels possess natural antibacterial properties and have shown activity against various inflammatory diseases.[21] Previous studies have demonstrated the effectiveness of banana peels against common pathogenic bacteria by biosorption and disruption of cell membrane activity.[22] Moreover, banana peels against *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*.[23]

To the best of our understanding, while previous research in Vietnam has focussed on utilising banana peels for compost, fertilisers, bio-ethanol, composites, and activated carbon production,[24] less attention has been given to extracting phytochemical compounds from these by-products for medicinal or functional food purposes. In recent years, Vietnamese scientists have optimised the extraction conditions for phytochemical compounds from banana peels for medicinal or functional food purposes.[9,25] It has been found that using aqueous ethanol solution as a solvent is the most efficient method for extracting these compounds. This is due to the low boiling point and safety for human consumption of ethanol and the ability to expand and soften plant material, making it easier to extract the desired compounds.[25] However, it should be noted that there is no universal extraction procedure for all types of materials, and the specific extraction parameters must be determined for each type of material. Therefore, this study was conducted to determine the optimal extraction process for phenolic compounds from CBP collected in Tien Giang province, Mekong Delta, Vietnam, using ethanol as an extraction solvent, as well as to evaluate the antioxidant and antibacterial properties of the obtained crude extract.

MATERIALS AND METHODS

Chemicals

Cemaco (Vietnam) supplied the ethanol solvent for extraction. Sigma-Aldrich (United States) supplied DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, gallic acid, and Folin-Ciocalteu reagent which were used for antioxidant tests and determination of phenolic content, DMSO (Dimethyl sulfoxide) dissolved crude extract. Himedia (India) supplied tryptic soy agar (TSA) was used to store organisms, while Mueller-Hinton broth and agar (MHB and MHA) were used to determine antibacterial activity. The purified water and other chemicals used in this work were of analytical reagent grade.

Preparation of Sample, Extraction and Crude Extract

Plant Material

The unripe fruits of Cau banana (*M. paradisiaca*) were harvested from home gardens in Chau Thanh district, Tien Giang province, Mekong Delta, Vietnam. After 5-7 days, the peel of fresh banana fruit at stage 6 of ripening (yellow peel) was thoroughly rinsed, cut into small pieces, and dried at 50°C until the moisture content was 12%. The dried peel was then blended and sifted through a 1.5-mm sieve to create a fine powder. Finally, the powdered banana peel sample was packed in vacuum-sealed PA bags and stored in refrigeration (4 ± 2°C) until needed for use, as shown in Figure 1.

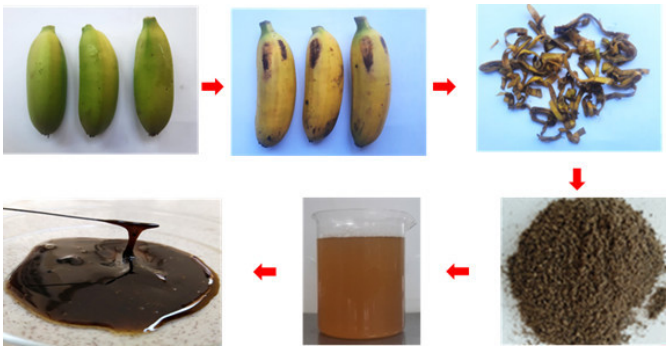

Figure 1. Crude extraction process from the CBP.

Crude Extract Collection

One hundred (100 grammes) of the CBP powder was used for extraction with ethanol (60%). The filtered transparent extract using Whatman number 1 philtre papers. The removed residues were re-extracted twice under the same conditions. The filtrates were combined and concentrated at 50°C using a rotary evaporator and then air-dried to obtain the crude extract. Finally, the extracted sample was kept in a clean brown bottle at 4 ± 2°C, before analysis.

$$Extraction\ yield(\%) = \frac{Weight\ of\ extract}{Weight\ of\ material}.100\% \quad (1)$$

Optimisation of Extraction Conditions of Phenolics from CBP Powder

The extraction process for phenolic compounds from the CBP powder was optimised using response surface methodology (RSM) with Box-Behnken design. This study examined the influence of three factors: the ratio of the CBP powder mass to ethanol volume (A), temperature (B), and time (C) on the total phenolic content-TPC (Y_1) and total flavonoid content-TFC (Y_2) of the transparent extracts (Table 1).

The extraction was performed with 1 g of dried sample in a flask, placed in a water bath, and shaken at a speed of 100 rpm. The transparent extract was then filtered using Whatman Number 1 philtre papers. The TPC and TFC of the extracts were analysed. The experiment was conducted in triplicate, and the data were analysed using multiple regression and analysis of variance (ANOVA) with Statgraphics Centurion XV software. The effect of the independent variables on the response (Y) model is described in the following section.

$$Y = b_0+b_1A+b_2B+b_3C+b_4AB+b_5AC+b_6BC+b_7A^2+b_8B^2+b_9C^2 \quad (2)$$

Table 1. Optimization experience plan using response surface methodology (RSM) and the research results.

No.	A (mL/g)	B (°C)	C (min)	Y_1 (mgGAE/g)		Y_2 (mgQE/g)	
				Experimental*	Predicted	Experimental*	Predicted
1	60 (0)	60 (0)	90 (0)	40.19 ± 0.06	40.18	9.52 ± 0.13	9.48
2	60 (0)	60 (0)	90 (0)	39.77 ± 0.20	40.18	9.44 ± 0.18	9.48
3	60 (0)	50 (-1)	120 (1)	37.33 ± 0.20	37.66	8.71 ± 0.07	8.71
4	60 (0)	60 (0)	90 (0)	40.11 ± 0.28	40.18	9.55 ± 0.14	9.48
5	60 (0)	60 (0)	90 (0)	40.67 ± 0.06	40.18	9.60 ± 0.07	9.48
6	70 (1)	70 (1)	90 (0)	38.34 ± 0.10	38.32	9.34 ± 0.05	9.45
7	60 (0)	60 (0)	90 (0)	40.18 ± 0.15	40.18	9.42 ± 0.08	9.48
8	50 (-1)	60 (0)	120 (1)	38.89 ± 0.33	38.55	8.34 ± 0.17	8.45
9	60 (0)	70 (1)	120 (1)	38.34 ± 0.96	38.63	8.58 ± 0.20	8.60
10	50 (-1)	70 (1)	90 (0)	38.96 ± 0.45	39.02	9.06 ± 0.19	8.94
11	70 (1)	60 (1)	60 (-1)	37.89 ± 0.17	38.24	9.31 ± 0.55	9.21
12	50 (-1)	60 (0)	60 (-1)	37.67 ± 0.04	37.94	9.17 ± 0.28	9.29
13	60 (0)	70 (1)	60 (-1)	38.30 ± 0.18	37.97	9.28 ± 0.20	9.28
14	60 (0)	50 (-1)	60 (-1)	39.21 ± 0.31	38.92	9.39 ± 0.56	9.37
15	50 (-1)	50 (-1)	90 (0)	38.91 ± 0.51	38.93	9.57 ± 0.14	9.46
16	70 (1)	60 (0)	120 (1)	37.29 ± 0.08	37.02	8.83 ± 0.14	8.71
17	60 (0)	60 (0)	90 (0)	40.16 ± 0.18	40.18	9.33 ± 0.63	9.48
18	70 (1)	50 (-1)	90 (0)	38.45 ± 0.12	38.39	9.01 ± 0.38	9.13

Note: "*" = Mean of duplicate runs.

where X_1, X_2, and X_3 are independent variables and b_0, b_1, b_2,... b_9 are offset, interaction, and squared effects. The selection model was mainly based on the R^2 value obtained from the regression.

Phytochemical Screening Assays

The CBP extract was tested for the presence of bioactive compounds, including tannin, flavonoid, alkaloid, saponin, and glycoside compounds, using the following standard procedures[26,27] (Table 2).

Total Phenol Content Analysis

The Folin-Ciocalteu assay was used for this determination.[28] The CBP extract (2 g) was dissolved in 100 mL of 99.99% ethanol to a concentration of 20 mg/mL. The gallic acid (ranging from 10 to 100 µg/mL) or diluted extract (1 mL) was added to 2.5 mL of 10% Folin-Ciocalteu reagent and mixed thoroughly for 1 min. To the solution, 2 mL of 2% (w/w) sodium carbonate was added, and the mixture was allowed to stand for 15 min at room temperature. The absorbances of the reaction solutions containing polyphenol compounds extracted from the extracts and Folin-Ciocalteu reagent were measured at 765 nm. TPC values were calculated as milligramme gallic acid equivalent per gramme of dry material (mgGAE/g) using a calibration curve ($y = 0.0117x + 0.0037$, $R^2 = 0.9969$).

Total Flavonoid Content Analysis

The total flavonoid content of the extracts was determined using the aluminium trichloride colorimetric method.[29] The quercetin

Table 2. Qualitative tests for phytochemical screening.

Compound	Procedure	Indicating Positive Test
Tannin	1 mL of diluted extract (200 mg/mL) + a few drops of 1% gelatin solution containing sodium chloride	A white precipitate
Flavonoid	1 mL of diluted extract (200 mg/mL) + a few drops of 10% lead acetate solution	A yellow colour precipitate
Alkaloid	1 mL of diluted extract (200 mg/mL) + a few drops of Mayer's reagent (Potassium Mercuric Iodide)	A yellow colour precipitate
Saponin	0.5 g of crude extract was shaken with 2 mL of purified water	The foam produced persists for 10 min
Glycoside	1 mL of diluted extract (200 mg/mL) + 1 mL of pyridine + 1 mL of sodium nitroprusside solution and made alkaline using 10% sodium hydroxide solution	A pink to blood red colour

(ranging from 20 to 200 µg/mL) or diluted extract (20 mg/mL) with 0.5 mL was added to 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride solution, 0.1 mL of 1M potassium acetate solution, and 2.8 mL of distilled water and mixed thoroughly for 1 min. The mixture was allowed to stand for 30 min at room temperature. The reaction formed a yellow solution, which was recorded at 415 nm. TFC values were calculated as mg of quercetin equivalent per gramme of dry material (mgQE/g) using a calibration curve ($y = 0.0049x + 0.0345$, $R^2 = 0.9953$).

Antioxidant Activity by the DPPH Radical Scavenging Assay

The free radical scavenging activity of the CBP extract was determined using the DPPH assay, according to the method described by Navghare and Dhawale.[30] The crude extract was diluted with methanol to concentrations of 200, 100, 50, and 25 µg/mL. As a positive control, an antioxidant standard, ascorbic acid (vitamin C), was prepared at the same concentrations. Then, 1 mL of the diluted extract or ascorbic acid solution was mixed with 3 mL of 0.1 mM DPPH in methanol. A blank sample was also prepared by mixing 3 mL of the methanol DPPH solution with 1 mL of methanol. The mixtures were incubated for 30 min in the dark at room temperature, and the decrease in absorbance was measured at 517 nm. The ability to scavenge DPPH radicals was calculated using the following formula:

$$\%Inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\% \qquad (3)$$

where A_{blank} is the absorbance of DPPH without a sample (control); A_{sample} is the absorbance of the sample and DPPH (test sample) at 517 nm wavelength.

Each experiment was run in triplicate, and the average of the three inhibition values at each concentration was calculated. The graphs of averaged inhibition values against extract concentrations were used to produce a linear regression line to determine the half-maximal inhibitory concentration (IC_{50}).

Antibacterial Activity

Bacterial Cultures

Two pure cultures of the pathogenic bacteria strains *Staphylococcus aureus* WDCM 00195 and *Escherichia coli* WDCM 00196 were obtained from the Vietnam Academy of Science and Technology in Hanoi, Vietnam. The organisms were stored in Tryptic soy agar (TSA) nutrient slants at 4°C until needed for use.

Preparation of Standard Culture Inoculums of Test Organisms

Five colonies of each bacterial strain were inoculated in 15 mL sterile Mueller-Hinton broth (MHB) for approximately 24 h at 37°C, adjusted to a McFarland scale 0.5 (~10^8 colony-forming units (cfu) per mL) with a spectrophotometer at 625 nm to reach an optical density of 0.08–0.10, and diluted 1:100 in sterile MHB to obtain final inoculums containing 10^6 cfu/mL. The standardised inoculum suspension was inoculated within 15-20 min.[31]

Antibiotic Susceptibility Testing

The paper disc diffusion method was used to determine the antibacterial activity of the CBP extract.[32] One hundred microliters (100 µL) of suspension of each bacterial strain was

swabbed onto Mueller-Hinton agar (MHA) media using a sterile cotton swab. Three sterile philtre discs of Whatman No. 1 paper (6 mm in diameter) was impregnated with the CBP extract at a concentration of 20 mg/mL in 5 µL volumes, placed on the inoculated agar plates, and gently pressed down with the help of a sterile forceps to ensure complete contact of the disc with the agar surface. Tetracycline at a concentration of 2 mg/mL was used as a positive control, whereas discs with a solution of 30% (v/v) DMSO served as a negative reference (5 µL each in separate Petri plates). Then, inoculated plates were incubated at 37°C for 24 h. The next day, the diameter of the inhibition zone was measured in millimetres around the discs in each plate. The size (diameter) of the inhibition zone was used to classify antibacterial activity, as shown in Table 3. The average inhibition zone diameter was categorised as very severe for diameters >20 mm; severe for diameters 10-20 mm, moderate for diameters 5-10 mm, and weak for diameters <5 mm.[33]

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the CBP extract was determined by the dilution method as described by Cheesbrough.[34] A double-fold serial dilution was performed using 30% DMSO solution (v/v). The following extract concentrations were obtained: 20, 10, 5, 2.5, and 1.25 mg/mL. Equal volumes of the extract solution and minimum bactericidal concentration (MHB) (2 mL) were dispensed into sterilised test tubes, and 100 µL of standardised inoculum (10^6 cfu/mL) was added to each test tube, which was aerobically incubated at 37℃ for 24 h. A tube containing only broth and inoculum without extract was used as the organism control, whereas a tube containing broth and extract without inoculum served as the extract control. The minimum inhibitory concentration (MIC) value is defined as the lowest dilution that showed no growth in the MHB medium.

Sterile MHA plates were transferred with 10 µL of sample from each test tube that showed a complete absence of growth in the MIC test. The agar plates were then incubated at 37 ℃ for 24 h. The lowest concentration of the extract that yielded no growth was considered the MBC value.

Statistical Analysis

The significance of differences was determined using one-way ANOVA and the Tukey test (p<0.05) with Statgraphics Centurion XV software.

RESULTS

Optimisation of Extraction of Phenolic Compounds from CBP Powder

Based on our previous study, a 60% ethanol concentration showed a high extraction efficiency of phenolics in banana peel extract. Its water content was sufficient to soften the cell walls, increase the extraction efficiency, be safe for human consumption, and easily evaporate. Tai et al.[25] for phenolic extraction from "Xiem" banana peel collected in Vietnam reported a similar result. Therefore, a solvent with 60% ethanol concentration was chosen for this experiment. The table below displays the optimisation experience plan and the corresponding test results as mean values (Table 1).

RSM was used to analyse the impact of extraction conditions on the extraction of phenolic compounds from the CBP powder. The results of this analysis are shown in Figures 2 and 3, which display the response surface plots. The regression equation for the selected model, along with the measured levels of phenolics and flavonoids and their adjusted R^2, is as follows:

TPC = 40.18 0.46AC + 0.48BC - 0.94A^2 - 0.58B^2 - 1.31C^2, with R^2 = 93.80%, R^2 (adj.) = 86.83%, SEE = 0.38,

TFC = 9.48 0.34C + 0.22AB - 0.16A^2 - 0.41C^2, with R^2 = 93.41%, R^2 (adj.) = 85.99%, SEE = 0.10,

On the basis of the response surface plots and regression model analysis results, the optimal extraction mixture condition for the total phenolic and flavonoid content was determined to be a ratio of 60% aqueous ethanol volume to the banana peel powder weight of 58:1 (mL/g), a temperature of 56°C, and a processing time of 76 min. A comparison between the maximum experimental values of phenolic compounds and extraction conditions and the predicted values (Table 4) revealed that the two sets of values were close. Indeed, the maximum values for TPC (prediction: 40.15 mgGAE/g, experimental: 38.42 mgQE/g) and TFC (prediction: 9.54 mgGAE/g, experimental: 9.75 mgQE/g) were obtained using the same extraction conditions in both the experimental and predicted models. These results demonstrate the validity of the experimental model, as there is a high degree of agreement between the observed values and those predicted by the regression model. Therefore, this response surface modelling approach can effectively predict the extraction of phenolic compounds from CBP powder.

Physical Properties, TPC, TFC, and Phytochemical Screening Results of the CBP Extract

Organoleptically, the crude extract obtained from the CBP powder was dark brown, with a slightly sticky texture, a bitter taste, and an intense odour of ripe banana. The equivalent yield of the extract was 13.01% (Table 5) compared with the mass of dried banana peel powder (100 g). In terms of total phenolic and flavonoid content, this extract sample showed high TPC and TFC values of 543.48 mgGAE/g and 158.96 mgQE/g, respectively. The results of phytochemical screening of the crude extract from the peel of Cau banana, as shown in Table 5, confirmed the presence of active compounds such as tannins, flavonoids, alkaloids, saponins, and glycosides. These compounds have beneficial effects on human health.

Table 3. Categorization based on the diameter of the inhibition zone formed.[31]

Average inhibition zone diameter (mm)	Inhibitory strength
>20	Very Severe
10–20	Severe
5–10	Moderate
<5	Weak

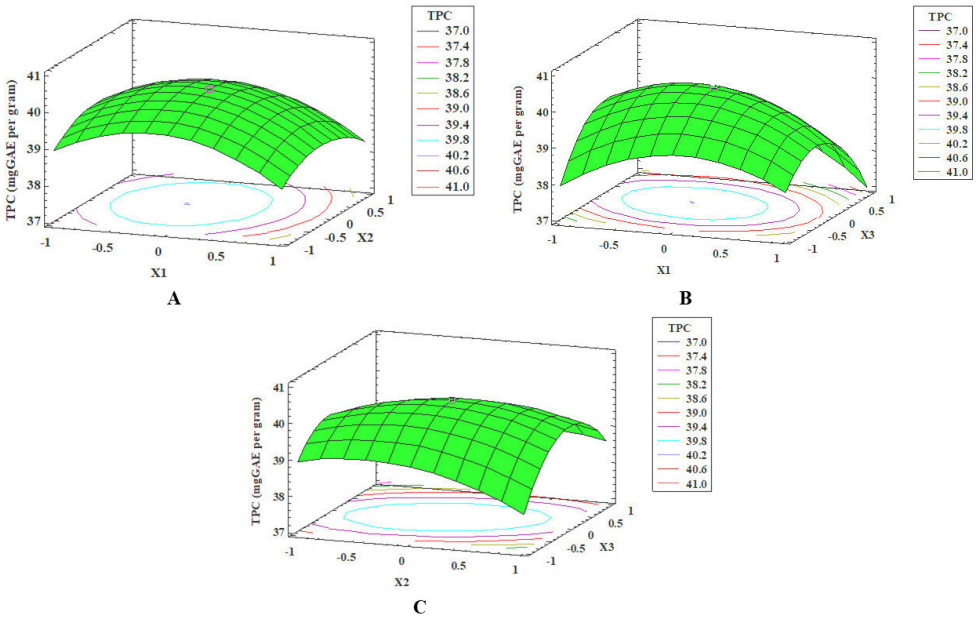

Figure 2. Impact of process conditions on the TPC of extraction. (A) Ethanol volume and temperature. (B) Ethanol volume and time. (C) Temperature and time.

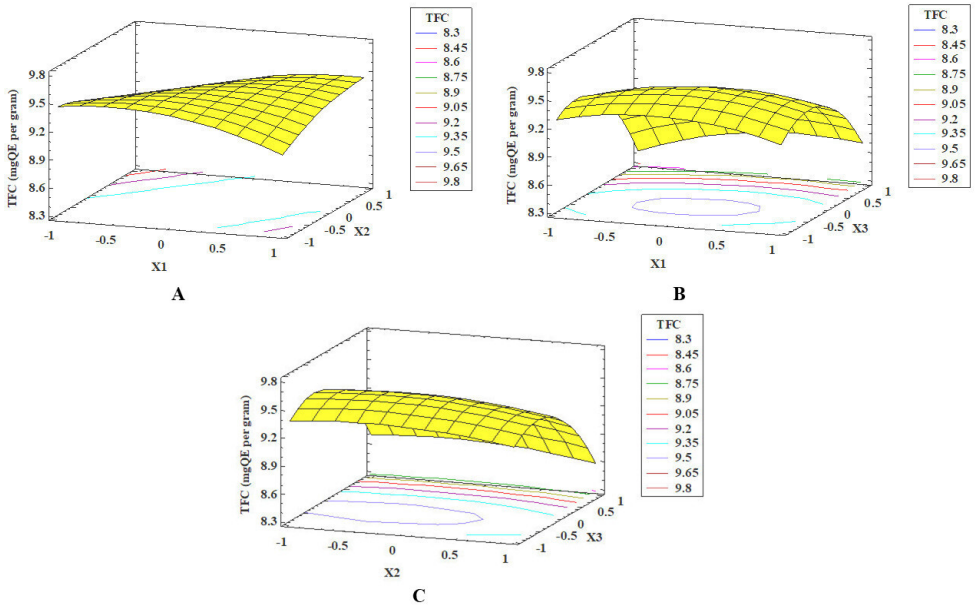

Figure 3. Impact of process conditions on the TFC of extraction. (A) Ethanol volume and temperature. (B) Ethanol volume and time. (C) Temperature and time.

Table 4. Experimental data of the validation of predicted values at optimal process conditions.

Response	Predicted value	Confidence interval (95%)		Experimental value
		Minimum	Maximum	
Y_1 - TPC (mgGAE/g)	40.15	39.79	40.51	38.42
Y_2 - TFC (mgQE/g)	9.54	9.41	9.67	9.75

TPC:total polyphenol content; TFC: total flavonoid content.

Table 5. The properties of the Cau banana peel extract.

Parameters	Result
Yield (%)	13.01
Moisture (%)	6.64 ± 0.47
TPC (mgGAE/g)	543.48 ± 1.08
TFC (mgQE/g)	158.96 ± 1.43
Tannins	+
Flavonoids	+
Alkaloids	+
Saponins	+
Glycosides	+

Note: "+" = Present. TPC:total polyphenol content; TFC: total flavonoid content.

Antioxidant Activity of the CBP Extract

The DPPH radical scavenging assay is a widely accepted method for determining the overall antioxidant capacity of plant extracts. In this study, the ethanolic extract from the CBP powder exhibited significant DPPH free radical scavenging activity, as illustrated in Figure 4.

Overall, the DPPH free radical scavenging activity of both the ethanolic extract of the CBP powder and ascorbic acid showed a dose-dependent relationship, with higher concentrations resulting in increased activity. Specifically, the percentage of inhibition of DPPH radicals for the four different concentrations of extract used in this study (25, 50, 100, and 200 µg/mL) was 24.64%, 32.55%, 54.89%, and 74.16%, respectively. In comparison, ascorbic acid at the same concentrations showed inhibition rates of 29.84%, 39.12%, 61.34%, and 90.24%. Based on the obtained percentage of inhibition, graphs were plotted to estimate the IC_{50} value using linear regression analysis. The linearity (R^2) of the extracted sample and ascorbic acid were 0.9616 and 0.9898, respectively. The IC_{50} values for quenching DPPH free radicals were found to be 105.85 µg/mL for the extract and 78.89 µg/mL for ascorbic acid. The CBP extract has approximately 1.34 times lower activity than ascorbic acid.

Antibacterial Activities of the CBP Extract

The results of the antibacterial activity of the CBP extract against the two tested pathogenic microorganisms are presented in Figure 5 and Table 6.

The zone of inhibition of bacterial growth was found to be dependent on the relative antimicrobial potency of the extract. At a concentration of 20 mg/mL, the extracted sample exhibited significant antimicrobial activity against only *S. aureus* by a larger zone of clearance observed as 15.00 mm compared with *E. coli* (0 mm) ($p < 0.05$). However, it was found to be less effective than the standard antibiotic tetracycline, whereas no inhibitory effect was noted for the negative reference (DMSO).

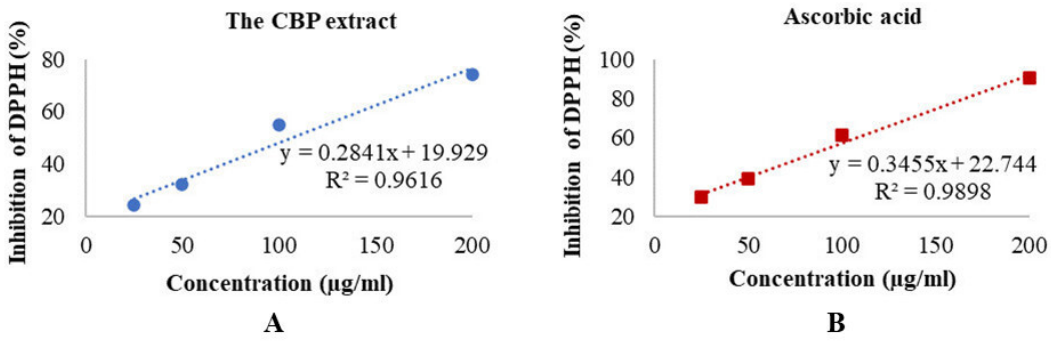

Figure 4. Percentage of DPPH inhibition by the CBP extract (A) and ascorbic acid (B) at different concentrations

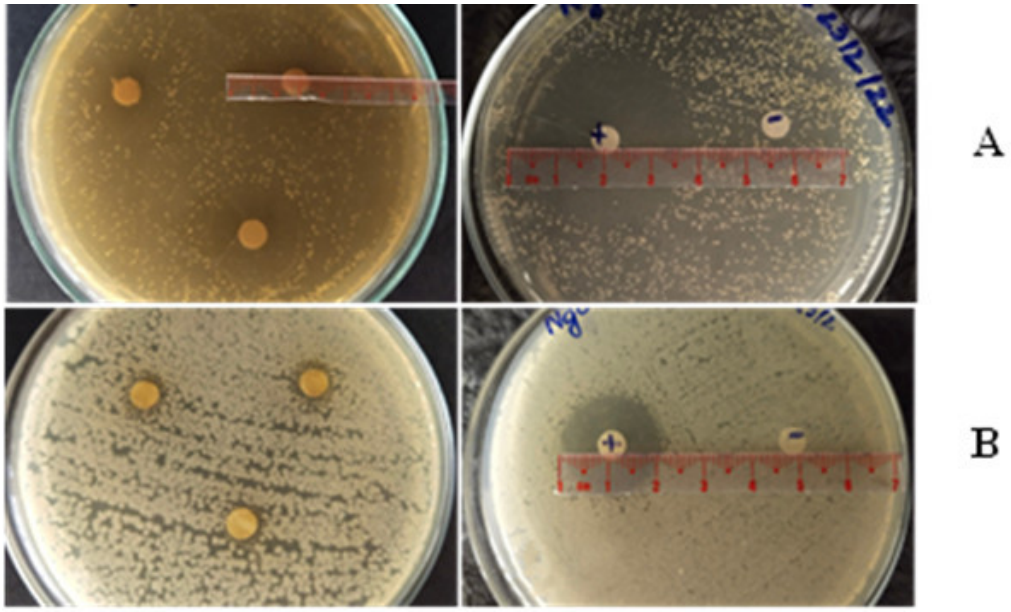

Figure 5. The inhibition of bacterial effects of the CBP extract at 20 mg/mL and controls A. The effect on *S. aureus* of the CBP extract (left) and the controls (right) B. The effect on *E. coli* of the CBP extract (left) and the controls (right).

Table 6. Antibacterial activity of the CBP extract at 20 mg/mL.

Test organisms	Zone of clearance (mm) ± SD			MIC (mg/mL)	MBC (mg/mL)
	The extract	Tetracycline	DMSO		
S. aureus	15.00 ± 0.50^{a}	33.83 ± 0.76^{a}	0^{a}	2.50^{a}	2.50^{b}
E. coli	0^{b}	21.47 ± 0.67^{b}	0^{a}	2.50^{a}	5.00^{a}

Note: Different letters (a, b,...) next to the mean values in each column indicate significant difference (Tukey test, $p<0.05$); MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.

Continuously, MIC and MBC values of the CBP extract was determined. The potent antibacterial effect of the extract is also shown in Table 6. An interesting result, the same MIC values of the extract against *S. aureus* and *E. coli* were 2.50 mg/mL, whereas the MBC values were different, they were 2.50 mg/mL for *S. aureus* and 5.00 mg/mL for *E. coli*. The MIC and MBC values obtained after susceptibility tests indicated the good antimicrobial activity of the CBP extract against both tested pathogens. The MBC/MIC ratios were calculated at 1.0 and 2.0 for extthe extractaint *S. aureus* and *E. coli*, respectively, which were both bactericidal agents.

DISCUSSION

In recent years, several reports have been published on the effects of various extraction techniques on the recovery of bioactive compounds from banana peels, as well as the antioxidant and antibacterial properties of the resulting extract.[35-38] However, the specific impact of using aqueous ethanol as an extraction method for phenolic compounds from has not yet been thoroughly investigated. Ishak et al.[36] optimised the periodic extraction of flavonoids from unripe Cavendish banana peel using aqueous ethanol solution with ultrasound-assisted techniques. Their research demonstrated a high recovery rate of flavonoids from banana peel (29 mgQE/g) and a reduced extraction time (only 30 min) when using ultrasound-assisted extraction. Although this method has many advantages, such as increased efficiency and reduced extraction time, it can also be relatively expensive due to the cost of equipment installation.

Our extraction yield was higher than the previous yield obtained from *M. acuminata Colla* AAA in Thailand (11.26%).[36] González-Montelongo et al.[1] discovered that banana peels contain considerable amounts of extractable compounds. In addition, the moisture content of the extracted sample did not exceed the reference value of the water content in the crude extracts from plants (<10%), this characterisation is necessary for safe storage and longer shelf life because it can inhibit the growth of bacteria and deteriorate the content of the bioactive compounds due to polyphenol oxidase activity, which adversely affects plant extract quality.[39,40] The TPC value was much higher than that previously reported by Anal et al.[38], who found the TPC in banana peel extract to range from 18.21 to 35.06 mgGAE/g, but it was lower than that extracted from peels of three banana varieties grown in West Java, Indonesia (1460-4630 mgGAE/g).[41] Next, the extracted sample had a TFC value lower than that in previous research of 196.05 mgQE/g.[38] However, they were higher than those discovered by Salim et al.[42], who recorded 10.92 mg QE/g. The TPC and TFC values in banana peel extracts vary depending on the variety, growing region, and ripeness as well as the methods used for sample preparation, extraction, and determination.[1,43] The high TPC and TFC values of banana peel are responsible for the strong antioxidant and microbial capacities.

Phenolic compounds, including tannins and flavonoids, are naturally occurring antioxidants and antibacterial agents found in plants. Tannins, in particular, are believed to be responsible for promoting haemostasis, treating diarrhoea, relieving pain, and promoting thrombolysis.[44] However, their low taste and odour can limit their use in the food industry. On the other hand, flavonoids have lipid-lowering, antiatherogenic, anti-inflammatory, and antimicrobial properties, making them beneficial in reducing the risk of atherosclerosis and related diseases.[45] The presence of alkaloids in these plants has been traditionally used in herbal medicines, teas, and potions.[46] One of the most notable biological properties of these active compounds is their cytotoxicity.[47] Saponins, known for their bitterness and ability to foam in aqueous solutions, had activities such as haemolysis and cholesterol-binding.[48] They have also been reported to have various pharmacological actions, including anti-inflammatory, anti-obesity, immunostimulant, hypocholesterolemic, hypoglycaemic, antifungal, and anticancer effects.[49] Glycosides, another active compound found in this plant extract, have shown great potential in treating a variety of illnesses, including cardiovascular, antibacterial, anticancer, anti-inflammatory, and neurodegenerative conditions.[50] These findings agree with previous research that identified tannins, flavonoids, alkaloids, saponins, and glycosides in banana peels from the Musa genus (*Musaceae*). Flavonoids, alkaloids, and saponins have antibacterial properties, whereas tannins and phenols have antioxidant potential.[51]

Our extract has strong antioxidant activity, as classified by the IC_{50} value given by Molyneux.[52] Our ethanolic extract from the CBP powder showed even greater DPPH scavenging power compared with other banana peels and fruit peels, such as the *M. cavendish* peel (IC_{50} = 232.08 µg/mL) and the *M. acuminata* peel (IC_{50} = 139.50 µg/mL),[30] as well as the Ambonese banana (*M. paradisiaca*) peel (IC_{50} = 114.00 µg/mL) and orange (*Citrus reticulata*) peel (IC_{50} = 222.00 µg/mL).[53] This is due to the high concentration of phytochemical compounds in banana peel, which are known for their antioxidant properties. Several previous studies have observed a positive association between the bioactive compound content and the DPPH radical scavenging activity of banana peel.[54] GC-MS analysis indicated the presence of potential antioxidant phenolic and flavonoid compounds in the ethanolic *M. paradisiaca* peel extract, including ellagic acid, gallic acid, rutin, myricetin, naringenin, epicatechin, gallocatechin, octadecenamide, β-sitosterol, stigmasterol, estragole, and vitamin E.[55,56] These bioactive compounds have the potential to remove free radicals and active oxygen species through active oxygen species (AOS) detoxifying enzymes, superoxide dismutase (SOD), and peroxidases, implying that they may play a protective role against oxidative damage.[11] The antioxidant activity found in CBP extract allows several potential uses to be suggested, including cosmetic use in the prevention of premature skin ageing[57,58] and chemoprevention of cancers and other chronic diseases at low cost.[59,60]

The 15.00 mm in diameter clear inhibition area to *S. aureus* was classified as severe actions.[61] Furthermore, plant extracts with activity at concentrations of 1000 µg extract per disc or lower are considered promising bioactive agents for further study.[62] MBC/MIC ratio $\leq$ 2.0 and $\leq$ 4.0 implies bactericidal and bacteriostatic effects, respectively.[63] Fortunately, the MBC/MIC ratios were calculated at to be 1.0 and 2.0 for extract against *S. aureus* and *E. coli*, respectively, which were both bactericidal agents. The results of this study indicated that the CBP extract is more effective against Gram-positive than Gram-negative bacteria at the same concentration. Similar findings have also been reported, and differences in sensitivity

could be attributed to the different morphological constitutions of Gram-positive and Gram-negative bacteria strains.[64,65] According to Fajrih et al.,[66] Gram-negative bacteria have an effective permeability barrier comprising a thick cell wall with three layers, including lipoprotein, outer membrane, and a thin lipopolysaccharide, and high lipid contents of 11-22%, which limited the penetration of antibacterial substances, while Gram-positive bacteria have lower lipid contents ranging from 1% to 4%. Another interesting point is that although Gram-positive bacteria have a thicker peptidoglycan layer than Gram-negative bacteria, this macromolecule is a mesh-like framework. As a result, antibacterial substances found it easier to penetrate.[67,68]

In the present study, the integration of phytochemicals in CBP extract was demonstrated, and their presence might be responsible for the potential antibacterial activity of CBP extract. Tannins are believed to have antibacterial activities by damaging components of cell membranes, cell walls, enzymes, genetic material, and other protein components of bacteria.[69] Flavonoids could play a role in forming complexes with soluble proteins or not on the cell surface and with the bacterial cell wall.[69,70] The more lipophilic a flavonoid its ability to damage the cell wall of the bacteria will be more powerful. Next, most alkaloids are strongly bactericidal agents with different mechanisms.[71] The alkaloids as pergularinine and tylophorinidine deactivated dihydrofolate reductase, an essential enzyme for bacterial nucleic acid synthesis. In addition, flavonoids and alkaloids can also kill bacteria by preventing the formation of their cell layers.[72] Meanwhile, saponins can disturb bacterial outer membrane permeability.[73] This result could be responsible for the popular use of banana peel to accelerate the wound-healing process and act as an inflammatory modulator in acne vulgarisbecause ofo the presence of antioxidant, antibacteria, and antiinflammatory substances.[74,75] They can suppress the growth of pathogenic bacteria and prevent infection of the wound.

The ethanolic extract from the CBP powder (*M. paradisiaca*) in this study showed antimicrobial activity greater than that in the previous study by Okorondu et al.[76] Reported that the water extract of *M. paradisiaca* peel did not exert any antimicrobial effect against the bacterial strains. In contrast, Asoso et al.[77] stated that the *M. paradisiaca* peel extract showed activity against *E. coli* ATCC 35218 (17 mm), *Salmonella typhi* ATCC 22648 (17 mm), *Shigella dysenteriae* ATCC 24162 (22 mm), *Klebsiella pneumonia* ATCC 34089 (17 mm), *S. aureus* ATCC 25923 (17 mm), and *Bacillus subtilis* ATCC 21332 (27 mm). Therefore, the antibacterial potency of *M. paradisiaca* extracts is controversial. It could depend on the nature of the varieties, extracted sample (solvent and concentration used), and microorganisms used.

CONCLUSION

In conclusion, the results of this study exceeded our expectations. The RSM was effectively and accurately applied to predict the TPC and TFC in the extract from the powder of Cau banana (*M. paradisiaca*) peel in Tien Giang province, Vietnam. The crude extract was confirmed to contain various chemical groups based on its phytochemical composition. The existence of all these compounds in the extracted sample was a major contributor to their potential to act as natural antioxidants and antibacterials against the growth of the tested pathogens *S. aureus* and *E. coli*. These results highlight the richness of Cau banana peel in secondary metabolites and its potential as a new-generation drug. Furthermore, it would be interesting to test more different bacterial pathogens, including *K. pneumoniae*, *Yersinia*, and *S. typhimurium*, as well as identify different phenolic molecules and test them *in vitro* and *in vivo by* exploiting animal models.

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