Optimization of Antioxidant Extraction on Banana Peels Using Response Surface Methodology

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ABSTRACT Banana peels are known as waste in the industry. Unutilized banana peels for other valuable purposes is also a disadvantage because banana peels contain a significant content of antioxidants. The purpose of the study is to produce antioxidants extract from banana peel waste. It also investigates the optimized condition for the extraction of antioxidants in banana peels under different parameters, which were ethanol concentration (20% to 80%), extraction period (5 min to 35 min) and extraction temperature (30°C to 50°C). The antioxidant activity of banana peel extract was assessed using DPPH radical scavenging assay. Results show that the highest antioxidant activity observed was 88.68%, and the lowest was 35.25% by experiment. The optimized conditions evaluated by Response Surface Methodology (RSM) are an ethanol concentration of 39.78%, an extraction period of 10.75 minutes and an extraction temperature of 43.99%. Under these optimized conditions, the antioxidant activity content was 89.82%, a 1.14% higher than the estimated value (88.68%). The parameters' effect study shows a relationship where a lower concentration of ethanol, a longer extraction period, and a high extraction temperature increase antioxidant activity. The IC50 concentration of banana peel extract is 0.0646 mg/mL. In conclusion, banana peel extracts had shown to have a good potential of antioxidant content.

KEYWORDS: Banana peel extract; Antioxidants activity; Response Surface Methodology; Optimization; ANOVA
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INTRODUCTION

Banana plants grows abundantly in many countries especially in countries with tropical climate (Aurore *et al*., 2009). Banana peel is approximately 30% of the whole banana fruit, which is considered as a waste (González-Montelongo *et al*., 2010). From environment perspective, plant by-products may lead to environment problem due to its high content of phosphorus and nitrogen and its high water content makes it easy to be modified by microorganisms (González-Montelongo *et al*., 2010). Other perspective is the unutilized part of the plant (by-product) where it actually can be transform/ re-use for other purpose that benefits to human. According to (Someya *et al*., 2009), banana peel contains significant antioxidant activity and phenolic compounds and higher in minerals content than the pulp.

There are several factors subjected to phenolic compounds extraction in plant material such as the adopted extraction technique, types of solvent, storage time, presence of unknown substances, sample particle size and their chemical nature (Silva *et al*., 2017; Uma *et al*., 2010). The favour extraction method should be efficient, feasible and sustainable (Apel *et al*., 2020). Ultrasonic-assisted extraction (UAE) is a sound wave extraction method which can improve the extraction process. by producing acoustic cavitation in the solvent. It offers an economic, environmental friendly, and relatively fast compared to the conventional extraction technique (Baqueiro-Pena & Guerrero-Beltran, 2017; Barrera Vazquez *et al*., 2014).

According Chakraborty *et al*. (2020), response surface methodology (RSM) has the capability of developing and optimizing processes which considers several variables using statistical methods,

ANOVA. It minimises the number of experiments to investigate multiple parameters and generates mathematical models for response prediction of varies conditions (Liu *et al*., 2000).

The main purpose of this study is to produce antioxidant extract of banana peel waste. The sub-objectives include a) to find the optimal conditions and study the effect of UAE parameters (solvent concentration, extraction temperature and extraction period) to obtain the maximum antioxidant activity of banana peel extracts using RSM, b) to verify the predicted optimum extraction conditions by experiment, and c) to determine the IC_{50} concentration of the optimized extract.

METHODOLOGY

Sample Collection, Chemicals, Apparatus and Instruments

The banana peels were collected around Kota Kinabalu, Sabah. The collected banana peels were identified based on the species (*Musa acuminata x balbisiana*) and sources (café, markets and disposal areas). The chemicals used were methanol, ethanol, DPPH and ascorbic acid obtained from Sigma-Aldrich. Apparatus used were weighing balance, ultrasonic cleaning bath, filter paper and rotary vacuum evaporator. Instruments used were UV-VIS spectrophotometer and Fourier Transform Infrared Spectrophotometer (FTIR).

Preparation of Samples and Sample Extracts

The banana peels were manually separated and sliced into smaller pieces. The peels were dried under direct sunlight for 48 hours and further in the oven at 40 °C until constant weight was achieved. Then, dried samples were pulverized using a commercial blender and the dried powder was sieved using 1 mm sieve and stored in polytene bags. The samples were properly stored in a sealed container at 18°C.

Extraction of banana peel was carried out using ultrasonic extraction method. Ten grams of banana peel sample powder were initially extracted with 100 mL of ethanol at selected concentration. The extraction mixture then was placed in the ultrasonic bath WUC-D10H at selected time and temperature with a frequency of 40 kHz and a power of 132 W. Then, the supernatant was separated through filter paper, and ethanol was removed using rotary evaporator with temperature ranging between 40°C to 50°C. The separated solvent left was considered as banana peel extracts and stored at 4°C until analysis. The total extraction process were done in triplicate.

Response Surface Methodology (RSM) Analysis

Box Behnken Design (BBD), three level design was applied in RSM to optimize the extraction parameters. This study carried out 17 experiments as suggested by the software. These 17 experiments were tested with different parameters which include the concentration of ethanol, extraction time, and temperature use during extraction. The antioxidant activity was determined based on 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity expressed in % inhibition.

Determination of Antioxidant Activity

Free radical scavenging assay adopted from Brand- Williams *et al*. (1995) with some modifications was used to determine the percentage of antioxidant activity (%) by using 2,2-diphenyl-2-picrylhydrazyl (DPPH). 24 mg DPPH was dissolved with 100 mL methanol to prepare the stock solution and stored at −20 °C. An aliquot of 1.0 mL of banana extracts at different concentration (0.016, 0.063, 0.125, and 0.250 mg/mL) were gently mixed with 3.0 mL methanol solution of DPPH in a test tube. The mixtures were kept in dark condition at 37°C for 40 minutes during incubation. The

absorbance of the mixture was determined at 517 nm in a UV-Vis spectrophotometer. Ascorbic acid of various concentrations (0.016, 0.031, 0.063, 0.0125, and 0.250 mg/mL) were used as standard. Every test was carried out three times and the percentage of antioxidant activity was calculated as scavenging percentage using the Equation (1).

$$DPPH\ Scavenging\ Activity\ (\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100 \quad (1)$$

where, $A_{control}$ is the absorbance of the control (standard solution) and A_{sample} is the absorbance in the presence of the tested sample.

Determination of IC_{50} of Banana peels Extracts at Optimized Condition

A plot of the % inhibitions/RSA against the extract concentrations (mg/mL) was generated and the IC_{50} value of plant extract was attained from a nonlinear regression analysis. The results for IC_{50} were displayed as mean and 95% confidence interval limits.

Statistical Analysis

Statistical analysis was carried out according to analysis done by Sulaiman *et al*. (2011) with slight modification. All data were expressed as means standard deviation (n=3) of triplicate measurements and analysed by Design-Expert software version 12. Analysis of Variance (ANOVA) was used to test the validity of the model and the effect of solvent concentration, time and temperature on the antioxidant activity of banana peel were also investigated. The p-value of less than 0.05 ($p<0.05$) is considered as statistically significant.

Fourier Transform Infrared Spectrophotometer (FTIR)

Infrared spectroscopy measures the molecular vibrations and the spectrum of an unknown compound is determined through comparison to a library of known compounds. The functional group refers to the characteristic of infrared absorption band, which corresponds to the basic vibration of the functional group (Colthup *et al*., 1975; Griffith & De Haseth, 1986). In this study, the banana peels extract samples were prepared by placing one drop of sample between two plates of sodium chloride and then analysed by FTIR. The molecular formula was observed to determine the presence of antioxidant compounds in the banana peel extract for validation.

RESULT AND DISCUSSION

Response Surface Methodology (RSM) Analysis

Table 1 details the antioxidant activity (expressed as % inhibition) of banana peel extracts obtained from 17 experiments.

Through analysis of variance (ANOVA), the experimental data was fitted to the quadric surface model for DPPH% of banana peel extract. The analysis indicates that the model inadequately represent the experimental data. The coefficient of multiple determinations (R^2) for the response of antioxidant activity is 0.4876. It shows that the model was only able to explain 48.76% of the results in the case of DPPH Radical scavenging activity (RSA). The p-value is 0.7794, indicates an insignificant lack of fit and F-value is 0.37. The regression equation (Equation (2)) is obtained for the independent and dependent variables for antioxidant activity was:

$$\%\ DPPH = 66.53 + 4.50A - 2.75B + 5.50C + 3.30A^2 - 0.70B^2 + 8.30C^2 - 8.50AB - 1.50AC + 12.15BC \quad (2)$$

Table 1. Response of antioxidant activity (DPPH %) for banana peel extract.

Run	Ethanol Concentration (% in water)	Time (min)	Temperature (°C)	DPPH (Average % ±SD)
1	50	5	30	78.32 ± 0.03
2	80	5	40	59.48 ± 0.12
3	80	20	50	72.41 ± 0.02
4	20	20	50	50.48 ± 0.15
5	80	35	40	56.34 ± 0.76
6	50	35	50	88.68 ± 0.06
7	50	20	40	35.25 ± 0.02
8	20	35	40	59.02 ± 0.34
9	80	30	30	57.47 ± 0.95
10	50	30	30	67.44 ± 0.43
11	50	50	50	76.86 ± 0.05
12	50	20	40	74.48 ± 0.08
13	50	20	40	85.66 ± 0.05
14	20	20	30	60.80 ± 0.05
15	50	20	40	83.89 ± 0.02
16	50	20	40	78.26 ± 0.01
17	20	5	40	48.92 ± 0.80

The effect of ethanol concentration on DPPH scavenging activity is shown on Figure 1a. The highest DPPH RSA can be obtained by using 20% of ethanol concentration (t=35 min). It indicates that the antioxidant activity is sensitive to the ethanol which explains the decrease of antioxidant activity at 80% ethanol concentration. More importantly, the water presence in the solvent allowed a good swelling of the sample and increase the surface area for solute-solvent contact eventually (Chirinos *et al*., 2007). In this study, the higher DPPH RSA obtained using 20% ethanol concentration shows that the active phenolic compounds presented in banana peel are moderately non-polar compounds.

The effect of time on DPPH RSA is observed on Figures 1a and 1c. Based on these figures, DPPH RSA increased with the increased of extraction time from 5 minutes to 35 minutes. No decrease in DPPH RSA was observed within this range of extraction time. However, prolonged extraction time beyond this range may result in decrease in DPPH RSA. This is caused by oxidation and degradation of the desired compound especially when extracted at high temperature.

The effect of temperature (30 °C to 50 °C) on DPPH scavenging activity is shown on Figures 1b and 1c. The rate of extraction of bioactive compound escalates as temperature increases due to solute diffusivity build-up. However, higher extraction temperature will degrade some biologically active compounds, such as phenolic acids, flavonoids and anthocyanins, resulting in the loss of their antioxidant activity (Peanparkdee, 2019). A decrease in antioxidant activity of the crude extract will occur after exceeding a maximum temperature due to thermal destruction of phenolic compounds and thus denatured the phenolic compound. Based on the model generated, the optimum conditions for banana peel extracts were ethanol solvent concentration of 39.78%, at temperature of 43.99 °C for 10.75 min.

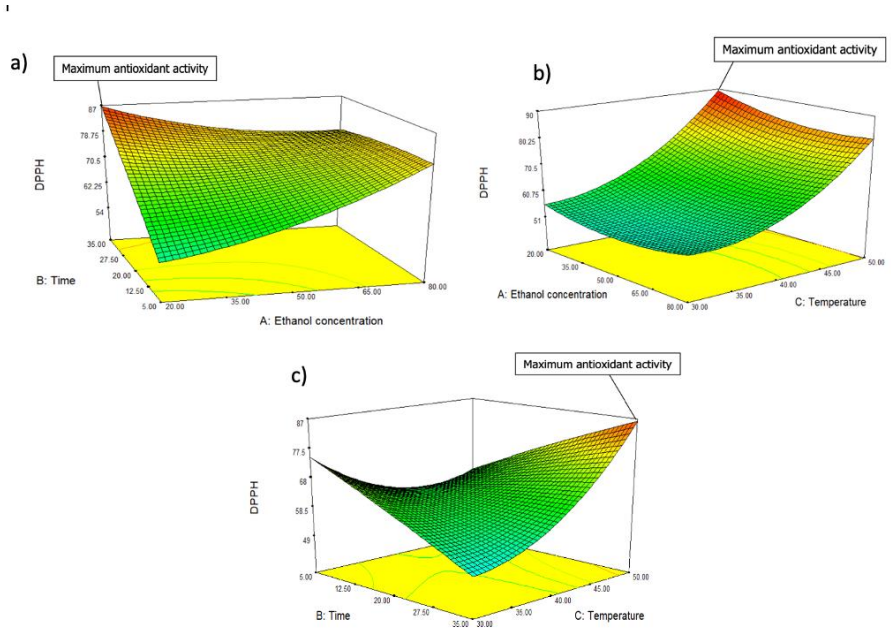

Figure 1. a) Effect of time and ethanol concentration on the antioxidant activity of the extract; b) Effect of temperature and ethanol concentration on the antioxidant activity of the extract; c) Effect of temperature and time on the antioxidant activity of the extract.

Experimental Validation of the Predicted Optimal Conditions

Table 2 shows the optimal extraction conditions and predicted-experimental values obtained. Based on the optimal conditions estimated experiments were carried out to compare between predicted and real experimental results. The close values of predicted-experimental (< 3%) show that the response surface model is sufficient to predict DPPH RSA of banana peel extract.

Table 2. Experimental confirmation of predicted value at optimal extraction conditions.

Optimal Levels	DPPH Radical Scavenging Activity (%)		Relative Error (%)
	Predicted	Experimental	
Ethanol concentration (39.78%); Time (10.75 min); Temperature (43.99 °C)	88	89.82 ± 0.1	2.13

According to Jadid *et al*. (2017), the inhibitory concentration, IC_{50} value determines the concentration of the sample required to inhibit 50% of radical. Lower IC_{50} value indicates higher antioxidant activity in the samples (Li *et al*., 2009). In this study, IC_{50} values for ascorbic acid and optimized banana peel extract were obtained from their non-linear regression equation. The IC_{50} value of ascorbic acid is 0.003 (0.0007059 to 0.01820) mg/mL while the IC_{50} value of optimized banana peel extract is 0.0646 (0.01120 to 0.3728) mg/mL.

FTIR Analysis

Figure 2 exhibits the IR spectrum appeared for banana peel extract by using FTIR. Table 3 summarizes the wavelength of several functional groups of organic compounds that presents in banana peel extract. The FTIR analysis shows high O-H bond possibilities in the extract, which indicates there is phenolic compounds presence in the extract (Coates, 2000).

Table 3. FTIR peaks value for banana peel extract

Functional Group	Wavelength range (cm^{-1})
O-H	3600-3200
C≡C	2250-2100
C=C	1680-1600
C-H	1480-1350
C-O	1300-1000
C-X (F,Cl,Br,I)	<667

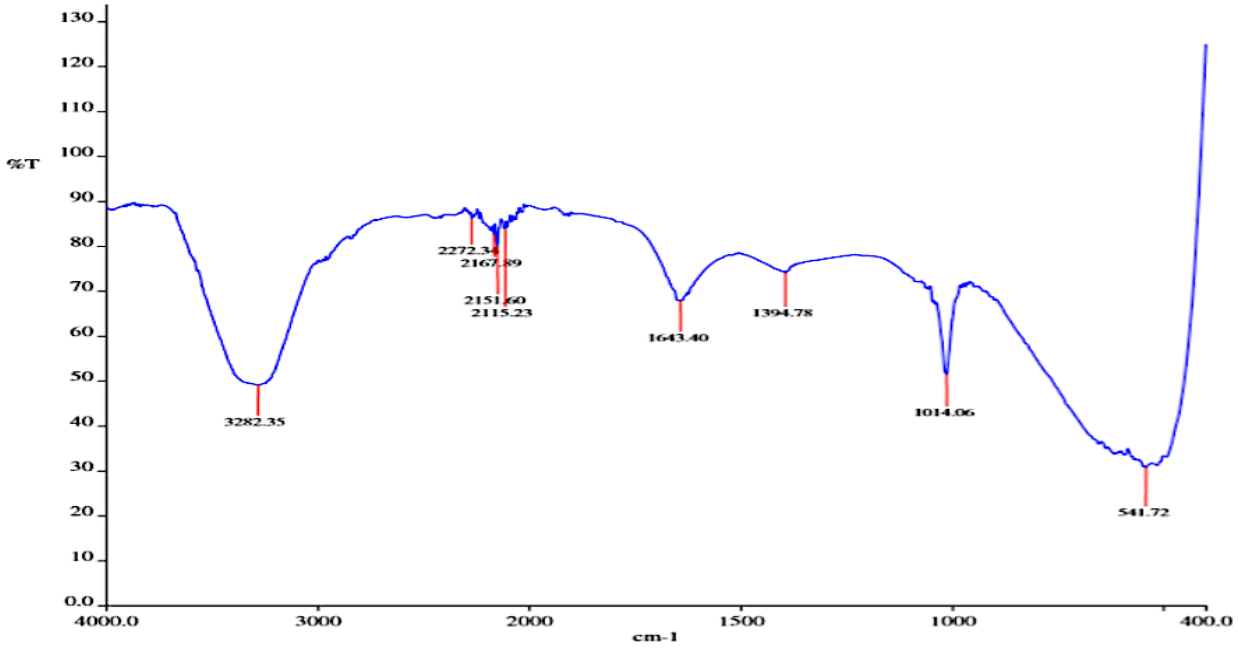

Figure 2. FTIR analysis of ethanolic extract of banana peel

CONCLUSION

Factors studied in this work include the concentration of ethanol, extraction temperature and time are significantly affected the process of extracting antioxidants from banana peel. The optimized conditions stated by RSM for the maximum antioxidant capacity were ethanol concentration of 39.78%, extraction time of 10.75 minutes and temperature of 44°C. Under these conditions, the maximum antioxidant activity is achieved with DPPH scavenging activity of 89.82% which is higher compared to the estimated DPPH scavenging activity of 88%.

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