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Extraction of *Musa acuminata* Peels: Response Surface Optimization, Phytochemical Screening and Antioxidant Activity

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Banana (*Musa acuminata*) is categorized as tasty fruit with high nutrient content. Significant quantities of banana peels are equivalent to 40% of the total weight of fresh bananas and are generated as waste products and disposed of in large amounts by households and industries. This study aims to determine the optimum extraction conditions to obtain the highest yield of *M. acuminata* peel extract and analyse its antioxidant capacity. The extraction was carried out via maceration technique using methanol with a solid-to-solvent of 1:30 g/mL. The extraction conditions of *M. acuminata* peels were optimized using a central composite design (CCD) of response surface methodology (RSM). The optimum extraction yield was predicted to be about 28.84% at an extraction temperature of 54.14°C and a time of 5 hours. The extraction was carried out at the optimum conditions; it was found that the average yield was 28.96 ± 0.5% revealing good correspondence with the predicted value. Qualitative phytochemical screening of the methanolic extract of *M. acuminata* peels obtained at optimized conditions revealed the presence of flavonoids, alkaloids, tannins, saponins, steroids, phenols, glycosides, and terpenoids. Total phenolic content of 32.91 ± 0.33 mg GAE/g and DPPH radical scavenging activity with 94.34% percentage of inhibition and IC_{50} value of 69.70 ± 1.08 ug/mL were recorded for the extract, respectively. High total phenolic content with a high percentage of inhibition in DPPH radical scavenging activity proved that *M. acuminata* peels might have a significant amount of bioactive constituents and can be a promising candidate for natural antioxidants.

Keywords: *Musa acuminata*; banana; phytochemical; antioxidant

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Approximately about 2000 medicinal plant species in Malaysia possess health benefit properties. Nutrition studies stated that all medicinal plants have diverse nutritional values, which contain various bioactive compounds that can act as antibacterial, antioxidant, and antimicrobial agents [1]. According to [2], an antioxidant is any substance that delays, prevent, or removes a target molecule's oxidative damage. Anti-oxidants have synergistic effects and protect against several degenerative disorders such as cancer, stroke, cardiovascular disease, Alzheimer's disease, and Parkinson's [3]. Nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) are widely used in most food and pharmaceutical products [4, 5, 6]. They were added to food to prolong product shelf life, mainly by preventing the oxidation of unsaturated double bonds of fatty acids. In pharmaceutical products, they are added to enhance the stability of therapeutic agents that are susceptible to chemical degradation by oxidation. However, it was reported that synthetic antioxidants could have carcinogenic effects on human cells, thus fuelling an intense search for new, natural, and efficient antioxidants [7].

The antioxidant present in fruits has the potential to reduce oxidative stress, where this oxidative stress can cause protein, DNA, and lipid damage. Plants usually contain various free-radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, and more [6]. Studies have proven that many of these antioxidant compounds exhibit anti-inflammatory, anticarcinogenic, antibacterial, antitumor, or antimutagenic effects in cells [8]. Nowadays, the intake of natural antioxidants is associated with reduced cancer risks, cardiovascular disease, and other diseases.

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Musa spp. (banana) is a plant grown worldwide throughout humid tropical and subtropical regions. It is often consumed as ripe fruit or used for culinary purposes. About 40% of the total weight of fresh bananas is accounted for banana peels [9]. In some countries, massive amounts of banana trees have been thrown after collecting the banana fruits or after the inner flesh is eaten, which can pose environmental problems due to their nitrogen and phosphorus quantity [10, 11]. Few studies have found that banana peels are rich in starch (3%), crude protein (6-9%), crude fat (3.8-11%), dietary fiber (43.2-49.7%), and vitamins [12]. The banana peels are also rich in a variety of bioactive compounds, including flavonoids, tannins, and phenols, whereby alkaloids, carbohydrates, reducing sugar, saponins, glycosides, phytosterols, proteins, anthroquinones, and steroids are the minor phytochemicals found in this plant [9, 10, 11, 13, 14, 15].

Musa acuminata, also known as lady finger banana or 'pisang mas,' belonging to the *Musaceae* family, is one of the most important tropical fruit in the world market. To date, the findings on the phyto-chemical constituents and antioxidant activity of *M. acuminata* peel extract are not well documented and are still unclear. This study utilized response surface methodology (RSM) to optimize the extraction conditions to obtain the highest extraction yield. The effect of two independent variables, temperature and extraction time, on the extraction yield was evaluated. The phytochemical content of the extract and its anti-oxidant activities were also further determined to investigate its beneficial value and to facilitate potential applications.

EXPERIMENTAL

Chemicals and Materials

Ripe fruits of *M. acuminata* of the same maturity (full yellow) were purchased from the local fruit stall in Kuala Pilah, Negeri Sembilan. The peels of *M. acuminata* were removed from the flesh and washed with tap water. After that, the peels were dried in a hot air oven at a temperature of 50°C for 6 hours. Then, the dried sample was ground into smaller particles using a grinder. The powdered peels (around 300 g) were stored in a plastic container and placed in a desiccator until further analysis [16]. The chemicals such as methanol, Wagner's reagent, chloroform, acetic acid, concentrated sulphuric acid, ferric chloride, sodium hydroxide, Folin-Ciocalteu, and ascorbic acid were purchased from R&M Chemicals, respectively. The *n*-hexane and sodium carbonate were purchased from Bendosen Laboratory Chemicals, whereby gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent were purchased from Sigma-Aldrich (M) Sdn. Bhd. All other chemicals used in this study were of analytical grade.

Extraction of *M. Acuminata* Peels

The powdered peels of *M. acuminata* were weighed about 5 g and placed into a 250 mL conical flask along with the methanol solvent. The mouth of the flask was sealed with aluminum foil to prevent the solvent from evaporating. The flask was placed in the water bath according to the conditions (temperature and time), total of 13 experimental runs, as suggested by the central composite response surface design. The 1:30 g/mL solid-to-solvent ratio was kept constant for all experiments. The extracts were filtered using filter paper, and all analyses were performed on the same day of the extraction. The solvent was removed by using a rotary evaporator at 52°C to obtain the crude extract, and the percentage yield of crude extract was calculated using Eq. 1 [17]:

$$\text{Percentage yield} = \frac{\textbf{Amount of extract (g)}}{\textbf{Amount of peel powder(g)}} \times 100\% \qquad \text{(Eq. 1)}$$

Experimental Design and Statistical Analysis

Response surface methodology (RSM) is a widely practiced approach to design an optimized process in a short time period and minimum trials. RSM with central composite design (CCD) was applied in the optimization process and evaluation of the effect of two different independent variables, namely temperature (30-50°C) and time (4-6 h) on the extract yield. These parameters were selected based on the study conducted by Anne and Nithyanandam [18] who have studied on the optimization of extraction of bioactive compounds from medicinal herbs. A two-factor-five-level rotatable CCD was chosen in the extraction of *M. acuminata* peels resulting in 13 experimental runs consisting of 5 central points, 4 factorial points, and 4 axial points. To determine if the constructed models were adequately fitted to the experimental data, an analysis of variance (*ANOVA*) was used. Design-Expert Software version 7.0.0 (Stat-Ease Inc., Statistics Made Easy, Minneapolis, MN, USA) was used to analyze and interpret the experimental data. The software numerical optimization function was utilized to establish the best conditions for extracting *M. acuminata* peels. The experiments were then carried out under the recommended conditions, and the resulting yields were compared with those predicted by the software.

Preliminary Qualitative Phytochemical Analysis

Several tests were carried out on the methanol peel extract of *M. acuminata* that was obtained at the optimize condition suggested from the optimization function of Design-Expert Software to determine the presence of secondary metabolites, including alkaloids, steroids, terpenoids, tannins, saponins, glycosides, flavonoids, and phenols. The phytochemical screening

test was conducted according to [10] with slight modifications. The alkaloid was tested by treating 0.5 g of an extract with a few drops of Wagner's reagent. The formation of brown or radish precipitate indicates the presence of alkaloids. As for the steroids test, about 0.5 g of extract was mixed with 2 mL chloroform, 2 mL acetic acid, and 1 mL concentrated sulfuric acid. A formation of blue-green indicates the presence of steroids. For the terpenoids test, about 0.5 g of extract was treated with 2 mL of chloroform, then a few drops of sulfuric acid were added to the mixture. Reddish brown coloration in the interface indicates the presence of terpenoids. In order to test for the presence of tannins, a few drops of 1 M ferric chloride solution were added into a test tube that contained 2 mL of methanolic extract. The formation of blue-black or greenish-black coloration shows the presence of tannins. As for the saponins test, about 0.5 g of extract was shaken with 5 mL of distilled water in a test tube. A form that persists for 10 min indicates the presence of saponins. In the test for triterpenes glycosides (Liebermann's test), about 0.5 g of extract was treated with 2 mL of chloroform and 2 mL of acetic acid. A green appearance shows glycosides' presence [19]. As adapted from [20], the flavonoids (lead acetate test) were carried out where a few drops of 10% lead acetate solution were added into 0.5 g of the extract. The formation of a yellow precipitate indicates the presence of flavonoids. Finally, for phenols (ferric chloride test), 2 mL distilled water was added into 0.5 g extract, and a few drops of 10% ferric chloride were added. The formation of green or blue colour indicates the presence of phenols [21].

Determination of Total Phenolic Content (TPC)

The total phenolic content in the peel extract of *M. acuminata* with different concentrations (120, 100, 80, 60, 40, and 20 g/mL) was determined using the Folin-Ciocalteu method developed by [22] with some modifications. About 0.2 mL of sample was added into 0.2 mL of Folin-Ciocalteu reagent and followed by mixing with 1.8 mL distilled water. Then the mixture was incubated for about 5 minutes in the dark. After that, 2 mL of sodium carbonate solution (7 w/v%) and 0.8 mL distilled water were added to the mixture and placed in the dark for another 30 minutes. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer, and the readings were taken in triplicate. The same procedure was repeated for the standard solution of gallic acid of various concentrations (120, 100, 80, 60, 40, 20 g/mL). The standard curve of the gallic acid was constructed, and the TPC value was calculated using the gallic acid equivalent (GAE) as shown in Eq. 2:

$$\text{TPC (mg GAE/ g dry weight)} = c \times V/m \qquad \text{(Eq. 2)}$$

Where : c is the concentration of gallic acid that was obtained from the calibration curve (mg/mL)
: V is the volume of extract (mL)
: m is the mass of extract (g)

Antioxidant Activity

A 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was used to determine antioxidant activity in the methanol extract of peels *M. acuminata* with some modifications. The standard solution of ascorbic acid and extract was prepared at various concentrations (1000, 500, 250, 125, 62.5, 31.3, 15.63, and 7.81 µg/mL). About 50 µM (3.8 mL) of DPPH solution in methanol was mixed with 0.2 mL extract. Afterward, the mixture was incubated in the dark for 30 minutes at room temperature. Then the absorbance was measured spectrophotometrically using a UV-Vis spectrophotometer at 517 nm [23]. DPPH blank was prepared by mixing 3.8 mL of DPPH solution and 0.2 mL of methanol. Ascorbic acid acted as a positive control, while a blank sample was prepared by mixing 0.2 mL of extract and 3.8 mL of methanol. The standard and extract were both tested in triplicate. The scavenging activity was calculated using Eq. 3:

$$\text{DPPH}\cdot \text{ scavenging effect (Inhibition \%)} = [(Ac - As / Ac) \times 100] \qquad \text{(Eq. 3)}$$

where : Ac is the absorbance of the DPPH solution without a sample (control).
: As is the absorbance in the presence of the plant extracts.

GraphPad Prism 6 was used to calculate the IC_{50} value (the concentration of the sample to scavenge 50% of the radicals). The result was reported as means ± standard deviation [24].

RESULTS AND DISCUSSION

Analysis of the Response Surface Methodology (RSM) Model

Design-Expert Software version 7.0.0 (Stat-Ease Inc., Statistic Made Easy. Minneapolis, MN, USA) was used to analyze and interpret the experimental data. Table 1 shows the design of experiments in terms of uncoded variables, experimental and predicted extraction yield for two-factor-five-level CCD response surface analysis of *M. acuminata* peels extraction.

Table 1. Design of experiments in terms of uncoded variables, experimental and predicted extraction yield for two-factor-five-level CCD response surface analysis

Std. run	Temperature, *A* (°C)	Time, *B* (h)	Experimental yield (%)	Predicted yield (%)
1	30.00	4.00	24.04	24.35
2	50.00	4.00	27.13	27.50
3	30.00	6.00	23.40	23.17
4	50.00	6.00	27.09	26.93
5	25.86	5.00	23.99	23.96
6	54.14	5.00	28.96	28.84
7	40.00	3.59	25.65	25.20
8	40.00	6.41	23.65	23.96
9	40.00	5.00	24.18	24.52
10	40.00	5.00	25.48	24.52
11	40.00	5.00	24.00	24.52
12	40.00	5.00	24.45	24.52
13	40.00	5.00	24.50	24.52

The predicted values were shown to agree with the experimental values using the model fitting technique. After fitting the data to various models such as linear, quadratic, and cubic, *ANOVA* revealed that the relationship between the yield (response) and independent variables (temperature and time) was best described by a quadratic polynomial model as follows:

Table 3 shows the *ANOVA* result of a regression model. The high model *F*-value of 22.84 with the values of "Prob > *F*" less than 0.0500 implies that the model is significant while the value of Prob > *F* was 0.64, indicating that the Lack of Fit is not significant and the greatest fit of the developed model. "Adequate precision" measures the signal-to-noise ratio. This ratio must be greater than 4 to navigate the design space, and the value of adequate precision was 15.844, indicating an adequate signal. Figure 1 illustrates the correlation between predicted and actual responses within the experiment range investigated, complemented by the information provided by statistical indicators. The graph shows a close fit between the actual and predicted values. As a result, the model generates a statistically significant relationship between independent variables and response. Thus, the developed RSM model could predict experimental results in the range of studied domains.

$$\text{Optimization yield (\%)} = 24.52 + 1.73A - 0.44B + 0.15AB + 0.94A^2 + 0.027B^2$$

where : *A* is the temperature
: *B* is the time

The results of model summary statistics are shown in Table 2. The results showed linear and interactive (2FI) models had lower R^2, adjusted R^2, and predicted R^2 than quadratic models, with the cubic model being aliased. The high R^2 value of 0.9422, adjustment R^2 of 0.9010, and predicted R^2 of 0.8061 and reasonable agreement between both predicted R^2 and adjustment R^2 values showed that the quadratic model could effectively study the interaction between the operating parameters [25].

Table 2. Model summary statistics

Source	Std. Dev.	*R*-Squared	Predicted *R*-Squared	*p*-value Prob > *F*	PRESS
Linear	0.91	0.7549	0.7059	0.5538	15.00
2FI	0.95	0.7576	0.6768	0.4946	16.99
Quadratic	0.53	0.9422	0.9010	0.8061	6.52
Cubic	0.52	0.9596	0.9031	0.8561	4.84

Table 3. The *ANOVA* result of the regression model

Source	Sum of Squares	Degree of freedom	Mean Square	*F*-Value	*p*-value Prob > *F*	Significant
Model	31.67	5	6.33	22.84	0.0003[a]	Significant
Residual	1.94	7	0.28			
Lack of fit	0.63	3	0.21	0.64	0.6291[b]	Not significant
Pure Error	1.31	4	0.33			
Cor Total	33.61	12				

[a]Significant at "Prob > *F*" less than 0.0500
[b]Insignificant at "Prob > *F*" greater than 0.0500

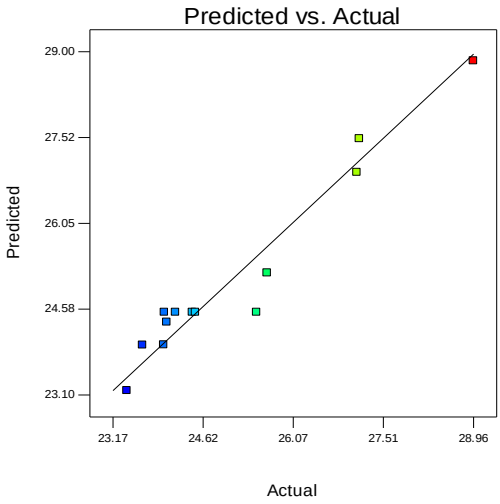

Figure 1. The correlation between predicted and actual responses.

Effect of Operating Variables

The coefficient of the empirical model is presented in Table 4. A higher regression coefficient and smaller Prob > *F* value (Prob > *F* less than 0.05) for those variables and their interaction demonstrated that they significantly impacted the response. Based on the results, the linear term of the temperature (*A*) and the quadratic term of temperature (A^2) were found to have a more significant influence on the extraction yield of *M. acuminata* peels compared to the linear team of time (*B*), a quadratic team of time (B^2) and their interaction of *AB*. Negative coefficient values denoted a negative influence of the parameter on the extraction yield. It was observed that the linear coefficient of temperature (*A*) had a positive effect on the extract yield, whereby the linear coefficient of the time (*B*) showed a negative effect on the extraction yield. However, the effect of time (*B*) was insignificant in influencing the percentage of extraction yield obtained as the Prob > *F* value was more than 0.05.

Figure 2 (a) and (b) show the effect of changing temperature and time (duration of extraction) on the percentage of extraction yield. From the findings, it was discovered that as the temperature was increased from 30°C to 50°C, the percentage yield of the

Table 4. Regression coefficient and *p*-value (Prob > *F*) of the model

Source	Sum of Squares	Degree of freedom	Mean Square	*F*-Value	*p*-value Prob > *F*
***A*-Temperature**	23.83	1	23.83	85.95	< 0.0001[a]
***B*-Time**	1.54	1	1.54	5.55	0.0507
AB	0.090	1	0.090	0.32	0.5867
A^2	6.14	1	6.14	22.15	0.0022[a]
B^2	5.118×10^{-3}	1	5.118×10^{-3}	0.018	0.8958[b]

[a]Significant at "Prob > *F*" less than 0.0500
[b]Insignificant at "Prob > *F*" greater than 0.0500

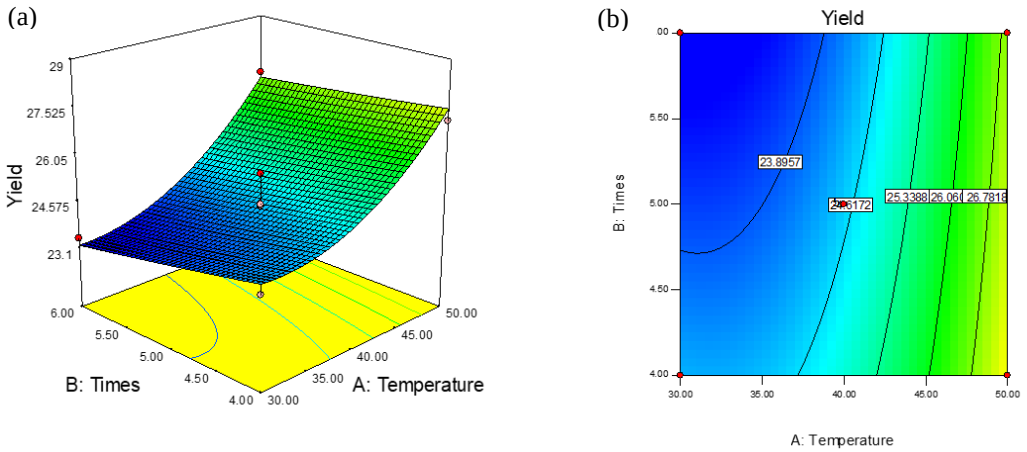

Figure 2. (a) Three-dimensional (3D) surface plot and (b) two-dimensional (2D) contour plot that shows the effect of changing temperature and duration of time on the percentage of extraction yield.

extraction was also increased. This finding was in agreement with those reported by [26], who reported that the percentage yield of banana peels (*M. acuminata* Colla AAA) and cinnamon bark (*C. varum*) extract increased as the temperature increased. This might be because the antioxidant and soluble compounds rapidly dissolve into the solvent at higher temperatures, thus, increasing the extraction yield. Ascorbic acid was discovered to have higher retention when the temperature was high and for a short duration [27]. Besides, the heating process may soften plant tissues and reduce phenol-protein and phenol-polysaccharide interactions, allowing more polyphenols to move into the solvent. Yet, more proteins and polysaccharides can be extracted at a higher temperature when water is used as an extraction solvent [28]. The temperature had a substantial influence on extraction yield, which agreed with those reported in the previous study where the maximum value of phenolic content of the banana peels (*M. acuminata* Colla AAA) and cinnamon barks (*C. varum*) extract using the ultrasonic method was obtained at high extraction temperature of 60°C with 30 minutes of extraction time [26]. However, some phenolic components are likely oxidized at higher temperatures and longer extraction times [29]. Theoretically, the extraction efficiency improves as the extraction duration increases. However, after the solute has reached equilibrium on both the inside and outside of the solid material, increasing the time will no longer affect the extraction yield [30].

Optimization of Extraction Conditions and Model Validation

The optimum condition for the *M. acuminata* peels extraction in methanol (1:30 g/mL solid-to-solvent ratio) to give the highest extraction yield was predicted using the optimization function of the Design Expert Software. Within the experimental range studied, the optimum conditions were found to be: temperature = 54.14°C and time = 5h, resulting in a predicted extraction yield of 28.84%. At optimum conditions, the experiments were carried out in triplicate, and the experimental data were compared with the predicted result. Experimentally, the percentage yield obtained was 28.96 ± 0.5%) and agreed with the predicted value. Well-agreed experimental and predicted results, thus confirming that the mathematical model derived from RSM can be used to adequately describe the relationship between the independent variables (temperature and time) and the response of the extraction yield.

Preliminary Qualitative Phytochemical Analysis

Phytochemical screening helps discover plant extract constituents and the one that predominates over the others, as well as the search for bioactive compounds that can be employed in developing therapeutic medications [31]. Phytochemicals are valuable bioactive elements in plants that work with fibers and nutrients to protect against various diseases. Table 5 shows the qualitative phytochemical screening result of the methanolic extract of *M. acuminata* peels. Methanol is a polar solvent frequently used as an extraction medium for phenolic compounds. In many studies, methanol extracts exhibited the highest level of phytochemicals, which are significant for therapeutic purposes. In this study, methanolic extracts of *M. acuminata* peels revealed the presence of alkaloids, saponins, terpenoids, tannins, phenols, glycosides, and phenols. However, steroids were absent in the methanolic extracts of *M. acuminata* peels.

Table 5. Qualitative phytochemical screening of *M. acuminata* peels extract.

Phytochemical	Result
Alkaloids	+
Saponins	+
Terpenoids	+
Steroids	-
Tannins	+
Glycosides	+
Phenols	+
Flavonoids	+

+ : Present, - : Absent

From the table above, alkaloids were detected in the methanolic extract of *M. acuminata* peels due to the presence of a brown or reddish precipitate. This is because the presence of potassium-alkaloid was predicted in the precipitate. Iodine reacts with the I^- ion from potassium iodide to produce I^{3-} ion (brownish solution) in Wagner's reagent preparation. The metal ion of K^+ will bind as covalent coordinate bonding with nitrogen to alkaloid in the Wagner test, resulting in a complex potassium-alkaloid precipitate [32]. It showed a positive result for saponins because saponins form miscellanea when shaken vigorously with water. Saponins consist of glycosyls as polar groups, while steroids and triterpenoids as nonpolar groups. Polar groups face the outside, and nonpolar groups face the inside [33].

However, in this study, the minimal foam was developed, which was in agreement with those reported by [32], who stated that less foam formation due to steroids and triterpenoids was insignificant. This is because saponins consist of two parts: a hydrophobic skeleton, known as the aglycone unit, and a hydrophilic saccharide, known as the glycosidic unit. Saponins can be classified into three types: triterpenoid saponins, steroidal saponins, and steroidal glycoalkaloids based on their aglycone structure [34]. The formation of greenish-black showed the presence of tannins in methanolic extract *M. acuminata* peels. The ferric test is specifically for phenolic chemicals, not just tannins [35]. It is based on the principle that phenolics react with iron salts to produce a blue or green black (or grey) substance. The phenolic content of the sample was found to be positive. As a result, the phenolics found can be reasonably assumed to be tannins. The formation of reddish brown colouration at the interface showed that the extraction of *M. acuminata* peels contained terpenoids. The green solution was detected in the extract, which shows that it consists of glycosides. The detection of yellow precipitate indicated the presence of flavonoids. Flavonoids can be classified into different subgroups including flavones, flavonols, isoflavones, flavanonols, flavanols or catechins, anthocyanidins and chalcones [14].

Total Phenolic Content (TPC)

According to [36] the Folin-Ciocalteu method is an electron transfer-based assay that determines reducing capacity and phenolic content. TPC was measured to identify the amount of phenolic content in the *M. acuminata* peels extract. The redox properties of this phenolic compound allow them to act as antioxidants. In this study, the total phenolic content of *M. acuminata* peels extract was 32.91 ± 0.33 mg GAE/g, with gallic acid used as a standard. The TPC value was calculated from the equation of the gallic acid solution of $y = 0.0035x + 0.0325$ with $R^2 = 0.9989$, as shown in Figure 3.

According to prior research, the total phenolic content of banana peels ranges from 4.95 to 47 mg garlic acid equivalent/g dry matter (mg GAE/g DM), indicating that it is a rich source of phenolic chemicals [37]. Thus, this extract is expected to perform well in antioxidant activities, which exhibit more significant free radical scavenging because the TPC value was in the ranges previous researchers have reported.

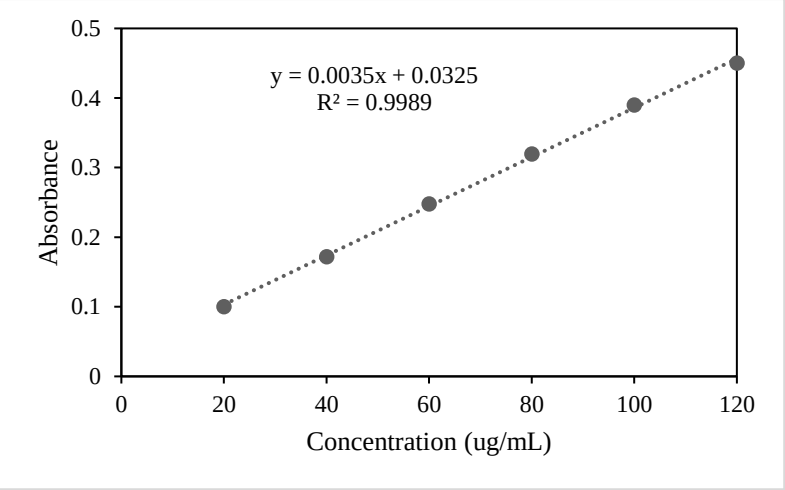

Figure 3. Gallic acid standard calibration curve.

Table 6. IC_{50} value of ascorbic acid and methanolic extract of *M. acuminata* peels

Sample	IC_{50} (μg/mL)
Ascorbic acid	19.23 ± 0.36
M. acuminata peels	69.70 ± 1.08

Antioxidant Activity

The DPPH assay is used to assess antioxidant activity based on the process by which antioxidants inhibit lipid oxidation, resulting in DPPH radical scavenging and thereby determining free radical scavenging capacity. The approach is extensively utilized because the analysis takes only a short time [38]. The DPPH scavenging activity is based on one-electron reduction, representing antioxidant-free radical scavenging activity [39]. The IC_{50} (concentration required for 50% inhibition) of a molecule is inversely proportional to its antioxidant capacity since it reflects the number of antioxidants necessary to reduce DPPH concentration by 50%, as determined by interpolation from a linear regression analysis. A compound with a lower IC_{50} has a higher antioxidant activity [40]. The highest concentration of extracts had the lowest absorbance value and the highest antioxidant resistance percentage [41]. In this study, the IC_{50} of the methanolic extract of *M. acuminata* peels and the ascorbic acid standard were calculated, and the values are shown in Table 6. Ascorbic acid was used as a positive standard in this study as it is the well known standard with strong antioxidant activities. Higher IC_{50} value indicates lower antioxidant activity. The results obtained were correlated with those reported by previous study [41] who have recorded the antioxidant activity of banana peel extract of 64.03 ± 2.78 μg/mL. The value of IC_{50} of banana peel extracted in 80% of methanol was found to be 56.22 ± 1.25 μg/mL with BHT as standard with IC_{50} value of 4.73 ± 0.72 μg/mL as discovered by [9].

Figure 4 shows the percentage of inhibition of standard ascorbic acid and *M. acuminata* peels extract at different concentrations. It was found that the percentage inhibition in different concentrations was increased. At 1000 ppm, the percentage of inhibition of ascorbic acid and *M. acuminata* peels extract were 97.03% and 94.34%, respectively. This study revealed that *M. acuminata* peels have a high radical scavenging activity which was in agreement with [42], who stated that the polarity-dependent increase in extraction yield, antioxidant activity, reducing properties, and free radical scavenging activity might attribute to the high affinity of antioxidant compounds towards more polar solvents.

CONCLUSION

The optimum yield of methanolic extract of *M. acuminata* peels with a solid-to-solvent ratio of 1:30 g/mL was obtained at an extraction temperature of 50°C and duration time of 4 h. Methanolic extract of *M. acuminata* peels showed the presence of flavonoids, alkaloids, tannins, saponins, steroids, phenols, glycolsides, and terpenoids. High amount of TPC play a major role in controlling oxidation. The methanolic extract of *M. acuminata* peels exhibited a TPC value of 32.91 ± 0.33 mg GEA/g and possessed high antioxidant activity with the high inhibition percentage of 94.34% and IC_{50} value of 69.70 ± 1.08 μg/mL. Another vital qualitative and quantitative analysis using various spectroscopic analysis and isolation of bioactive compound in *M. acuminata* can be further

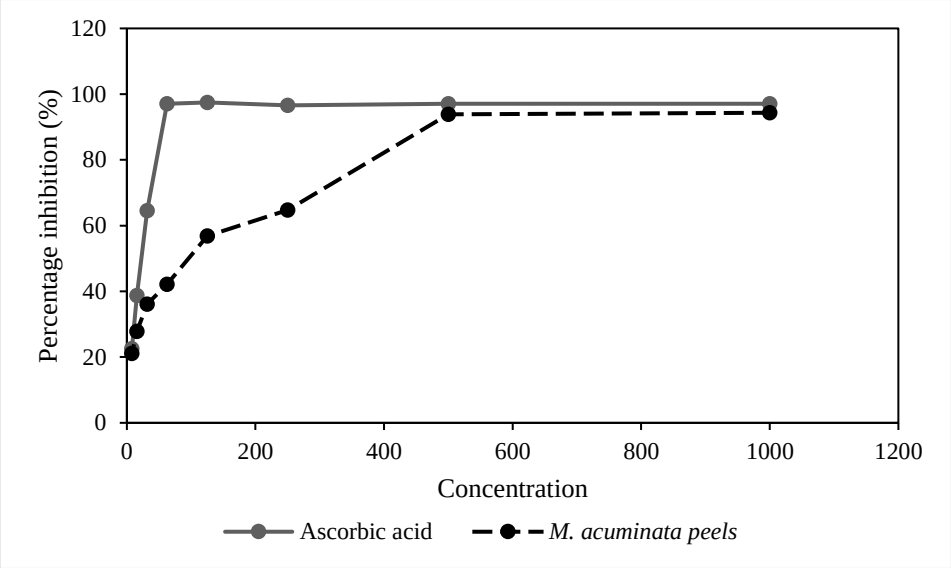

Figure 4. The percentage inhibition methanolic extract of *M. acuminata* peels and ascorbic acid at different concentrations.

verified for future works. The presence of biologically active molecules in frequently discarded material suggests that *M. acuminata* peels could be a valuable source of therapeutic substances that can act as antioxidants.

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