Ultrasonic-aided Optimisation of Phytochemicals from *Ananas comosus* Peel

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Pineapple (*Ananas comosus*) peels contain various phytochemicals, such as antioxidants and polyphenols. In this study, ultrasound-assisted extraction (UAE) was applied to optimise the extraction of *A. comosus* peels by using the Central Composite Design (CCD). The extraction of ground *A. comosus* peels with aqueous methanol was optimised for factors including extraction time and solvent: sample and methanol: water ratios in order to identify the responding percentage yield (PY), total phenolic content (TPC), total flavonoid content (TFC), and free radicals scavenging activity by DPPH. This study also compared the extraction via Soxhlet extraction for all three responses. The UAE extracts, under conditions E18 and E20, exhibited 50% scavenging activity of DPPH free radicals with a corresponding SC_{50} value of 549.535±17.277 and 669.744±0.955 μg/mL, respectively. Meanwhile, Soxhlet extraction outcomes revealed that only the chloroform extract gave an SC_{50} value of 631.238±3.126 μg/mL. Additionally, the UAE and Soxhlet-generated extracts employed in the antibacterial assay were weakly active against Gram-positive and Gram-negative bacteria, respectively. Henceforth, this study highlights the *A. comosus* peels as a good source of different minor compounds, warranting further exploration using the UAE method.

Key words: *Ananas comosus* peels; response surface methodology; optimization; ultrasonic-assisted extraction

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Pineapples (*Ananas comosus* L. Merr.) are one of the most popular tropical fruits worldwide, and the state of Johor is the largest producer of this crop in Malaysia with a plantation area of 6,455.51 hectares. It was reported that 274,284.36 metric tons of pineapples were produced in 2017, with an estimated value of RM 538,283,000.06 [1]. The fruit belongs to the Bromeliaceae family and is the most economically significant fruit crop in the family. Large-scale production typically generates large quantities of by-products. Inedible parts of the fruit include the peel, seeds, crown and bagasse, which are traditionally discarded as waste after fruit processing [2]. By-products such as peels and seeds have been reported to contain more functional compounds compared to the pulp [3].

Ultrasound-assisted extraction (UAE) is one of the novel methods developed by the scientific community to overcome the limitations of conventional methods such as Soxhlet extraction and maceration, among others. The acoustic cavitation caused by the ultrasound increases surface contact between sample and solvent. This action enhances the mass transport process, leading to a shortened extraction time [4, 5]. Furthermore, its low-temperature operation can minimise any potential damage to the structural and molecular attributes of thermolabile plant compounds [6]. Concurrently, the method's low usage of solvents minimises the cost of managing waste while providing higher percentage yields [7].

The purpose of this study was to investigate the interactions between the independent variables and optimise the extraction conditions (i.e. extraction time, solvent:sample ratio, and methanol:water ratio) of UAE. This was done by employing Response Surface Methodology (RSM) using Central Composite Design (CCD) to maximise the yield of responses investigated, which include percentage yield (PY), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. *In vitro* bioactivity of the extracts was assessed accordingly against Gram-negative bacteria (*Escherichia. coli* ATCC 11775 and *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive bacteria (*Bacillus subtilis* ATCC 21332 and *Staphylococcus aureus* ATCC 25923).

EXPERIMENTAL

Materials

Yellow-coloured MD2 pineapple peels used in this study were bought from a local market (KipMart Sdn. Bhd) in Pasir Gudang, Johor. Organic solvents used included chloroform, *n*-hexane, methanol, and dimethyl sulfoxide (DMSO) while inorganic solvents

were sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), and aluminium chloride ($AlCl_3$). These were all procured from Merck, as well as nutrient agar (NA), silica gel 60 (0.040-0.063 mm), and TLC Silica gel 60 F_{254}.The Folin-Ciocalteu reagent, gallic acid, quercetin, and 2,2-Diphenyl-1-picrylhydrazyl were sourced from Sigma-Aldrich.

Preparation of Extracts

The pineapple peels were cut into small pieces of uniform size before being oven-dried overnight at ~40 ºC, following which the sample was ground into powder form using a blender and passed through a 300-mesh sieve. The powder sample was packed into a container and stored in a refrigerator at 4ºC until analysis commenced.

Ultrasound-assisted extraction: 1.0g of ground *A. comosus* peel was extracted using a probe ultrasonicator (20kHz, 130W) under different conditions, including extraction time (min), methanol to water ratio (v/v), and sample to solvent ratio (w/v). Post-ultrasonication, filtration of the crude extracts was done using Whatman No.1 filter paper, following which they were concentrated using a vacuum rotary evaporator. This step would yield different extracts across varying extraction conditions. Each extract was then kept in a desiccator and weighed until a constant weight was obtained. The extracts were then stored in zip-locked plastic bags in the refrigerator.

Soxhlet extraction: The powdered *A. comosus* peel (195 g) was consecutively extracted with hexane, chloroform, and methanol (150 ml) in a Soxhlet extractor for 18 hours. Next, the crude extracts were concentrated *in vacuo* using a rotary evaporator, whereby those obtained subsequently were stored for further tests.

Experimental Design

The extraction of phytochemicals from *A. comosus* peels was optimised by RSM implementation. This was done using different methanol:water ratios (0, 20, 40, 60, and 80, v/v), solvent:sample ratios (20:1, 30:1, 40:1, 50:1 and 60:1, v/w (mL/g)), and extraction times (10, 15, 20, 25, and 30 min). Here, the CCD method was selected for parameter optimisation purposes, i.e. extraction time (X_1), solvent to sample ratio (X_2), and methanol to water ratio (X_3). The experimental data were then fitted to the second-order polynomial equation (Equation 1).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{j=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} \beta_{ij} X_i J_i + \varepsilon \quad (1)$$

Y is the measured response variable, β_0 is a constant, β_i, β_{ii}, and β_{ij} are the linear, squared, and interactive effects coefficients, respectively, and ε is the error. Regression analysis and analysis of variance (ANOVA) were performed using the Design-Expert software (Version 8.0.6, Stat-Ease Inc., Minneapolis, MN). Here, the statistical significance test was based on the total error criteria with a confidence level of 95.0% ($p < 0.05$). The three-dimensional (3D) surface response plots generated were obtained by changing two variables within the experimental range while the remaining variable was kept constant at the central point [8].

Total Phenolic Content (TPC)

The total TPC value for pineapple peel extracts tested was quantified using the Folin-Ciocalteu assay as described by Sulaiman and Balachandran [9]. An aliquot (500 µL) of each extract (i.e. hexane, chloroform, and methanol) was placed in a graduated test tube containing 8 mL of distilled water, to which the Folin-Ciocalteu solution (500 µL, 10%) was added, and then homogenised. After 5 min, 1 mL of 7.5% Na_2CO_3 solution was added to the mixture, and each sample was then incubated for 30 min at room temperature. The absorbance value was measured in triplicate at 760 nm against the reagent blank. Here, the TPC value was expressed as milligrams of gallic acid equivalents per gram of sample or extract (mg GAE/g) [10].

Total Flavonoid Content (TFC)

The TFC value of pineapple peel extracts in this study was obtained via aluminium chloride colourimetric assay. In brief, 200 µL of each extract generated was added into a graduated test tube containing 4 mL of distilled water. Then, another 300 µL of 5% $NaNO_2$ was introduced into the mixture, which was shaken and incorporated with 300 µL of 10% $AlCl_3$ after 5 min. After 6 min, 2 mL of 1 M NaOH was added. The solution was then made up to 10 mL with distilled water and mixed well. The absorbance was subsequently measured in triplicate at 510 nm against the reagent blank. The TFC value was expressed as milligrams of quercetin equivalents per gram of sample or extract (mg QCE/g) [11].

Scavenging Activity on DPPH Free Radicals

The antioxidant capacity of pineapple peel extracts was assessed in this study by evaluating their free radical-scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH). A 3 mL aliquot of each pineapple peel extract was mixed with 1 mL of 0.83 mM DPPH methanolic solution. The mixture was then thoroughly vortexed and kept in the dark for 30 min. Next, the absorbance was measured at 517 nm with a spectrophotometer, using a blank reagent containing only methanol and DPPH. The activity test was carried out in triplicate [12, 13] and the percentage of radical-

scavenging ability was calculated using Equation (2). Here, A_0 is the absorbance of the blank while A_S denotes the absorbance of the sample.

$$\text{Radical scavenging activity (\%)} = \frac{(A_0 - A_S)}{A_0} \times 100 \quad (2)$$

Antimicrobial Activity

The antimicrobial assay was assessed in triplicate using the agar well diffusion method in which each concentrated extract was dissolved in 5% DMSO. Nutrient agar (NA) plates were spread uniformly with 100 µL of respective bacteria using sterile cotton swabs. Five wells were made in each dish using a sterile cork borer, whereby three wells were loaded with 100 µL of 150 mg/mL *A. comosus* peel extracts. Meanwhile, one well contained 100 µL of 5% DMSO without any fruit extract as the negative control, and the last well incorporated streptomycin (50 mg/mL) and served as the positive control. The plates were maintained at room temperature for 1 hr to allow solution diffusion into the medium. Accordingly, all bacterial plates were incubated in an upright position at 37°C for 24 hr before any antibacterial activity was assessed by measuring the inhibition zone diameter (i.e. in mm, including the well diameter) [14].

RESULTS AND DISCUSSION

Optimisation of Ultrasound-assisted Extraction

The experimental results of 20 randomised runs obtained for pineapple peel extracts and the selected responses (i.e. PY, TPC, TFC, and antioxidant activity) under different experimental conditions are displayed in Table 1. In essence, the optimum conditions for each response in order to generate a high percentage of yield (32.06%) were 20 min extraction time, 40:1 solvent:sample ratio (v/w, mL/g), and 40:60 MeOH:H_2O ratio (v/v). Furthermore, high values for the TPC (38.61 mg GAE/g) and TFC (35.29 mg QCE/g) were obtained with 20 min extraction time, 40:1 solvent:sample ratio (v/w, mL/g) and 80:20 MeOH:H_2O ratio (v/v). However, 10 min extraction time, 40:1 solvent:sample ratio (v/w, mL/g), and 40:60 MeOH:H_20 ratio (v/v) were required to produce high antioxidant activity (71.42%).

Subsequently, ANOVA validated statistical models for significance were generated in the study by assessing the effects of independent variables tested and their interaction with the abovementioned responses. In particular, the models' suitability for predicting the responses were affirmed upon accounting for their p-values for lack-of-fit ($p>0.05$) and the R^2 values.

The effects of variables X_1 (extraction time), X_2 (solvent:sample ratio), and X_3 (MeOH:H_2O ratio) under test were further explored by determining the significance of coefficients according to the p-value of the F-test ($p<0.05$). The regression equations of the above-coded variables for the pineapple peel extracts in this study are written with the significant regression terms as in Table 2. The table confirms the levels of probability are significant, with a range between 0.0001 and 0.0002. This data conveys the model's adequacy as supported by the values of R^2 and adjusted-R^2, which are above 75%. Moreover, the lack-of-fit of the model across all responses as indicated by $p > 0.05$ signified the model's good fit for predicting the interactions of the variables studied.

As observed in Equations 3 and 4 in Table 2, the solvent:sample ratio (X_2) displays a positive linear correlation with PY and TPC, indicating its increment as the solvent:sample ratio increases. In Equation 4, MeOH:H_2O (X_3) ratio and extraction time (X_1) also presented a positive linear correlation with TPC.

In contrast, Equations 5 and 6 (Table 2) depict the solvent:sample ratio (X_2) showing a negative linear correlation with TPC and DPPH radical scavenging activity. This indicates that a low solvent:sample ratio (X_2) could yield high flavonoids content, thereby displaying a high radical scavenging activity.

Equation 5 also shows the extraction time (X1) and MeOH:H_2O ratio (X3), conveying the factors influencing the TFC obtained in the experiment. The information was presented by the positive linear and quadratic effects. The data implies the plausibility of high flavonoids content following the high MeOH:H_2O ratio (X_3), long extraction time (X_1), and low solvent:sample ratio (X_2). Additionally, extraction time (X_1) denotes the other factor influencing the scavenging activity in Equation 6, which shows a negative linear correlation with DPPH radical scavenging activity.

Effect of Extraction Parameters on Percentage Yield

The perturbation plot displays the influence exerted by each variable on the percentage yield (Figure 1(a)). In particular, the extraction time (A) and MeOH:H_2O ratio (C) did not affect the percentage yield, whereas the solvent:sample ratio (B) rendered a positive effect. Here, the 2D contour and 3D plots depict the solvent:sample ratio's significant influence on the percentage yield for the UAE process ($p < 0.05$). Such an outcome could be ascribed to the high proportion of solvent, thus reducing the energy needed to separate the molecules and enhancing the solute's mobility towards the solvent [15]. The maximum values of percentage yield are set along the entire X-axis for extraction time (15 to 25 min) and MeOH:H_2O ratio (20 to 60 v/v), thereby underlining the two factors' insignificant influence on percentage yield as seen in Figures 1(c) and 1(e). Thus, one may conclude that high percentage yields can be obtained by utilizing

either long or short extraction times, in combination with water or aqueous methanol, and a high solvent:sample ratio.

Effect of Extraction Parameters on TPC

The perturbation plot shows a steep positive slope as it passes through the reference point. Such a trend implies that all variables significantly influence the TPC of the UAE process (Figure 2(a)). In particular, the 2D contour and 3D graph plots depicted in Figures 2(b) and 2(c) reveal a linear model, leading to high TPC yields following the high values for all three variables tested. A long extraction period, for example, would impart adequate time for the ultrasonic cavitation force to disrupt the cell walls of the pineapple peel. This action enhances the permeability and mass transfer rate of the compounds produced. Moreover, high MeOH:H_2O and high solvent:sample ratios could improve efficiency in yielding high TPC from the extracts. This is based on literature describing the amalgamation of methanol and water as a good contributor to the greater recovery of phenolic compounds. Such a combination creates a medium due to the polarity between water and MeOH, ensuring better extraction from both ends of the polarity spectrum [16]. Here, a high amount of solvent can penetrate the cell wall and facilitate the release of phenolic compounds in larger amounts.

Table 1. Experimental design conditions and PY, TPC, TFC, and antioxidant activity from pineapple peel extracts

	Extraction conditions			Experimental responses			
Experiments	**X_1**	**X_2**	**X_3**	**Percentage yield (%)**	**TPC (mg GAE/g)**	**TFC (mg QCE/g)**	**DPPH (%)**
1	20	40	40	27.32	23.53	12.74	54.65
2	20	40	40	26.81	27.43	12.61	56.37
3	15	30	20	19.34	18.81	17.99	64.81
4	30	40	40	14.66	29.91	28.51	61.45
5	20	40	40	29.12	27.42	17.79	55.77
6	20	60	40	23.99	31.09	15.34	57.26
7	10	40	40	20.61	23.32	14.46	71.42
8	15	50	20	23.28	25.12	12.80	57.45
9	20	40	0	18.04	19.82	18.74	55.22
10	15	50	60	23.35	27.83	13.68	64.12
11	25	30	20	20.48	25.37	17.48	61.64
12	20	40	40	32.06	26.91	14.91	52.42
13	20	40	40	25.31	26.64	13.35	55.21
14	20	20	40	13.11	23.25	28.07	66.85
15	20	40	40	29.96	27.64	12.62	51.68
16	25	50	60	19.01	31.04	30.93	52.89
17	25	30	60	18.89	29.62	29.54	57.45
18	20	40	80	15.53	38.61	35.29	61.45
19	15	30	60	14.96	25.37	27.27	65.27
20	25	50	20	19.96	30.73	23.95	56.15

Table 2. Coded quadratic polynomial equations

Responses	Regression equation	Regression (p-value)	R^2	R^2 (adj)	Lack-of-fit
% Yield	$28.35+2.11X_2-1.59X_1X_2-2.74X_1^2-2.51X_2^2-2.95X_3^2$ (3)	0.0002	0.9213	0.8505	0.7774
TPC	$26.97+2.05X_1+1.95X_2+3.21X_3$ (4)	0.0001	0.8022	0.7652	0.1860
TFC	$14.12+3.64X_1-2.27X_2+3.89X_3+3.33X_1X_2-1.68X_2X_3+1.93X_1^2+1.99X_2^2+3.31X_3^2$ (5)	0.0001	0.9629	0.9295	0.5738
% DPPH	$54.29-2.72X_1-2.36X_2-1.82X_1X_3+2.99X_1^2+1.90X_2^2+0.97X_3^2$ (6)	0.0001	0.9404	0.8867	0.5724

* X_1 = extraction time, X_2 = solvent:sample ratio, X_3 = methanol:water ratio

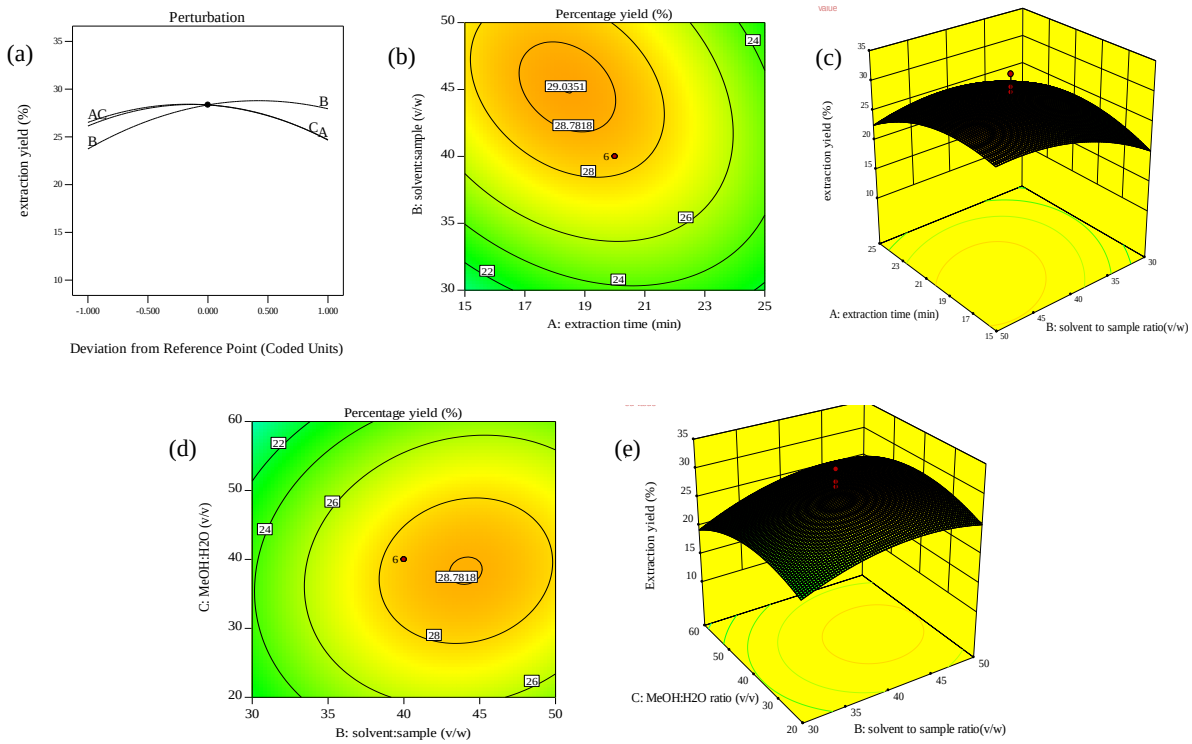


Figure 1. The perturbation (a), contour (b & d), and 3D (c & e) plots' response surface showing the effects of variables (X_1: extraction time, X_2: solvent:sample ratio, X_3: MeOH:H_2O ratio) on the PY.

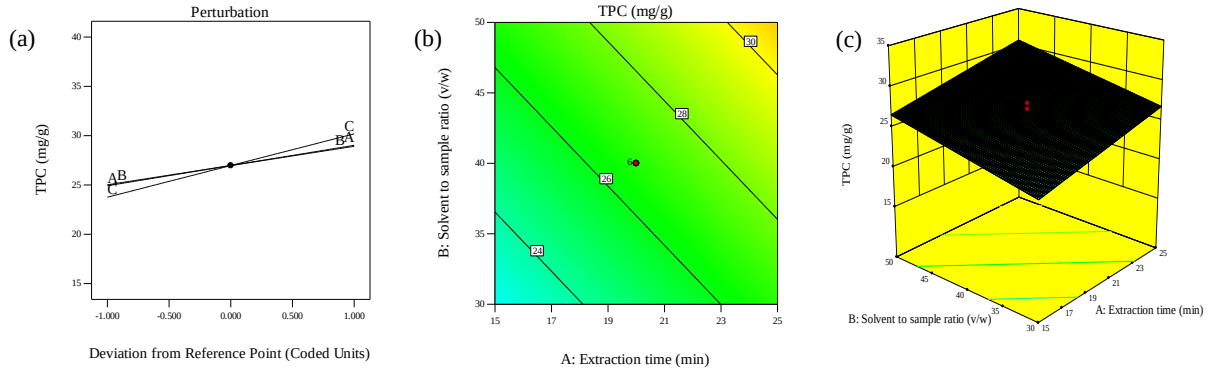
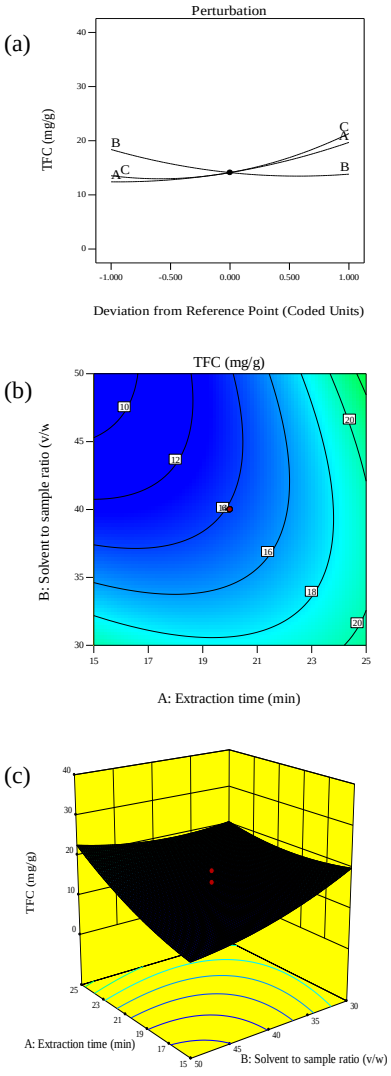


Figure 2. The perturbation (a), contour (b), and 3D (c) plots response surface showing the effect of variables (X_1: extraction time, X_2: solvent:sample ratio, X_3:MeOH:H_2O ratio) on the TPC.



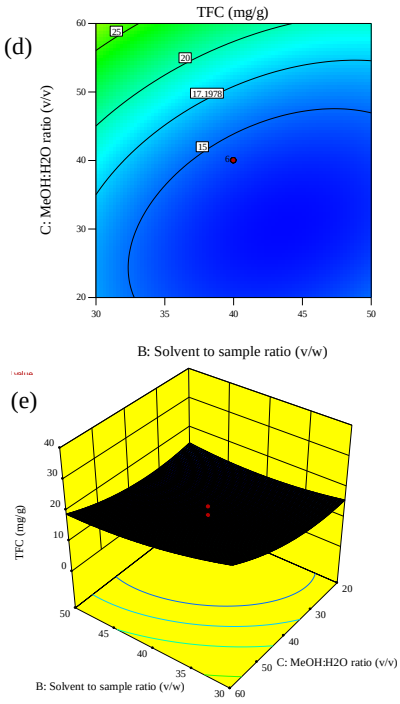


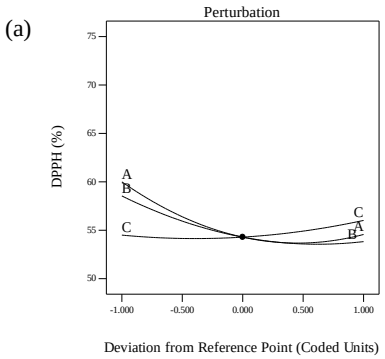
Figure 3. The perturbation (a), contour (b & d) and 3D (c & e) plots response surface showing the effects of variables (X_1: extraction time, X_2: solvent:sample ratio, X_3: MeOH:H_2O ratio) on the TFC.

Effect of extraction parameters on TFC

The perturbation plot in Figure 3(a) displays the solvent:sample ratio and its negative effect on TFC, whereas the extraction time and MeOH:H_2O ratio positively impacted the same variable. This implied that a high flavonoid content would possibly be achieved in UAE by applying a low solvent:sample ratio, a high MeOH:H_2O ratio, and a long extraction time. Meanwhile, the 2D contour and 3D graph plots show the influence of the two variables towards the TFC following the UAE process. Here, a long extraction time (23 to 25 min), a low solvent:sample ratio (30 to 40 v/w), and a high MeOH:H_2O ratio (50 to 60 v/v) yield a high amount of TFC as seen in Figures 3(d) and 3(e).

Effect of Extraction Parameters on Antioxidant Activity

The perturbation plot displays a negative impact on the extraction time and solvent:sample ratio (Figure 4(a)). The trend suggests that higher values of the variables would translate into a lower antioxidant activity. In contrast, the MeOH:H_2O ratio show an insignificant influence on antioxidant activity. In Figure 4(c), the maximum values of this ratio on the antioxidant activity are set along the entire X-axis (20 to 60 v/v). The resulting outcomes show that the extraction of compounds with antioxidant properties could be attained using water or aqueous methanol as the solvent in combination with a shorter extraction time (15 to 17 min) and an optimum solvent:sample ratio (30 to 35 v/w).



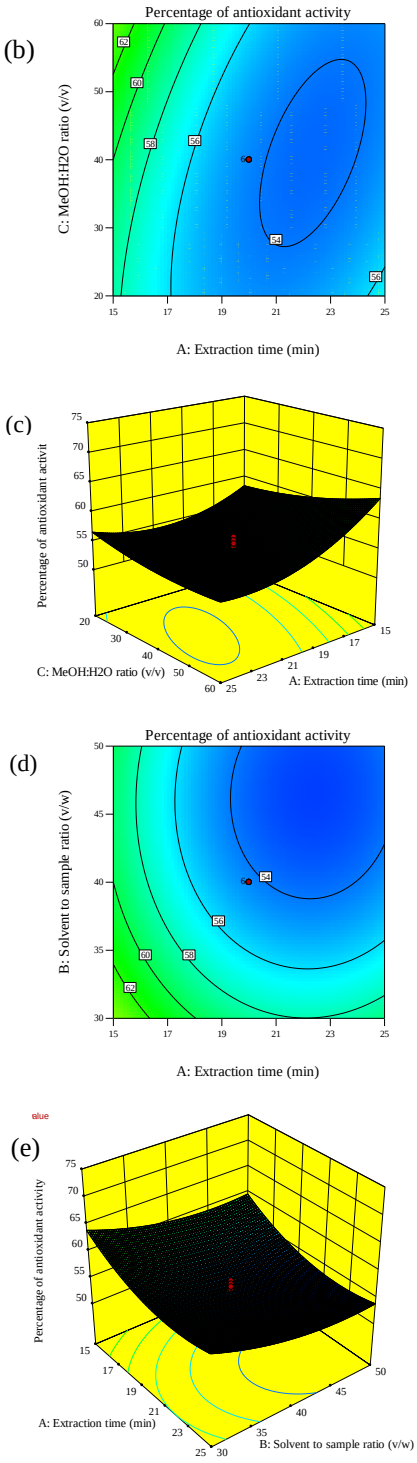


Figure 4. The perturbation (a), contour (b & d), and 3D (c & e) plots response surface showing the effects of variables (X_1: extraction time, X_2: solvent:sample ratio, X_3: MeOH:H_2O ratio) on the percentage of DPPH radical scavenging activity (%DPPH).

DPPH radical scavenging activity of *A. comosus* peel extracts

Based on the TPC values, extracts generated from the application of three UAE conditions (i.e. highest, E18; moderate, E20; and lowest, E3) were selected to assess their antioxidant potential in scavenging DPPH free radicals. Concurrently, extracts obtained via Soxhlet extraction were also evaluated for their DPPH scavenging potential.

The antioxidant activity obtained demonstrated that the E18 and E20 UAE extracts were capable of scavenging 50% of the DPPH free

radicals (Figure 5), yielding corresponding SC_{50} values of 549.535±17.277 and 669.744±0.955 µg/mL, respectively (Table 4). These values indicate the presence of antioxidant compounds [17]. Carotenoids and vitamin C typically display similarly strong antioxidant properties [18, 19], whereas monophenols are regarded as weak counterparts [17, 20].

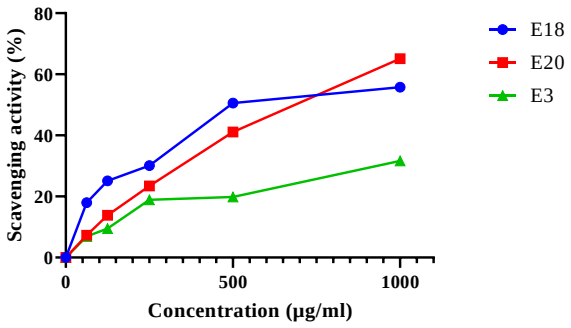


Figure 5. Percentage of DPPH free radical scavenging activity for UAE-subjected pineapple peel extracts.

Contrarily, extracts yielded via Soxhlet extraction revealed that only the chloroform extract displayed potential for scavenging 50% of the DPPH free radicals, with an SC_{50} value of 631.238±3.126 µg/mL (Table 5). The hexane and methanol extract, however, showed low antioxidant potentials as the compounds could not scavenge 50% of the DPPH free radicals. These results imply that the extracts contained a low amount of antioxidant compounds (Figure 6).

Table 4. SC_{50} values for DPPH free radical scavenging activity of the UAE extracts.

UAE conditions	DPPH SC_{50} (µg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)
E3	-	18.810 ±0.589	17.994 ±1.551
E18	549.535 ±17.277	38.608 ±0.621	35.287 ±4.021
E20	669.744 ±0.955	30.725 ±0.570	23.948 ±2.296

It should be noted that some of the SC_{50} values produced by the pineapple peel extracts in this study were higher while others were lower compared to prior studies. For example, a survey of the Bali pineapple peels extracted via the reflux method has obtained methanolic extracts with an SC_{50} value of 1.13 ± 0.03 mg/mL [21].

Moreover, another study has reported a higher SC_{50} value (266.02 µg/ mL) for aqueous pineapple peel extracts compared to methanol-based extracts (281.33 µg/ mL) [22]. Nevertheless, several factors can influence the differences observed across the abovementioned extract concentration and activity, such as cultivars, natural fruit variations, soil conditions, types of fertiliser used, climatic conditions, or geographical origin [23]. Similarly, sampling, preparing, and determining the sample matrix may greatly affect the phytochemical concentrations in pineapples and its by-products [24].

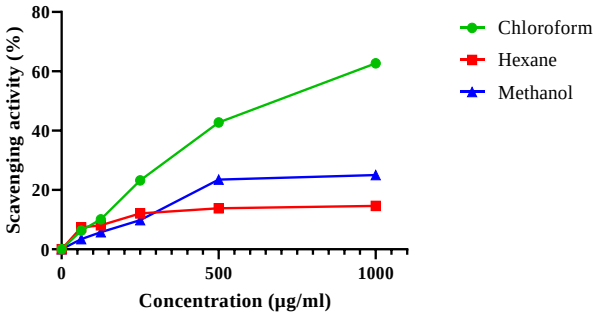


Figure 6. Percentage of DPPH free radical scavenging activity for the Soxhlet-generated pineapple peel extracts.

Table 5. SC_{50} value for DPPH free radical scavenging activity of Soxhlet extracts.

Soxhlet extract	DPPH SC_{50} (µg/mL)	TPC (mg GAE/g)	TFC (mg CAE/g)
Hexane	-	7.162 ±0.521	2.873 ±0.320
Chloroform	631.238 ±3.126	1.037 ±0.004	4.804 ±0.205
Methanol	-	27.475 ±0.081	1.866 ±1.333

Correlation between TPC, TFC, and antioxidant activities of *A. comosus* peel extract

The Pearson product-moment correlation coefficient was computed to determine the relationship between TPC and SC_{50} for DPPH free radical scavenging activities. The value was presented as r/R = 0.8388 and p = 0.366 for the UAE-generated extracts, while its Soxhlet counterparts recorded r/R = -0.6793 and p = 0.525, respectively. The r/R value documented for both methods displayed a considerable strength of association between TPC and SC_{50} value. The positive value of Pearson (r) would indicate that both variables increase or decrease simultaneously,

Table 6. Antibacterial activity of extracts generated via the UAE and Soxhlet methods

Extract	Zone of Inhibition (mm)			
	E. c	*P. a*	*S. a*	*B. s*
UAE				
E3	-	-	-	8.33 ± 0.58
E18	-	-	-	-
E20	-	-	-	-
Soxhlet				
HE	-	-	-	-
CE	-	8.00 ± 0.00	-	-
ME	8.00 ± 0.00	8.67 ± 0.58	-	-

**E. c = E. coli, P. a = P. aeruginosa, S. a = S. aureus, B. s = B. subtilis,* HE = hexane extract, CE = chloroform extract, ME= methanol extract, E3 = extract from experiment 3, E18 = extract from experiment 18, E20 = extract from experiment 20.

whereas a negative value is suggestive of an inverse relationship between them. However, the correlation was not statistically significant based on the p-values obtained by both methods ($p > 0.05$); even though the Pearson (r) value is large, this would not necessarily suggest that one variable could directly influence the other.

Furthermore, a previous study reported a non-significant correlation between TPC ($r/R = -0.28$, $p > 0.05$) and TFC ($r/R = -0.45$, $p > 0.05$) with their respective DPPH antioxidant activity in pineapple peel extracts [25]. In particular, Rahiman and colleagues stated that several factors contribute to such a non-correlation in which the antioxidant capacity observed may not be solely attributable to the phenolic contents. Contrarily, it can also be due to other phytochemicals present in the extracts, such as ascorbic acid, tocopherol, vitamin C, pigments, carotenoids, terpenoids, organic acids, and sugar, among others [26, 19, 18, 27, 28]. Theoretically, the synergistic effect between compounds is contributory to the total antioxidant capacity. Besides, the Folin-Ciocalteu method is highlighted as one that does not ensure an absolute measurement for TPC as the activity depends on the compound structure. Accordingly, different types of phenolic compounds would exhibit dissimilar antioxidant activities [28].

Antibacterial activity of *A. comosus* peel extracts

Based on the data obtained, *A. comosus* peel extracts actively demonstrated antibacterial activities towards *B. subtilis, P. aeruginosa,* and *E. coli.* Among the three extracts obtained via UAE, the E3 extract actively inhibited *B. subtilis* while E18 and E20 extracts were not active against the tested bacteria (Table 6). In contrast, Soxhlet's MeOH and chloroform extracts showed antibacterial activity by inhibiting both *P. aeruginosa* and *E. coli* (Table 6). Furthermore, streptomycin was used in this study as a positive test, which displayed inhibition zones ranging between 14−16 mm for the respective bacteria species. A previous study reported that 50 mg/mL of the methanolic and ethanolic extracts generated from *A. comosus* peels displayed a high antibacterial activity against *P. aeruginosa*, *B. subtilis*, *Azotobacter*, *Klebsiella*, and *Xanthomonas* [29]. Nevertheless, the lack of procedural standardisation in assessing antibacterial activity is noted, thus creating diversity in research data [30].

CONCLUSION

This study documented the optimal conditions for the UAE process to maximise variable responses. In particular, a high percentage yield (32.06%) was obtained with an extraction time of 20 min, a 40:1 solvent:sample ratio (v/w, mL/g), and a 40:60 MeOH:H_2O ratio (v/v). Meanwhile, high TPC (38.61 mg GAE/g) and TFC (35.29 mg QCE/g) values were produced with 20 min extraction time, a 40:1 solvent:sample ratio (v/w, mL/g), and an 80:20 MeOH:H_2O ratio (v/v). Alternatively, the combination of 10 min extraction time, 40:1 solvent:sample ratio (v/w, mL/g), and 40:60 MeOH:H_20 ratio (v/v) yielded the best result for high antioxidant activity (71.42%). In the present study, *A. comosus* peel extracts obtained from UAE and Soxhlet methods both displayed antioxidant activity and antibacterial activity against *B. subtilis*, *E.coli*, and *P. aeruginosa*.

In brief, this study confirmed *A. comosus* peel as a good source of different kinds of minor chemical compounds. This merits further exploration for in-depth clarification, especially in view of the limited information available regarding the isolation of such compounds from *A. comosus* peels.

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