Optimization Microwave-assisted Extraction (MAE) for Extracting Antioxidant Compounds and 2acetyl-1pyrroline (2AP) from *Pandan (Pandanus amarillyfolius* Roxb) Leaf

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Abstract. The purpose of this study was to identify the ideal circumstances for antioxidant component extraction and 2acetyl1pyrroline (2AP) from Pandan leaf using Microwave-assisted Extraction (MAE) through the use of Box-Behnken Design Response Surface Methodology (BBD-RSM), with three factors: ethanol concentration (65, 80, and 95%), temperature (55,65, and 75 C), and time (15,25, and 35 min).The spectrophotometry method determined total phenolic content (TPC) and antioxidant activity (IC50). Gas chromatography (GC) was used to quantify 2acetyl1pyrroline 2AP) content compared with the 2acetyl1pyrroline (2AP) standard. The findings indicated that the extraction parameters significantly impacted the total phenolic content, antioxidant activity (IC50), and 2AP content of pandan leaf extracts. Furthermore, the ideal extraction condition strongminded were 80% ethanol concentration, 65 C extraction temperature, and 25 minutes of extraction time, resulting in a total phenolics content of 0.34 mg GAE/g, antioxidant activity (IC50) of 14.27 ppm, and 2AP content of 7.78 ppm.

1 Introduction

The Pandanus amaryllifolius Roxb, a member of the Pandanaceae family, is widely distributed in Southeast Asian nations including Indonesia, Malaysia, Thailand, Vietnam, and the Philippines [1; 3; 18]. Commonly, In Southeast Asia, the leaves of this plant, known as *pandan* leaves, are used for the flavor and natural coloring in many foods and beverages, including baked goods, desserts, drinks, and even home cooking [2] . *Pandan* leaves are rich in volatile chemicals such as 2acetyl-1pyrroline (2AP) [26] and chlorophyll [16], a green pigment that can impart unique and aromatic flavor and color to food products. Numerous studies have also shown that *pandan* leaves present valuable bioactive compounds, including tannins, anthraquinone glycoside, cardiac glycoside, flavonoids, terpenoids, and alkaloids [5; 13]. Numerous studies have also demonstrated that *pandan* leaves traditionally have several uses as a diuretic to treat headaches, fever, arthritis, and other conditions. Furthermore, because of their antibacterial, antiviral, anti-diuretic, anti-neoplastic, antioxidant, and

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neuroprotective qualities, pandan leaves have been used for a long time as a medicinal herb and also for food preservatives [5; 8] .

Based on the above fact, extracting *pandan* leaves is essential to obtain beneficial bioactive and aromatic compounds and utilize them in multiple sectors, including food and pharmaceutical. The selection of the extraction method is crucial because the extraction results reflect the success rate of the technique [11]. Commonly, pandan leaf extraction is performed using conventional extraction, using applied thermal, and it takes a long time, thus damaging the bioactive compound, including phenolic compounds, during the extraction [19]. An alternative for extraction of *pandan* leaves was microwave-assisted extraction (MAE) with more advantages, like short time, less solvent requirement, saving cost, and produced high yield compared with the different methods, such as soxhlet extraction techniques and supercritical fluid extraction ultrasonic-assisted extraction [6; 14; 23]. [10] conducted extraction of *pandan* leaves using MAE with water as a solvent, with three observed factors, including microwave power, extraction time, and temperature, and they concluded the condition for maximum yield (23.53%) were found at microwave power of 450 W, temperature extraction of 91-92 °C and time extraction of 20 min. Furthermore, [31] also evaluated the effect of ethanol concentration, soaking temperature, and microwave power during pandan leaf extraction using MAE, with observed responses that were antioxidant activity and total phenolic content (TPC) . Zaki and his colleagues discovered that the total phenolic content (TPC) was influenced by the soaking temperature, microwave power, and ethanol concentration and pandan leaf extract's antioxidant activity, with a high TPC (1.56 mg/g GAE) seen at 450 W of microwave power and 75% ethanol concentration.

Moreover, several researchers also conducted the extracting of 2AP from *pandan* leaf with various methods, such as ultrasound [3], using headspace-solid phase microextraction (HS- SPME) [26] , and the extraction of supercritical carbon dioxide (SC-CO2) [29], with various results of 2AP content in the ranges of 0.04 t0 4.873 ppm. To date, prior research has not explored the optimum parameters for extracting *pandan* leaves utilizing the MAE (Microwave-assisted Extraction) method, including determining the optimal to achieve a high total phenolic content, adjust the extraction temperature, extraction time, and ethanol concentration and 2-acetyl-1-pyrroline (2AP) content, while minimizing IC_{50} levels. To obtain the desired responses from the extraction of *pandan* leaves using MAE, The ideal extraction parameters, including the temperature, duration, and ethanol concentration, must be ascertained. Microwave-Assisted Extraction offers significant advantages over conventional techniques due to its efficiency, speed, reduced solvent use, and improved selectivity, making it a preferred choice in many analytical applications. The common method to achieve this is response surface methodology (RSM) [27] . RSM is a robust, powerful, and valuable tool widely used for developing, enhancing, and optimizing processes, including the extraction process, which combines mathematical and statistical techniques [4] to obtain the optimal condition of the process. Thus, the purpose of this study is to ascertain the ideal temperature, extraction duration, and ethanol concentration to produce *pandan* leaf extracts with high total phenolic content and 2-acetyl-1-pyrroline (2AP) content and low IC_{50} extracted by the MAE method using the Response Surface Methodology.

2 Materials and methods

2.1 Chemicals

The chemical reagents in analytical grade and the standard, including ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-ciaocalteau reagent, sodium carbonate, toluene, gallic acid standard, and 2-acetyl-1-pyrroline standard, were acquired from Merck KgaA (Darmstadt,

Germany) and Sigma-Aldric (St Louis, USA). The local chemicals store in Malang City, Indonesia, supplied the distillate water. Equipment specification for *Microwave assisted extraction* (*Anton Paar*). Anton Paar systems typically feature adjustable microwave power settings ranging from 100 W to 1000 W. The systems generally operate at a frequency of 2.45 GHz.

2.2 Materials

2.2.1 Pandan (Pandanus amarillyfolius Roxb) leaf samples

Pandan wangi leaves were bought from the local market in Malang City, Indonesia. specific variety (Pandanus amaryllifolius), optimal growth conditions (tropical climate with adequate moisture and nutrients), and careful timing of harvest (6–12 months post-planting). Then, it is transferred to the Central Laboratory, Universitas Brawijaya, Malang, Indonesia, for further processing.

2.2.2 Preparation of Pandan (Pandanus amarillyfolius Roxb) leaf extracts

Briefly, running water was used to properly wash the fresh pandan leaves, and tissue paper was used to dry their surface. After being chopped into 3x1 cm pieces, the pandan leaves were crushed in a blender for 1.5 minutes. The vessel was then filled with 30 ml of various ethanol concentrations (65%, 80%, and 95%) and 3 g of crushed pandan leaves. Then, the mixture was extracted using MAE (Anton Paar brand) at different extraction temperatures (55, 65, and 75 °C) for various extraction times (15, 25, and 35 min) according to the design of the experiment (DoE) in the Design Expert 13.0 software trial version (Tabel 1 and Tabel 2). Filter paper was used to filter the resultant pandan leaf extract, which was then kept at 4 °C until additional analysis.

2.2.3 Determination of total phenolic content (TPC)

The total phenolic content in *pandan* leaf extract was determined using the spectrophotometric method described by [22], with slight modifications. Shortly, the 0.5 ml of *pandan* leaf extract was put into the tube test and combined with added 2.5 ml of Folin-Ciocalteau reagent (1:10 v/v), and homogenized using vortexed and incubated for 5 min, then in 6^{th} minutes were added 2 ml of 7.5% Na_2CO_3 solution, vortexed, then incubated in a dark environment at room temperature for 30 minutes. The absorbance of the sample solution was then measured at a wavelength (λ) of 751 nm. Then, the total phenolic content was determined using gallic acid as the curve standard at concentrations ranging from 0 to 100 ppm in *pandan* leaf extract and expressed as mg GAE/g.

2.2.4 Determination of Antioxidant Activity (IC_{50})

The Molyneux method [17] was utilized to measure the antioxidant activity in *pandan* leaf extract, and DPPH (1,1-diphenyl-2-picrylhydrazyl) was used as a reagent. In short, in a 50 ml volumetric flask, 50 mg of pandan leaf extract was dissolved with ethanol and homogenized to create a stock solution with 1000 ppm. Then, the *pandan* leaf extract stock solution was re-diluted with ethanol to different concentrations of 10, 20, 30, 40, and 50 ppm. For testing, the 2 ml of the various concentrations of *pandan* leaf extract solution, as prepared previously, were mixed with 2 ml of 0.2 mM DPPH in a test tube and then incubated for 30 minutes at room temperature and in a dark environment before being homogenized. Then,

the absorbance in each concentration was assessed using a UV-VIS spectrophotometer at a wavelength of 517 nm. Absorbances of different concentrations of *pandan* leaf extract and percent inhibitory were plotted on the X and Y axes, thus obtaining a linear regression equation y = a(x) + b, by specifying the y value as 50 and the x value as IC_{50}.

2.2.5 Determination of 2Acetyl-1pyrroline Content

Gas Chromatography was used to examine 2Acetyl-1pyrroline, which reflected the aroma of the pandan leaf extract [32]. Shortly, a 0.5 ml *pandan* leaf extract sample was combined with 0.5 ml of toluene and shaken until homogeneous. Then, the 1 µL of the prepared sample was injected into the Gas Chromatography with the following conditions: the injector and detector temperature was set to 150 °C, and the column temperature was set to 80°C with FID (Flame ionization detector) using helium as a carrier gas. For quantification of 2AP (2acetyl-1pyrroline) in the *pandan* leaf extract, the chromatogram peak of *pandan* leaf extract compared with the chromatogram peak of 2AP (2acetyl-1pyrroline) internal standard in the same concentration.

2.3 Designing experiments, analyzing statistics, and confirming optimal conditions

The Response Surface Methodology (RSM) was used to optimize the ethanol concentration, extraction temperature, and extraction duration with a Box Behnken design (BBD) using Design Expert 13.0 software trial version (State-Ease, Inc., USA) for extraction of *pandan* leaf, with three responses, like total phenolics content, antioxidant activity (IC_{50}), and 2AP content. Table 1 lists the upper, center, and lower limits for concentration of ethanol, temperature of extraction, and duration of extraction in this study, and these ranges are based on our preliminary investigation.

The specific ranges for concentration of ethanol, temperature of extraction, and duration of extraction in Microwave Assisted Extraction (MAE) are chosen based on their noteworthy influence on the extraction process' efficacy and efficiency. That factors are derived from empirical studies aimed at optimizing extraction efficiency while preserving the integrity of bioactive compounds. These parameters are carefully selected based on their effects on solubility, energy transfer, and kinetics of extraction.

Table 1. The range of independent parameters of extraction conditions of *pandan* leaf

Independent Parameters	**Unit**	**Upper Limit (+1)**	**Center (0)**	**Lower Limit (-1)**
Ethanol concentration	%	95	80	65
Extraction temperature	°C	75	65	55
Extraction time	min	35	25	15

Tabel 2. Design *Box Behnken Design* (BBD) response surface methodology

Std	Run	Variabel code			Variabel actual			Response		
		X_1	X_2	X_3	X_1	X_2	X_3	Y1	Y2	Y3
9	1	0	-1	-1	80	55	15			
13	2	0	0	0	80	65	25			
12	3	0	+1	+1	80	75	35			
14	4	0	0	0	80	65	25			
17	5	0	0	0	80	65	25			
6	6	+1	0	-1	95	65	15			
15	7	0	0	0	80	65	25			
16	8	0	0	0	80	65	25			
8	9	+1	0	+1	95	65	35			
4	10	+1	+1	0	95	75	25			
7	11	-1	0	+1	65	65	35			
10	12	0	+1	-1	80	75	15			
5	13	-1	0	-1	65	65	15			
2	14	+1	-1	0	95	55	25			
11	15	0	-1	+1	80	55	35			
3	16	0	+1	0	80	75	25			
1	17	0	-1	0	80	55	25			

Keterangan: X_1 = Ethanol concentration (%)
X_2 = Extraction temperature (°C)
X_3 = Extraction time (menit)
Y_1 = Total fenol (mg GAE/g)
Y_2 = Atioxidan activity(% inhibisi)
Y_3 = Aroma (ppm)

A second-order polynomial equation was used to fit the responses from the pandan leaf extract, and multiple data regression was used to build an empirical model based on the independent parameters. Equation 1 represents the general second-order polynomial equation. The accuracy of the polynomial model for each response, including total phenolics content, antioxidant activity (IC_{50}), and 2AP content, was assessed using Design-Expert software to determine the significance model, R^2, adjusted-R^2 adequate precision, and lack of fit. Three times of experiments in the laboratory to verify and confirm the optimum conditions of the software prediction. Then, utilizing the Minitab 17 software trial version (Minitab, Inc., USA), the program prediction and verification results were compared using a paired t-test at p-value < 0.05.

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

Noted: Y is the predicted responses (total phenolics content, antioxidant activity (IC_{50}), and 2AP content); Intercept coefficient β0, independent variables xi and xj, and the linear, quadratic, and interaction coefficients of variables i and j are denoted by βi, βii, and βij, respectively.

3 Results and discussion

3.1 Fitting models and statistical evaluation

This study used response surface methods to assess the effects of ethanol concentration, extraction temperature, and extraction duration on the total phenolic content, antioxidant activity, and 2acetyl-1pyrroline (2AP) content of *pandan* leaf extract. The response data of *pandan* leaf extracts, the second-order polynomial fit, and the quadratic coefficient regression for all responses were presented sequentially in Tables 3, 4, and 5. Table 4 demonstrates that the selection of the mathematical model for *pandan* leaf extract responses, including the total phenolic content, antioxidant activity (IC_{50}), and 2AP content, were all affected by ethanol concentration, extraction temperature, and extraction duration, and all *pandan* leaf extract responses fit into a quadratic model with a p-value of less than 0.0001. A low p-value for a constructed model suggests that the model was highly significant in a specific response [3]. Moreover, the R^2 in the quadratic model for total phenolic content, antioxidant activity (IC_{50}), and 2AP content of *pandan* leaf extract were 0.9966, 0.9981, and 0.9952, respectively. Furthermore, in a sequence of the adjusted-R^2 of 0.9923, 0.9957, and 0.9891 for total phenolic content and IC50 for antioxidant activity and 2AP content. Due to its high R^2 and adjusted R^2, the quadratic model can explain all response variances in the *pandan* leaf extract [28]. All responses have an adequate precision greater than 4, in Table 4 Mathematical model selection each response for each response of *pandan* leaf extracts, indicating preference and classification as an adequate precision [20]. Additionally, the quadratic model selected was accurate and precise up to pure error for total phenolic and 2acetyl-1pyrroline content, according to the lack of fit value greater than 0.05 [12].

Table 3. Total phenolic content, antioxidant activity (IC_{50}), and 2Acetyl-1pyrroline (2AP) content of pandan leaf extracts with various extraction conditions

Std	Independent Factor			Response		
	Ethanol Concentration (%)	Extraction temperature (^{0}C)	Extraction time (min)	Total Phenolic Content (mg GAE/g)	Antioxidant Activity (IC_{50}) (ppm)	2AP content (ppm)
1	66	55	25	0.11	45.38	0.67
2	95	55	25	0.18	33.94	3.44
3	65	75	25	0.13	47.88	0.80
4	95	75	25	0.18	39.80	3.17
5	65	65	15	0.12	47.00	0.52
6	95	65	15	0.17	39.11	3.52
7	65	65	35	0.13	47.40	0.83
8	95	65	35	0.18	39.95	3.83
9	80	55	15	0.36	13.25	8.30
10	80	75	15	0.35	15.11	8.18
11	80	55	35	0.32	14.59	8.05
12	80	75	35	0.36	15.11	8.20
13	80	65	25	0.35	14.33	8.06
14	80	65	25	0.34	14.32	8.05
15	80	65	25	0.34	14.33	8.05
16	80	65	25	0.34	14.14	7.31
17	80	65	25	0.35	14.24	7.41

Prediction	80.00	65.00	25.00	0.34±0.00[a]	14.27±0.00[b]	7.78±0.00[c]
Verification *	80.00	65.00	25.00	0.34±0.02[a]	14.27±0.58[b]	7.78±0.62[c]

Explanation: *average of 3 replicates ± SD

Table 4. Mathematical model selection each response for each response of *pandan* leaf extracts

Parameters	Total Phenolic Content	Antioxidant Activity (IC_{50})	2-Acetyl-1-pyrroline content
Mathematical Model	Quadratic	Quadratic	Quadratic
Significance model	<0.0001[s]	<0.0001[s]	<0.0001[s]
R^2	0.9966	0.9981	0.9952
Adjusted-R^2	0.9923	0.9957	0.9891
Adequate Precision	36.1155	47.2874	30.4131
Lack of Fit	0.0732[ns]	<0.0001[s]	0.7472[ns]

Explanation: [s]: significant, and [ns]: not significant

Table 5. Significance responses of the *pandan* leaf extracts in quadratic models

Coefficient	Total Phenolic Content	Antioxidant Activity (IC_{50})	2-Acetyl-1-pyrroline content
Intercept	0.3429	14.00	7.78
X_1-Ethanol Concentration	0.0277***	-4.36***	1.39***
X_2- Extraction temperature	0.0047[ns]	1.34**	-0.0150[ns]
X_3- Extraction time	-0.0021 [ns]	0.3225 [ns]	0.0471 [ns]
$X_1 X_2$	-0.0042 [ns]	0.8412 [ns]	-0.1033[ns]
$X_1 X_3$	-0.0024 [ns]	0.1100 [ns]	0.0014[ns]
$X_2 X_3$	0.0123*	-0.3345 [ns]	0.0679 [ns]
X_1^2	-0.1968***	28.17***	-5.88***
X_2^2	0.0030 [ns]	-0.6841 [ns]	0.1258 [ns]
X_3^2	0.0026 [ns]	0.9296 [ns]	0.2802[ns]

Significance remarks: [ns]P>0.05; *0.01<P<0.05; **0.001<P<0.01; ***P<0.001
Explanation: [ns]: not significant

3.2 Total phenolic content

The total phenolic content (TPC) in *pandan* leaf extract ranges from 0.11 to 0.36 mg GAE/g. This result was lower than the TPC of *pandan* leaf extract produced by reflux extraction (3.12 to 6.58 mg/g DW) [12], conventional soaking (0.428 to 0.979 mg/g GAE), and microwave-

assisted extraction (MAE) methods with different soaking time and microwave power (0.484 to 1.557 mg GAE/g) [31] . In this work, the ethanol concentration in both linear and quadratic models influenced the total phenolic content of pandan leaf extract and interaction extraction time and extraction temperature, as listed in Table 5. Figures 1A and 1B exhibited that the increasing ethanol concentration from 65 to 80% significantly increases the TPC in *pandan* leaf extract, after which the TPC decreased significantly. This report aligned with [31] , who reported increased ethanol concentrations from 50 to 75% at various temperatures and microwave powers, raising the TPC in Malaysian *P. amaryllifolius* leaves extract. However, increasing the ethanol concentration by 100% decreased the TPC. In this study, the decline of TPC at high ethanol concentration (95%) might be correlated with the solvent's dielectric properties toward microwave heating because ethanol solvent plays an essential role in microwave extraction by facilitating heat distribution throughout the *pandan* leaves sample to extract phenolic compounds completely [9]. [31] also claimed that certain phenolic compounds can create complexes that dissolve in solvents like ethanol. Additionally, Figure 1C and Table 5 exhibited that increasing the interaction of extraction duration and extraction temperatures significantly increases the TPC in the *pandan* leaf extract. This outcome was consistent with the earlier research published by [24] reported that the increase in extraction time from 80 to 120 minutes and the rise in extraction temperature from 60 to 70 °C significantly increased the total phenolic content in *Clinacanthus nutans* Lindau leaves extracts. However, the rising temperature extraction to 80 °C declined the TPC due to the heat sensitivity of some phenolic components contained therein. Another study reported that increasing the temperature to 60 °C enhances the solubility of phenolic compounds and their diffusion rate from cells into the solvent, so the mass transfer rate also rises [7].

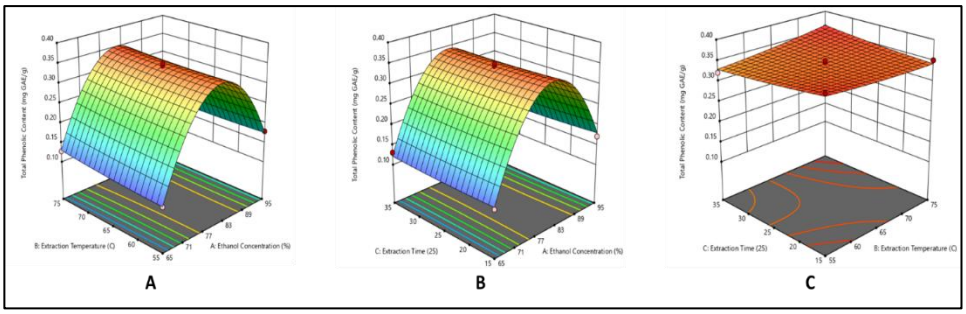

Fig. 1. 3D-response surface plot of the effect of independent factors on the total phenolic content of *pandan* leaf extract. (A) Interaction of ethanol concentration and extraction temperature, (B) Interaction of ethanol concentration and extraction time, and (C) Interaction of extraction temperature and extraction time

3.3 Antioxidant Activity (IC_{50})

The antioxidant activity of *pandan* leaf extract, in terms of $IC_{50,}$ ranges from 13.25 to 47.88 ppm. This result was lower than that of *pandan* leaf powder, which has IC_{50} in 181.51 to 264.97 ppm [1], and in the ranges of the ethanolic *pandan* leaf extract from Thailand (12.57 ppm) [30] . The ethanol content in this study had an impact on the antioxidant activity (IC50) of pandan leaf extract in linear and quadratic models and extraction temperature in linear models, as listed in Table 5. The IC_{50} in *pandan* leaf extract was significantly decreased at ethanol concentrations from 65 to 80% and rose again when concentrations were up to 95%, as exhibited in Figures 2A and 2B. The IC_{50} was correlated with the bioactive compounds in *pandan* leaf extract, including TPC. According to [15], the TPC and antioxidant activity using DPPH positively correlate with R^2 of 0.9756. In a recent study, the decrease of IC_{50} at ethanol

concentration of 65 to 80% was associated with an increase of TPC in *pandan* leaf extract, and in contrast, the increases of ethanol concentration up to 95% correlated with the decrease of the TPC therein, as presented in Figure 1A and 1B, and also explained before. The lowest IC_{50} was found at an ethanol concentration of around 85%, indicating the strong antioxidant activity of the *pandan* leaf extract. According to [25], higher total phenolic content (TPC) leads to higher antioxidant activity, indicated that the color solution during testing lost its brightness and showed a lower absorbance value due to the hydrogen atoms of TPC attached to DPPH radicals. In contrast, the extraction temperature had significant linear increases of IC_{50} in *pandan* leaf extract, as revealed in Figures 2A and 2C. This result was in agreement with the earlier study reported by [21], who reported the increasing extraction temperature from 30 to 70 °C at fixed time extraction, ethanol concentration, and the ratio solvent to the material increased the IC_{50} of *Hypericum perforatum* L. extract, due the degradation of the certain bioactive compounds, including phenolics compounds. Another independent factor is that the extraction time did not impact the IC_{50} of *pandan* leaf extract.

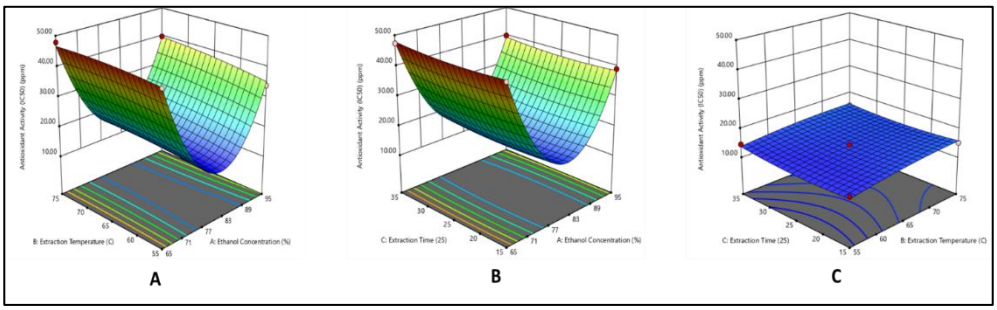

Fig. 2. 3D-response surface plot of the effect of independent factors on the antioxidant activity of *pandan* leaf extract. (A) Interaction of ethanol concentration and extraction temperature, (B) Interaction of ethanol concentration and extraction time, and (C) Interaction of extraction temperature and extraction time

3.4 2acetyl-1pyrroline content

The 2acetyl-1pyrroline (2AP) content represents the aroma of pandan leaf extract. The 2-acetyl-1-pyrroline content of *pandan* leaf extract in this study was 0.52 to 8.30 ppm, as listed in Table 3. This result was in the range of the 2AP content of *pandan* leaf extract produced using headspace-solid phase microextraction (HS- SPME), ultrasonic, and supercritical carbon dioxide (SC-CO_2) extraction, with the amount of 0.09 to 4.98 ppm [26] , 0.591 to 4.873 ppm [3], and 0.04 to 0.45 ppm [29], respectively. The 2acetyl-1pyrroline content of *pandan* leaf extract only affected ethanol concentration in linear and quadratic models Table 5. Figures 3A and 3B exhibited that the significant increase of ethanol concentration from 65 to 80% was a rise in the 2AP content, and the application of the high ethanol concentration up to 95% significantly declined the 2AP content of *pandan* leaf extract. According to [3], there is an upward trend between the concentration of ethanol and the content of 2AP in pandan leaf extract. In contrast, the extraction time and extraction temperature did not impact the 2AP content of *pandan* leaf extract. The significant effect of ethanol concentration on the extraction of 2acetyl-1pyrroline (2AP) compared to the lesser influence of extraction duration and temperature can be attributed to several key factors related to solvent properties, compound solubility, and extraction dynamics. the substantial effect of ethanol concentration on the extraction of 2acetyl-1pyrroline is primarily due to its polar nature and optimal solubility characteristics that favor the dissolution and release of this compound from plant

matrices. In contrast, while extraction time and temperature are relevant factors, their effects are often overshadowed by the choice of solvent concentration in achieving efficient extraction outcomes. Figure 3C revealed that the increasing extraction duration and extraction temperature increase the 2AP content of pandan leaf extract insignificantly.

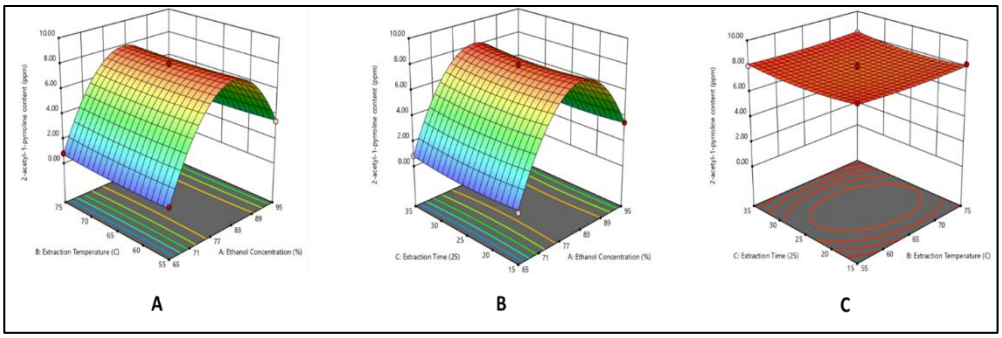

Fig. 3. 3D-response surface plot of the effect of independent factors on the aroma of *pandan* leaf extract. (A) Interaction of ethanol concentration and extraction temperature, (B) Interaction of ethanol concentration and extraction time, and (C) Interaction of extraction temperature and extraction time

The observed trends in the extraction of 2-acetyl-1-pyrroline from pandan leaves using MAE can be attributed to a combination of solvent properties (ethanol concentration), energy transfer dynamics (temperature), and kinetic factors (extraction time). Ethanol concentration significantly affects solubility and selective extraction efficiency; temperature enhances energy transfer but must be controlled to prevent degradation; and time impacts yield but shows diminishing returns beyond optimal periods. Understanding these mechanisms allows for more effective optimization of MAE parameters to maximize yield and quality of desired bioactive compounds like 2-AP from pandan leaves.

3.5 Optimization and Verification of Optimum Condition

In this study, the *pandan* leaf extract produced is expected to have a high total phenolic content, 2acetyl-1pyrroline (2AP) content, and low antioxidant activity (IC_{50}). Hence, for the optimization, the total phenolic content and 2acetyl-1pyrroline (2AP) content were set to *maximize*, and the antioxidant activity (IC_{50}) was set to *minimize*. Meanwhile, the independent factors were set in range, such as the temperature, extraction duration, and concentration of ethanol. An ethanol concentration of 80%, an extraction temperature of 65°C, and an extraction duration of 25 minutes were found to be the ideal extraction parameters**.** The resulting responses were a total phenolics content of 0.34±0.00 mg GAE/g, antioxidant activity (IC_{50}) of 14.27±0.00 ppm, and 2acetyl-1pyrroline (2AP) content of 7.78±0.00 ppm. To verify these findings, three replications were conducted and yielded a total phenolics content of 0.34±0.02 mg GAE/g, antioxidant activity (IC_{50}) of 14.27±0.58 ppm, and 2 acetyl-1-pyrroline (2AP) content of 7.78±0.62 ppm. The experimental and projected outcomes did not differ statistically significantly, according to a paired t-test, as presented in Table 3. These insignificant differences highlight the quadratic model's precision and suitability for optimizing *pandan* leaf extraction conditions.

4 Conclusion

1. In this study, we found that the ethanol concentration was affected in all responses of *pandan* leaf extract, including total phenolic content, antioxidant activity (IC_{50}), and 2acetyl-1pyrroline content.
2. The extraction temperature only affected antioxidant activity (IC_{50}), and the extraction time only had a little effect on all responses of *pandan* leaf extract.
3. The optimum extraction conditions were found at an 80% ethanol concentration, 65 °C extraction temperature, and 25 min extraction time, with a total phenolics content of 0.34±0.02 mg GAE/g, antioxidant activity (IC_{50}) of 14.27±0.58 ppm, and 2acetyl-1pyrroline (2AP) content of 7.78±0.62 ppm.
4. Microwave-assisted Extraction (MAE) is a promising method for producing *pandan* extract with high antioxidant compounds and 2-acetyl-1-pyrroline (2AP).
5. Further research is needed on factors that influence extraction other than ethanol concentration, temperature and time, such as material ratio, type of solvent and extraction method.

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