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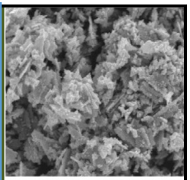

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Application of Different Aqueous Ultrasound-assisted Extraction Process Parameters on the Quality of Pandan (*Pandanus amaryllifolius* Roxb) Leaves Extract

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ABSTRACT

Pandan of *Pandanaceae* family, known for its aromatic foliage, vibrant green color, and rich in bioactive compounds, requires green extraction technology to stabilise compounds. Hence, this study aims to optimise process parameters of aqueous ultrasound-assisted extraction (UAE) on the color, yield, and antioxidant content of pandan extract using the Response Surface Method (RSM), and compare the volatile compounds present in optimised ultrasonicated pandan extract with macerated extract (control). The analysis includes DPPH and FRAP assays, total phenolic content (TPC), color, and yield. Optimally validated parameters ($R^2 \geq 0.90$, p-value lack-of-fit ≥ 0.05) were: 80°C extraction temperature, 90 min ultrasonic time, 2.60 % of solid in 20 mL distilled water yielded promising antioxidant activities: 277.64 mg/g AAE, 19.90 mg/g GAE, and 1.48 g/g AAE for DPPH, TPC, and FRAP assays, respectively). UAE technique has significantly ($p<0.05$) higher yield of extract (86.24%) and DPPH scavenging activity (277.64 mg/g AAE) compared with control. The 3-methyl-2(5H)-furanone is the most abundant volatile compound found in both extracts. The cavitation in ultrasonication generates currents, increasing pandan's mass transfer rate into the solvent medium. The optimised ultrasonication condition contributes to the increased quality of the pandan extract.

INTRODUCTION

The majority of pandan is produced in South Asian countries like Indonesia, Thailand, Vietnam, India, and Madagascar. These countries produce 200,000 to 300,000 tons per year [1]. Pandan leaves contain many bioactive constituents such as phenolic compounds, tannins, essential oils, terpenoids, alkaloids, flavonoids, saponins, and steroids, exhibiting promising health advantages. A study reported that aqueous pandan extract enhanced thermal stability and reduced gel hardness and adhesiveness in both waxy and normal rice starches [2]. Ultrasound-assisted extraction (UAE) utilises low-temperature (30 °C) and preserves the quality and bioactivity of extracted compounds better than Soxhlet at above 80 °C for 8 h.

Additionally, UAE requires less solvent, which is a win for both cost-effectiveness and environmental sustainability. However, limited studies have been conducted on the extraction of high-antioxidant pandan extract using the UAE technique at varying extraction temperatures. Therefore, this study aims to optimise process parameters of aqueous UAE on pandan extract's color, yield, and antioxidant content using the Response Surface Method (RSM) and compare the volatile compounds present in optimised ultrasonicated pandan extract with macerated extract. The optimised ultrasonication condition contributes to the increased quality of the pandan extract.

MATERIALS AND METHOD

Materials

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by Thermo scientific USA, Folin-Ciocalteu reagent, 3,4,5-trihydroxybenzoic acid (Gallic acid) was purchased from Merck Germany.

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Sample preparation

Pandan leaves were harvested from Serdang, Selangor in July 2023. The leaves were cleaned from debris, sorted, and selected for the mature leaves (the 15th leaves from the shoot and below) for higher levels of antioxidant content [3]. The leaves were then dried at 105℃ for 24 h [4]. The temperature of the drying was determined via a preliminary study, using three different drying temperatures: 35 °C, 65 °C and 105℃ [5], which corresponded to the optimum value of antioxidant level (52.39%, 16.87 g/g AAE and 27.52 mg/g GAE for DPPH, FRAP, TPC assays respectively), moisture content (6.77%) and color (L value 45.32, a value -4.79 and b value 21.35). The pandan leaves powder was dried at 105 ℃. The dried leaves were then ground using a stainless-steel grinder (model RT-02A from Good and Well Sdn. Bhd, Malaysia) at a maximum of 425 nm particle size and sieved by a siever (Endecotts Ldt, London). The powder was kept in an airtight container at room temperature until further analysis.

Experimental design for optimisation

The ultrasound-assisted extraction was done using Elmasonic S100H ultrasonic cleaner, USA, at 37 kHz with a time range of 20 to 90 mins. The range of temperature used was 30 to 80 °C. The tank dimension is 30 × 23.9 × 15 cm, with a capacity of 9.5 L, using sweep ultrasound mode. The extraction of the pandan was done [4]. The range of parameters used is listed in Table 1. The temperature, time and solid-liquid ratio were selected as manipulated variables (process parameters) with 20 runs altogether. The model involves two-levels, three variables using central composite face-centred design.

Extraction of pandan extract via maceration technique

The maceration of pandan leaves was conducted [6] and the independent variables value and the corresponding levels for optimisation of process parameters of UAE pandan extract is shown in **Table 1**. The yield of extract was measured using the formula:

$$Yield = \frac{X_1}{X_2} x100\%$$

Where X_1 is the mass of extract in grams, while X_2 is the total mass of pandan leaves and solvent used in the extraction.

Table 1. Independent variables value and the corresponding levels for optimisation of process parameters of UAE pandan extract.

Independent variables	Units	Symbols	Coded levels -1	0	1
Extraction temperature	°C	X_1	30	55	80
Extraction time	min	X_2	20	55	80
Percentage of solid in solvent	%	X_3^*	2.50	6.25	10.00

Note: X_3 is the percentage of solid (%) in 20 mL solvent to make up the solid-liquid ratio

Types of analysis

The physicochemical analysis includes moisture (MA35 Sartorius, Germany) and color by Konica Minolta Inc., Japan. The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay [7], total phenolic count and ferric reducing antioxidant potential (FRAP) assay were also conducted [8]. Gas chromatography-flame ionisation detector (Thermo Fisher Scientific, USA) technique was employed to determine the volatiles present in both optimised and macerated pandan extract [9].

Statistical analysis

Response Surface Methodology (RSM), Analysis of Variance (ANOVA): Single-Factor and Tukey's test, were done via Minitab Statistical Application version 21, USA. The ANOVA method was done at the 0.05 significance level.

RESULTS AND DISCUSSION

Table 2 shows the experimental data ultrasound-assisted extraction of pandan. The validated optimum parameters and their responses: yield of pandan extract, the L, a, b value, and antioxidant activities: DPPH scavenging activity, Total Phenolic Content (TPC), and Ferric Reducing Power (FRAP) were tabulated in Table 3. All experimental responses show no significant difference from the predicted responses. Based on **Table 3**, the UAE technique has a significantly ($p<0.05$) higher yield of extract (86.24%) and DPPH scavenging activity (277.64 mg/g AAE) in comparison with the control, which yielded 80.63% in yield, and 149.20 mg/g AAE.

Temperature increases the yield of UAE extract by enhancing solute desorption and solubility, decreasing solvent viscosity, and increasing solvent diffusivity in the tissue matrix [10]. However, a weakened cavitation effect may occur due to the temperature increase, hence decreasing the extract yield. Higher antioxidant activity (DDPH assay) was found in the UAE extract than in the macerated sample, which might be due to the extraction temperature above 70 ℃ that triggers chemical reactions and leads to the formation of new antioxidant compounds [11]. Conversely, maceration relies on diffusion, a relatively slow process where target chemicals passively migrate from cells into the solvent.

The UAE pandan extract is significantly ($p<0.05$) lighter, greener and yellower in color compared to the macerated pandan extract which utilises a gentler technique, facilitating a gradual and regulated dispersion of pigments. In a study on grape skin anthocyanins, UAE using temperature demonstrated significantly higher color lightness (L value) than maceration [12]. Nine volatile compounds were detected in both samples, with eight compounds from macerated (control) pandan extract and 4 compounds from the optimised ultrasound-assisted pandan extract (**Fig 1**).

Table 2. Experimental data of pandan ultrasound-assisted extraction using different process parameters.

Run	Yield (%)	L value	b value	DPPH (mg AAE/ g)	TPC (mg GAE / g)	FRAP (g AAE / g)
1	80.10	62.29	42.04	77.07	10.76	0.30
2	81.60	55.50	37.66	74.83	10.76	0.34
3	80.70	56.63	39.31	82.88	11.00	0.31
4	82.41	58.19	42.59	99.77	10.36	0.30
5	79.70	56.59	41.76	73.17	10.76	0.29
6	81.10	59.83	39.88	128.73	11.00	0.28
7	79.90	60.04	40.77	127.80	10.76	0.30
8	85.10	64.16	31.07	201.60	21.02	0.72
9	77.60	52.43	40.86	52.17	7.25	0.19
10	81.60	60.33	42.12	77.61	10.55	0.30
11	88.80	65.95	35.48	236.37	21.83	0.77
12	71.20	51.61	41.24	47.91	7.05	0.19
13	85.20	55.89	40.30	94.36	10.76	0.30
14	87.60	63.79	37.78	277.03	20.08	0.76
15	72.70	49.39	37.91	39.79	6.87	0.20
16	88.70	67.41	33.28	217.27	19.11	0.76
17	86.40	50.11	36.59	56.76	6.87	0.20
18	85.70	58.96	39.20	63.45	10.55	0.29
19	83.60	62.45	31.86	201.37	19.41	0.74
20	80.70	48.42	35.62	45.51	6.87	0.17

Table 3. Comparison between yield, color, antioxidant activity analysis and total phenolic content of ultrasound-assisted extract and macerated extract.

Responses		Ultrasound-Assisted Extract (n=9) Predicted	Experimental	Macerated Extract (control)
Yield (%)		88.73±0.00[a]	86.24±1.164[a]	80.63±0.351[b]
Color	L value	63.52±0.00[a]	63.79±0.823[a]	46.57±0.57[b]
	b value	36.52±0.00[a]	37.177±0.932[a]	31.836±0.60[b]
Antioxidant Activity	DPPH (mg/g AAE)	277.03±0.00[a]	277.64±14.01[a]	149.20±39.10[b]
	TPC (mg/g GAE)	20.08±0.00[a]	19.95±0.09[b]	27.74±0.55[a]
	FRAP (g/g AAE)	0.51±0.00[a]	0.50±0.01[a]	0.48±0.01[a]

Note: Amount of solid in the extract makes up the solid-liquid ratio. Different letters within a row are significantly different ($p < 0.05$). n=9, number of replicates

The compounds include 1 alcohol, 1 alkane, 1 ketone, 2 unknown compound, and 4 acids. The 3-methyl-2(5H)-furanone is the most abundant compound in both extracts, making up 68.5% of the ultrasound-assisted extract, and 22.6% of the macerated extract. This is in correlation with the findings who found the same compound in a large amount of pandan extract. It shows that 3-methyl-2(5H)-furanone can also be found in various processed foods such as fermented soy hydrolysate, Finnish birch syrup, cheese and dried bonito [9]. Due to the fruity aroma characteristic, the 3-hexenoic acid is commonly used as fragrance or flavouring.

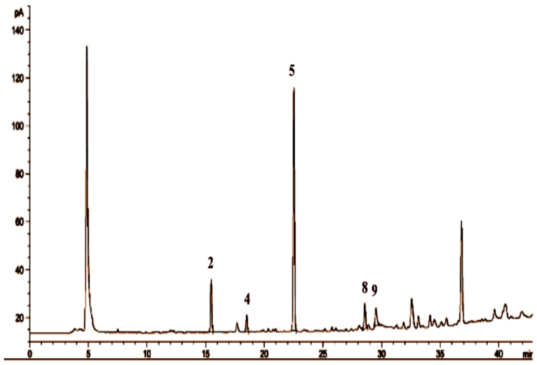

Fig. 1. Gas chromatography/flame ionisation detection (GC/FID) of pandan extract prepared using ultrasound-assisted extraction (UAE).Note: Peak 1= (E)-2-penten-1-o1, Peak 2= acetic acid, Peak 4= unknown, Peak 5= 3-methyl-2(5H)-furanone, Peak 8 = 3-hexenoic acid, Peak 9 = eicosane (IS).

CONCLUSION

The experimental results show a strong conformity to a second-order polynomial equation, validating the accuracy and dependability of the model. At the optimum point, the antioxidant activity determined via DDPH, TPC and FRAP assays were 277.64 mg/g AAE, 19.95 mg/g GAE and 0.50 g/g AAE, respectively. The study determined that the solid-liquid ratio had a substantial impact on the extraction yield, color parameters (L and b values), and antioxidant activities as assessed by DPPH scavenging activity, total phenolic content (TPC), and Ferric Reducing Power (FRAP) studies. Ultrasound-assisted extraction (UAE) yielded significantly ($p<0.05$) higher amounts of DPPH scavenging activity (277.64 mg/g AAE) compared to conventional maceration extraction (149.20 mg/g AAE). Nevertheless, maceration demonstrated a greater overall phenolic content (TPC) of 27.74 mg/g GAE, compared to the UAE extract (19.95 mg/g GAE), as it is especially appropriate for compounds sensitive to heat. This study may assist UAE extraction strategies in enhancing the quality of pandan extract.

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