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Optimization of Microwave Assisted Hydro Distillation for Enhanced Essential Oil Extraction and Antioxidant Activity from *Alpinia blepharocalyx K. Schum.* Leaves

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ABSTRACT

Background: This study aimed to optimize the conditions of microwave assisted hydro distillation (MAHD) for maximizing the essential oil yield and DPPH radical scavenging activity from *Alpinia blepharocalyx* K. *Schum.* leaves.

Methods: Using the central composite design (CCD) of response surface methodology (RSM) with factors that affect the yield of essential oil and DPPH obtained: Microwave power (W), liquid-to-material ratio (mL/g) and extraction time (min).

Result: The optimal extraction conditions determined by the RSM model were a microwave power of 600 W, a liquid-to-material ratio of 7.0:1 mL/g and an extraction time of 45 min. Under these conditions, the experimental essential oil yield was 3.35±0.02% and the DPPH antioxidant activity was 87.05±0.05%. The chemical composition of the essential oil was also characterized by gas chromatography-mass spectrometry (GC-MS), revealing β-Pinen, 1,8-Cineol and α-Pinen as the major compounds.

Key words: *Alpinia blepharocalyx*, Antioxidant activity, Essential oil, Response surface methodology, Yield.

INTRODUCTION

Essential oils are gaining significant importance in food, cosmetic and pharmaceutical sectors due to their diverse biological effects (Bakkali *et al.,* 2008). They are commonly obtained from different parts of plants, such as leaves, flowers and stems and roots (Ali *et al.,* 2015). The genus *Alpinia* (Zingiberaceae family), found abundantly in tropical Asia, is known for its high content of volatile oils and terpenoids (Padalia *et al.,* 2010). Recent studies have highlighted the antioxidant, antihypertensive and antimicrobial properties of essential oils from this genus (Zhang *et al.,* 2016).

Traditional methods like hydro-distillation (HD) and steam distillation are widely used for essential oil extraction (Cassel *et al.,* 2009; Tongnuanchan *et al.,* 2014). However, these methods are often limited by long processing times and high energy consumption (Rostagno *et al.,* 2013). Furthermore, the high temperatures can lead to the degradation of unsaturated compounds and the loss of volatile active ingredients, thereby reducing the quality and value of the essential oil.

Optimized essential oil extraction methods demonstrate notable efficiency. For instance, the optimization of essential oil extraction from *Cinnamomum camphora* and *Rumex Crispus* using microwave-assisted hydro-distillation (MAHD) has been reported (Hundie *et al.,* 2023; Shang *et al.,* 2020). Furthermore, other studies from the field of Agricultural Science and comparisons between MAHD and traditional distillation methods highlight the potential advantages of MAHD (War and Highland 2022; Moradi *et al.,* 2018).

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To address these limitations, microwave-assisted extraction (MAE) has emerged as a superior alternative (Nagahata *et al.,* 2019). MAE is a rapid and efficient technique that utilizes microwave energy to heat the plant material, leading to faster cell rupture and more effective extraction of compounds (Dao *et al.,* 2020). This method not only improves the electrical energy conversion to thermal energy but also accelerates chemical processes (Chanthai *et al.,* 2012).

This study utilized MAHD with the aim of enhancing the yield of essential oil extraction from the leaves of *Alpinia blepharocalyx* K. Schum. The research aimed to determine the optimal conditions for maximizing both essential oil yield and DPPH radical scavenging activity. By employing response surface methodology (RSM) with a central composite design (CCD), we systematically focused on how three specific parameters-microwave power, liquid-to-material ratio and extraction time affected the process (Bezerra *et al.,* 2008).

MATERIALS AND METHODS

Material and reagents

Alpinia blepharocalyx leaves were gathered from Ky Son District, Nghe An Province, Vietnam. The samples were cleaned to remove pests and old parts, then washed and dried at 40°C. The dried leaves were pulverized and passed through a 1.0 mm mesh screen. The treated samples were kept refrigerated at a temperature range of 0°C to 4°C for later experiments.

Microwave-assisted hydro-distillation

The microwave-assisted hydro-distillation (MAHD) system consists of a microwave oven and a Clevenger apparatus (Fig 1). First, 50 g of dried *A. blepharocalyx* leaf samples were transferred to a distillation flask, followed by the addition of deionized water. The mixture was then heated with microwave radiation. After a set period, the essential oil fraction was collected in a separate bottle. To remove any remaining water, anhydrous sodium sulfate was added to the essential oil, followed by centrifugation.

The yield of essential oil is calculated *via* the ratio of the the dried essential oil mass (DMO) to dried leaf sample mass (DML) as shown in the Equation 1:

$$Y(\%) = \frac{DM_O}{DM_L} \times 100 \quad \text{....(1)}$$

DPPH radical scavenging assay (RSA)

A. blepharocalyx leaf extract was evaluated using the the DPPH radical scavenging test. This method measures the capacity of the extract to scavenge 1,1-diphenyl-2-picrylhydrazyl radicals. The experimental procedure was performed by combining 0.2 mL of the sample extract and 3.8 mL of a DPPH ethanolic solution (5.9 mg DPPH in 100 mL ethanol). The mixture was stirred for 1 minute and then left to stand for 30 minutes at ambient temperature and protected from light. Absorbance readings were taken at 517 nm using an Agilent 8453 UV-visible Spectrophotometer.

The experiment was replicated with triplicate with all determination's samples. The DPPH (%) was calculated according to Equation 2:

$$DPPH\ (\%) = 1 - \frac{Abs_{sample}}{Abs_{control}} \times 100 \quad \text{....(2)}$$

Abs_{sample}, $Abs_{control}$ are noted for the absorbance of *A. blepharocalyx* leaf extract and the absorbance of blank control, respectively.

Experimental design

To optimize the extraction conditions, using three-factor response surface method analysis using a central composite design (CCD) with parameters (Microwave power (W), Liquid-to material ratio (mL/g) and extraction time (min)). In which, each experiment using the total amount of water is 100 g. Table 1 displays the coded independent variables for the RSM design.

In this research, using the program Design-Expert® to the evaluated effects of the extraction parameters. The quadratic polynomial model of response variable was ditermined as Equation 3:

$$y = \beta_0 + \sum_{i=1}^{i=k} \beta_i X_i + \sum_{i=1}^{i=k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{i<j} \beta_{ij} X_i X_j \quad \text{....(3)}$$

Where,

Yi = Predicted response.

β_0, β_i, β_{ii} = Regression coefficient.

x_i, x_j = Different independent variables.

X_i and X_j = Coded independent variables.

Determine the chemical composition of A. blepharocalyx leaves essential oil by Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oil was chemically characterized by a Trace GC Ultra - ITQ900 GC-MS system (Thermo, USA). The oven temperature was programmed as follows an initial hold at 60°C for 2 min, a ramp to 200°C at 4°C/min, followed by another ramp to 360°C at 10°C/min, with a final 10 min hold for column clean-up. The injector temperature was 280°C. A 1.0 µL sample, diluted in n-hexane (1/50, v/v), was injected in split mode (split ratio: 1/15) (Yim *et al.,* 2011).

RESULTS AND DISCUSSION

Regression model analysis

Using the central composite design with 20 actual experiments with 3 factors to optimize the yield of *A. blepharocalyx* leaves essential oil and the DPPH radical scavenging activity values of extract fluids, are shown in Table 2.

From the experimental results and linear regression analysis of 20 experiments, regression equations were proposed of two responses (essential oil yield and DPPH):

$$Y_1 = 3.28 + 0.24X_1 + 0.024X_2 + 0.29X_3 - 0.071X_1X_2 - 0.034X_1X_3 - 0.056X_2X_3 - 0.13X_1^2 - 4.36X_2^2 - 0.46X_3^2 \quad \text{....(4)}$$

$$Y_2 = 87.68 - 3.9X_1 - 0.18X_2 - 0.52X_3 - 01.67X_1X_2 + 4.82X_1X_3 + 0.39X_2X_3 - 1.71X_1^2 - 0.26X_2^2 - 0.14X_3^2 \quad \text{....(5)}$$

The experiments were conducted using a central composite design with 20 runs to optimize the essential oil yield (Y_1) and DPPH radical scavenging activity (Y_2). The analysis of variance (ANOVA) for the quadratic regression model (Table 3) confirmed that the models were highly significant for both responses ($p<0.0001$) (Rezzoug *et al.,* 2005). The coefficients of determination (R^2) were 0.9596 for essential oil yield and 0.9933 for DPPH activity, indicating that the models could explain 95.96% and 99.33% of the variability in the responses, respectively (Chanthai *et al.,* 2012).

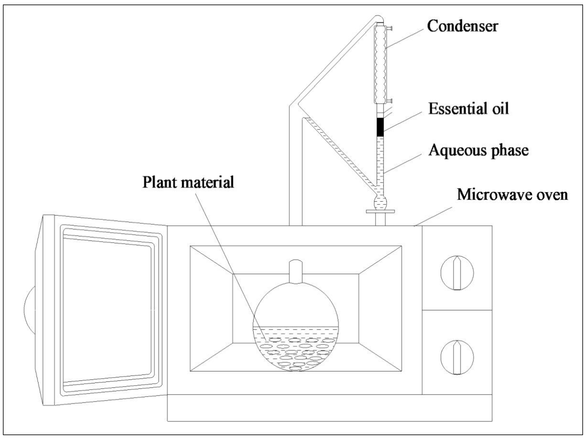

Fig 1: Schematic diagram of MAHD method.

Response surface analysis

The three-dimensional plots of the response surface (Fig 2 and Fig 3) clearly illustrate the effects and interactions of the independent variables on the responses.

Essential oil yield (Y_1)

- **Effect of microwave power and liquid-to-material ratio:** At a constant extraction time of 40 min, the oil yield increased with higher microwave power. The yield also increased as the liquid-to-material ratio increased from 5:1 to 7:1 mL/g but decreased with further increases, suggesting an optimal point around this ratio.
- **Influence of microwave power and extraction time:** With a fixed liquid-to-material ratio of 7:1 mL/g, the oil yield was positively correlated with an increase in both microwave power and extraction time, reaching a peak at the central levels.

Table 1: Coded level in the central composite design.

Factors	Symbols	Units	Range and level				
			-1.68	-1	0	+1	+1.68
Microwave power	X_1	W	431.82	500	600	700	768.18
Liquid-to-material ratio	X_2	mL/g	3.64	5	7	9	10.36
Extraction time	X_3	min	23.18	30	40	50	56.82

Table 2: Experimental design and response values.

Run	X_1 (Microwave power - W)	X_2 (Liquid to material ratio - mL/g)	X_3 (Extraction time - min)	Y_1 (Essential oil yield - %)	Y_2 (DPPH - %)
1	500.00	5.00	30.00	2.17	89.92
2	700.00	5.00	30.00	2.52	74.91
3	500.00	9.00	30.00	2.32	91.38
4	700.00	9.00	30.00	2.96	71.46
5	500.00	5.00	50.00	2.92	77.01
6	700.00	5.00	50.00	3.14	83.02
7	500.00	9.00	50.00	2.85	81.78
8	700.00	9.00	50.00	3.35	79.36
9	431.82	7.00	40.00	2.41	89.41
10	768.18	7.00	40.00	3.36	76.36
11	600.00	3.64	40.00	2.63	75.87
12	600.00	10.36	40.00	2.39	74.91
13	600.00	7.00	23.18	2.35	86.62
14	600.00	7.00	56.82	3.38	86.23
15	600.00	7.00	40.00	3.21	86.72
16	600.00	7.00	40.00	3.24	87.88
17	600.00	7.00	40.00	3.28	88.12
18	600.00	7.00	40.00	3.33	88.22
19	600.00	7.00	40.00	3.25	87.76
20	600.00	7.00	40.00	3.41	87.38

- The increase in essential oil yield with higher microwave power (from 500 W to 700 W) can be attributed to the efficient heating mechanism of microwaves. Microwave radiation directly interacts with polar molecules, primarily the intracellular water within the plant matrix. This interaction causes rapid, localized superheating, leading to a dramatic increase in internal pressure that ruptures the cell walls and oil glands. Consequently, the essential oil is released more rapidly and completely into the surrounding solvent, thus enhancing the extraction yield.

Table 3: ANOVA analysis of the quadratic regression model (Essential oil yield and DPPH).

Source	Y_1 - Essential oil yield		Y_2 - DPPH	
	F- value	p- value	F- value	p-value
Model	26.40	<0.0001[c]	165.43	<0.0001[c]
X_1	56.51	<0.0001[c]	430.23	<0.0001[c]
X_2	0.55	0.4753[NS]	0.94	0.3545[NS]
X_3	83.56	<0.0001[c]	7.76	0.0193[a]
X_1X_2	2.86	0.1214[NS]	46.03	<0.0001[c]
X_1X_3	0.64	0.4413[NS]	383.78	<0.0001[c]
X_2X3	1.79	0.2111[NS]	2.49	0.1460[NS]
X_1^2	17.13	0.0020[a]	87.65	<0.0001[c]
X_2^2	69.99	<0.0001[c]	567.99	<0.0001[c]
X_3^2	19.05	0.0014[c]	6.39	0.0300[a]
Lack of fit	4.34	0.0664[NS]	2.13	0.2129[NS]
R^2	0.9596		0.9933	

[a]p<0.05; [b]p<0.01; [c]p<0.001; NS: non-significant.

DPPH radical scavenging activity (Y_2)

- **Effect of microwave power:** DPPH activity showed an inverse relationship with microwave power, decreasing as the power increased. This could be due to the degradation of some antioxidant compounds at higher energy levels.
- **Influence of liquid-to-material ratio and extraction time:** DPPH activity reached a maximum at a liquid-to-material ratio of approximately 7.5:1 mL/g, then decreased. The activity also decreased as the extraction time increased, suggesting that prolonged exposure to microwave radiation may reduce the antioxidant capacity of the extract.
- Interestingly, an inverse relationship was observed between microwave power and DPPH radical scavenging activity. While higher power enhances yield, the associated increase in temperature likely promotes the degradation of thermolabile antioxidant compounds. Components such as certain oxygenated monoterpenes or phenolic compounds, which are known contributors to antioxidant capacity, may have been partially decomposed or volatilized at higher energy levels, leading to a reduction in the overall radical scavenging ability of the extracted oil.

Optimization and model verification

The optimal conditions predicted by the RSM model were a microwave power of 602.49 W, a liquid-to-material ratio

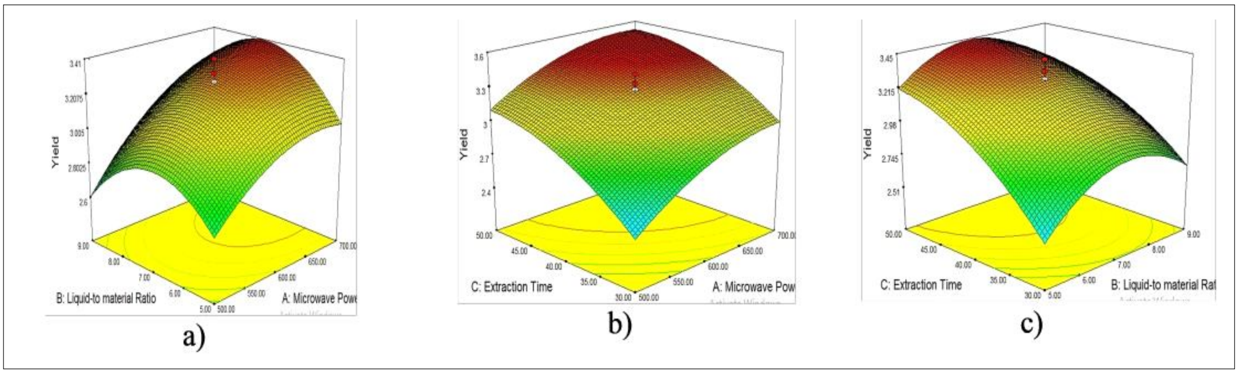

Fig 2: Response surface of oil yield.

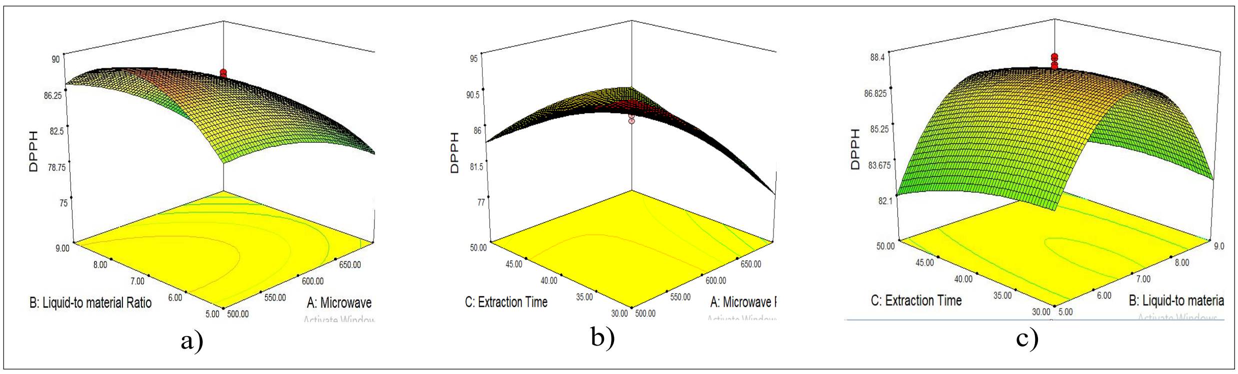

Fig 3: Response surface of DPPH.

Table 4: Optimum conditions of responses of *A. blepharocalyx* leaves extraction.

Variables			Response	Optimum value	
X_1	X_2	X_3		Experimental value	Predicted value
600.0	7.0:1	45.0	Y_1 (%)	3.35±0.02	3.41
(W)	(mL/g)	(min)	Y_2 (%)	87.05±0.05	87.22

Table 5: The chemical components of essential oil mixture extracted from *A. blepharocalyx* leaves at the found optimal condition.

No	Compound	RI[a]	%[b]
1	Tricyclen	926	0.6
2	α-Pinen	939	13.2
3	Camphen	953	6.7
4	β-Pinen	980	20.9
5	β-Myrcen	990	1.9
6	α-Phellandren	1006	2.2
7	δ-3-caren	1011	0.3
8	α-Terpinen	1017	0.2
9	1,8-Cineol	1034	20.8
10	Linalool	1100	2.3
11	α-Thujon	1105	4.3
12	Fenchyl alcohol	1120	0.4
13	Camphor	1145	2.4
14	Borneol	1167	3.2
15	Terpinen-4-ol	1177	0.9
16	α-Terpineol	1189	0.5
17	Myrtenal	1200	0.3
18	Fenchyl axetat	1222	0.4
19	Bornyl axetat	1289	1.1
20	α-Copaen	1377	0.7
21	Geranyl axetat	1381	0.6
22	β-Caryophyllen	1419	2.6
23	γ-Elemen	1437	0.7
24	Aromadendren	1441	0.6
25	α-Humulen	1454	1.3
26	γ-Muurolen	1480	0.7
27	Germacren D	1485	0.3
28	α-Amorphen	1485	1.5
29	β-Selinen	1486	0.6
30	α-Selinen	1493	0.7
31	Cadina-1,4-dien	1496	0.3
32	α-Muurolen	1500	0.4
33	δ-Cadinen	1525	1.2
34	α-Cadinen	1539	0.5
35	α-Calacoren	1546	0.3
36	(E)-Nerolidol	1563	0.7
37	Caryophyllen oxit	1583	1.1
38	Humulene epoxit II	1625	0.5
39	α-Cadinol	1654	0.8

[a]Retention indices on HP-5MS capillary column; [b]Percentage content of total essential oil.

of 6.99:1 mL/g and an extraction time of 45.44 min. These predicted values were highly consistent with the experimental results obtained from a validation experiment conducted under slightly modified, practical conditions: A microwave power of 600 W, a liquid-to-material ratio of 7.0:1 mL/g and an extraction time of 45 min (Table 4). The experimental values for essential oil yield (3.35±0.02%) and DPPH activity (87.05±0.05%) were very close to the predicted values, validating the model's reliability.

Chemical profile of essential oils

The GC-MS analysis identified 39 chemical components in the essential oil extracted under optimal conditions (Table 5). The major compounds were β-Pinen (20.9%), 1,8-Cineol (20.8%) and α-Pinen (13.2%).

The chemical profile of *A. blepharocalyx* essential oil in our study was dominated by β-Pinene (20.9%), 1,8-Cineol (20.8%) and α-Pinene (13.2%). This composition shows some similarities to a previous report on the same species from Vietnam by Hung *et al.* (2018), who also identified 1,8-Cineol and α-Pinene as major constituents, although in different proportions. However, our finding of β-Pinene as a primary component differs from their report, suggesting that factors such as geographical location, harvest season or the extraction method (MAHD vs. traditional hydrodistillation) could signi-ficantly influence the chemical chemotype of the essential oil.

CONCLUSION

This study successfully demonstrated the effectiveness of MAHD for extracting essential oil from *Alpinia blepharocalyx* leaves. By using response surface methodology, we were able to optimize the extraction process and identify the most favorable conditions for maximizing both essential oil yield and antioxidant activity. An optimal microwave power of 600 W was achieved, a liquid-to-material ratio of 7.0:1 mL/g and an extraction time of 45 min. These findings provide a robust scientific foundation for the efficient and high-quality production of essential oil from *A. blepharocalyx* on a larger scale.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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