ORIGINAL ARTICLE

# Optimization ultrasonic–microwave-assisted extraction of phenolic compounds from *Clinacanthus nutans* using response surface methodology

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## ABSTRACT

*Clinacanthus nutans (C. nutans)* is an edible profitable herb with high phenolic content that recognized herb relieves skin disorder, antityrosinase, and anticancer. Along with these health benefits *C. nutans*, however, there is no study on the factors that influence the phenolic content of C. nutans extraction by water-based ultrasonic-microwave-assisted extraction (UMAE). The aim of this study evaluates UMAE conditions (ultrasonic power, microwave power, and extraction time) on responses using response surface Box–Behnken design and compared with the hydrothermal extraction. The findings found that the caffeic acid and ferulic acid content decrease with increasing the microwave power and long extraction time ($P<0.05$). The combination factors significant impact on the phenolic compound are microwave power with a time of extract and ultrasonic with microwave power ($P<0.05$). The optimization UMAE of *C. nutans* was ultrasonic power 150 W, microwave power 50 W, and time of extraction 3 min ($P < 0.05$), and final temperature after extraction should be <60°C. UMAE was a four-fold greater target response and a sixty-fold lower extraction time compared to conventional hydrothermal extraction. The synergistic of ultrasonic and microwave power encourages extraction efficiency, which is advantageous to prepare the high-quality *C. nutans* extracted raw materials to apply in the nutraceutical, pharmaceutical, and cosmetic industry.

**Key words:** Box–Behnken design, design of experiments, microwave-assisted extraction, polyphenols, ultrasonic-assisted extraction

## INTRODUCTION

*Clinacanthus nutans (C. nutans)* are included in Thai National List of Essential Medicine (NLEM) because of *C. nutans* have a high efficacy and cost-effectiveness for skin disorder disease.[1] Phenolic compounds normally found in *C. nutans* that they have benefit health effect like antioxidants, anti-inflammatory, antibacterial, antiviral, antihyperlipidemic, antidiabetic and anticancer.[2] Polyphenols are classified to be nutraceutical that shows the biological benefit effects and disease prevention.[3] Many studies have investigated the type and content of phenolic compounds in *C. nutans* which are powerful for disease prevention. As total phenolic content (TPC), caffeic acid, ferulic acid, and gallic acid.

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Phenolic compounds are predominant in antioxidants.[4,5] Thus, *C. nutans* with high phenolic contents can decrease oxidative stress-related chronic diseases such as cancer cardiovascular disease, diabetes, and other noncommunicable chronic diseases.[6] The acceptance of cunsumers on the *C. nutans* can be seen by the variety of commercialized *C. nutans* extract on health and wellness products including teas, soap, cream, solution and product in cosmetic fields.[7] The high yield of active contents from *C. nutans* extracted could improve the efficacy and bioavailability of finished products. Therefore, the technique of extraction is extremely influential to *C. nutans* value added.

In a previous study, the authors reported that the highest TPC of *C. nutans* was 640 mg GAE/100 g DM by MAE 300 W, 81°C for 3.6 min in 50% ethanol, and the results found that the pretreatment by stirring for 3 min to make sure the sample was immersed in the solvent that can increase the polyphenol content.[2] It is noteworthy that MAE showed the potential to reduce extraction time while UAE gave the longer extraction time and higher polyphenol content compared to MAE by 7 and 2 folds, respectively. The best conditions of C. nutans water extract by MAE were microwave power 90 W and extraction time at 75 s to reach the highest polyphenol content at 8.9 mg/d. Although, this study lack results on the optimize of ultrasonic power, extraction temperature, and other methods of desired active compound determination.[7] For the present study design the systematic experiment using design, the systematic experiment using response surface response surface Box Behnken (BB) to optimize conditions parameters of extraction in water-based UMAE for the highest amount of phenolics compounds from *C. nutans* and compared with the conventional hydrothermal extraction. *C. nutans* extract with the high active compound using BBD is accurate and reliable results that make a valuable raw material and easy to apply in pharmaceuticals, dietary supplements, and plant-based cosmetics industrial scale.

## MATERIALS AND METHODS

### Plant material preparation

*C. nutans* fresh leaves were collected from Thai Traditional Medicine College Organic Botanical Garden, Pathum Thani, Thailand. Voucher specimens BK No. 070077 were prepared and authenticated by botanists from the Department of Agriculture, Thailand. *C. nutans* leaves aged 6–8 months were selected before extraction because that period represents the highest phytochemical contents.[1] The samples were dried in an oven at 40°C for 24 h and then ground and sieved through a sieve no. 80. The moisture content determination for a screen the raw material for further experiment.

### Experimental design and process by ultrasonic–microwave-assisted extraction

The extraction of *C. nutans* by combined ultrasonic and microwave had designed by response surface Box-Behnken design (BBD). BBD statistically uses for optimizing processes. The experiment has designed in three independent variables (X) and three levels (total experiment 30 runs) [Table1]. The target response of extraction was optimized by the maximized % yield of crude extract and phenolic content and minimized extraction temperature. *C. nutans* powder 10 g mixed with distilled water 200 g (1:20 w/w) in a four-neck round-bottom flask, then after stirring by magnetic stirrer (IKA MAG HS7) for10 min (pretreatment). Uwave-1000 extraction reactor machine (Sineo Microwave Chemistry Technology Co. Ltd, China; the maximum capacity microwave-power of 1000W at a frequency of 2450 MHz, and an ultrasonic-power of 800W at a frequency 26-28 KHz) was setting in the power-time model according to Table 2. For conventional hydrothermal extraction, the same ratio sample solution was boiled for 3 h. Sample solutions were centrifuged at 1500 rpm for 15 min, then collected supernatant (triplicates), and prepared powder by freeze-drying for further analysis.

### Determination of total phenolic content

The total phenolic content (TPC) determination by Folin-

**Table 1: Design of experiment, independent variables, and three levels employed in the optimization process**

| Independent variables | Code | Level | | |
|---|---|---|---|---|
| | | – l (low) | 0 (center) | l (high) |
| Ultrasonic power (W) | X1 | 0 | 75 | 150 |
| Microwave power (W) | X2 | 50 | 150 | 250 |
| Extraction time (min) | X3 | 3 | 6.5 | 10 |

**Table 2: Experimental design by Minitab software version 17**

| Runs | Independent variables | | |
|---|---|---|---|
| | Ultrasonic power (W) $X_1$ | Microwave power (W) $X_2$ | Extraction time (min) $X_3$ |
| 1/16 | 0 | 50 | 6.5 |
| 2/17 | 150 | 50 | 6.5 |
| 3/18 | 0 | 250 | 6.5 |
| 4/19 | 150 | 250 | 6.5 |
| 5/20 | 0 | 150 | 3 |
| 6/21 | 150 | 150 | 3 |
| 7/22 | 0 | 150 | 10 |
| 8/23 | 150 | 150 | 10 |
| 9/24 | 75 | 50 | 3 |
| 10/25 | 75 | 250 | 3 |
| 11/26 | 75 | 50 | 10 |
| 12/27 | 75 | 250 | 10 |
| 13/28 | 75 | 150 | 6.5 |
| 14/29 | 75 | 150 | 6.5 |
| 15/30 | 75 | 150 | 6.5 |

Ciocalteu method. About 1 mL of leaf extract (10%) was added into the test tube containing 5 mL Folin–Ciocalteu reagent, and the mixture was left to stand for 3 min before mixing 4 mL of sodium carbonate (7.5% w/v). The Folin–Ciocalteu reagent and sodium carbonate were transferred into the aliquot using a 10-mL measuring pipet. The solutions were allowed to stand for 30 min in the dark at room temperature, and then, the absorbance was observed at 765 nm of absorbance single-beam UV-VIS spectrophotometer (SP-3000nano, Japan). All determination was conducted in triplicates (mg GAE/100 g DM). This protocol was followed with some modifications.[8]

### Analysis of selected phenolic compounds

The content of desired active constituents of *C. nutans* leaf extracts was performed using an Agilent HPLC 1200 system pump linked with a diode array detector (California, USA). The separation of phenolic compounds was used ZORBAX Eclipse Plus C18 Column (100 mm × 4.6 mm i.d., particle size 5 µm). The mobile-phase solvents were composed of (A) 1% glacial acetic acid and (B) acetonitrile with gradient elution. A flow rate of 0.8 mL/min was used, and 20 µL of the sample was injected. The solutions were scanned with a UV detector at wavelength 280–360 nm. Analyses were performed in quintuplicate.[9]

### Statistical analysis

Data were designed and analyzed with the design of experiment using Minitab 18 statistical software (Minitab Inc., State College, Pennsylvania, USA). RSM was used to analyze the optimum UMAE condition, when the coefficient of determination ($R^2$ adjusted value) was used to consider the fitness of the polynomial model equation, where the significance was determined when the $P < 0.05$.

## RESULTS AND DISCUSSION

### The effect of independent variables on the response variables

The moisture content of drying the ground *C. nutans* was 11.10%. The results show in Table 3 indicate that when increasing microwave power and extraction time result in statistically significant increased temperature of extraction and decreased % crude extract, and desired active constituents (caffeic acid, ferulic acid). An explanation is related to the previous study. When increasing microwave power with increasing extraction temperature, that was resulted to destroy the thermal sensible active compounds such as caffeic acid, ferulic acid.[10,11]

### The effect of independent variables on target response

The results from BBD shown the power of determination $R^2$ (adj) 86.5%, lack-of-fit >0.05, meaning that the experimental data were acceptable and reliable.[12] BBD analyzed the optimized conditions by maximizing the water-based extraction of TPC, gallic acid, caffeic acid, and ferulic acid of the extracts obtained. The target response in this study was given in equation[1] classified by maximizing the % extracted yield, the maximum yield of phenolic compounds, and minimizing the temperature of extraction temperature [Table 3]. The prolonged extraction time with extraction temperature higher than 55°C will degrade the molecular structure of bioactive substances, especially TPC, flavonoid, antioxidant activity, and left toxic compounds.[8]

$$\text{The target response equation } (Y_7) = 3089 + 2.151X_1 + 0.018X_2 - 30.5X_3 - 0.00472X_1 \times X_1 + 0.00169X_2 \times X_2 + 1.52X_3 \times X_3 - 0.00681X_1 \times X_2 - 0.0848x_1 \times X_3 - 0.1932x_2 \times X_3$$

where $X_1$ = Ultrasonic power, $X_2$ = Microwave power, and $X_3$ = Extraction time (1)

The results implied the significance of ultrasonic power ($P < 0.05$) on the target response. Besides, when ultrasonic power increase resulted in increasing active compounds content. A possible explanation for this might be that the energy from ultrasonic promote the solvent permeates to plant cell walls, then they are rupture and release bioactive solute to solvent.[11] The relation was on the contrary. The two factors interaction effect of microwave power and extraction time, ultrasound and microwave power were significantly impacted to target response ($P<0.05$) and suggest in Figure 1a-c. The optimized extraction temperature should be <60°C and a short extraction period of only 3 min. The finding confirms that the phenolic compound yields increase with decreasing the time of extraction.[13] High temperature and a long extraction time can be enhancing heat/mass transfer with microwave power, resulting in rapid plant cell wall rupture to increase the targets active yields in vice versa this conditions in thermal degradation bioactive, and when using higher microwave power with excessive temperature can be leak impurities into the solvent.[9] Longer extraction time plus high temperature during extraction could degrade phenolic compounds, especially gallic acid.[14]

### Optimization of the ultrasonic power, microwave power, and extraction time

The optimized method of *C. nutans* extraction is ultrasonic 150 W, microwave 50 W, and 3 min (95% confidence interval: 3008.1–3221.3; $P < 0.05$). The optimization reaches the highest phenolic content and lowest extraction temperature through UMAE of *C. nutans* [Figure 2]. The percentage of extracted yield significantly decrease with increasing microwave energy and extraction time ($P<0.05$). These results consistently with Yu *et al*., 2017, reported that extraction of *C. nutans* with a microwave power of more than 80 W can cause an increase to destroy the plant cell wall and bioactive compounds.[15] Noteworthy results were synergistic of the ultrasonic- and microwave-assisted method to *C. nutans* extraction that complied with previous research.[16] This study confirmed that the synergistic effect

**Table 3: Response variables of the Clinacanthus nutans leaf extract from ultrasonic-microwave-assisted extraction and conventional hydrothermal extraction**

| Run | Response variables (Y) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Final temperature (°C) $Y_1$ | Crude extract (%) $Y_2$ | TPC (mg GAE/100 g DM) $Y_3$ | Gallic acid (mg/g DM) $Y_4$ | Caffeic acid (mg/g DM) $Y_5$ | Ferulic acid (mg/g DM) $Y_6$ | Target $Y_7$ |
| 1 | 64 | 1375 | 1202.08 | 6.25±4.59 | 217.04±1.75 | 7.89±2.36 | 3946.34 |
| 2 | 60 | 1594 | 1237.45 | 15.10±1.35 | 205.68±5.74 | 14.64±0.63 | 4244.32 |
| 3 | 90 | 1448 | 1135.46 | 11.90±1.22 | 197.71±4.74 | 15.09±0.46 | 3853.62 |
| 4 | 86 | 1375 | 1183.98 | 8.12±6.17 | 209.89±5.49 | 8.83±0.09 | 3883.80 |
| 5 | 65 | 1584 | 1238.21 | 11.65±0.65 | 203.03±5.68 | 7.74±1.21 | 4217.84 |
| 6 | 57 | 1510 | 1270.77 | 5.82±1.28 | 209.44±4.59 | 8.68±1.02 | 4218.47 |
| 7 | 90 | 1343 | 1162.96 | 12.88±5.39 | 212.24±4.34 | 3.66±0.09 | 3807.70 |
| 8 | 88 | 1335 | 1093.18 | 13.49±5.02 | 209.58±3.23 | 4.19±1.66 | 3660.61 |
| 9 | 40 | 1628 | 1284.92 | 13.25±0.11 | 210.68±2.10 | 9.46±0.09 | 4391.23 |
| 10 | 64 | 1569 | 1209.60 | 4.61±0.46 | 218.56±4.87 | 9.58±0.22 | 4156.96 |
| 11 | 64 | 1546 | 1169.40 | 4.49±0.09 | 208.91±1.69 | 8.95±0.231 | 4043.15 |
| 12 | 92 | 1216 | 1119.94 | 15.18±3.71 | 212.63±5.57 | 3.49±0.16 | 3595.18 |
| 13 | 86 | 1487 | 1133.08 | 14.89±0.01 | 211.14±2.22 | 9.35±0.90 | 3902.54 |
| 14 | 87 | 1503 | 1193.28 | 14.29±3.82 | 220.58±3.98 | 10.22±1.26 | 4047.65 |
| 15 | 84 | 1487 | 1155.72 | 16.73±1.24 | 224.58±3.47 | 10.95±0.39 | 3966.69 |
| 16 | 57 | 1557 | 1250.53 | 5.79±2.0 | 212.16±6.03 | 8.09±2.25 | 4227.10 |
| 17 | 62 | 1588 | 1204.00 | 13.12±3.93 | 211.77±4.62 | 9.72±0.18 | 4138.62 |
| 18 | 92 | 1508 | 1162.59 | 14.96±1.32 | 197.19±7.80 | 11.33±3.76 | 3964.66 |
| 19 | 92 | 1396 | 1131.97 | 7.03±0.14 | 183.47±6.91 | 5.89±0.45 | 3764.33 |
| 20 | 64 | 1627 | 1173.25 | 14.59±0.55 | 194.80±4.60 | 6.98±1.51 | 4125.86 |
| 21 | 59 | 1577 | 1227.74 | 7.88±0.99 | 219.47±8.02 | 6.31±0.46 | 4207.14 |
| 22 | 95 | 1422 | 1237.81 | 12.01±0.71 | 198.60±3.51 | 6.02±0.19 | 4019.25 |
| 23 | 93 | 1430 | 1128.13 | 14.30±1.00 | 191.01±1.28 | 5.16±2.13 | 3803.72 |
| 24 | 37 | 1622 | 1302.16 | 11.74±1.40 | 213.15±3.30 | 8.39±3.20 | 4422.59 |
| 25 | 65 | 1587 | 1250.99 | 4.47±0.06 | 177.37±3.80 | 5.66±3.32 | 4211.49 |
| 26 | 63 | 1555 | 1170.94 | 6.41±9.31 | 250.94±6.34 | 6.52±5.87 | 4097.75 |
| 27 | 92 | 1216 | 1142.39 | 12.71±0.33 | 181.89±1.42 | 4.43±0.09 | 3607.81 |
| 28 | 80 | 1471 | 1214.00 | 12.27±1.36 | 192.29±2.85 | 4.46±0.25 | 4028.02 |
| 29 | 84 | 1510 | 1200.65 | 15.83±0.57 | 230.23±8.95 | 9.16±4.09 | 4082.52 |
| 30 | 83 | 1459 | 1197.95 | 16.29±1.33 | 213.14±0.18 | 9.99±1.60 | 4011.32 |
| **Conventional hydrothermal extraction** | | | | | | | |
| 1 | 100 | 380 | 428.58 | ND | 140.848±9.438 | 2.323±0.558 | 850.9 |

*ND: Non detect data, TPC: Total phenolic content, DM: Dry matter, GAE: Gallic acid equivalent

of UMAE through ultrasonic can speedily disintegrate cell wall and release polyphenol compounds.[7] However, the high power of microwave and long extraction time will be increases temperature, which has to avoid in thermal sensitivity active extract. The interaction effect of ultrasonic and microwave power base on phenolic contents was significant ($P < 0.05$).[17]

### Ultrasonic–microwave-assisted extraction and conventional hydrothermal extraction compared

UMAE demonstrates the highest all targeted phenolic compounds over the conventional hydrothermal extraction. UMAE was a four-fold greater target response compared to conventional hydrothermal extraction, while the time of extraction was around 60 times less than the time used by conventional from 180 to 3 min. This study affirms the previous study that UMAE indicates much higher phenolics than the classical extraction method.[18] The synergistic application of ultrasound and microwave power encourages extraction efficiency by extraction time saving with increased recovery at milder temperatures, extraction yield and desired phenolic compounds increasing green environment by water-based solvent extraction, solvent usage, and decrease raw material volume.

## CONCLUSION

UMAE produces the benefit of each extraction method. Microwave creates a short extraction time, while ultrasonic

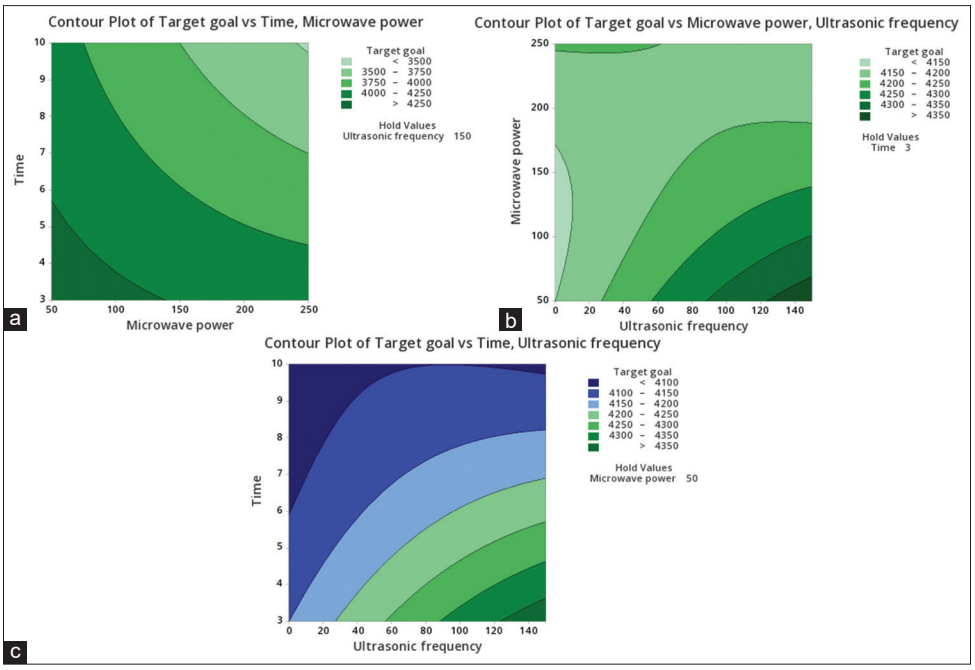


**Figure 1: (a-c)** Contour (holding value: Ultrasonic 150 W, microwave 50 W, and 3 min of extraction)

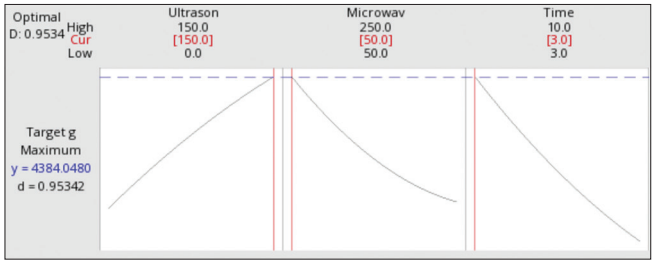


**Figure 2:** Response optimization for the target response

decreases the extraction temperature and effect to reach higher TPC content. The optimal conditions for UMAE of *C. nutans* are 150 W of ultrasonic, 50 W of microwave power, and 3 min of extraction period. The overall extraction efficiency by UMAE was higher than conventional or their individual when used separately. The results from the design of the experiment are obvious to apply in the pharmaceutical, cosmetic, and nutraceutical industry. However, future research should use gas chromatography–mass spectrometry analysis to confirm and expand the type of active compound in extracted *C. nutans*.

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## Conflicts of interest

There are no conflicts of interest.

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