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บทความวิจัย

Optimization, Bioactivity and Composition of *Curcama aromatica* Salisb. Extraction Oils Extracted by Microwave-assisted and Hydro-distillation

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Abstract: *Curcama aromatica* Salisb. (*C.aromatica*) rhizomes were extracted by hydro-distillation (HD) and microwave-assisted extraction (MAE). The response surface method (RSM) and analysis of variance (ANOVA) were carried out to optimize the statistical data of MAE. The extraction oils (Eos) were analyzed by the compositions by GC/MS and were compared to the antioxidant activity, total phenolic contents (TPC), and total flavonoid contents (TFC). The highest yields (dry weight basis) of HD and MAE were 1.96% and 2.22% (w/w) respectively. From the optimization result, the highest yield for MAE was 2.33% at 795 W power, time 44 mins, and a solid/liquid ratio of 100 g/583 ml. The compositions detected from HD and MAE were 41 and 45 contents respectively. Cedrene and copaene were the major compositions, detected in the extraction oils. Cedrene was detected in HD (25.54%) and MAE (23.69%). Copaene was detected in HD (22.97%) and MAE (24.71 %). MAE presented DPPH (%inhibition) at 82.68 ± 0.31 %, TPC at 4227.62 ± 28.72 (mgGAE/g Dry weight), and TFC at 3.92 ± 28.72 (mgRE/g Dry weight) higher than HD. The extraction oils obtained in this study contained many potential compositions, and MAE was an effective method to extract the essential oil from *C.aromatica*.

Keywords: Cedrene; copaene; response surface method; isolation oil; antioxidant

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1. Introduction

Curcuma aromatica Salisb. (*C.aromatica*) is an aromatic medicinal herb that belongs to the Zingibereceae family. *C.aromatica* (wild turmeric) is widely cultivated in many countries, such as India, China, Bangladesh, Australia, and a few countries in Southeast Asia (e.g., Thailand and Myanmar) [1]. Many parts of *C.aromatica* such as leaves, flowers, and rhizomes are commonly used as folk and modern medicines due to their pharmacology, antioxidant, and bioactivity [2, 3]. However, the extraction oils (EOs) extracted from *C.aromatica* rhizomes, which were cultivated in different areas, were identified with different major compositions [4]. Xanthorrhizol, camphor, curdione, and curcumene were the typical major compositions detected in the EOs obtained from *C.aromatica* rhizomes grown in India, Thailand, and China [3, 5-8].

There are many methods used to extract *C.aromatica* rhizomes for their compositions and bioactivities benefits. Hydro-distillation (HD) and heat refluxing were typical methods of extracting the EOs from *C.aromatica* rhizomes. HD is one of the conventional methods to extract EOs from plants. Compositions among forty-four contents were identified in the EOs obtained from HD [3]. Heat refluxing with ethanol is used to extract the EO from *C.aromatica* fresh rhizomes. *C.aromatica* presented a very high percent inhibition (% inhibition) compared with other Curcuma species [8]. *C.aromatica* rhizomes obtained from different areas in China were also extracted by steam distillation (SD) to investigate their compositions and bioactivities [9]. The different compositions among seventy-eight contents were identified in the EOs obtained from *C.aromatica*, grown in different areas. The EO yields and results obtained from SD were around 3.12% to 4.23%.

Microwave-assisted extraction (MAE) is also known as one of the green extraction methods using less power, time, and solvent [10]. During extraction with microwave irradiation, the temperature of the plant sample and solvent increased due to the dipole rotation and ionic conduction [11]. Therefore, the electric field changes over time, and the polar molecules of the samples are reoriented by repeating their orientation in a field polarity. The process variables of MAE have been studied in many reports to investigate the EO yields and compositions [12]. Different combinations of variables in MAE, such as power, time, and a solid/liquid ratio, produced different results of the EO yields, composition contents, and bioactivities [13, 14]. The plant samples extracted by MAE reached the boiling temperature faster than conventional methods due to the heat generated by microwave irradiation. Consequently, the

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sample degradation due to a very high temperature was less than a conventional method. Furthermore, the microstructures of plant samples were more ruptured and damaged. It caused a higher rate of isolation oil released from the oil glands when compared with a conventional method.

The antioxidants and bioactivities of the EOs from plant samples were studied in the reports as well. *C.aromatica* fresh and dry rhizomes were extracted to compare the EO. From the previous report, the antioxidant activity results (DPPH/inhibitory concentration at 50%, IC_{50}) of the EOs from *C.aromatica* rhizomes obtained from different areas (e.g., India, Thailand, and China) were in a range of 3.5-10.03 mg/mL; meanwhile, the antioxidant activity results (DPPH/% inhibition) was at 62 % [15]. The phenolic contents and the antioxidant activity results of the EOs obtained from Curcuma species growth in Thailand (17 species) were also studied [16]. The total phenolic content and the antioxidant activity of the EO from *C.aromatica* were 11.0 ± 0.2 (mg GAE/g Dry Weight) and 20.8 ± 0.1 (% inhibition), respectively. The relationship between the radical scavenging assay results and the total phenolic contents of Curcuma species was not necessarily correlated. The total flavonoid content was investigated in the EO obtained from *C.aromatica* fermented with a common fungus (*Rhizopus oligosporus*) [17]. The fermented *C.aromatica* showed higher antioxidant activities and flavonoid contents than a conventional method. The interaction between a fungus and *C.aromatica* in a fermentation process increased the number of enzymes and lead to more ruptures in the microstructure of the plant sample.

In the underlying study, the *C.aromatica* rhizomes were extracted to obtain the EOs. Resulted yields, chemical compositions, and bioactivities of the EOs obtained by HD and MAE were used to compare in this study. The response surface method (RSM) based on Box-Behnken Design (BBD) was carried out to illustrate the relationship between the process variables of MAE. The statistical data was analyzed using analysis of variance (ANOVA). The chemical compositions of EOs from HD and MAE were analyzed by gas chromatography mass-spectrometer (GC/MS). The DPPH radical scavenging assay, the total phenol content (TPC), and the flavonoid content (TFC) for EOs obtained by HD and MAE, and used in this study.

2. Materials and methods

2.1 Plant sample preparation

The *C.aromatica* fresh rhizomes (5000 g) were collected and purchased from the local herbs market (Prachinburi, Thailand). The fresh *C.aromatica* rhizomes were cut into small pieces using a cutting machine and dried in a hot air oven at 65 °C for 5 hrs to reduce moisture. The dried

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rhizomes were grounded via a blade grinder and sieved through a standard 50-mesh size screen. The voucher specimen (code: SL-NC209) was kept at Laboratory for Medicinal Cannabis Research, Kamphaeng Saen, Nakhon Pathom, Thailand. In this study, distilled water was used as a non-chemical reagent in the extraction methods. A Clevenger-type apparatus (1000 mL) round bottom flask was used in this study.

2.2 The extraction oil (EO) yield of HD and MAE

The *C.aromatica* dried powder (100 g) was prepared in each experiment. The weighted *C.aromatica* powder was mixed with distilled water, followed by the experimental design based on BBD in Table 2. for MAE and 600 mL for HD. The EOs derived by HD and MAE were separated from the hydrosol. weighted, and calculated EO yield using the equation (1).

$$\text{Yield \% (w/w)} = \frac{\text{weight of EO (g)}}{\text{weight of sample (g)}} \times 100\% \quad (1)$$

2.3 Hydro distillation (HD)

A Clevenger-types apparatus (1000 mL) with an adjustable temperature heating mantle was prepared for HD. A solid/liquid ratio for HD was constant at 100 g/600 mL. The cThe clevenger apparatus condensing unit was connected to a water-cooled chiller. The water temperature of a chiller was set at 8.0 °C, meanwhile, a heating mantle temperature was set at 120 °C. The extraction time of HD was set at 6.0 hrs. The extraction oil yield results for the HD were performed in triplicate and used the average data values.

2.4 Microwave-assisted extraction (MAE)

The MAE apparatus as shown in Fig. 1 was modified from a microwave oven (Model: ER-SM20(W), Toshiba, Thailand). The MAE oven chamber was redesigned from the original design to increase the chamber capacity from 20 to 35 liters. The new chamber was fabricated from a stainless-steel sheet (grade: SUS304, a thickness of 1.5 mm). A magnetron and other electrical components were reassembled in the new chamber. For operational safety, the irradiation leakage was measured by a microwave leak detector (Model: EMF300), EXTECH instruments, Thailand. The temperature setting of MAE was controlled in the same manner as HD. The process parameters of MAE followed the experimental design in Table 1.

Table 1 Independent variables and coded setting levels of MAE

Independent variables	Code setting levels		
	-1	0	1
Microwave power (W) (X1)	400	600	800
Microwave time (min) (X2)	15	30	45
Solid/liquid ratio (g/mL) (X3)	1:4	1:5	1:6

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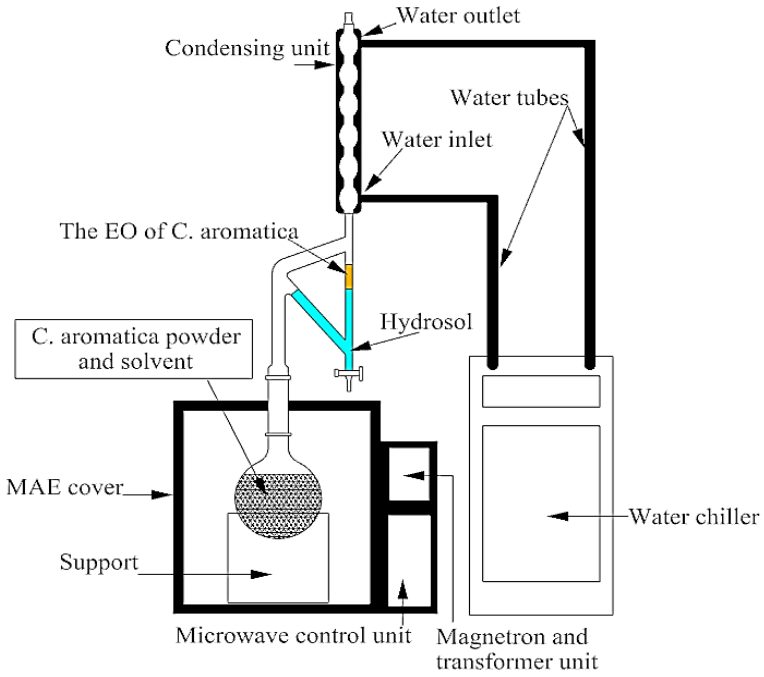

Fig. 1 MAE apparatus and control unit

2.5 Yield optimization for MAE

The response surface methodology (RSM) based on Box-Behnken Design (BBD) was performed in the experimental design for MAE. The BBD with three numeric parameters at three levels (-1, 0, 1) was used to determine the number of experimental and process variables of MAE. Three main independent parameters consisted of power (400, 600, and 800 W), time (20, 30, and 40 min), and solid/liquid ratio (1:4, 1:5, and 1:6 g/mL). The actual parameters and values are shown in Table 1. The total experiment number of MAE was fifteen experiments, including the center points (three replicated) shown in Table 2. The second-order polynomial and quadratic model equation were carried out to indicate the correlation between MAE process variables and the EO of *C.aromatica*. The quadratic model equation of MAE is expressed in equation (2).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_j + \sum_{i=1}^{k} \beta_i X_j + \sum_{i=1}^{k-1} \sum_{j>1}^{k} \beta_{ij} X_i X_j \quad (2)$$

Where Y is the dependent variable, β_0, β_i, β_{ii} and β_{ij} are the regression coefficients of the intercept, linear, quadratic, and interaction between variables respectively, meanwhile, and are the independent variables.

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Table 2 Experimental design with BBD and the response values

No.	Code setting level			Actual setting level			%Yield	
	X1	X2	X3	X1 (W)	X2 (min)	X3 (g/mL)	Predicted	Actual
1	0	0	0	600	30	1:5	1.27	1.3
2	1	-1	0	800	15	1:5	1.26	1.22
3	1	0	-1	800	30	1:4	2	1.97
4	0	1	1	600	45	1:6	1.96	1.89
5	1	0	1	800	30	1:6	1.94	1.93
6	-1	0	-1	400	30	1:4	0.41	0.42
7	-1	-1	0	400	15	1:5	0.39	0.38
8	0	-1	-1	600	15	1:4	0.63	0.67
9	0	0	0	600	30	1:5	1.27	1.23
10	-1	1	0	400	45	1:5	1.09	1.12
11	1	1	0	800	45	1:5	2.14	2.22
12	0	-1	1	600	15	1:6	0.6	0.63
13	0	1	-1	600	45	1:4	0.73	0.69
14	0	0	0	600	30	1:5	1.27	1.29
15	-1	0	1	400	30	1:6	1.69	1.72

The experiment was repeated in triplicate and the value data was expressed as the means ± standard deviation (SD). The correlations between the response value and process variables were calculated using computer software, and shown as 2D contours and 3D surface plots.

2.6 Chemical compositions analysis

The compositions of the EOs were analyzed by gas chromatography-mass spectrometer (GC/MS). Shimadzu model GCMS-QP2020 assembled with DB-WAX column (30 m × 0.25 mm × 0.25 μm, film thickness) and mass detector in the full scan mode was carried out to identify the compositions of EOs. Helium was used as a carrier gas, and it was set constant flow rate of 1.0 mL/min. The samples were diluted in dichloromethane (1μL) and were injected into an oven with a split ratio of 1:200. The temperature was set constant initially at 60 °C for 2.0 min, and it was increased continuously to 2 °C/min until it

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reached a maximum point at 250 °C for a 5- minute holding time. The MS quad was set a temperature at 140 °C with maximum temperature control of 190 °C, meanwhile, the MS source temperature was set at 220 °C with maximum temperature control of 250 °C. The full scan mode of MS was set to 40 to 350 m/z using an ionization mode (EI) with 70-eV. In each composition analysis, the total run time was approximately 65.0 min. The analysis data was kept and analyzed by the LabSolutions DB/CS software. The compositions of EOs were compared with the NIST17 M/S library by computer matching and were identified by their retention time (RT) relative to Kovat's index (DB-WAX column) in the literature data [6, 7, 17-18].

2.7 DPPH radical scavenging assay

The EOs mixed methanol (2 mg/mL) and butylated hydroxytoluene (BHT) mixed methanol (200 µg/mL) were prepared in this study. The sample and control solutions (50 µL) were prepared in a well plate and mixed with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) of 950 µL (0.1 mM in methanol). The sample solutions were incubated a room temperature (28 ± 2 °C) for 30 min. Spectrophotometer (ONILAB, model SP-UV1100) was used to measure the absorbance at 517 nm. The results were repeated in triplicates, and the value data was expressed as the mean ± SD. The results of percent inhibition were calculated followed by equation (3).

2.8 Total phenolic content determination

The total phenolic content of EOs was determined using a Follin-Clocalteu method. The sample solution (250 µg/mL) and gallic acid solution (10-100 µg/mL) of 0.2 mL were dissolved with the Follin-Clocalteu reagent (0.5 mL) with a concentration of 1:10 in deionized water and sodium bicarbonate solution (0.8 mL) with 7.5 % w/v. The sample solution was incubated at room temperature (28 ± 2 °C) for 30 min. The spectrophotometer was used to measure the absorbance of the sample at 765 nm. The results were repeated in triplicates and expressed as milligrams of gallic acid equivalent in 1 g of the sample and dried powder (mgGAE/g).

2.9 Total flavonoid content determination

The total flavonoid content of EOs was investigated by the aluminum chloride assay. In brief, the sample solution (500 µL) was diluted in methanol (2.0 mL) and 10 % aluminum chloride (0.1 mL). Sodium acetate (0.1 mL) was added to the prepared sample solution. Distilled water (5 mL) was added into the prepared sample solution to increase volume, shacked, and incubated in

$$\%Inhibition = \left[\frac{A_0 - A_1}{A_0} \right] \times 100 \qquad (3)$$

Where A_0 and A_1 are the absorbances of a control and sample, respectively.

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room temperature for 30 min. Rutin was used as a control, and the absorbance was measured at 415 nm. The results were carried out in triplicates and expressed in terms of rutin equivalents (mgRE/g).

3. Results and discussion

3.1 The EOs comparison between HD and MAE

The color of the EO obtained from HD provided a lighter clear yellowish than MAE. The highest yields of HD and MAE were 1.96 ± 0.06 % and 2.22 ± 0.09 %, respectively. The highest yield of MAE was obtained from experimental run no. 11 of power (800 W), time (45 min), and a solid/liquid ratio (100 g/500 mL) as shown in Table 2. Meanwhile, the lowest yield of MAE was 0.42 % which was obtained from run no. 6 of power (400 W), time (30 min), and a solid/liquid ratio (100 g/400 mL). The increasing power affected the results of the EO yield of *C.aromatica*. At a lower microwave power, the temperature of the immersed sample was heated up and reached the boiling point slower than at higher power. Due to the heat generation mechanism of microwave irradiation, the microstructures of *C.aromatica* were disrupted by the electromagnetic field change. Therefore, the cell walls of oil glands were ruptured and leading to the release of EOs. The extraction time of MAE also affected the EO yield of *C.aromatica*.

A longer extraction time and a higher solid/liquid ratio presented a better result of the EOs than the lower. A longer extraction time affected the penetration rate of the solvent to take place in isolation oil microstructures, meanwhile, a higher solid/liquid ratio affected the viscosity rate of a solvent. The temperature of immersed *C. aromatica* and the solvent was increased immediately by the heat generation mechanism of microwave irradiation as described previously [10]. Hence, the temperature of MAE reached boiling point faster than the heat convection mechanism from HD. However, the highest amount of solid/liquid ratio in this study showed a slight decrease in the EO yield. A higher solid/liquid ratio takes a longer time to reach a boiling point than a lower ratio. Therefore, the highest yield was obtained from a solid/liquid ratio at 100g/500 mL.

3.2 Statistical data analysis and optimization

From the ANOVA results in Table 3, the model of RSM presented a statistically significant ($p < 0.0001$) to the independent variables (power, time, and solid/liquid ratio). The value of the determination coefficient (R^2) and the adjusted determination coefficient (R^2_{Adj}) were 0.993 and 0.980, respectively. It was indicated that the model of the second-order response surface model was appropriate with the predicted data

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and actual experimental. Furthermore, the lack of fit test also showed a non-significant value relative to the pure error (p > 0.05) at 0.115. Linear (X_1, X_2, and X_3) and interaction (X_1X_3 and X_2X_3) of process variables which were power, time, and solid/liquid ratio showed a highly significant (p < 0.0001) to the response value. Meanwhile, the interaction between power and time (X_1X_2) and the quadratic solid/liquid ratio (X_3^2) showed a non-significant value. The predicted value and actual data are shown in Table 2. The correlation between process variables of MAE is shown in Fig 2. The temperature of immersed *C.aromatica*. was increased as the result of dipole rotation and ionic conduction [12, 14]. The immersed *C.aromatica* at a higher power could reach a boiling point faster. Meanwhile, increasing the extraction time affected the thermal accumulation between the immersed sample and solvent. Moreover, a more prolonged extraction time provided a better result for a continuous process of solvent vapor to take the EO from a heating-immersed sample to a condensing unit. Hence, the highest yield was obtained by the highest power and time, as shown in Fig 2 (a). The EO yield results from the interaction between a power and a solid/liquid ratio are shown in Fig 2 (b). The EO yield of *C.aromatica* increased by increasing power and solid/liquid ratio. The amount of solvent affected the efficiency of the solvent flow rate and a viscosity rate of the immersed sample. Meanwhile, in the interaction between time and solid/liquid ratio shown in Fig. 2 (c), the highest yield was found at a higher solid/liquid ratio and time. Microwave irradiation increased the sample temperature from room to a boiling point. A longer time showed a better result in the solvent performance taking the EO from immersed sample to a condensing unit. A higher amount of solvent required a higher power and a longer time to reach a boiling point. However, a higher amount of solvent improved the solubility rate between the EO and solvent. Therefore, the EO yield increased as the time and solid/liquid ratio increased. For the optimal result, the highest yield obtained from MAE was obtained at 2.33% of microwave power (795 W), time (44.0 min), and a solid/liquid ratio (100 g/583 mL). The model regression equation of MAE in this study is shown in equation (4).

$$Yield = 1.27 + 0.4606X_1 + 0.3767X_2 + 0.3041X_3 + 0.0635X_1X_2 - 0.3347X_1X_3 + 0.3130X_2X_3 + 0.2525X_1^2 - 0.2878X_2^2 - 0.0115X_3^2 \quad (4)$$

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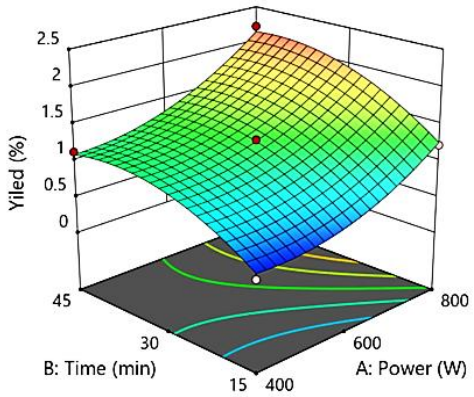
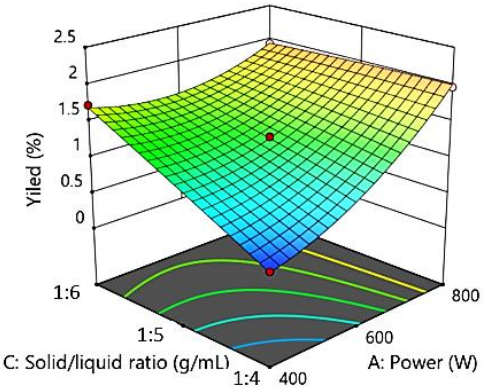
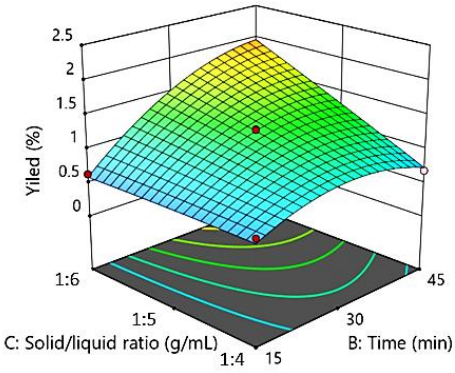


(a) Graph plot between yield, power and time



(b) Graph plot between yield, power and solid/liquid ratio



(c) Graph plot between yield, time and solid/liquid ratio

Fig. 2 3D surface plots and 2D contours of process variables and response value

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Table 3 Chemical compositions of *C.aromatica* extracted by HD and MAE

S.No	RT	Composition contents	HD	MAE	RI a	RI b
1	3.55	α-Pinene	0.22	0.08	940	943
2	4.54	β-Pinene	0.41	0.69	962	971
3	5.75	D-limonene	0.97	0.72	1015	1018
4	5.92	Eucalyptol	0.20	tr	1058	1059
5	8.42	Ethylcinnamate	0.18	0.65	1367	1367
6	9.13	Cedrene	**25.54**	**23.69**	1394	1399
7	10.72	Sesquithujene	2.15	1.88	1563	1560
8	11.46	Chavibetol	1.99	1.06	1566	1392
9	11.58	4-(p-Tolyl) pentanal	-	0.19	1410	1406
10	12.74	Caryophyllene	0.75	0.34	1583	1587
11	12.95	Copaene	**22.97**	**24.71**	1591	1475
12	13.21	Curcumene	2.07	2.16	1516	1503
13	14.06	Citrylideneacetone	0.24	0.23	1532	1511
14	14.86	(Z)-beta-Sesquisabinene hydrate	1.79	1.64	1538	1523
15	15.22	Camphor	4.17	3.60	1540	1535
16	15.37	ar-Turmerol	0.39	0.38	1551	1511
17	16.02	(E)-β-elemene	0.74	0.66	1623	1384
18	17.14	Bergamotene	0.32	0.55	1432	1430
19	19.38	Epicurzerenone	3.52	3.41	1580	1584
20	23.81	Atractylon	0.20	0.17	1654	1655
21	24.12	trans-β-Farnesene	3.73	2.91	1662	1661
22	24.68	Isoborneol	2.15	2.03	1669	1672
23	25.01	β-Humulene	-	1.26	1703	1675
24	25.74	α-Terpineol	0.19	0.11	1712	1680
25	27.16	α-Selinene	0.93	0.87	1719	1703
26	27.47	Zingiberene	-	0.40	1720	1728
27	27.93	β-(Z)-Curcumen-12-ol	0.60	0.65	1722	1730

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Table 3 continued

S.No	RT	Composition contents	HD	MAE	RI a	RI b
28	28.45	Xanthorrhizol	**10.40**	**13.97**	1744	1730
29	28.82	Ethyl p-methoxycinnamate	0.13	0.52	1556	1760
30	29.11	Selina-3,7(11)-diene	0.73	0.61	1507	1793
31	29.64	β-Germacrene	1.98	2.08	1603	1802
32	29.86	Cuparene	0.40	-	1822	1825
33	30.21	Curcumenone	0.14	0.16	1840	1844
34	30.67	Curzerene	1.31	1.36	1902	1898
35	32.42	Gerany-p-cymene	0.39	0.39	2006	1980
36	32.95	Zederone	0.14	0.18	2012	2009
37	33.57	Cinnamaldehyde	0.95	0.13	2025	2025
38	33.96	α-Nerolidol	0.17	0.18	2040	2042
39	34.08	Elemol	0.22	0.11	2055	2058
40	34.24	Globulol	0.29	tr	2080	2083
41	34.47	Cubenol	-	0.23	2085	2085
42	34.53	trans-β-elemenone	-	0.18	2092	2099
43	35.01	Geranyl-alpha-terpinene	0.12	0.22	2103	2142
44	35.11	Tumerone	1.18	0.56	2211	2245
45	36.29	Isospathulenol	0.81	0.80	2220	2222
46	35.23	Farnesol	-	0.15	2376	2378
Monoterpene (Sr. No. 1-4, 30, 32)			4.14	3.63		
Monoterpenoid (Sr. No. 15)			4.17	3.60		
Sesquiterpene (Sr. No. 6-7, 10-12, 21, 23, 25-29,31, 42)			71.25	75.44		
Sesquiterpenoid (Sr. No. 14, 16-17, 19-20, 30, 32-34,36, 38-40, 42, 44-46)			12.03	10.55		
Others (Sr. No. 5, 8-9, 13,18, 35, 37, 41, 43)			4.19	3.65		

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3.3 Composition analysis of the EOs between HD and MAE

The compositions were detected in the EOs from HD and MAE at 40 and 45, respectively. The total identified compositions obtained from HD and MAE were 95.78 % and 96.87 %, as shown in Table 3. The majorities of compositions detected in the EOs from HD and MAE were cedrene, copaene, and xanthorrizol. Cedrene at 25.54 % was the highest composition detected in HD; meanwhile, MAE was detected at 23.69 %. Copaene at 24.71 % was the highest composition detected in MAE; meanwhile, HD was detected at 22.97 %. Xanthorrizol, a sesquiterpene was detected in the EOs from HD at 10.40 %, with MAE at the higher rate of 13.97 %. Cubenol (0.23 %), zingiberene (0.40 %), farnesol (0.15 %), trans-β-element (0.18 %), 4-(p-Tolyl) pentanal (0.19 %) and β-humulene (1.26 %) were only found in the EO from MAE. Cuparene (0.40 %), sesquiterpenoid was only detected in the EO from HD. Camphor, equicurzerenone, curcumene, trans-β-farnesene, and β-germacrene were the minor composition contents detected in the EOs from HD and MAE.

3.4 Antioxidant activity, total phenolic contents (TPC), and total flavonoid contents (TFC)

In antioxidant activity, TPC and TFC results of the EOs were shown in Table 4. The percent inhibition (% inhibition, 50 mg/mL) of the EOs from HD and MAE were 81.21 ± 0.31 % and 82.68 ± 0.40 %, respectively. The antioxidant activity of HD and MAE was higher than that of a control (BHT) of 16.04 ± 1.63 %. The TPC results of the EO obtained from HD were 3975.04 ± 25.82 (mg GAE/g Dry weight), and MAE was 4227.62 ± 28.72 (mg GAE/g Dry weight). Meanwhile, the TFC result of the EO obtained from HD was higher than MAE. The EO from HD presented the TFC result at 3.92 ± 0.01 (mg RE/g Dry weight) and MAE was 3.70 ± 0.08 (mg RE/g Dry weight). In this study, MAE presented better results of the antioxidant activity and TPC than HD. Due to the composition contents of the EOs, the MAE showed higher composition numbers than HD. It revealed a positive correlation between the antioxidant activity and TPC of the EOs from HD and MAE. Meanwhile, TFC results showed a non-positive linear correlation between antioxidant activity and TPC.

4. Conclusion

Microwave-assisted extraction (MAE) and hydro-distillation (HD) were performed to extract the EOs from *C.aromatica* rhizomes. The relationships between process variables of MAE for the response value were analyzed by response surface method (RSM) and analysis of variance (ANOVA). MAE presented the advantages of a shorter extraction time, composition contents, antioxidant activity, and TPC. Meanwhile, HD

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presented a higher TFC result. The EOs obtained from *C.aromatica* contained various compositions. From the results, MAE and RSM were the potential methods to extract the EOs from *C.aromatica* and to optimize the process variables.

Table 4 ANOVA result of MAE

Source	Adj SS	DF	Adj MS	F-Value	P-Value	Significant
Model	5.02	9	0.5573	79.62	< 0.0001	significant
A-Power	1.7	1	1.7	242.51	< 0.0001	***
B-Time	1.14	1	1.14	162.23	< 0.0001	***
C-Solid/liquid ratio	0.7399	1	0.7399	105.72	0.0001	**
AB	0.0161	1	0.0161	2.3	0.1895	*
AC	0.4482	1	0.4482	64.04	0.0005	**
BC	0.3919	1	0.3919	55.99	0.0007	**
A^2	0.2353	1	0.2353	33.62	0.0021	**
B^2	0.3058	1	0.3058	43.69	0.0012	**
C^2	0.0005	1	0.0005	0.0703	0.8015	*
Residual	0.035	5	0.007			
Lack of Fit	0.0322	3	0.0107	7.82	0.1155	Non-significant
Pure Error	0.0027	2	0.0014			
Cor Total	5.05	14				

R^2 = 0.987, R^2_{Adj} = 0.964, ***($p < 0.001$); **($p < 0.01$); *($p < 0.05$)

Table 5 Bioactivity results of the EOs obtained from HD and MAE

Methods	Antioxidant activity (%inhibition) [1]	Total phenolic contents (mgGAE/g Dry weight)	Total flavonoid contents (mgRE/g Dry weight)
HD	81.21 ± 0.40	3975.04 ± 25.82	3.92 ± 0.01
MAE	82.68 ± 0.31	4227.62 ± 28.72	3.70 ± 0.08

[1] Antioxidant activity (%): final sample concentration = 50 mg/mL, BHT (%inhibition) = 16.04 ± 1.63

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