Arabian Journal of Chemistry (2021) **14**, 103328

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Cinnamomum camphora leaves as a source of proanthocyanidins separated using microwave-assisted extraction method and evaluation of their antioxidant activity *in vitro*

Zaizhi Liu [a,*], Hualan Li [a], Yanlong Qi [b], Zheng Zhu [a], Dai Huang [a], Ke Zhang [a], Jun Pan [a], Lan Wen [a], Zhengrong Zou [a,*]

[a] *College of Life Sciences, Jiangxi Normal University, Nanchang 330022, China*
[b] *Key Laboratory of High-Performance Synthetic Rubber and Its Composite Materials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Renmin Road, Changchun 130022, China*

Received 8 June 2021; accepted 9 July 2021
Available online 17 July 2021

KEYWORDS

C. camphora;
Proanthocyanidins;
Microwave-assisted extraction;
Optimization;
Antioxidant activity

Abstract *Cinnamomum camphora* is a notable tree species of great significance to human beings. Proanthocyanidins (PCs) were separated from *C. camphora* leaves using microwave assisted extraction (MAE) method. Four variables of the MAE procedure were studied using the Box-Behnken design of response surface method. The results demonstrated that the optimal extraction conditions were: ethanol concentration of 77%; liquid/solid ratio of 20 mL/g, microwave time of 18 min, and microwave power of 530 W to achieve an actual yield of 81.56 ± 2.03 mg/g for *C. camphora* PCs. Extraction kinetic study results indicated that MAE is the most efficient method for *C. camphora* leaf PCs extraction under the obtained conditions in comparison to the reference methods (heat reflux extraction, ultrasound extraction, and Soxhlet extraction), by which the highest yield of PCs and the volumetric mass transfer coefficient were obtained in the shortest extraction time. Antioxidant activity study revealed that *C. camphora* leaf PCs could be used as a natural alternative to the synthetic commercial antioxidants.

1. Introduction

Proanthocyanidins (PCs) are polyphenolic compounds with high molecular weight, which derivate from the polymerization of flavan-3-ols (Neto et al., 2020). Thanks to their potential antioxidant activity (Jayaprakasha et al., 2001; Liu & Yang, 2018), antimicrobial activity (Allaker & Douglas, 2009; de Moura Martins et al., 2020), and low toxicity (Yamakoshi et al., 2002), PCs have been used as food additives in the food industry to replace the traditional synthetic chemicals as

* Corresponding authors at: College of Life Sciences, Jiangxi Normal University, Nanchang 330022, China.
E-mail addresses: zaizhiliu@hotmail.com (Z. Liu), zouzrjxnu@163.com (Z. Zou).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2021.103328

stabilizers added in cooking oils and meats (Taghvaei & Jafari, 2015; Lau & King, 2003). Moreover, PCs have exhibited great beneficial effects against various diseases such as anti-tumor (Olaku et al., 2015), cardiovascular diseases (Pons et al., 2014), asthma (Li et al., 2017), anti-obesity (Pascual-Serrano et al., 2018).

Cinnamomum camphora is a large broad-leaved evergreen tree species belonging to the Lauraceae family, which has been extensively cultivated in China for over two thousand years as a valuable timber and aromatic tree (Babu et al., 2003). *C. camphora* has been used in Chinese folk medicine to treat diseases (Shi et al., 2016; Hamidpour et al., 2013). Nowadays, it has attracted much attention on the utilization of *C. camphora* leaf essential oil, as this essential oil is widely applied as a food additive and the major ingredient in pharmacy, domestic chemical, flavors and perfume (Chen et al., 2020; Tian et al., 2021). In return, large amount of deoiled leaf residues produced as by-products every year and generally disposed in a way that bring about a higher environmental impact (Ritchie and Roser, 2017). Meanwhile, a previous study has pointed out that reasonably harvesting *C. camphora* leaves could facilitate sustaining good conditions for growth (Chen et al., 2016). Therefore, the deoiled *C. camphora* leave residues are ideal materials for PCs extraction. Osawa et al. (2011) have identified the chemical structures of *C. camphora* PCs, however, there is no study on the separation of PCs from *C. camphora* leaves and evaluation of their bioactivities. Herein, it is of great significance to extract *C. camphora* leaf PCs and explore their antioxidant activities.

The techniques employed to recover PCs from plant materials can be concluded into two categories: traditional and untraditional methods. The traditional methods include heat reflux extraction (HRE), Soxhlet extraction (SE), and maceration extraction, while the later includes microwave assisted method (MAE), ultrasound assisted method (UAE), and supercritical fluid extraction (Papagiannopoulos et al., 2002; Liu et al., 2017; Neto et al., 2020; Da Porto et al., 2014). The MAE method is commonly applied among these techniques thanks to its easy operation, saving energy and time, and reduction in solvent (Qin et al., 2021; Sánchez-Camargo et al., 2021). Nonetheless, the study regarding the isolation of PCs from *C. camphora* leaves using MAE is yet to be reported. Many parameters in MAE process can affect the target compounds yield, including solvent concentration, extraction time and temperature, liquid/solid ratio, and microwave power (Sharma et al., 2020; Liu et al., 2021). Herein, it is to screen and optimize the performing conditions during the MAE process to obtain the desirable responses (Pinela et al., 2016). Response surface methodology (RSM) is a statistical-based methodology that employs quantitative data to analyze and fit multinomial quadratic equation to optimize the condition of factors and predict the desirable response (Hwang et al., 2002). RSM has been employed to optimize the separation process and predict PCs' maximum extraction yield from plant materials (Bhuyan et al., 2015; Zhao et al., 2020).

In the present study, MAE was employed to separate PCs from *C. camphora* leaves. Four process factors (ethanol concentration, liquid/-solid ratio, and microwave time and power) were investigated via a Box-Behnken design (BBD) of RSM to obtain the optimal conditions for PCs extraction. Meanwhile, the antioxidant activity of PCs was studied *in vitro*. The results would provide the basis for the further practical applications of *C. camphora* leaf PCs.

2. Materials and methods

2.1. Chemicals and material

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-car boxylic acid (Trolox) used for the antioxidant assays are purchased from Sigma–Aldrich (St. Louis, MO, USA). All the other chemicals and reagents were procured from Aladdin Reagent (Shanghai, China) and used in the experiments without further purification.

C. camphora leaves were obtained from Jiangxi Normal University campus (Nanchang, China) and identified by Prof. Zhengrong Zou (Jiangxi Normal University, China). Fresh *C. camphora* leaves were dried in the darkness for 7 days at around 25 °C and then grinded. Before MAE of PCs, the leaf powders were decreased three times by macerating for 4 h with the addition of 2 L petroleum ether (boiling range 60–90 °C) and then filtered. The supernatant was condensed under a vacuum to recover petroleum ether while the defatted materials were dried again and stored in a closed desiccator for further experiments.

2.2. MAE apparatus

A domestic WP700SL23 microwave oven (Guangdong Galanz Enterprise Co., Ltd., China) was employed in the MAE process to extract *C. camphora* leaf PCs. The preestablished microwave time and power can be changed by rotating the control panel as introduced in our previous research (Liu et al., 2018), as shown in Fig. 1. The laboratory used MAE apparatus was equipped with a Clevenger condenser and connected with a glass flask by a hole pre-dug on the top of the microwave oven. During the MAE process, polytetrafluoroethylene was used to avoid microwave leakage by wrapping the external upper part of the glass flask around the hole. The rectangular of the microwave oven bath's interior size was 215 mm × 350 mm × 330 mm. The extraction apparatus worked at a normal pressure operating at a frequency of 40 kHz. Based on the previous study (Lucchesi et al., 2007; Feki et al., 2021), nominal microwave powers (120, 230, 385, 540, and 700 W) were employed to describe the parameters in the MAE process.

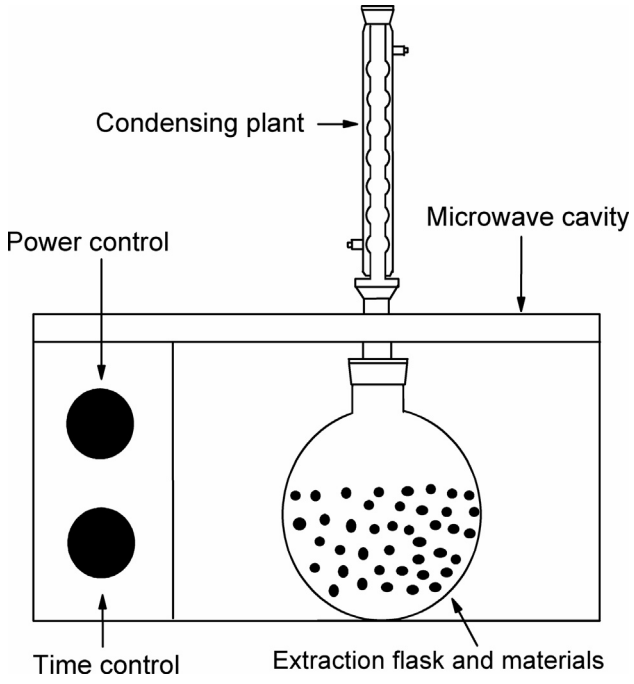

Fig. 1 The apparatus of MAE.

2.3. Extraction and determination of C. camphora leaves PCs

2.3.1. Extraction of PCs of C. camphora leaves

The de-oiled leaf powders of *C. camphora* leaves (5 g) were transferred into glass flask and then extracted under different conditions by MAE. The effects of MAE factors, including ethanol concentration, liquid/solid ratio, microwave time, and microwave power on the yield of PCs from *C. camphora* leaves were studied using single experimental trials to obtain the suitable ranges for the following optimization by BBD. Afterwards, the suspension liquids were filtered and then stored in a refrigerator at 4 °C before measuring the PCs contents in the extracts.

2.3.2. Experimental design and optimization

The independent or interactive effects of variables in the extraction process could determine the extraction efficiency (Sabino et al., 2021). Therefore, BBD was applied to investigate the factors: ethanol concentration (*A*), liquid/solid ratio (*B*), microwave time (*C*), and microwave power (*D*). Table 1 demonstrated the levels of the code and the real variables and the complete experimental design, which contains 29 runs (5 replicates are the central points). The experimental data was fitted using a second-order polynomial equation (Eq. (1)):

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

where β_0, β_i, β_{ii}, and β_{ij} are variables, k is the number of the tested variables, X_i and X_j are the independent variables, and Y is the predicted response.

2.3.3. Determination of PCs

The Vanillin assay method was employed to determine PCs content with some modifications (Sun et al., 1998). The sample solution (1 mL) was blended with 1.5 mL of hydrochloric acid and 3 mL of 40 mg/mL vanillin in methanol (w/v). A control group was performed by replacing the vanillin solution with methanol to calibrate the absorbance by non-vanillin reactive substances to exclude the effect of the interference effect (e.g., anthocyanins). After incubation in water bath (20 ± 1C°) for 15 min, the absorbance of the samples and control groups was determined at 500 nm and their difference was applied to measure the PCs in *C. camphora* leaves, which was conveyed as mg grape PCs/g samples (dry weight). Calculation of PCs was on the basis of a generated calibration curve using grape PCs ($y = 3.284 \times -0.0046$, $R^2 = 0.9979$).

2.4. Extraction kinetic model of MAE and the reference methods for C. camphora leaf PCs

On the basis of previous studies (Wongkittipong, et al., 2004; Handayani et al., 2008), mass transfer is the mechanism control for the solid–liquid extraction process. Herein, the extraction kinetic model of Fick's law, namely the following first-

Table 1 Coded and real levels of BBD and the predicted and observed responses.

Run[a]	*A*	*B*	*C*	*D*	Yield (mg/g)	
					Predicted	Actual
1	100 (+1)	20 (0)	20 (+1)	385 (0)	75.83	75.51
2	75 (0)	20 (0)	20 (+1)	230 (-1)	65.45	66.61
3	50 (-1)	25 (+1)	15 (0)	385 (0)	73.61	73.23
4	50 (-1)	20 (0)	15 (0)	540 (+1)	73.55	73.44
5	75 (0)	20 (0)	20 (+1)	540 (+1)	77.67	78.37
6	50 (-1)	20 (0)	20 (+1)	385 (0)	68.35	67.05
7	50 (-1)	15 (-1)	15 (0)	385 (0)	68.44	70.06
8	75 (0)	25 (+1)	15 (0)	540 (+1)	78.18	79.18
9	75 (0)	15 (-1)	15 (0)	540 (+1)	72.96	72.38
10	50 (-1)	20 (0)	10 (-1)	385 (0)	69.99	70.06
11	100 (+1)	25 (+1)	15 (0)	385 (0)	73.03	71.61
12	100 (+1)	20 (0)	15 (0)	230 (-1)	66.96	67.11
13	75 (0)	15 (-1)	10 (-1)	385 (0)	69.34	69.31
14	75 (0)	25 (+1)	10 (-1)	385 (0)	71.84	72.22
15	100 (+1)	20 (0)	15 (0)	540 (+1)	72.47	72.42
16	75 (0)	20 (0)	10 (-1)	230 (-1)	65.24	64.73
17	75 (0)	20 (0)	15 (0)	385 (0)	75.68	74.41
18	100 (+1)	20 (0)	10 (-1)	385 (0)	66.08	67.14
19	75 (0)	20 (0)	15 (0)	385 (0)	75.68	75.43
20	75 (0)	20 (0)	15 (0)	385 (0)	75.68	76.40
21	75 (0)	20 (0)	15 (0)	385 (0)	75.68	75.91
22	50 (-1)	20 (0)	15 (0)	230 (-1)	62.32	62.42
23	75 (0)	20 (0)	10 (-1)	540 (+1)	69.76	68.79
24	100 (+1)	15 (-1)	15 (0)	385 (0)	72.58	73.16
25	75 (0)	20 (0)	15 (0)	385 (0)	75.68	76.24
26	75 (0)	25 (+1)	15 (0)	230 (-1)	67.4	67.74
27	75 (0)	25 (+1)	20 (+1)	385 (0)	76.21	76.29
28	75 (0)	15 (-1)	20 (+1)	385 (0)	73.09	72.76
29	75 (0)	15 (-1)	15 (0)	230 (-1)	67.00	65.75

[a] *A*: Ethanol concentration, %; *B*: Liquid/solid ratio, mL/g; *C*: Microwave time, min; *D*: Microwave power, W.

order equation Eq. (4) was employed to plot the extraction kinetics:

$$Y = Y_e[1 - \exp(-K_L \cdot \alpha \cdot t)] \quad (2)$$

where Y and Ye (mg/g) are the yields of *C. camphora* leaf PCs at any time in bulk liquid and reaching the extraction equilibrium; K_Lśa is the volumetric mass transfer coefficient; t (min) represents the extraction time.

HRE, UAE, and SE were employed as the reference methods for *C. camphora* leaf PCs extraction, and they were compared to MAE. HRE and SE were carried out at 1000 W for 4 h in a digital heating unit, jacket at an operation power of 1000 W for 4 h. The UAE was carried out in a digital water bath oscillator (Kunshan, Jiangsu) at a nominal power of 250 W for 1 h. The other extraction conditions were the same as the obtained optimal conditions.

2.5. Antioxidant activity

2.5.1. Scavenging ability on DPPH free radicals of PCs

The scavenging activities on DPPH radicals of PCs were performed based on the method proposed in the previous study (Liu & Yang, 2018). A 25 mg/L DPPH solution was prepared using anhydrous ethanol. 3.9 mL of DPPH solution was mixed with 0.1 mL of sample solutions of different concentrations (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL), respectively. Afterward, the mixtures were placed at 20 °C for 30 min and then measured at 517 nm. The blank group included 3.9 mL of DPPH solution and 0.1 mL of ethanol. Vitamin C (V_C), butylated hydroxytoluene (BHT) and butylated hydroxytoluene (BHA) were investigated as the positive controls. The formula of DPPH radical scavenging activity of samples was presented as follows:

$$\text{Scavenging activity}(\%) = (A_0 - A_1)/A_0 \times 100\% \quad (3)$$

where A_1 refers to the absorbance of the sample group, A_0 refers to the absorbance of the blank group.

2.5.2. Ferric reducing antioxidant power (FRAP) of PCs

The FRAP assay is performed based on the previously published study (Oyaizu, 1986). The FRAP stock solution was prepared by mixing of 300 mM acetate buffer (25 mL, pH 3.6), 10 mM TPTZ solution (2.5 mL) and 20 mM $FeCl_3$ś$6H_2O$ solution (2.5 mL) and was incubated at ambient temperature (37 ± 2 ○C) for 30 min. The sample solution (0.15 mL) was blended with the FRAP stock solution (2.85 mL), placed in the darkness for 30 min, and determined at 593 nm. VC, BHT, and BHA were used as the positive controls. Trolox

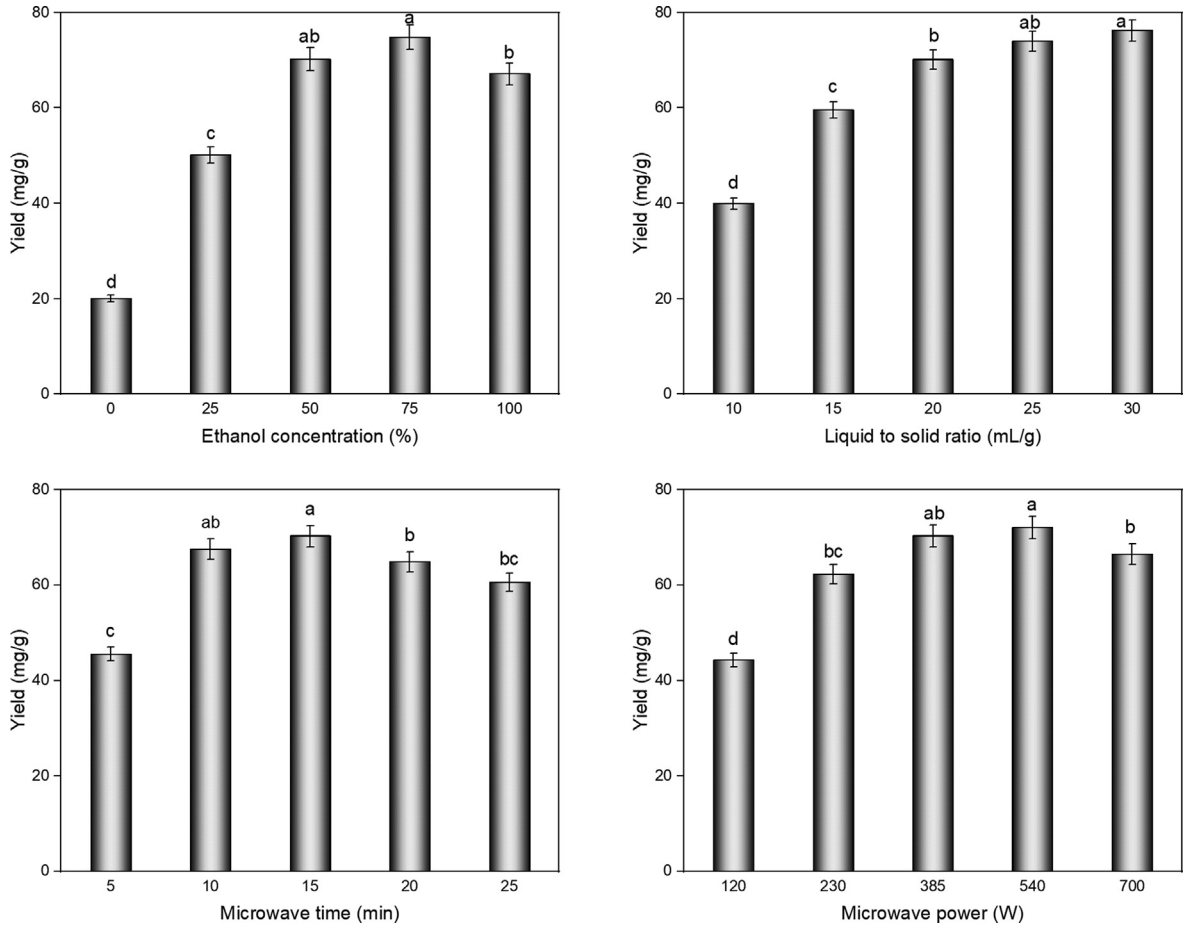

Fig. 2 Effects of ethanol concentration (a), liquid/solid ratio (b), microwave time (c) and microwave power (d) on the yield of PCs from *C. camphcra* leaves.

was employed to plot a standard curve with concentrations ranging from 37.5 to 600 mM. The FRAP was conveyed as mM Trolox equivalents per gram PCs.

2.5.3. Reducing power of PCs

The reducing power of PCs was investigated on the basis of a previous study (Oyaizu, 1986). One milliliter of the sample solutions (0.03–0.15 mg/mL) was combined with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ and then the tubes were capped and incubated at 50 °C for 20 min. Afterward, 2.5 mL of 10% trichloroacetic acid was added in reaction mixtures and then the mixtures were centrifuged (3000*g*) for 10 min. The upper supernatant (2.5 mL) was blended with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ and then measured at 707 nm. The blank groups were prepared using ethanol solution to replace the sample solutions, VC, BHT, and BHA were investigated as the positive controls.

2.6. Statistical analysis

Design Expert 8.0 software (Stat-Ease, Minneapolis, USA) was employed to carry out BBD. Analysis of variance (ANOVA) investigated the significance of differences in the *C. camphora* leaf PCs yield. All assays were proceeded in triplicate (n = 3). The actual values of each run input into BBD were conveyed as the mean values based on the built-in default settings in Design Expert 8.0 software. The other data was given as the mean values ± standard deviation.

3. Results and discussion

3.1. Single factor experimental analysis

3.1.1. Effect of ethanol concentration on PCs yield

The solubility of target compounds in the solvent strongly connects to the extraction efficiency in separation science (Lovrić et al., 2017). Hence, ethanol concentration was investigated to clarify its effect on the procedure for plant PCs extraction. Ethanol concentration was varied between 0 and 100% against water as liquid/solid ratio, microwave time, and power were kept at 20 mL/g, 15 min, and 385 W, respectively. The results demonstrated that the highest yield of PCs was obtained at an ethanol concentration of 75% (74.96 ± 2.50 mg/g) as depicted in Fig. 2a. The further increase in ethanol concentration led to a significant decrease in *C. camphora* leaf PCs yield. This is likely due to two reasons: on the one hand, an increase in ethanol concentration led to the decrease of polarity, and hence giving rise to the insufficient extraction of oligomeric PCs (Murga et al., 2000); on the other hand, a high concentration of ethanol can promote the conversion of proanthocyanidins into anthocyanidins (Gao et al., 2020). A similar phenomenon was also observed in the previous studies (Downey & Hanlin, 2010; Bosso et al., 2016). Therefore, the range of ethanol concentration was determined from 50% to 100% in the subsequent optimization study.

3.1.2. Effect of liquid/solid ratio on PCs yield

The effect of liquid/solid ratio on the *C. camphora* leaf PCs yield was also investigated. The yield of PCs was measured

Table 2 Analysis of variance (ANOVA) for the experimental results.

Source	Sum of squares		Degrees of freedom	Mean square	*F* value	*P* value Prob > *F*	Inference[f]
Model[a]	504.90		14	36.06	29.72	< 0.0001	***
A	9.52		1	9.52	7.85	0.0141	*
B	23.66		1	23.66	19.50	0.0006	***
C	49.37		1	49.37	40.69	< 0.0001	***
D	210.17		1	210.17	173.22	< 0.0001	***
AB	5.57		1	5.57	4.59	0.0502	ns
AC	32.38		1	32.38	26.68	0.0001	***
AD	8.15		1	8.15	6.72	0.0213	*
BC	0.096		1	0.096	0.079	0.7825	ns
BD	5.78		1	5.78	4.77	0.0465	*
CD	14.82		1	14.82	12.22	0.0036	**
A^2	64.78		1	64.78	53.39	< 0.0001	***
B^2	2.34		1	2.34	1.93	0.1869	ns
G^2	39.14		1	39.14	32.26	< 0.0001	**
H^2	88.51		1	88.51	72.95	< 0.0001	***
Residual	16.99		14	1.21			
Lack of fit	14.42		10	1.44	2.25	0.2262	ns
Pure error	2.57		4	0.64			
Cor total[b]	521.89		28				
Std. Dev.[c]	Mean	C.V.[d] %	Press	R^2	Adjusted R^2	Predicted R^2	Adequate precision
1.1	71.58	1.54	87.07	0.9675	0.9349	0.8332	20.014

[a] *A*: Ethanol concentration, %; *B*: Liquid/solid ratio, mL/g; *C*: Microwave time, min; *D*: Microwave power, W.
[b] Cor Total: Totals of all information corrected for the mean.
[c] Std. Dev.: Standard deviation.
[d] C.V.: Coefficient of variation.
[f] * Signifcant at $P \leq 0.05$, ** Signifcant at $P \leq 0.01$, *** Signifcant at $P \leq 0.001$, [ns] Not signifcant.

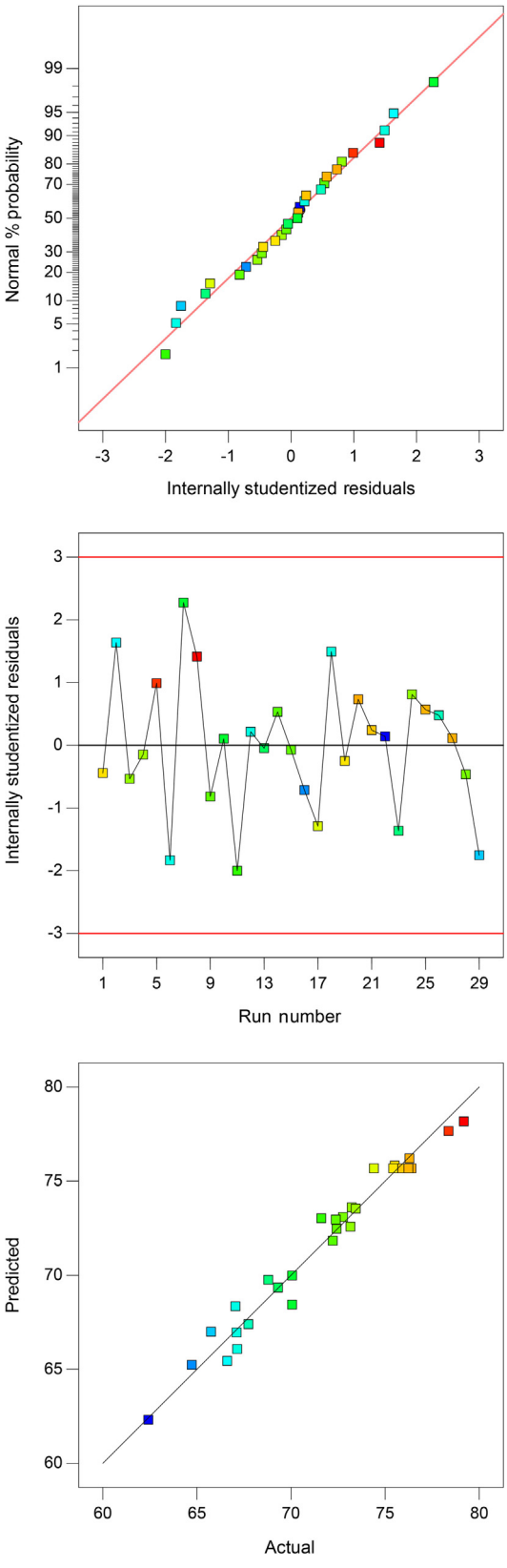

Fig. 3 Diagnostic plots for model adequacy checking. Normal plot (a), residuals vs. run number (b), and predicted vs. actual (c).

by varying liquid/solid ratio between 10 and 30 mL/g at fixed 50% of ethanol concentration, 15 min of microwave power, and 385 W of microwave power. As presented in Fig. 2b, it was seen that the PCs significantly raised with initially increasing of liquid/solid ratio. Nevertheless, when the liquid/solid ratio increased from 25 mL/g to 30 mL/g, the PCs yield showed a nonsignificant increase. Hence, the range between 15 and 25 mL/g was used for the next optimization investigations.

3.1.3. Effect of microwave time on PCs yield

Microwave time is a crucial parameter in the extraction process of MAE. Inadequate microwave time, either shorter or longer microwave time, can change the yield of target compounds from the plant matrix. This is because the underexposure or overexposure of plant materials under microwave radiation could decrease the extraction yield of target components. Thus, the effect of microwave time on *C. camphora* leaf PCs yield was investigated within the range of 5 to 25 min as shown in Fig. 2c; and the other variables including ethanol concentration, liquid/solid ratio, and microwave power were maintained at 50%, 20 min, and 385 W, respectively. PCs yield was observed and obviously increased as microwave time function increased from 5 to 15 min and followed by a significant decline when the microwave time exceeded 15 min. This phenomenon demonstrated the dissolution process of target compounds from solid to the solvent stopped as the longtime exposure to microwave radiation triggered degradation (Alara and Abdurahman, 2019). The range of microwave time was then determined from 10 to 20 min for further optimization.

3.1.4. Effect of microwave power on PCs yield

On the basis of the previous study (Dahmoune et al., 2014), microwave irradiation is triggered by the dissemination of electromagnetic energy and can promote the mass transfer coefficient between the extraction solvent and the plant materials. For that reason, microwave power was changed within 120 W, and 700 W was kept at 50% of ethanol concentration, 20 mL/g of liquid/solid ratio, and 20 min of microwave time. The PCs yield increased with an enhancement of microwave power from 120 W to 385 W, and a further increase in microwave power did not significantly enhance PCs yield. Still, an apparent decrease of over 540 W of microwave power (Fig. 2d). The reduction in PCs beyond 540 W could be attributed to the effect of the excessive microwave irradiation originated from improved power. Therefore, the range of microwave power from 230 to 540 W was screened for the subsequent optimization process.

3.2. Process optimization of experiments by BBD

3.2.1. Model fitting and regression coefficients

BBD is autonomous without any influences on the abovementioned screening proposals. Three levels of each variable are usually recommended, whereas runs in which all variables simultaneously at lower or higher points are not included, building a design with fewer trials and thus more economical

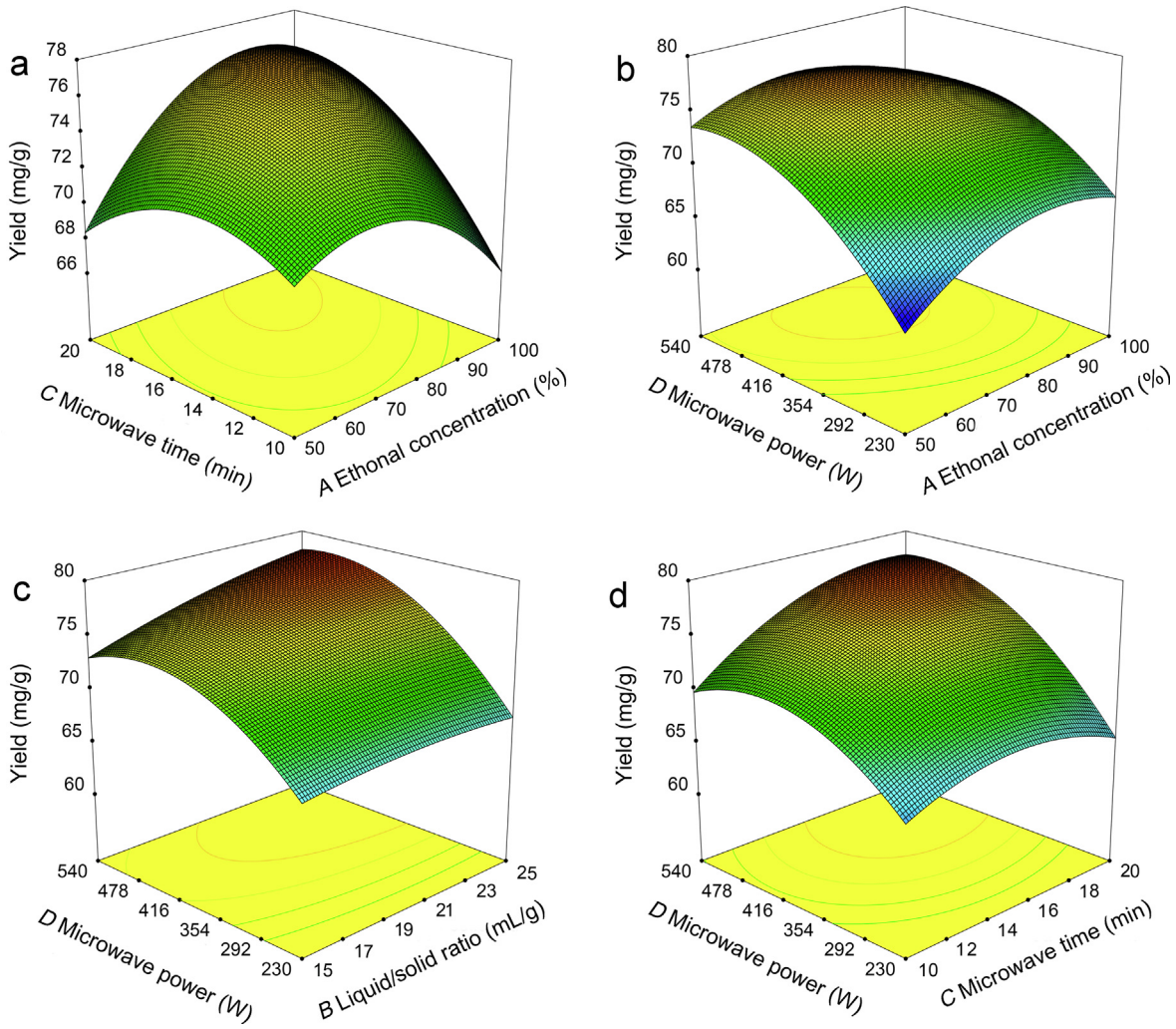

Fig. 4 3-D response surface plots from BBD. (a) The interactive influences of ethanol concentration (*A*) and microwave time (*C*) on PCs yield from *C. camphcra* leaves while fixing liquid/solid ratio and microwave power at the middle level of 20 mL/g and 385 W; (b) the interactive influences of the ethanol concentration (*A*) and microwave power (*D*) on PCs yield from *C. camphcra* leaves while at the average liquid/solid ratio (20 mL/g) and microwave time (15 min); (c) the interactive influences of liquid/solid ratio (*B*) and microwave power (*D*) on *C. camphcra* leaf PCs yield while fixing ethanol concentration (*A*) and microwave time at the middle level of 75% and 15 min; and (d) the interactive influences of microwave time (*C*) and power (*D*) on *C. camphcra* leaf PCs yield while fixing ethanol concentration (*A*) and liquid/solid ratio (*B*) at the middle level of 75% and 20 mL/g.

(Massart et al., 1997). Twenty-nine runs associated with four variables, namely ethanol concentration (*A*), liquid/solid ratio (*B*), microwave time (*C*), and microwave power (*D*) of MAE, were carried out for the further optimization of the PCs extraction process using BBD to obtain the optimal conditions as presented in Table 1. The regression analysis was carried out to study the actual responses acquired from the experiments, and a quadratic regression model equation was finally generated as given in Eq. (4):

$$\begin{aligned} Y = {} & 2.62 + 0.74 \times A + 1.02 \times B + 0.60 \times C + 0.11 \times D \\ & + 0.02 \times AC - 3.68 \times 10^{-4} \times AD + 1.55 \times 10^{-3} \\ & \times BD + 2.48 \times 10^{-3} \times CD - 5.05 \times 10^{-3} \times A^2 \\ & - 0.02 \times B^2 - 0.10 \times C^2 - 1.54 \times 10^{-4} \times D^2 \end{aligned} \quad (4)$$

The model significance and suitability were tested by analysis of variance (ANOVA) for the response. The regression coefficients of the developed model including the intercept, linear, quadratic, and interaction terms were investigated and submitted to a significance test. *P* value was applied to examine the significance of each coefficient. *P* values less than 0.05 demonstrate the corresponding term is significant. The ANOVA analysis results of the PCs yield are given in Table 2. A good connection was observed between the PCs yield and the process variables as evidenced by a coefficient of determination (R^2) of 0.9675; the values of adjusted R^2 (0.9349) also showed an excellent agreement to that of predicted R^2 (0.8332). Furthermore, coefficient of variance (C.V.% = 1.54) implied a good reproducibility of the developed model. The model *F* value of 29.72 with a *P* value of 0.2262 for lack

of fit all implied that the developed model is significant, indicating that the model can be applied to further analyze of the correlations of variables within the boundaries used. ANOVA results revealed that all four individual factors were significant model terms, while with respect to the interactions between variables and only the interactions of liquid/solid ratio (*B*) versus ethanol concentration (*A*) and microwave time (*C*) were nonsignificant terms.

3.2.2. Investigation of model adequacy

Three diagnostic plots of the developed model, including normal plot, residuals vs. run number, and predicted vs. actual. Fig. 3a showed that the residuals follow a normal distribution and were distributed mainly in the straight line. Therefore, the error of the developed secondary model was normally and identically distributed (Sekhar et al., 2021). The residuals vs. run number plot (Fig. 3b) showed a random distribution of data points in the range of −3 to 3 surround the central line, which implied that the developed quadratic model built interaction between the causal parameters of the MAE method and the PCs yield. The actual responses obtained based on experimental trails were depicted against the predicted response (Fig. 3c). This plot aims to seek out a value or a cluster of values that the developed model could not easily predict. It was observed that the predicted values by the developed model have a linear relation with the actual values. Briefly, the three diagnostic plots indicated that the developed model has a good capacity to optimize the MAE process for PCs extraction from *C. camphora* leaves.

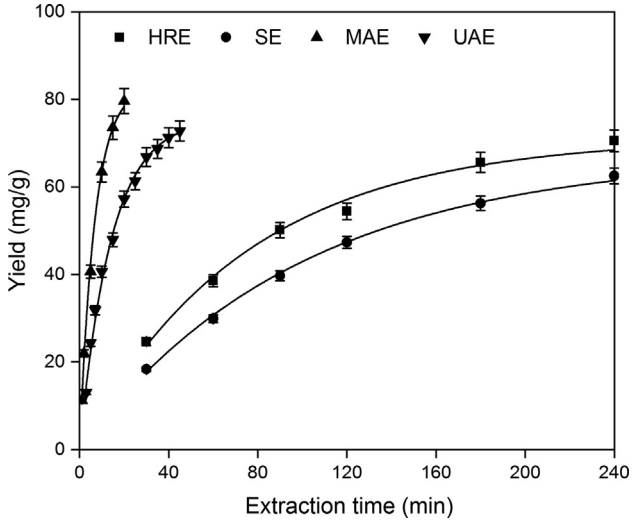

Fig. 5 Mass transfer kinetics curves of microwave-assisted extraction (MAE) and reference methods for extraction of *C. camphcra* leaf PCs.

3.2.3. Effects of MAE conditions on PCs through RSM

Fig. 4a showed the 3-D response surface plots for the yield of PCs with varying ethanol concentration (*A*) and microwave time (*C*) while fixing liquid/solid ratio (*B*) and microwave power (*D*) at the middle level of 20 mL/g and 385 W, respectively. The depicted plot showed that the PCs yield increased gradually as the ethanol concentration and microwave time increased, but they all exhibited a curvature as further increased. The highest extracted PCs yield was 76.55 mg/g and occurred at an ethanol concentration of 84% and at a microwave time of 18 min. The 3-D response surface plot was obtained for the yield of PCs with varying ethanol concentration (*A*) and microwave power (*D*) at the average liquid/-solid ratio (*B*) and microwave time (*C*) as shown in Fig. 4b. It can be observed that ethanol concentration and microwave power showed a quadric effect on PCs yield and the maximum yield (76.86 mg/g) of PCs can be obtained when ethanol concentration and microwave power were 75% and 472 W, respectively. Fig. 4c depicted the interactive influence of liquid/solid ratio (*B*) and microwave power (*D*) on the PCs yield; by improving microwave power from 230 to 500 W, the *C. camphora* leaf yield of PCs tends towards increase with liquid/solid ratio enhancing to optimum values and then gradually decreased. Fig. 4d and Table 2 showed a strong interaction between microwave time (*C*) and power (*D*) significantly influencing PCs yield. By increasing the microwave time to 18 min and microwave power to 501 W, the optimum PCs yield of 77.97 mg/g was achieved from *C. camphora* leaves. The synergetic effect between microwave time and power can be illustrated by improving the extraction system temperature, increasing the extraction yield. Based on the previous study (Alara et al., 2021), an increase of microwave power or microwave time enables keeping the temperature in the microwave oven at high levels, giving rise to the maximum extraction yields.

3.2.4. Method validation

RSM has performed the process optimization of the PCs extraction process on the developed quadric regression equation. The optimal MAE conditions with a predicted yield of 79.79 mg/g for *C. camphora* leaf PCs were obtained as: 76.90% of ethanol concentration, 19.68 mL/g of liquid/solid ratio, 18.39 min of microwave time, and 525.59 W of microwave power. The validation experiments were performed three times with the slight modifications of the optimal conditions (77% of ethanol concentration, 20 mL/g of liquid/solid ratio, 18 min of microwave time, and 530 W of microwave power) to verify the accuracy and acceptance of the MAE process. A mean yield of 81.56 ± 2.03 mg/g was obtained for *C. camphora* leaf PCs extraction, which indicated that the obtained conditions by BBD are reliable.

Table 3 Experimental data, the fits of mass transfer kinetic model and the fitted parameters *Ye* and $K_L{\cdot}a$.

Method	Y fitted (mg/g)	Y experiment (mg/g)	$K_L{\cdot}a$	R^2	Adjust R^2
MAE	84.39 ± 1.55	81.56 ± 2.03[a]	0.1629	0.9992	0.9990
UAE	75.54 ± 2.06	73.91 ± 1.94[b]	0.0710	0.9922	0.9914
HRE	72.59 ± 1.72	69.91 ± 1.67[b]	0.0133	0.9969	0.9961
SE	67.30 ± 1.99	65.25 ± 1.88[c]	0.0102	0.9975	0.9969

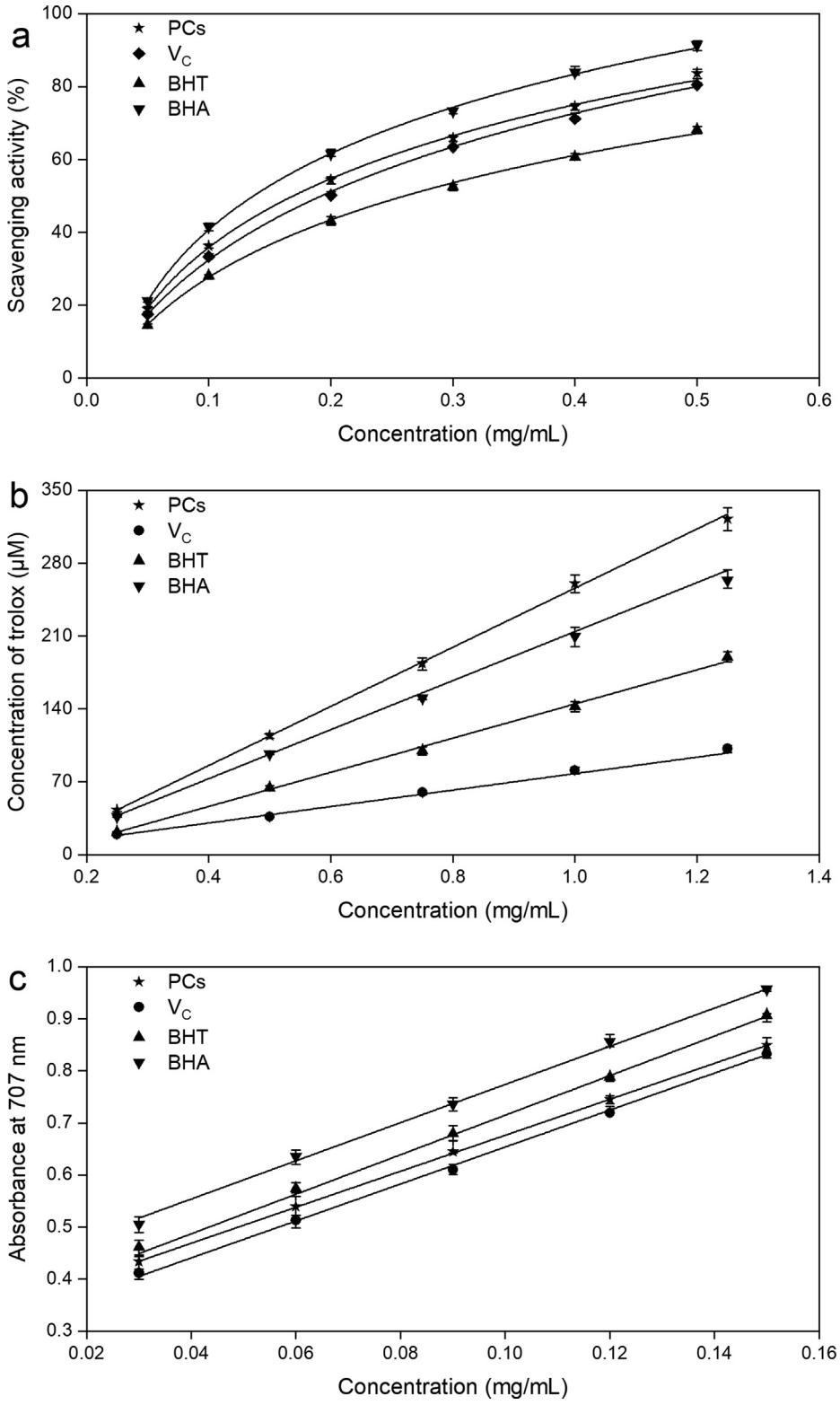

Fig. 6 Antioxidant activity of PCs from *C. camphcra* leaves. (a) DPPH radical scavenging activity, (b) ferric scavenging activity, and (c) reducing power.

3.3. Comparison with the reference methods and kinetic study

Extraction kinetic curves were depicted to compare the behaviors of different methods on *C. camphora* PCs extraction as presented in Fig. 5. MAE possessed significantly higher PCs yield in the function of shortest extraction time than those of SE, HRE, and UAE. The results also demonstrated that MAE is the most effective method, with the extractive effec-

tiveness improving 10.35%, 16.67%, and 25.00% compared to UAE, HRE, and SE, respectively. The fitted parameters of Y fitted and k_L•a were investigated using the nonlinear fit of Eq. (3) to the actual experimental data and summarized in Table 3. High R^2 and adjust R^2 (>0.99) for the MAE and three reference methods denoted that the selected first-order kinetic model could fit the extraction processes for *C. camphora* PCs. The parameter k_L•a of MAE was dramatically higher than those of the three reference methods. This is probably owing to that the extraction system temperature influences the diffusivity coefficient, and the microwave oven temperature increase quickly in a short time under microwave irradiation (Feki et al., 2021). Meanwhile, the increased diffusivity coefficient increased the mass transfer rate of PCs from solid to liquid. The majority of PCs are isolated within 20 min by MAE, while the reference methods needed a longer time to reach the extraction equilibrium. Hence, the proposed approach is a potential candidate for the separation of PCs from *C. camphora* leaves.

3.4. Antioxidant activity

3.4.1. DPPH radical-scavenging activity

The DPPH free radical could receive an electron or hydrogen radical and transform into a stable diamagnetic molecule (Soares et al., 1997). The DPPH stock solution was prepared with a maximum absorbance of 0.84 at 517 nm. Its purple color usually fades when DPPH solution is mixed with the antioxidant reagents, leading to a decrease in absorbance at 517 nm (Ma et al., 2012). As depicted in Fig. 6a, the scavenging activity of all the samples increased with the increase of their concentrations, and the DPPH scavenging influence decreased in that order: BHA > PCs > V_C > BHT.

3.4.2. FRAP

The antioxidant potential of *C. camphora* leaf PCs was investigated for their ability to convert the TPTZ-Fe^{3+} complex into the PTZ-Fe^{2+} complex. The FRAP conveyed by Trolox equivalent antioxidant capacity and the FRAP of PCs, BHA, BHT and V_C were given in Fig. 6b. The results obviously demonstrated that the PCs from *C. camphora* leaves possessed the highest FRAP to those of BHA, BHT, and VC. The results indicated that *C. camphora* leaf PCs had strong FRAP in comparison to the commercial antioxidants.

3.4.3. Reducing power

Reducing power trials were performed as an indicator to estimate the antioxidant activity of *C. camphora* leaf PCs. The existence of reductants in the mixed solution gives rise to the conversion of Fe^{3+} or ferricyanide into Fe^{2+} and then Fe^{2+} is detected by measuring the absorbance at 707 nm (Ardestani and Yazdanparast, 2007). As shown in Fig. 6c, the reducing power of *C. camphora* leaf PCs and the reference groups all improved linearly with an increase in their concentration. PCs showed a little bit weaker reducing power than the reference samples.

4. Conclusion

In this study, MAE was applied for the separation of *C. camphora* leaf PCs. The RSM of BBD was employed to optimize the MAE process. The optimal extraction conditions were achieved with a predicted yield of 79.79 mg/g as follows: ethanol concentration of 77%; liquid/solid ratio of 20 mL/g, microwave time of 18 min, and microwave power of 530 W. Extraction kinetic curves were depicted using a first-order kinetic model and the results implied that MAE possessed the highest yield and volumetric mass transfer coefficient than the reference methods (SE, HRE, and UAE) for PCs extraction from *C. camphora* leaves under the obtained conditions. Compared to the commercial antioxidants, *C. camphora* leaf PCs showed strong antioxidant activity *in vitro* which could be applied as promising natural antioxidants in the food industry.

CRediT authorship contribution statement

Zaizhi Liu: Conceptualization, Methodology, Funding acquisition. **Hualan Li:** Investigation, Visualization, Data curation, Writing – original draft. **Yanlong Qi:** Investigation, Data curation, Methodology. **Zheng Zhu:** Investigation, Data curation, Methodology, Writing – original draft. **Dai Huang:** Software, Investigation, Writing – original draft. **Ke Zhang:** Investigation, Visualization, Data curation. **Jun Pan:** Investigation, Data curation, Software. **Lan Wen:** Software, Writing – original draft. **Zhengrong Zou:** Software, Validation, Supervision.

Declaration of Competing 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.

Acknowledgements

The authors thank the financial supports by Natural Science Foundation of Jiangxi Province (20202BAB213024) and National Natural Science Foundation of China (31860189 and 31760099).

References

Alara, O.R., Abdurahman, N.H., 2019. Microwave-assisted extraction of phenolics from *Hibiscus sabdariffa* calyces: kinetic modelling and process intensification. Ind. Crops Prod. 137, 528–535.

Alara, O.R., Abdurahman, N.H., Ali, H.A., Zain, N.M., 2021. Microwave-assisted extraction of phenolic compounds from *Carica papaya* leaves: An optimization study and LC-QTOF-MS analysis. Future Foods 3, 100035. https://doi.org/10.1016/j.fufo.2021.100035.

Allaker, R.P., Douglas, C.W.I., 2009. Novel anti-microbial therapies for dental plaque related diseases. Int. J. Antimicrob. Agents 33 (1), 8–13.

Ardestani, A., Yazdanparast, R., 2007. Antioxidant and free radical scavenging potential of Achillea santolina extracts. Food Chem. 104 (1), 21–29.

Babu, K.N., Sajina, A., Minoo, D., John, C.Z., Mini, P.M., Tushar, K.V., Rema, J., Ravindran, P.N., 2003. Micropropagation of camphor tree (*Cinnamomum camphora*). Plant Cell Tissue Organ Cult. 74, 179–183.

Bhuyan, D.J., Van Vuong, Q., Chalmers, A.C., van Altena, I.A., Bowyer, M.C., Scarlett, C.J., 2015. Microwave-assisted extraction of *Eucalyptus robusta* leaf for the optimal yield of total phenolic compounds. Ind. Crop. Prod. 69, 290–299.

Bosso, A., Guaita, M., Petrozziello, M., 2016. Influence of solvents on the composition of condensed tannins in grape pomace seed extracts. Food Chem. 207, 162–169.

Chen, C., Zhong, Y., Yu, F., Xu, M., 2020. Deep sequencing identifies miRNAs and their target genes involved in the biosynthesis of

terpenoids in *Cinnamomum camphora*. Ind. Crop. Prod. 145, 111853. https://doi.org/10.1016/j.indcrop.2019.111853.

Chen, T., Quan, Q., Duan, L., Yang, M., Yang, J., Cong, L., 2016. Extraction and content variation of essential oils from Cinnamomum camphora (L.) leaf in Yongzhou. Nat. Prod. Res. Dev. 28, 83–89.

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., Madani, K., 2014. *Pistacia lentiscus* leaves as a source of phenolic compounds: microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. Ind. Crops Prod. 61, 31–40.

Downey, M.O., Hanlin, R.L., 2010. Comparison of ethanol and acetone mixtures for extraction of condensed tannin from grape skin. S. Afr. J. Enol. Vitic. 31, 54–159.

Feki, F., Klisurova, D., Masmoudi, M.A., Choura, S., Denev, P., Trendafilova, A., Chamkha, M., Sayadi, S., 2021. Optimization of microwave assisted extraction of simmondsins and polyphenols from Jojoba (*Simmondsia chinensis*) seed cake using Box-Behnken statistical design. Food Chem. 356, 129670. https://doi.org/10.1016/j.foodchem.2021.129670.

Gao, N., Sun, X., Li, D., Gong, E., Tian, J., Si, X.u., Jiao, X., Xing, J., Wang, Y., Meng, X., Li, B., 2020. Optimization of anthocyanidins conversion using chokeberry pomace rich in polymeric proanthocyanidins and cellular antioxidant activity analysis. LWT - Food Sci. Technol. 133, 109889. https://doi.org/10.1016/j.lwt.2020.109889.

Hamidpour, R., Hamidpour, S., Hamidpour, M., Shahlari, M., 2013. Camphor (*Cinnamomum camphora*), a traditional remedy with the history of treating several diseases. Int. J. Case Rep. Image 4 (2), 86. https://doi.org/10.5348/ijcri-2013-02-267-RA-1.

Handayani, A.D., Sutrisno, Indraswati, N., Ismadji, S., 2008. Extraction of astaxanthin from giant tiger (*Panaeus monodon*) shrimp waste using palm oil: studies of extraction kinetics and thermodynamic. Bioresour. Technol. 99 (10), 4414–4419.

Hwang, K.T., Jung, S.T., Lee, G.D., Chinnan, M.S., Park, Y.S., Park, H.J., 2002. Controlling molecular weight and degree of deacetylation of chitosan by response surface methodology. J. Agric. Food Chem. 50 (7), 1876–1882.

Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem. 73 (3), 285–290.

Lau, D.W., King, A.J., 2003. Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. J. Agric. Food Chem. 51 (6), 1602–1607.

Li, Y., Yu, Q., Zhao, W., Zhang, J., Liu, W., Huang, M., Zeng, X., 2017. Oligomeric proanthocyanidins attenuate airway inflammation in asthma by inhibiting dendritic cells maturation. Mol. Immunol. 91, 209–217.

Liu, Z., Deng, B., Li, S., Zou, Z., 2018. Optimization of solvent-free microwave assisted extraction of essential oil from *Cinnamomum camphora* leaves. Ind. Crop. Prod. 124, 353–362.

Liu, Z., Li, H., Cui, G., Wei, M., Zou, Z., Ni, H., 2021. Efficient extraction of essential oil from *Cinnamomum burmannii* leaves using enzymolysis pretreatment and followed by microwave-assisted method. LWT - Food Sci. Technol. 147, 111497. https://doi.org/10.1016/j.lwt.2021.111497.

Liu, Z., Mo, K., Fei, S., Zu, Y., Yang, L., 2017. Efficient approach for the extraction of proanthocyanidins from *Cinnamomum longepaniculatum* leaves using ultrasonic irradiation and an evaluation of their inhibition activity on digestive enzymes and antioxidant activity *in vitro*. J. Sep. Sci. 40 (15), 3100–3113.

Liu, Z., Yang, L., 2018. Antisolvent precipitation for the preparation of high polymeric procyanidin nanoparticles under ultrasonication and evaluation of their antioxidant activity *in vitro*. Ultrason. Sonochem. 43, 208–218.

Lucchesi, M.E., Smadja, J., Bradshaw, S., Louw, W., Chemat, F., 2007. Solvent free microwave extraction of *Elletaria cardamomum* L.: a multivariate study of a new technique for the extraction of essential oil. J. Food Eng. 79 (3), 1079–1086.

Lovrić, V., Putnik, P., Kovačević, D.B., Jukić, M., Dragović-uzelac, V., 2017. Effect of microwave-assisted extraction on the phenolic compounds and antioxidant capacity of blackthorn flowers. Food Technol. Biotechnol. 55, 243–250.

de Moura Martins, C., Morais, S.A.L.d., Martins, M.M., Cunha, L.C. S., V. da Silva, C., Teixeira, T.L., B. Santiago, M., de Aquino, F.J. T., Nascimento, E.A., Chang, R., Martins, C.H.G., de Oliveira, A., 2020. Antifungal and cytotoxicity activities and new proanthocyanidins isolated from the barks of *Inga laurina* (Sw.) Willd. Phytochem. Lett. 40, 109–120.

Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., Jong, S.D., Lewi, P.G., SmeyersVerbeke, J., 1997. Handbook of chemometrics and qualimetrics: part A, data handling in science and technology, vol. 20 A, Elsevier, Amsterdam, pp. 643–738.

Ma, C., Yang, L., Zu, Y., Liu, T., 2012. Optimization of conditions of solvent-free microwave extraction and study on antioxidant capacity of essential oil from Schisandra chinensis (Turcz.) Baill. Food Chem. 134, 2532–2939.

Murga, R., Ruiz, R., Beltrán, S., Cabezas, J.L., 2000. Extraction of natural complex phenols and tannins from grape seeds by using supercritical mixtures of carbon dioxide and alcohol. J. Agric. Food Chem. 48 (8), 3408–3412.

Neto, R.T., Santos, S.A.O., Oliveira, J., Silvestre, A.J.D., 2020. Biorefinery of high polymerization degree proanthocyanidins in the context of circular economy. Ind. Crop. Prod. 151, 112450. https://doi.org/10.1016/j.indcrop.2020.112450.

Olaku, O.O., Ojukwu, M.O., Zia, F.Z., White, J.D., 2015. The role of grape seed extract in the treatment of chemo/radiotherapy induced toxicity: a systematic review of preclinical studies. Nutr. Cancer 67 (5), 730–740.

Osawa, H., Endo, I., Hara, Y., Matsushima, Y., Tange, T., 2011. Transient proliferation of proanthocyanidin accumulating cells on the epidermal apex contributes to highly aluminum-resistant root elongation in camphor tree. Plant Physiol. 155, 433–446.

Oyaizu, M., 1986. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet 44 (6), 307–315.

Papagiannopoulos, M., Zimmermann, B., Mellenthin, A., Krappe, M., Maio, G., Galensa, R., 2002. Online coupling of pressurized liquid extraction, solid-phase extraction and high-performance liquid chromatography for automated analysis of proanthocyanidins in malt. J. Chromatogr. A 958 (1-2), 9–16.

Pascual-Serrano, A., Bladé, C., Suárez, M., Arola-Arnal, A., 2018. Grape seed proanthocyanidins improve white adipose tissue expansion during diet-induced obesity development in rats. Int. J. Mol. Sci. 19 (9), 2632.

Pinela, J., Prieto, M.A., Carvalho, A.M., Barreiro, M.F., Oliveira, M.B.P.P., Barros, L., Ferreira, I.C.F.R., 2016. Microwave-assisted extraction of phenolic acids and flavonoids and production of antioxidant ingredients from tomato: a nutraceutical-oriented optmization study. Separ. Purif. Technol. 164, 114–124.

Pons, Z., Guerrero, L., Margalef, M., Arola, L., Arola-Arnal, A., Muguerza, B., 2014. Effect of low molecular grape seed proanthocyanidins on blood pressure and lipid homeostasis in cafeteria diet-fed rats. J. Physiol. Biochem. 70 (2), 629–637.

Da Porto, C., Natolino, A., Decorti, D., 2014. Extraction of proanthocyanidins from grape marc by supercritical fluid extraction using CO_2 as solvent and ethanol–water mixture as co-solvent. J. Supercrit. Fluid. 87, 59–64.

Qin, Z., Liu, H., Ma, Y., Wang, X., 2021. Chapter 9 - Developments in extraction, purification, and structural elucidation of proanthocyanidins (2000–2019). Stud. Nat. Prod. Chem. 68, 347–391.

Ritchie, H., Roser, M., 2017. Comparative analysis of environmental impacts of agricultural production systems, agricultural input efficiency, and food choice. Environ. Res. Lett. 12, 064016.

Sabino, L.B.d.S., Filho, E.G.A., Fernandes, F.A.N., de Brito, E.S., Júnior, I.J.d.S., 2021. Optimization of pressurized liquid extraction and ultrasound methods for recovery of anthocyanins present in jambolan fruit (*Syzygium cumini* L.). Food Bioprod. Process. 127, 77–89.

Sánchez-Camargo, A.D.P., Ballesteros-Vivas, D., Buelvas-Puello, L. M., Martinez-Correa, H.A., Parada-Alfonso, F., Cifuentes, A., Ferreira, S.R.S., Gutiérrez, L.-F., 2021. Microwave-assisted extraction of phenolic compounds with antioxidant and anti-proliferative activities from supercritical CO_2 pre-extracted mango peel as valorization strategy. LWT-Food Sci. Technol. 137, 110414. https://doi.org/10.1016/j.lwt.2020.110414.

Sekhar, S.C., Karuppasamy, K., Kumar, M.V., Bijulal, D., Vedaraman, N, Sathyamurthy, R., 2021. Rain tree (Samanea saman) seed oil: Solvent extraction, optimization and characterization. https://doi.org/10.1016/j.jobab.2021.04.005.

Sharma, B.R., Kumar, V., Kumar, S., Panesar, P.S., 2020. Microwave assisted extraction of phytochemicals from Ficus racemose. Curr. Res. Green Sustain. Chem. 3, 100020.

Shi, X., Zhang, C., Liu, Q., Zhang, Z., Zheng, B., Bao, M., 2016. De novo comparative transcriptome analysis provides new insights into sucrose induced somatic embryogenesis in camphor tree (*Cinnamomum camphora* L.). BioMed Cent. Genomics 17, 26–43.

Soares, J.R., Dins, T.C.P., Cunha, A.P., Ameida, L.M., 1997. Antioxidant activity of some extracts of *Thymus zygis*. Free Radical Res. 26, 469–478.

Sun, B., Ricardo-da-Silva, J.M., Spranger, I., 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 46 (10), 4267–4274.

Taghvaei, M., Jafari, S.M., 2015. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. J. Food Sci. Technol. 52 (3), 1272–1282.

Tian, Z., Luo, Q., Zuo, Z., 2021. Seasonal emission of monoterpenes from four chemotypes of *Cinnamomum camphora*. Ind. Crop. Prod. 163, 113327. https://doi.org/10.1016/j.indcrop.2021.113327.

Wongkittipong, R., Prat, L., Damronglerd, S., Gourdon, C., 2004. Solid–liquid extraction of andrographolide from plants experimental study, kinetic reaction and model. Sep. Purif. Technol. 40 (2), 147–154.

Yamakoshi, J., Saito, M., Kataoka, S., Kikuchi, M., 2002. Safety evaluation of proanthocyanidin-rich extract from grape seeds. Food Chem. Toxicol. 40 (5), 599–607.

Zhao, C., Yang, X., Tian, H., Yang, L., 2020. An improved method to obtain essential oil, flavonols and proanthocyanidins from fresh *Cinnamomum japonicum* Sieb. leaves using solvent-free microwave-assisted distillation followed by homogenate extraction. Arab. J. Chem. 13 (1), 2041–2052.