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Original Article

An optimization approach of dynamic maceration of *Centella asiatica* to obtain the highest content of four centelloids by response surface methodology

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ABSTRACT

Centella asiatica (L.) Urb., Apiaceae, is commonly used as food, food supplement, and medicine. Development of the extraction process to obtain the high extent of the active compound is necessary. So, the response surface methodology was used in this work to optimize the dynamic maceration of *C. asiatica* to obtain the highest content of the four centelloids including asiatic acid, madecassic acid, asiaticoside, and madecassoside. Two factors: extraction temperature and extraction time, were studied. The content of four centelloids was observed. After the extraction of *C. asiatica* using ethanol, the content of four centelloids was analyzed using validated high performance liquid chromatography. The optimization result showed that madecassoside and asiaticoside had a similar pattern of the contour plots and response surfaces. These two centelloids were highly extracted at a high extraction time and high extraction temperature. The other two centelloids had the same pattern, they had a high content at a high temperature and time as well as at a low temperature and time. The simultaneous highest content of four centelloids was achieved when extracted at 60 °C for 120 min. The optimal condition could be used as standard condition for extraction of *C. asiatica* to provide the highest content of four centelloids.

Introduction

Centella asiatica (L.) Urb. is a plant in the family Apiaceae. It is an herbal medicinal plant which exhibits several biological and pharmacological activity including anti-ulcer (Sarma et al., 1995), antioxidation (Singh et al., 2014; Sultan et al., 2014), antimicrobial (Ahmed et al., 2006; Oyedeji and Afolayan, 2005; Sultan et al., 2014), anti-inflammation (Sultan et al., 2014), analgesic (Sultan et al., 2014), anti-hyperglycemic (Kabir et al., 2014), anti-diarrheal (Ahmed et al., 2006), wound healing (Azis et al., 2017; Ruszymah et al., 2012; Saeidinia et al., 2017; Shetty et al., 2006; Somboonwong et al., 2012; Suguna et al., 1996) etc. *C. asiatica* composed of several chemical constituents, while important chemical markers of *C. asiatica* were pentacyclic triterpenoids called centelloids such as madecassoside, asiaticoside, madecassic acid, and asiatic acid (Bylka et al., 2014). There are formerly reported the wound healing activity of centelloids and *C. asiatica* extract. Tenni et al. (1988) report that total triterpenoid fraction from *C. asiatica* can stimulate collagen and fibronectin production in human skin fibroblast. Maquart et al. (1990) report asiaticoside and Titrated Extract from *C. asiatica* (TECA) containing asiatic acid (30%), madecassic acid (30%), and asiaticoside (40%) stimulate collagen production in human foreskin fibroblast. Bonté et al. (1995) report that asiaticoside and madecassoside stimulate type I collagen production in human fibroblast. In addition, madecassoside can stimulate type III collagen production as well. However, Lu et al. (2004a) report asiaticoside increase both type I and type III collagen level in human dermal fibroblast. Furthermore, there are some studies that also explain about the stimulation of collagen production of *C. asiatica* extract (Bonte et al., 1994; Nowwarote et al., 2013). Other wound healing mechanisms of *C. asiatica* are also described, such as increase intracellular free proline in human foreskin fibroblasts (Maquart et al., 1990), changing of gene expression related to angiogenesis and wound healing (Coldren et al., 2003), changing of gene related to cell proliferation, cell cycle, extracellular matrix synthesis (Lu et al., 2004a; Lu et al., 2004b), and increase migration and proliferation of fibroblast (Lee et al., 2012).

In vivo studies show that it exhibits antioxidant activity (Liu et al., 2008; Shukla et al., 1999a, 1999b), increase hydroxyproline level (Maquart et al., 1999; Shetty et al., 2006; Shukla et al.,

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1999a), increase tensile strength of the wound (Brinkhaus et al., 2000; Shukla et al., 1999a; Somboonwong et al., 2012; Sunilkumar et al., 1998), increase dry weight, DNA, protein of the wound (Maquart et al., 1999), increase collagen synthesis (Brinkhaus et al., 2000; Liu et al., 2008; Maquart et al., 1999; Sunilkumar et al., 1998), decrease scar tissue (Brinkhaus et al., 2000), increase breaking strength, wet and dry granulation tissue (Shetty et al., 2006), increase epithelization (Shetty et al., 2006b; Somboonwong et al., 2012), increase wound contraction (Shetty et al., 2006), increase angiogenesis (Kimura et al., 2008; Liu et al., 2008), stimulation of the production of vascular endothelial growth factor, monocyte chemoattractant protein-1, interleukin-1 (Kimura et al., 2008), and anti-inflammation (Wan et al., 2013).

The extraction method of plant raw material is an important step to yielding the high content of plant active compound that perhaps influence their activity. The aim of this work was to optimize the dynamic maceration of *C. asiatica* to obtain the highest content of the four centelloids *i.e.* madecassoside, asiaticoside, madecassic acid, and asiatic acid using response surface methodology. Furthermore, the high performance liquid chromatography (HPLC) method used for determination of four centelloids content was also validated to confirm the reliability of the analysis.

Materials and methods

Materials

Madecassoside was purchased from Sigma–Aldrich Inc., USA. Asiaticoside, madecassic acid, and asiatic acid were purchased from Chengdu Biopurify Phytochemicals Ltd., China. Solvents used as a mobile phase for HPLC analysis including acetonitrile and orthophosphoric acid were purchased from Honeywell-Burdick & Jackson, USA, and Carlo-Erba, France, respectively. Ultrapure water was produced in-house by Puris-Expe UP system, Korea. Ethanol (95%) was purchased from Samchai Chemical Co., Ltd., Thailand. The other chemicals were analytical grade.

Plant sample

Arial parts of *Centella asiatica* (L.) Urb., Apiaceae, were bought from the local market at Rangsit, Pathum Thani Province in March 2017, which *C. asiatica* originated from Sai Noi District, Nonthaburi Province. The plant sample was authenticated by Chair Prof. Dr. Nijsiri Ruangrungsi, Department of Pharmacognosy, College of Pharmacy, Rangsit University. A voucher specimen (CM-CA001-1-03-2017) was deposited at Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University.

Preparation and extraction of Centella asiatica

Arial parts of *C. asiatica* were cleaned and dried for 24 h at 50 °C. Dried *C. asiatica* was ground using a grinder and passed through a 60-mesh sieve. Dried *C. asiatica* powder (20 g) was extracted by 100 ml of 95% ethanol in 250-ml erlenmeyer flask using a water bath (WNB-14, Memmert, Germany) with agitation by shaker (SV 1422, Memmert, Germany). The extraction temperature and extraction time were set as the following specific condition (Table 1). After the extraction process, the filtrate was collected and the marc was re-extracted for two times with same solvent volume. The filtrates were pooled and dried in a vacuum condition by a rotary evaporator (Büchi Labortechnik AG, Switzerland) follow by drying in a hot air oven at 50 °C for 24 h. The yield of the extraction was reported as plant dry weight basis.

Response surface methodology

The circumscribed central composite experimental design was used in this work. Extraction temperature (X_1) and extraction time (X_2) were two investigated independent factors. Each factor was varied in five levels. Extraction temperatures were coded as $-\sqrt{2}$, -1, 0, 1, and $\sqrt{2}$ which the actual values were 40, 42.9, 50, 57.1, and 60 °C, respectively. Extraction times were also coded as similar as extraction temperature which the actual values were 60, 68.8, 90, 111.2, and 120 min, respectively.

The four dependent factors including madecassoside content (Y_1), asiaticoside content (Y_2), madecassic acid content (Y_3), and asiatic acid content (Y_4), were monitored. The individual centelloids content was predicted using Design-Expert® software version 11 (Stat-Ease, Inc., USA) and quantified by HPLC. Contour plots and response surfaces of the model conditions were reported. The reliability of the estimation was confirmed by the correlation plot between the predicted value and the actual value. The coefficients and *p*-value of individual centelloids in different polynomial terms were reported. Finally, the mathematics model, the coded equation, and the actual value obtained from the optimal condition were also reported. Furthermore, the desirability function was used to select the optimal condition that provided the high content of four centelloids.

Determination of individual centelloids content

The HPLC method was used for analysis of individual centelloids content. The HPLC instrument (Agilent 1260 infinity, Agilent, USA) equipped with an autosampler and photodiode array detector was used in this work. The ACE C18-PFP column (250 × 4.6 mm, i.d., 5 μm) connected with C18 guard column was used for the separation with temperature controlled at 25 °C. The gradient system comprised of acetonitrile (A) and 0.01% phosphoric acid aqueous solution (B). The gradient elution went from 2.5 min of 20% A, increased to 37.5% A within 9.5 min, increased to 45% A within 3 min and holding for 10 min, and decreased to 20% A within 1 min and holding for 2 min before the next injection. The flow rate of the mobile phase was 1 ml/min. The injection volume was 20 μl. The signal was detected at 210 nm.

The extract with a concentration of 400 μg/ml was used in the analysis. The methanolic solution of the extract was filtered and injected into the HPLC instrument. The content of individual centelloids was calculated from the calibration curve of standard madecassoside, asiaticoside, madecassic acid, and asiatic acid. The mean and standard deviation (SD) of the content of individual centelloids were reported.

Method validation

Method validation was investigated based on the ICH guideline. Five topics were studied *i.e.* linearity and range, detection limit (DL) and quantitation limit (QL), specificity, precision, and accuracy.

Linearity and range

The mixed standard of madecassoside, asiaticoside, madecassic acid, and asiatic acid were dissolved in methanol to obtain the concentration of 1 mg/ml of each compound. Then, it was diluted to seven concentration levels from 0.78 to 50 μg/ml. They were filtered through a syringe filter with 0.45 μm pore size and injected into the HPLC instrument ($n = 3$). A calibration curve of each centelloids was constructed. The three parameters *i.e.* linear equation, coefficient of determination (R^2), and test range were reported.

Table 1
Model conditions of circumscribed central composite experimental design.

Factor	Condition									
	1	2	3	4	5	6	7	8	9	10
X_1[a]	−1	1	−1	1	$-\sqrt{2}$	$\sqrt{2}$	0	0	0	0
X_2[b]	−1	−1	1	1	0	0	$-\sqrt{2}$	$\sqrt{2}$	0	0

[a] X_1 was extraction temperature; $-\sqrt{2}$ = 40 °C, −1 = 42.9 °C, 0 = 50 °C, 1 = 57.1 °C, and $\sqrt{2}$ = 60 °C.
[b] X_2 was extraction time; $-\sqrt{2}$ = 60 min, −1 = 68.8 min, 0 = 90 min, 1 = 111.2 min, and $\sqrt{2}$ = 120 min.

DL and QL

DL and QL were calculated from the slope of calibration curve of each centelloids and SD of y-intercepts of the regression lines. DL and QL were calculated as equations below:

$$DL = \frac{3.3\sigma}{S}$$

$$QL = \frac{10\sigma}{S}$$

where σ = the SD of *y*-intercepts of the regression lines
S = the slope of the calibration curve

Specificity

The UV spectrum at the upslope, top, and downslope of the peak of each centelloids in the extract were collected. The specificity was achieved when UV spectrums of the peak were similar for three regions and similar to the UV spectrum of standard centelloids.

Precision

The mixed standard of madecassoside, asiaticoside, madecassic acid, and asiatic acid in a concentration of 1.5625, 12.5, and 25 μg/ml were prepared. They were filtered through a syringe filter with 0.45 μm pore size and injected into the HPLC instrument (n = 3). The content of each centelloid was calculated to obtain the mean and SD value of each compound. The percent relative SD (%RSD) of the analysis in the same day and in three different days was reported as intraday precision and inter-day precision, respectively.

Accuracy

Accuracy was investigated using a spike technique. The mixed standard of madecassoside, asiaticoside, madecassic acid, and asiatic acid in a concentration of 1.5625, 12.5, and 25 μg/ml were added into *C. asiatica* extract with a known amount of each centelloids. They were filtered through a syringe filter with 0.45 μm pore size and injected into the HPLC instrument (n = 3). Percent recovery was reported.

Results and discussion

Validation of the HPLC method is an important step to ensure the reliability of the analysis. Previously, we published a research article related to the HPLC method validation to determine the content of two centelloids *i.e.* madecassoside and asiaticoside in herbal formulation (Monton et al., 2018). Then, in this work we validated the HPLC method for simultaneous determination of four centelloids containing in the plant raw material. Method validation results of this work are shown in Tables 2 and 3. Results showed that the response of the analysis and concentration of the standard compounds had a good linear correlation (R^2 close to 1.0). The UV spectrum at the upslope, top, and downslope of the peak of each centelloid in the extract were similar to those of standard centelloids (data not shown), indicating the specific of the analysis. The precision and accuracy results are shown in Table 3. The precision of the analysis presented as percent RSD of intraday and inter-day was less than 2% and 5%, respectively, indicating that the analysis of all four centelloids was precise. Accuracy represented as percent recovery was 93.03–98.19%, 97.88–109.40%, 102.39–105.08%, and 93.30–107.01% for madecassoside, asiaticoside, madecassic acid, and asiatic acid, respectively.

Extraction yield and content of individual centelloids are shown in Table 4. Extraction of *C. asiatica* using 10 model conditions provided extraction yield of 2.20–5.20%. Ranges of each centelloids content found in this work were 0.473–0.949%, 0.096–0.193%, 0.017–0.039%, and 0.003–0.022% for madecassoside, asiaticoside, madecassic acid, and asiatic acid, respectively based on raw data. However, we mentioned that the extraction procedure used in this work was not exhausting extraction. Extraction yield in this work was lower than the previous report by Niamnuy et al., as they reported the extraction yield of 11.41–17.87% (Niamnuy et al., 2013). Randriamampionona et al. (2007) showed *C. asiatica* in Madagascar contained madecassoside, asiaticoside, madecassic acid, and asiatic acid of 0.46–1.40%, 0.58–1.78%, not detect–0.29%, and 0.03–0.36%, respectively. James et al. (2008) reported *C. asiatica* in Southern Africa had content of four centelloids of 2.76–3.62%, 3.13–3.68%, 0.11–0.59%, and 0.05–0.64%, respectively. Rafamantanana et al. (2009) reported *C. asiatica* in Madagascar had content of four centelloids of 1.27–1.70%, 1.63–2.00%, not detect–0.95%, and not detect–0.98%, respectively. The variation of the centelloids content of *C. asiatica* was dependent on cultivation location and harvesting period which was investigated by Puttarak and Panichayupakaranant (2012). Furthermore, Niamnuy et al. (2013) reported *C. asiatica* from Pathum Thani Province, Thailand had a summation of the content of asiaticoside and asiatic acid higher than those of madecassoside and madecassic acid. However, our work found that summation of the content of asiaticoside and asiatic acid was lower than those of madecassoside and madecassic acid.

Fig. 1 shows contour plots and response surfaces of the model conditions of four centelloids content. Results showed similar contour plots and response surfaces between madecassoside and asiaticoside, while madecassic acid had a similar pattern to asiatic acid. In case of madecassoside and asiaticoside, extraction temperature and extraction time had a positive effect on their content. Increasing of extraction temperature or extraction time, the content of madecassoside and asiaticoside was increased. The mathematics model of the model conditions of madecassoside and asiaticoside were fitted to the quadratic model. According to madecassic acid and asiatic acid, high content of these two centelloids achieved at two conditions: low extraction temperature with low extraction time as well as high extraction temperature with high extraction time. The mathematics model of the model conditions of madecassic acid and asiatic acid were fitted to 2 factor interaction (2FI) model. The correlation plots between the predicted and actual value of the model conditions of madecassoside, asiaticoside, madecassic acid, and asiatic acid are shown in Fig. 2. This result could be used to confirm the reliability of the prediction

Table 2
Linearity parameters (linear equation and R^2), range, DL and QL of the analysis.

Compounds	Equation	R^2	Range (μg/ml)	DL (μg/ml)	QL (μg/ml)
Madecassoside	$y = 3922.5x + 1354.7$	0.9999	0.78–50	0.21	0.61
Asiaticoside	$y = 5199.6x + 1398.3$	0.9999	0.78–50	0.20	0.60
Madecassic acid	$y = 16018x + 2342.6$	0.9999	0.78–50	0.19	0.59
Asiatic acid	$y = 22659x + 5060.4$	0.9998	0.78–50	0.22	0.65

Table 3
Precision and accuracy of the analysis.

Compounds	Conc. (μg/ml)	Precision (%RSD)		Spike amount (μg/ml)	Accuracy
		Intraday	Inter-day		Recovery (%)
Madecassoside	1.5625	1.47	2.22	1.5625	93.68 ± 0.72
	12.5	0.33	1.41	12.5	93.03 ± 0.44
	25	0.45	0.98	25	98.19 ± 0.04
Asiaticoside	1.5625	0.84	1.87	1.5625	109.40 ± 1.64
	12.5	0.15	1.36	12.5	97.88 ± 2.55
	25	0.16	0.91	25	98.74 ± 3.11
Madecassic acid	1.5625	0.66	2.20	1.5625	105.08 ± 0.55
	12.5	1.05	0.62	12.5	102.39 ± 0.08
	25	1.07	1.18	25	104.91 ± 0.04
Asiatic acid	1.5625	0.15	1.76	1.5625	93.30 ± 0.36
	12.5	0.18	0.64	12.5	104.66 ± 0.10
	25	0.06	0.52	25	107.01 ± 0.06

Table 4
Extraction yield and content of individual centelloids of model conditions.

Condition	Yield (%w/w)	Content (%w/w)			
		Madecassoside	Asiaticoside	Madecassic acid	Asiatic acid
1	3.30	0.475 ± 0.002	0.096 ± 0.001	0.039 ± 0.000	0.022 ± 0.000
2	4.55	0.818 ± 0.004	0.166 ± 0.001	0.028 ± 0.000	0.005 ± 0.001
3	3.25	0.810 ± 0.017	0.156 ± 0.002	0.025 ± 0.000	0.008 ± 0.000
4	5.20	0.904 ± 0.006	0.182 ± 0.001	0.037 ± 0.000	0.014 ± 0.000
5	3.20	0.661 ± 0.060	0.151 ± 0.016	0.020 ± 0.001	0.005 ± 0.001
6	4.65	0.941 ± 0.005	0.192 ± 0.000	0.034 ± 0.000	0.008 ± 0.001
7	2.20	0.617 ± 0.052	0.127 ± 0.013	0.018 ± 0.001	0.007 ± 0.000
8	3.75	0.856 ± 0.080	0.169 ± 0.018	0.034 ± 0.002	0.011 ± 0.001
9	2.30	0.623 ± 0.059	0.122 ± 0.013	0.020 ± 0.001	0.006 ± 0.001
10	2.90	0.743 ± 0.002	0.151 ± 0.000	0.032 ± 0.000	0.012 ± 0.000

(Duangjit et al., 2012, 2014a, 2014b). The significant correlation between predicted and actual value of model conditions of four centelloids ($p < 0.05$) with relatively high R^2 for madecassoside and asiaticoside and moderate R^2 for madecassic acid and asiatic acid was observed in this work. However, correlation plots of the model conditions and R^2 of madecassic acid and asiatic acid indicated poor fitting between predicted and actual value. The assumption of this occurrence was degradation of madecassoside and asiaticoside into madecassic acid and asiatic acid during the extraction process, respectively. Puttarak et al. (2016) revealed that glycoside form of centelloids were less stable in alkaline pH and accelerated condition compared to aglycone form. In our work, *C. asiatica* was extracted at high temperature in some condition with slightly alkaline pH of the solvent (approximately 8.0). The extraction condition might induce the degradation of both madecassoside and asiaticoside, so the content of analyzed madecassic acid and asiatic acid could be varied from madecassic acid and asiatic acid composed in *C. asiatica* in their nature with additional degradation of both madecassoside and asiaticoside during the extraction process.

Table 5 shows the term of the significant model, regression coefficient, and *p*-value for the independent variable. According to the madecassoside and asiaticoside, *p*-value less than 0.05 indicated model terms are significant. In this case, $X_1, X_2, X_1X_2, {X_1}^2$ were significant model terms. The Lack of Fit was not significant relative to the pure error, which non-significant Lack of Fit was good because we want the model to fit. In case of madecassic acid, X_1 and X_1X_2 were significant model terms. Furthermore, X_1X_2 was a significant model term for asiatic acid. The Lack of Fit is also significant for both madecassic acid and asiatic acid. These results indicated that the model could be affect by the pure error. The Lack of Fit data was related to the result of the correlation plots between the predicted and actual value of the model conditions above. Furthermore, the multiple regression analysis was investigated and adjusted R^2 was reported as an indicator of the suitability of each linear regression equation to estimate the coefficient of the determination (Duangjit and Kraisit, 2018). The predicted R^2 of four centelloids was in reasonable agreement with the adjusted R^2 because of the difference between two data less than 0.2. In addition, adequate precision measures the signal to noise ratio, a ratio greater than 4 was desirable for four centelloids.

As the coefficient value in Table 5, the coded equations used for estimation of the content of four centelloids could show as the equation in Table 6. According to these equations, extraction temperature and extraction time play a positive effect on the content of four centelloids, except extraction temperature had a negative effect on asiatic acid content. Extraction temperature and extraction time affected the content of chemical constituents in the extract which was reported in previous reports. The high content of phenolic compound, flavonoid, and condensed tannins of *C. asiatica* was achieved when temperature increased. But, the extraction time had the optimum value due to prolongation of extraction time possibly decomposed of plant chemical compounds

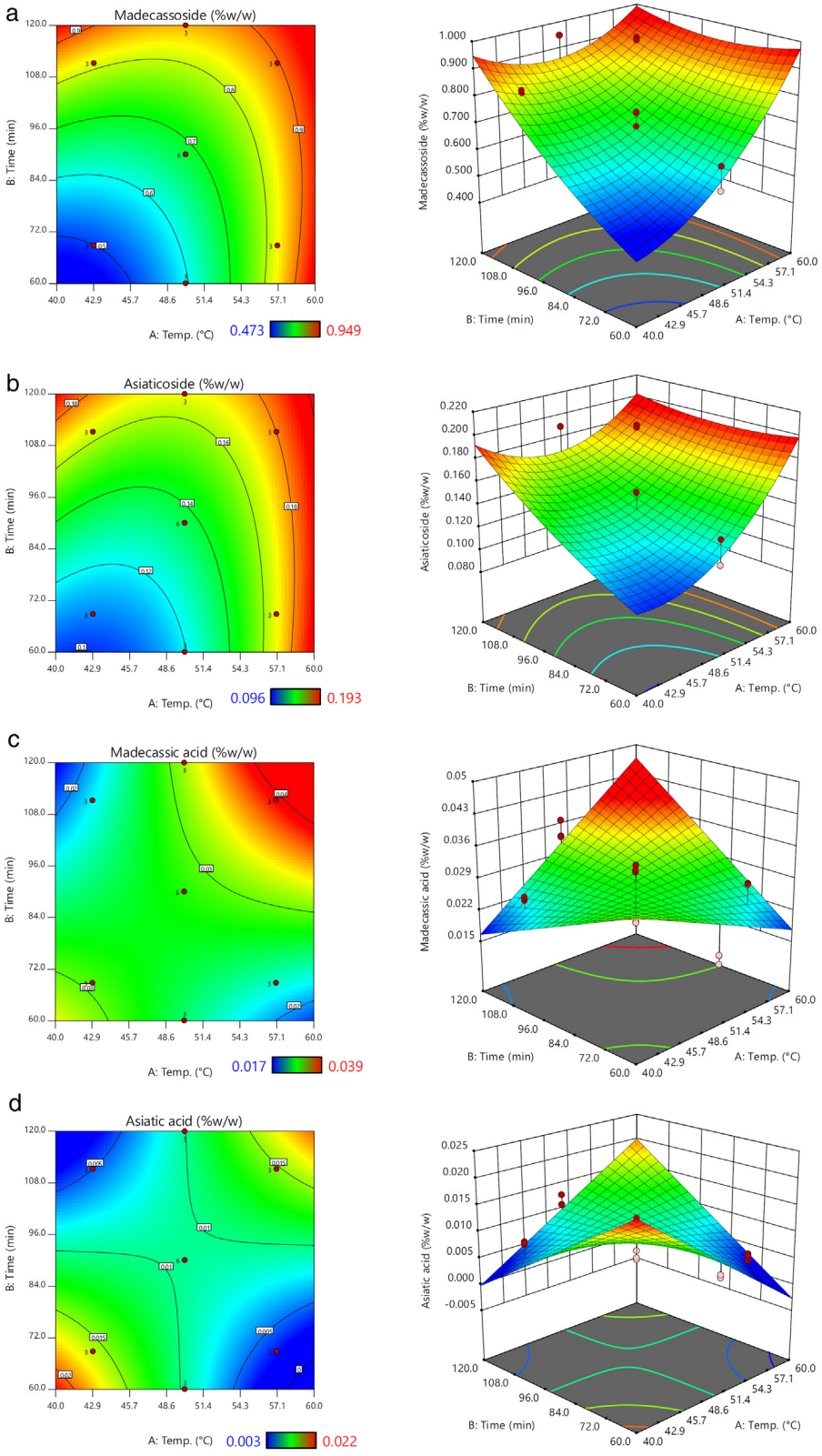

Fig. 1. Contour plots (left) and response surfaces (right) of the model conditions of the content of (a) madecassoside, (b) asiaticoside, (c) madecassic acid, and (d) asiatic acid.

(Chew et al., 2011). The effect of extraction temperature and extraction time was also reported in the extraction of other plants. Lv et al. (2005) investigated the effect of extraction temperature and extraction time on the content of amygdalin of Apricot-kernel and *Prunus tomentosa* Thunb. using reflux extraction. The content of amygdalin increased when temperature and duration time was increased. After the maximum content reached, amygdalin content gradually decomposed. The longer extraction time leads to thermal instability and degradation of plant compound, which was reported in other publications. Polysaccharides from

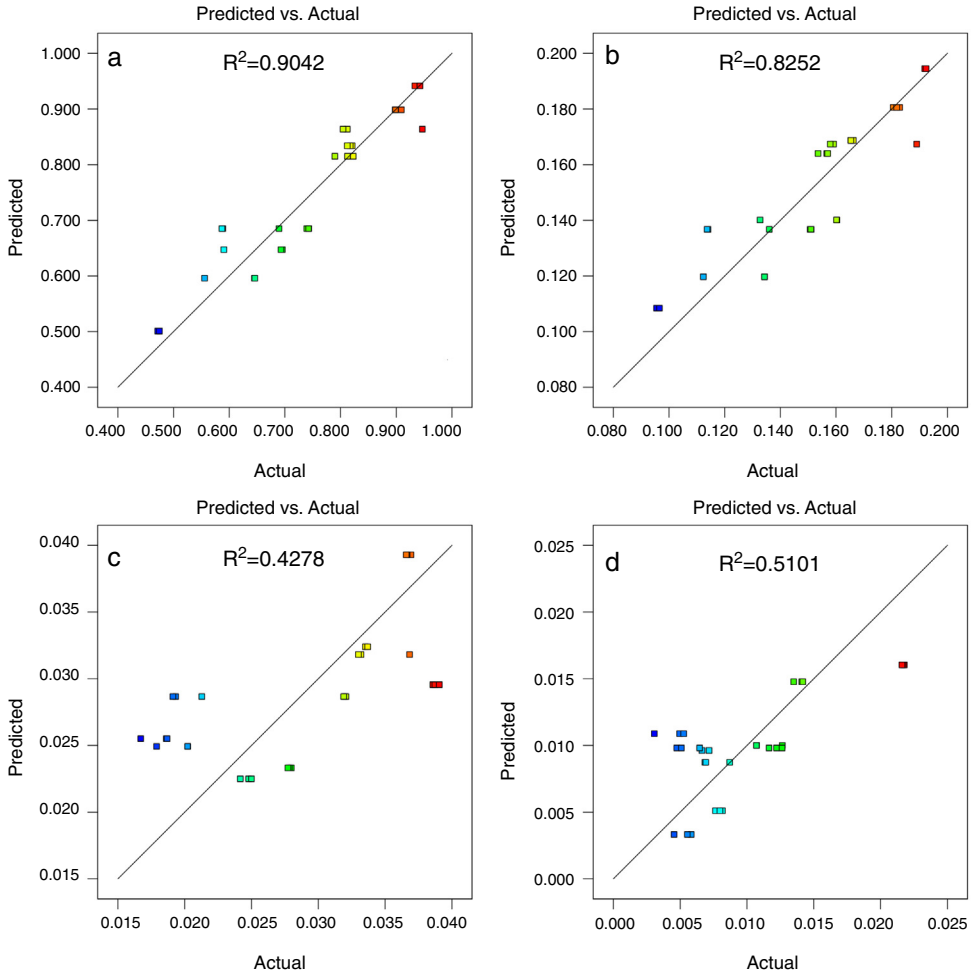

Fig. 2. The correlation plots between predicted vs actual value of model conditions of the content of (a) madecassoside, (b) asiaticoside, (c) madecassic acid, and (d) asiatic acid.

Table 5
The term of the significant model, regression coefficient, and *p*-value for the independent variable.

Polynomial term	Madecassoside		Asiaticoside		Madecassic acid		Asiatic acid	
	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value
Model	–	<0.0001[a]	–	<0.0001[a]	–	0.0020[a]	–	0.0003[a]
Intercept	0.6833	–	0.1364	–	0.0286	–	−.0097	–
X_1: temp.	0.1041	<0.0001[a]	0.0192	<0.0001[a]	0.0026	0.0384[a]	−0.0008	0.3373
X_2: time	0.0947	<0.0001[a]	0.0168	<0.0001[a]	0.0022	0.0770	0.0001	0.8674
X_1X_2	−0.0624	0.0002[a]	−0.0109	0.0096[a]	0.0058	0.0024[a]	0.0056	<0.0001[a]
X_1^2	0.0547	0.0005[a]	0.0153	0.0003[a]	–	–	–	–
X_2^2	0.0224	0.1086	0.0034	0.3596	–	–	–	–
R^2	0.9042	–	0.8252	–	0.4278	–	0.5101	–
Adjusted R^2	0.8843	–	0.7888	–	0.3618	–	0.4536	–
Predicted R^2	0.8543	–	0.7315	–	0.2376	–	0.3401	–
Adequate precision	19.7686	–	14.3129	–	7.7524	–	9.1687	
Lack of Fit	–	0.6034	–	0.0675	–	<0.0001[a]	–	<0.0001[a]

[a] Significant value.

Table 6
Mathematics model and coded equation of the model conditions.

Compound	Model	Coded equation
Madecassoside	Quadratic	$Y_1 = 0.6833 + 0.1041(X_1) + 0.0947(X_2) - 0.0624(X_1)(X_2) + 0.0547(X_1)^2 + 0.0224(X_2)^2$
Asiaticoside	Quadratic	$Y_2 = 0.1364 + 0.0192(X_1) + 0.0168(X_2) - 0.0109(X_1)(X_2) + 0.0153(X_1)^2 + 0.0034(X_2)^2$
Madecassic acid	2FI	$Y_3 = 0.0286 + 0.0026(X_1) + 0.0022(X_2) + 0.0058(X_1)(X_2)$
Asiatic acid	2FI	$Y_4 = 0.0097 - 0.0008(X_1) + 0.0001(X_2) + 0.0056(X_1)(X_2)$

Table 7
Predicted value, actual value, percent error, and range of 95% CI.

Response	Predicted value (%w/w)	Actual value (%w/w)	Error (%)[a]	95% CI (lower-upper)
Y_1	0.993	0.855 ± 0.0005	−16.1	0.854–1.132
Y_2	0.203	0.174 ± 0.0001	−16.7	0.165–0.240
Y_3	0.047	0.053 ± 0.0001	11.3	0.032–0.062
Y_4	0.020	0.025 ± 0.0003	20.0	0.010–0.030

[a] %Error = (actual value − predicted value) × 100/actual value.

Hovenia dulcis peduncles degraded when longer extraction time was used (Liu et al., 2015) as well as polysaccharide from *Angelica sinensis* (Tian et al., 2017). Furthermore, the higher extraction temperature and extraction time lead to degradation of *Astragalus cicer* polysaccharides (Shang et al., 2018), *Tricholoma mongolicum* polysaccharides (Wang et al., 2015), and mulberry fruit polysaccharides (Chen et al., 2015). In case of pectin isolated from sugar beet pulp, when pH of the extraction medium was constant, extraction time had a greater effect on pectin yield compared to extraction temperature (Yapo et al., 2007). Vergara-Salinas et al. (2012) studied the effect of temperature (50–200 °C) and time (5–30 min) on the polyphenolic content of deodorized thyme (*Thymus vulgaris*) using pressurized hot water extraction. They found that the content of polyphenols was diverse in different extraction temperature and extraction time. The maximum yield of polyphenols *i.e.* hydroxycinnamic acids, flavones, flavonols/flavanones, and total polyphenols achieved at 100 °C and 5 min. The higher extraction temperature and longer extraction time could reduce the diversity of polyphenols. The 3,4-dihydroxyphenyllactic acid was the only phenolic compounds that mostly extracted at high temperature. However, the nonphenolic antioxidant was favored at the higher extraction temperature and extraction time. Dent et al. (2013) established extraction temperature had a positive effect on mass fractions of sage (*Salvia officinalis*) total polyphenols, rosmarinic acid, and luteolin-3-glucuronide, while extraction time had a positive effect only on mass fractions of luteolin-3-glucuronide. Ultrasound-assisted extraction of rosmarinic acid and caffeic acid from *Apeiba tibourbou* showed that extraction time had a significant effect, while extraction temperature had a non-significant effect on both rosmarinic acid and caffeic acid (Martins and da Conceicao, 2015). Kuzmanović et al. (2015) using high temperature and high pressure reactor to extract the phenolic compound from corn silage. They varied extraction temperature, extraction time, etc. The optimization results showed that temperature was the most significant factor affecting phenolic compound content. These above results indicated that optimal temperature and time of the extraction should be optimized to obtain the maximum content of plant compounds that might affect their biological and pharmacological activity.

Optimal condition provided the simultaneous highest content of four centelloids was extraction temperature of 60 °C and extraction time of 120 min with desirability value of 0.980. The predicted values and actual values of the content of four centelloids predicted follow the optimal condition, percent error, and the range of 95% CI are shown in Table 7. The *C. asiatica* extracted follow the optimal condition provided the content of madecassoside, asiaticoside, madecassic acid, and asiatic acid of 0.855%, 0.174%, 0.053%, and 0.025%, respectively (Table 7). The actual values of the four centelloids were slightly different from the predicted value, however, they were still within 95% CI. These results indicated that the prediction by the computer software was accurate.

Conclusions

The response surface methodology could be used to optimize the dynamic maceration of *C. asiatica* to obtain the simultaneous high of four centelloids content with the reliable result. The simultaneous high content of madecassoside, asiaticoside, madecassic acid, and asiatic acid achieved when extraction was at 60 °C for 120 min. The contents of these four centelloids analyzed by validated HPLC were 0.855%, 0.174%, 0.053%, and 0.025%, respectively. This optimal condition could be used as a standard condition for extraction of *C. asiatica* to provide the highest content of four centelloids.

Authors' contributions

CM is a project leader, design the experiment, contributed to experimental part, analyzed and interpreted of the data, and draft the manuscript. SS contributed to experimental part and analyzed of the data. CL and TS analyzed and interpreted of the data. All authors have read and approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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