Food and Applied Bioscience Journal, 2020, 8(2): 14−26

Optimization of phenolic extraction from coffee by−product using response surface methodology and their antioxidant activities

Nisakorn Saewan*, Wanisa Vichit and Ampa Jimtaisong

Abstract

Coffee is one of the most popular beverage and important agricultural commodities. During the processing of coffee bean by wet process, coffee pulp (CP) which is a fibrous mucilagenous material was removed as a waste and become an environmental problem. The coffee pulp contained high amount of phenolic compounds which exhibited a wide range of physiological properties. This study aimed to optimize the extraction of phenolic compounds from CP using response surface methodology (RSM) and determine their antioxidant. The independent factors included microwave power (X_1), concentration of ethanol in water (X_2) and solvent to raw material ratio (X_3). The optimal condition parameters predicted were microwave power of 540 watt, 41% ethanol in water and solvent to raw material ratio at 22:1 with corresponding phenolic content 0.653 mg GAE/mL. These parameters were validated with an experiment carried out under optimal condition. The obtained phenolic 0.661 ± 0.003 mg GAE/mL was comparable with the optimized condition, which indicated that the model was adequate to optimize extraction of phenolic from coffee by−products. The CP extract under optimum condition (10 mg/mL) showed good antioxidant activities in Ferric reducing power (4.38 ± 0.01 mg AAE/mL), DPPH radical scavenging (97.62 ± 0.26%), nitric oxide inhibition (90.41 ± 4.72%), and superoxide dismutase activity (85.73 ± 3.72%).

Keywords: Antioxidant activity, coffee pulp, microwave−assisted extraction, phenolic compounds, response surface methodology

School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand

**Corresponding author E−mail: nisakorn@mfu.ac.th*

Received: 27 February 2020, Accepted: 2 June 2020

Food and Applied Bioscience Journal, 2020, 8(2): 14−26

1. Introduction

Coffee (*Coffea* sp.) is one of the most important agricultural commodities and widely consumed beverage in the world. In processing of coffee, husk and pulp is removed from coffee bean which is further roasted to obtain coffee aroma. The husk and pulp are produced as wastes and become an environmental problem. Fortunately, coffee pulp contains high amount of phenolic compounds which exhibit a wide range of physiological properties such as antioxidant, anti−inflammatory, anti−carcinogenic, anti−microbial, and anti−aging properties (Carocho and Ferreira, 2013; Sergent *et al.,* 2010). The coffee phenolic compounds include chlorogenic acid (42.2%), epicatechin (21.6%), isochlorogenic acid I (5.7%), isochlorogenic acid II (19.3%), isochlorogenic acid III (4.4%), catechin (2.2%), rutin (2.1%), protocatechuic acid (1.6%), and ferulic acid (1.0%) (Ramirez−Martinez, 1988). Thus, coffee pulp (CP) has potential to utilize in many fields such as food, pharmaceutical and cosmetics production.

The extraction of phenolic compounds from plant materials was accomplished by traditional methods (e.g. maceration, and soxhlet extraction) which require relatively large quantities of solvent and may be operated at high temperature and long extraction periods. These conditions might degrade the phenolic compounds. The alternative extraction techniques have been developed for reducing the extraction time, decreasing the solvent consumption, increasing the extraction yield, and enhancing the quality of extracts (Wang and Weller, 2006). Microwave−assisted extraction is a process of using microwave energy to heat solvents in contact with plant matrix and partition phenolic compound from the plant matrix into the solvent resulting in an increase of the kinetic of extraction (Delazar *et al.,* 2012).

Response surface methodology (RSM) is an effective tool for optimizing the extraction of active compound from plant by evaluating the effect of the variables factor and their interaction. The objective of this study was to optimize the microwave−assisted extraction condition of phenolic compounds from CP using RSM. A first set of experiments performed to identify the suitable extraction solvent (hexane, dichloromethane, ethyl acetate, ethanol, 50% aqueous ethanol and water), extraction technique (maceration, shaking, sonication, and microwave-assisted extraction) and time duration (1−5 h). RSM was designed to determine the effects of microwave power (180−900 watt), ethanol concentration (0−80%), and solvent to raw material ratio (10:1 – 30:1) on total phenolic content. Finally, antioxidant activities of CP extract (under optimum condition) was determined through ferric reducing power, DPPH radical scavenging, nitric oxide inhibition, and superoxide dismutase activities assays.

Food and Applied Bioscience Journal, 2020, 8(2): 14–26

2. Materials and Methods

2.1 CP Materials

CP was obtained as waste material from a coffee bean manufacturing in Chiang Rai, Thailand. The CP was dried at 40°C for 24 h in hot air oven (UNE 600, Memmert, Germany). Then, the dried CP was ground into fine powder (< 0.5 mm) and stored at room temperature until further analysis.

2.2 Selection of appropriate extraction conditions

Before the optimisation of phenolic compounds extraction through response surface methodology (RSM), the preliminary experiment was performed to select appropriate factors (extraction solvents, technique and time) which were effective on phenolic extraction yield. At a first step, the effect of different polarities of extraction solvents; hexane, dichloromethane, ethyl acetate, acetone, ethanol, 50% ethanol in water, and water on phenolic extraction was examined. Dried CP (1 g) was macerated with 20 mL of each solvent at room temperature for 2 h. The second step, the effect of extraction techniques including maceration, shaking (150 rpm), sonication (37 Hz), and microwave (540 watt) by using the best solvent chosen in the first preliminary step was studied. Final step of the preliminary experiment was to select the appropriate duration of extraction. The CP was extracted during various extraction time ranging from 1 – 5 h using the solvent and technique from the previous step.

2.3 Experimental design

Optimization of extraction conditions of phenolic from CP was carried out using response surface methodology (RSM) with Box–Behnken experiment. The effect of independent variables of microwave power (X_1, watt), ethanol concentration (X_2, %) and solvent to raw material ratio (X_3) at three variation levels were evaluated in the extraction process (Table 1). The sixteen experiments were conducted to establish models for phenolic extraction and antioxidant activity. The responses function (Y) was partitioned into linear, quadratic and interactive components. The variables were coded according to the following equation:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{m=i+1}^{3} b_{im}\, x_i X_m$$

Where Y is the dependent variable, b_0 is the model constant of the intercept, b_i, b_{ii} and b_{im} are the model coefficients which represent the linear, quadratic and interaction effects of the variables, and X_i and X_m are independent variable.

Food and Applied Bioscience Journal, 2020, 8(2): 14–26

Table 1 Variables and experimental design levels for response surface

Independent variables	Coded Symbols	Levels		
		–1	**0**	**+1**
Microwave power	(X_1)	180	540	900
Ethanol concentration	(X_2)	0	40	80
Liquid–to–solid ratio	(X_3)	10	20	30

2.4 Measurement of total phenolic content

Total phenolic content of samples were determined according to the procedure described by using the Folin–Ciocalteu assay (Vichit and Saewan, 2015). In brief, a 12.5 µL of sample were mixed with 50 µL of distilled water, 12.5 µL of folin ciocalteu reagent, and 125 µL of sodium bicarbonate solution. The mixtures were incubated for 60 min at 25ºC and then measured the absorbance at 750 nm. The total phenolic contents of samples were determined using the standard gallic acid calibration curve and expressed as gallic acid equivalents (mg GAE/mL).

2.5 Ferric reducing antioxidant power assay (FRAP)

The antioxidant activity was determined by ferric reducing antioxidant power method (Vichit and Saewan, 2015). The extract (25 µL) were mixed with 50 µL of 1% potassium ferricyanide and stood for 60 min at room temperature. Trichloroacetic acid (25 µL) and deionized water (75 µL) were added to the mixture and the absorbance was measured at 700 nm as absorbance 1 (A1). Then, 25 µL of 0.1% ferric chloride was added and the absorbance measured at 700 nm again as absorbance 2 (A2). The optical density of sample was calculated using the following equation:

$$\text{Optical density} = (A2 - A1)_{sample} - (A2 - A1)_{control}$$

Where, $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the tested sample. The results were expressed as ascorbic acids equivalent (mg AAE/mL extract).

2.6 DPPH radical scavenging activity assay (DPPH)

The scavenging of 1,1–diphenyl–2–picrylhydrazyl (DPPH) free radicals activity was determined according to Vichit and Saewan (2015). The reaction mixture containing 5 µL of extract and 195 µL of 0.1 mM of DPPH solution was incubated for 30 min. Absorbance was measured at 515 nm and the scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

Food and Applied Bioscience Journal, 2020, 8(2): 14–26

2.7 Nitric oxide inhibition assay

NHEK (20,000 cells/mL) were supplemented with 100 µL of CP extract before being stimulated with 1 µg/mL lipopolysaccharide (LPS) and incubated for 24 h (Vichit and Saewan, 2016). The nitric oxide production was assessed using Griess reagent system (Promega, USA). The percentage of nitric oxide inhibition was calculated as follows:

$$\text{Inhibition of nitric oxide (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

2.8. Superoxide dismutase activity

NHEK (20,000 cells/ml) were supplemented with 100 µL of CP extract and incubated for 24 h. Then, cells were harvested with 0.05 % typsin solution. Cells were lysed with lysis buffer and centrifuged at 3,000 rpm for 15 min (Vichit and Saewan, 2016). The SOD activity in supernatants was measured by using SOD assay Kit–WST (Sigma–Aldrich, USA). The SOD activity was calculated using the following equation:

$$\text{SOD activity (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

2.9 Statistical analysis

Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (Version 7.1.6, Stat–Ease, Inc., Minneapolis, MN, USA). The model adequacies were checked in terms of the R^2 values and adjusted R^2. Analysis of variance (ANOVA) was employed to determine the significance of the models. Verification of optimized conditions and predicted values were done in triplicate to confirm the validity of the model.

3. Results and Discussion

3.1 Effect of extraction solvents

Extraction efficiency of bioactive compound from natural source depends on the polarity of solvents which should closely match to the polarity of target compounds (Kaneria *et al.,* 2012). In order to select the extraction solvent, the CP sample was extracted with various solvents including hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc), ethanol (EtOH), 50% aqueous ethanol (50% EtOH) and deionized water (DI) and determined total phenolic content as a potential indicator for extraction efficiency. The mixture of ethanol and water showed significantly the highest total phenolic content (0.593 ± 0.021 mg GAE/mL), followed by ethanol (0.434 ± 0.052 mg GAE/mL) and water (0.197 ± 0.008 mg GAE/mL,

Food and Applied Bioscience Journal, 2020, 8(2): 14–26

as shown in Fig 1a. Therefore, 50% ethanol in water was selected as extraction solvent for the next step in the study.

3.2 Effect of extraction techniques

Extraction technique is an important step involved in the discovery of bioactive components from natural materials. Biological activities of natural extracts showed significant differences depending on extraction methods, emphasizing the importance of selecting the suitable extraction method (Hayouni *et al.,* 2007). In this study, CP was extracted with various extraction techniques; maceration, shaking, sonication, and microwave-assisted extraction in order to investigate the effect of extraction techniques on phenolic content. The highest content of phenolic compound was found in the microwave and ultrasound with 0.685 ± 0.018 and 0.672 ± 0.050 mg GAE/mL, followed by shaking and maceration with 0.630 ± 0.008 and 0.588 ± 0.004 mg GAE/mL, respectively, as shown in Fig 1b. Therefore, the microwave-assisted was selected as extraction technique for the next step in the study.

3.3 Effect of duration of extraction

The irradiation time is a relevant parameter effect on microwave-assisted extraction efficacy (Falleh *et al.,* 2008). Various extraction durations (1, 2, 3, 4 and 5 h) were performed in order to estimate the appropriate extraction periods for further RSM experiment. The result showed that total phenolic content significantly improved when extraction time increased from 1 to 3 h (Fig 1c). Beyond 3 h, total phenolic content slightly increased and remain constant during 4 to 5 h (0.382–0.386 mg GAE/mL). Thus, the microwave irradiation duration of CP was 3 h in further study.

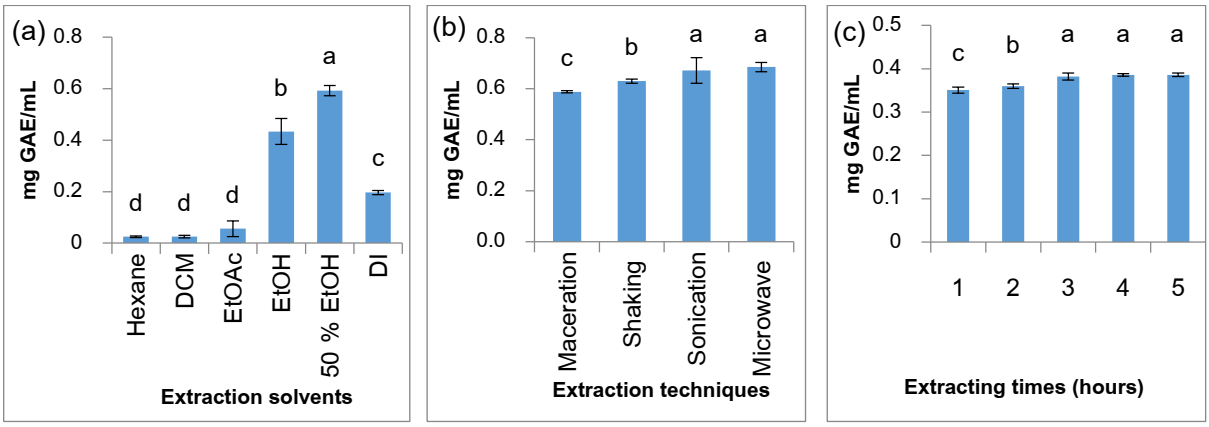

Fig 1 Effect of (a) extracting solvents, (b) extraction techniques and (c) duration of microwave–assisted extraction on phenolic content

Food and Applied Bioscience Journal, 2020, 8(2): 14–26

3.4 Optimization of phenolic compounds extraction by RSM

From the investigation of the extraction solvents, techniques and duration, the results showed that 50% ethanol in water, microwave-assisted technique and 3 h showed highest extraction efficacy on phenolic compound from CP. Therefore, optimization of phenolic extraction conditions using response surface methodology (RSM) was carried out with variables of microwave power (X_1, watt), ethanol concentration (X_2, %) and solvent to raw material ratio (X_3) at three variation levels as shown in Table 1. The sixteen experiments with four center points were conducted to establish models for phenolic extraction. The effect of microwave power (180, 540 and 900 watt), ethanol concentration (0, 40 and 80%) and solvent to raw material ratio (10:1, 20:1 and 30:1) on total phenolic content were reported in Table 2. The total phenolic content ranged from 0.354 to 0.667 mg GAE/mL. The highest values of total phenolic content were observed in the extraction with microwave power of 540 watt at 40% ethanol in water and solvent to raw material ratio at 20:1. The ANOVA of quadratic regression model of total phenolic content showed that this model was significant ($P<0.01$). Total phenolic content was obtained as

$$Y = 0.65 + 0.016X_1 - 0.10X_2 + 0.025X_3 + 5.179\text{x}10^{-3}X_1X_2 - 0.059X_1X_3 - 0.035X_2X_3 - 0.053X_1^2 - 0.13X_2^2 - 0.067X_3^2$$

Table 2 Responses surface design for combination of the independent variables and experiment data

Experiment number	Coded variables			Actual variables			Total phenolic content (mg GAE/mL)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	-1	-1	0	180	0	20	0.566
2	-1	0	-1	180	40	10	0.438
3	-1	0	+1	180	40	30	0.599
4	-1	+1	0	180	80	20	0.337
5	0	-1	-1	540	0	10	0.486
6	0	-1	+1	540	0	30	0.614
7	0	0	0	540	40	20	0.656
8	0	0	0	540	40	20	0.667
9	0	0	0	540	40	20	0.638
10	0	0	0	540	40	20	0.652
11	0	+1	-1	540	80	10	0.366
12	0	+1	+1	540	80	30	0.354
13	+1	-1	0	900	0	20	0.590
14	+1	0	-1	900	40	10	0.587
15	+1	0	+1	900	40	30	0.510
16	+1	+1	0	900	80	20	0.381

Food and Applied Bioscience Journal, 2020, 8(2): 14-26

A summary of ANOVA of the experimental results of the Box-Behnken design is provided in Table 3. The F-test was used to check the statistical significance of the regression equation. P-values were used as a tool to check the significance of each coefficient, which in turn might indicate the pattern of the interactions between the variables. The high F-value (140.90) and low P-value (P<0.001) indicated highly significant and good prediction of the present model.

The quadratic terms of all parameters were significant with at least P<0.05 on the phenolic content. The phenolic content of CP was significantly affected by ethanol concentration (P<0.001) followed by the liquid-to-solid ratio (P<0.01) and microwave power (P<0.05). Moreover, the interaction between parameters has significantly affected on total phenolic content with microwave power and liquid-to-solid ratio (X_1X_3) (P<0.001) and ethanol concentration and liquid-to-solid ratio (X_2X_3) (P<0.01). Therefore, it could be stated that the linear and quadratic effects of parameters were the main terms that produce significant effect to the response of phenolic content.

Table 3 Analysis of variance (ANOVA) for the regression equation of total phenolic content

Source	Sum of squares	df	Mean square	F Value	P Value	Remarks
Model	0.21	9	0.023	140.90	< 0.0001	***
X_1	0.002061	1	0.002061	12.55	0.0122	*
X_2	0.084	1	0.084	510.02	< 0.0001	***
X_3	0.004988	1	0.004988	30.39	0.0015	**
X_1X_2	0.0001073	1	0.0001073	0.65	0.4497	
X_1X_3	0.014	1	0.014	86.17	< 0.0001	***
X_2X_3	0.004904	1	0.004904	29.88	0.0016	**
X_1^2	0.011	1	0.011	68.69	0.0002	***
X_2^2	0.069	1	0.069	421.01	< 0.0001	***
X_3^2	0.018	1	0.018	108.72	< 0.0001	***
Residual	0.000985	6	0.0001642			
Lack of fit	0.0005568	3	0.0001856	1.30	0.4171	
	R^2=0.9953		R^2_{Adj}=0.9882	C.V.%=2.43		

Note: Remark column represent that statistically by *** is significant at P<0.001, ** is P<0.01, and * is P<0.05

To investigate the interactions of the variables and determine the optimal level of each variable, the response surface curves were plotted. Fig 2-4 show three independent response surface plots and their respective contour plots, which reveal information about the interactions between two variables, while the third variable fixed.

Food and Applied Bioscience Journal, 2020, 8(2): 14–26

Fig 2 shows the effect of microwave power (X_1), ethanol concentration (X_2), and their reciprocal interaction on phenolic content (%), when solvent to raw material ratio (X_3) was fixed at 20:1. The result revealed that the phenolic content increased with the increase in microwave power (X_1) and decreased with increase in ethanol concentration (X_2).

Fig 3 shows the effect of liquid–to–solid ratio (X_3) and microwave power (X_1), when ethanol concentration (X_2) was fixed at 40%. The phenolic content increased with increasing microwave power (X_1) and solvent to raw material ratio (X_3).

Fig 4 shows the effects of ethanol concentration (X_2) and liquid–to–solid ratio (X_3), when microwave power (X_1) was fixed at 540 watt. The phenolic content decreased with an increase in ethanol concentration (X_2) and increased with an increase in solvent to raw material ratio (X_3). With a further decrease of ethanol concentration, the phenolic content showed a slight decrease.

The optimal values of the selected variables were obtained by solving the regression equation. After calculation by Design Expert software, the optimal condition of phenolic extraction was microwave power of 540 watt at 40.90% ethanol and solvent to raw material ratio at 22.01:1. Under this condition, the highest phenolic content was 0.653 mg GAE/mL.

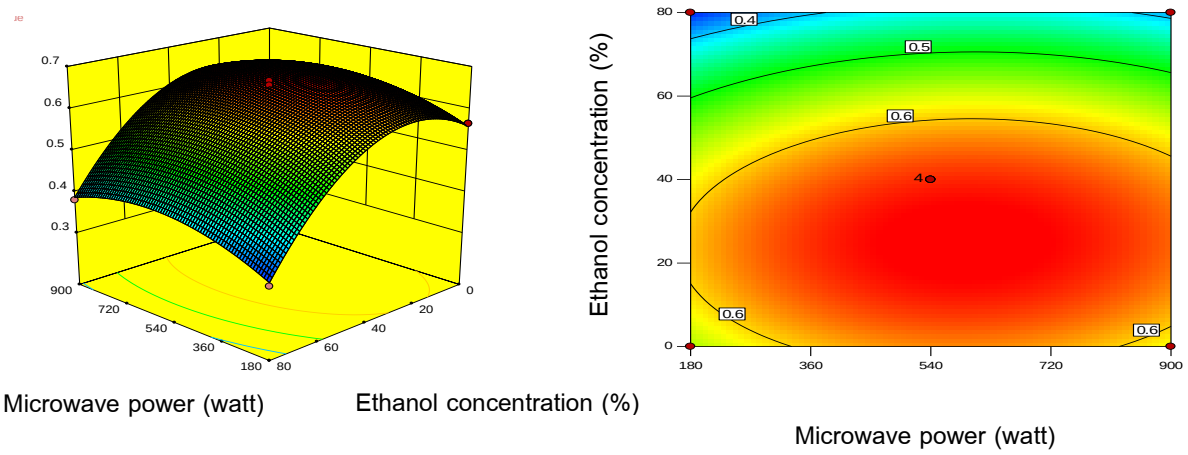

Fig 2 Response surface and contour plots for the effects of microwave power and ethanol concentration on total phenolic content

Food and Applied Bioscience Journal, 2020, 8(2): 14–26

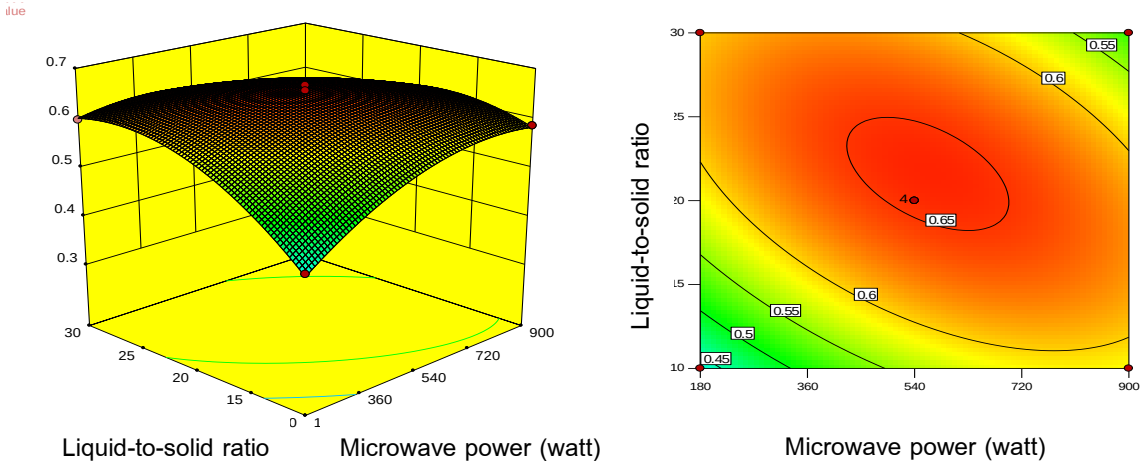

Fig 3 Response surface and contour plots for the effects of liquid–to–solid ratio and microwave power on total phenolic content

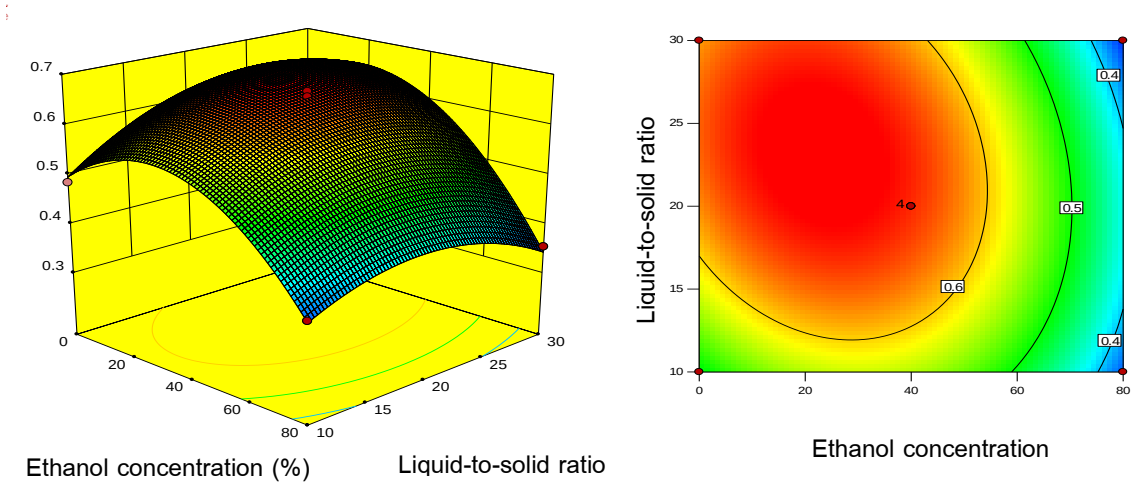

Fig 4 Response surface and contour plots for the effects of ethanol concentration and liquid–to–solid ratio on total phenolic content

3.5 Determination and experimental validation of the optimal conditions

In order to verify the predictive capacity of the model, a triplicate experiment was set up to validate with the optimized condition (540 watt at 41% ethanol and ratio at 22:1) and total phenolic content of the extract was also investigated. The experimental values agree with the predicted from the regression model with 95% confidence interval (Table 4). This result confirms the predictability of the model for the extraction of phenolic compounds from CP in the experimental condition used.

Food and Applied Bioscience Journal, 2020, 8(2): 14−26

Table 4 Comparison between the predicted value and observed value

Optimized condition		Total phenolic content (mg GAE/mL)	
		Predicted value	Experimental value
Microwave power (watt)	540		
Ethanol concentration (%)	41	0.653	0.661± 0.003
Solvent to raw material ratio	22:1		

3.6 Antioxidant activities of CP extract

The antioxidant activities of CP extract from optimal condition was evaluated by four assays: ferric reducing power, DPPH radical scavenging activity, nitric oxide inhibition and superoxide dismutase activity. It was found that all antioxidant activities of CP extract were increased with increasing concentration.

Ferric reducing antioxidant power assay measures the reduction of ferric ion (Fe^{3+})-ligand complex to ferrous (Fe^{2+}) complex by antioxidants in an acidic medium which can be monitored by measuring the Prussian blue color at 700 nm (Prabha and Vasantha, 2011). The CP extract showed good FRAP (4.38 ± 0.01 mg AAE/mL extract).

DPPH assay is a widely used method to evaluate the free radical scavenging ability of sample. The molecule of $DPPH^{\cdot}$ (1,1−diphenyl−2−picrylhydrazyl) is characterized as a stable free radical that can be reduced to DPPHH by antioxidant substances and cause of loss violet color to be detected at 515 nm (Alam *et al.,* 2013). The CP extract showed good DPPH radical scavenging activity (97.62 ± 0.26%) which was equivalent to 250 µg/mL ascorbic acid (96.43 ± 0.31%) as positive standard compound.

Nitric oxide is free radical that generated in biological tissues by specific nitric oxide synthases in various types of inflammatory disorders (Coleman, 2001). In this study, nitric oxide inhibition property was evaluated in human keratinocytes which activated by bacterial lipopolysaccharide (LPS) and detected by using Griess reagent. The CP extract showed high level of nitric oxide inhibition (90.41 ± 4.72%) with comparable to 500 µg/mL ascorbic acid (88.97 ± 4.20%).

Superoxide dismutase (SOD) is a primary antioxidant enzyme which protects organisms against the toxic effect of superoxide radicals (O^{2-}) by catalyzing their dismutation to hydrogen peroxide (H_2O_2) and oxygen (O_2) (Pieme *et al.,* 2010). In this study, the experiment was investigated for SOD induction effect in human keratinocyte cells with WST solution. The result showed that the CP extract was good antioxidant activity by inducting SOD effect 85.73 ± 3.72% which higher than 500 µg/mL of ascorbic acid (56.13 ± 1.24%).

4. Conclusion

The results showed that the operation power of microwave, ethanol concentration and solvent to raw material ratio had significant effects on extraction of active compounds from CP. The optimal condition of phenolic compounds extraction from CP was microwave−assisted of 540 watt at 41% ethanol and solvent to raw material ratio at 22:1 by showing the highest phenolic content which corresponding to 0.660 mg GAE/mL. The extract exhibited good antioxidant activities in all studied assay. Thus, CP is interesting to increase economic value and utilization in cosmetic industry.

Acknowledgements

The authors would like to acknowledge research funding from National Research Council of Thailand (NRCT). The authors also would like to thanks Mae Fah Luang University for providing scientific equipment and facilities for this work.

References

Alam, M. N., Bristi, N. J. and Rafiquzzaman, M. 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal. 21(2): 143−152.

Carocho, M. and Ferreira, I. C. 2013. The role of phenolic compounds in the fight against cancer–a review. Anti−Cancer Agents in Medicinal Chemistry. 13(8): 1236−1258.

Coleman, J. W. 2001. Nitric oxide in immunity and inflammation. International Immunopharmacology. 1(8): 1397−1406.

Delazar, A., Nahar, L., Hamedeyazdan, S. and Sarker, S. D. 2012. Microwave-assisted extraction in natural products isolation. Methods in Molecular Biology. 864: 89−115.

Falleh, H., Ksouri, R., Chaieb, K., Karray−Bouraoui, N., Trabelsi, N., Boulaaba, M. and Abdelly, C. 2008. Phenolic composition of Cynara cardunculus L. organs, and their biological activities. Comptes Rendus Biologies. 331: 372−379.

Hayouni, E. A., Abedrabba, M., Bouix, M. and Hamdi, M. 2007. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chemistry. 105(3): 1126−1134.

Kaneria, M., Kanani, B. and Chanda, S. 2012. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. Asian Pacific Journal of Tropical Biomedicine. 2(3): 195−202.

Pieme, C. A., Penlap, V. N., Ngogang, J. and Costache, M. 2010. In vitro cytotoxicity and antioxidant activities of five medicinal plants of Malvaceae family from Cameroon. Environmental Toxicology and Pharmacology. 29(3): 223−228.

Prabha, M. R. and Vasantha, K. 2011. Antioxidant, cytotoxicity and polyphenolic content of *Calotropis procera* (Ait.) R. Br. flowers. Journal of Applied Pharmaceutical Science. 1(7): 136−140.

Ramirez−Martinez, J. R. 1988. Phenolic compounds in coffee pulp: Quantitative determination by HPLC. Journal of the Science of Food and Agriculture. 43(2): 135−144.

Sergent, T., Piront, N., Meurice, J., Toussaint, O. and Schneider, Y.J. 2010. Anti−inflammatory effects of dietary phenolic compounds in an in vitro model of inflamed human intestinal epithelium. Chemico−Biological Interactions. 188(3): 659−667.

Vichit, W. and Saewan, N. 2015. Antioxidant activities and cytotoxicity of Thai pigmented rice. International Journal of Pharmacy and Pharmaceutical Sciences. 7(7): 329−334.

Vichit, W. and Saewan, N. 2016. Effect of germination on antioxidant, anti−inflammatory and keratinocyte proliferation of rice. International Food Research Journal. 23(5): 2006−2015.

Wang, L. and Weller, C. L. 2006. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science and Technology. 17(6): 300−312.