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ORIGINAL ARTICLE

Ultrasound assisted extraction of antioxidative phenolics from cashew (*Anacardium occidentale* L.) leaves

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Abstract Optimization of ultrasound-assisted extraction (UAE) of antioxidative phenolics from the leaves of cashew (*Anacardium occidentale* L.) was carried out by response surface methodology along with the central composite design. Two independent variables were considered: amplitude (30–77%) and time (7–31 min). The highest extraction yield was 23.61% when the optimal extraction condition (77% amplitude for 31 min) was implemented. The extract containing total phenolic content of 579.55 mg GAE/g dry extract possessed radical scavenging activities and reducing power. The experiment values were in line with the predicted counterparts. Extract contained gallic acid, isoquercetin, tannic acid, quercetin, catechin, apigenin, hydroquinin, eriodictyol, and rutin. The extract with increasing levels inhibited AAPH-induced DNA damage to a higher extent. Thus, UAE was demonstrated to potentially increase the extraction efficacy of phenolics from cashew leaves and the extract could be applied as a natural antioxidant.

Keywords Optimization · Antioxidant activity · Ultrasound-assisted extraction · Cashew · DNA damage

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Introduction

Lipid oxidation plays the adverse effect in biological system as well as foods. Reactive oxygen species could promote various diseases namely neurodegenerative disorders cardiovascular disease and cancer (Zhao et al. 2015). In recent years, several studies have been performed to search for the efficient radical scavengers to conquer the oxidative stress (Mohadjerani and Roodgar 2016; Yoshioka et al. 2017). Numerous antioxidants, particularly from plant extracts, have drawn attention for health promotion. Furthermore, the uses of natural antioxidants from plants are of increasing interest in food industry owing to their availability, biodegradability and low toxicity (Yingngam et al. 2015).

Cashew (*Anacardium occidentale* L.) is an economic plant, which is abundant in the southern part of Thailand. Several parts of cashew tree, especially cashew leaves possess phenolics with bioactivities (Kamath and Rajini 2007). Apart from consumption, it has been extensively used in folk medicine for therapy of mouth ulcers, throat problems as well as gastrointestinal disorders (Kudi et al. 1999). Phenolic compounds have been reported for their antioxidative activity by donating electrons and metal chelation, thereby preventing the occurrence of diseases and the loss in food quality (Sindhi et al. 2013).

The extraction process of phenolic compounds is a significant step prior to further isolation, purification and application of the extracts. Many techniques, especially conventional method, is limited due to toxicological effect associated with solvent used and long extraction time. To tackle these problems, ultrasound-assisted process has been employed to improve the extraction efficiency with short extraction time, simplicity and low cost (Wang et al. 2013).

Ultrasound having a frequency ranging from 20 kHz to 100 MHz shows cavitation and mechanical effects, which lead to implosion bubbles (Ince et al. 2014). This phenomenon causes disruption of plant cell and decrease in particle size and intensification of mass transfer (Sharmila et al. 2016). Thus, non-toxic solvent in combination of ultrasound can be implemented to enhance the extraction efficiency of plant antioxidant.

Response surface method (RSM) is useful for optimizing various parameters in industrial process (Shekarchizadeh et al. 2009). Therefore, the purposes of this investigation were (1) to optimize the extraction of phenolics from cashew leaves by ultrasound-assisted process using RSM and (2) to examine antioxidant activity and protective effect of the resulting extract against plasmid DNA damage.

Materials and methods

Chemicals

Ferric chloride ($FeCl_3.6H_2O$), potassium persulfate, 2,2′-azinobis (2-ethyl benzothiazoline-6-sulfonate (ABTS) and ferrous sulfate ($FeSO_4.7H_2O$) were procured from Sigma (MO, USA). Gallic acid and 2,2,-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma (MO, USA). Ethanol was purchased from Merck (Darmstadt, Germany). Standards (catechin, isoquercetin, gallic acid, tannic acid, apigenin, hydroquinin, eriodictyol, rutin and quercetin) were obtained from Sigma-Aldrich, Inc. (Chemie GmbH. Steinheim, Germany). Plasmid DNA (pUC18) was procured from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

Collection of cashew leaves

Cashew (*Anacardium occidentale*, L.) leaves were gifted by an orchard in Songkhla province, Thailand. The samples, collected during November and December 2016, were prepared and dried as tailored by Chotphruethipong et al. (2017). Dried samples were subjected to blending and sieved through a screen with a size of 80 mesh. Thereafter, the obtained powder was subjected to chlorophyll removal by mixing the powder with chloroform at (1:20; w/v) (Chotphruethipong et al. 2017), followed by filtration through Whatman filter paper No.1 (Whatman International Ltd., Maidstone, UK). The retentate was dried at 105 °C for 1 h using a hot air oven (Memmert, Schwabach, Germany). Prepared cashew leaf powder was collected.

Preparation of cashew leaf extracts

Ultrasound-assisted extraction was performed using an ultrasonic equipment (Sonics, Model VC750, Sonica & Materials, Inc., Newtown, USA, 20 kHz ± 50 Hz, 750 W). To 10 grams of cashew leaf powder placed in a 250-mL beaker, 200 mL of 80% ethanol were added. The sonication time was controlled via the equipment panel, in which the external water was used to keep temperature at 35 ± 5 °C. After extraction, the mixtures were prepared as guided by Chotphruethipong et al. (2017). The resulting extracts were kept in a desiccator until further analysis.

Optimization of extraction of antioxidative phenolics by ultrasound-assisted process using RSM

Experimental design

Ultrasound-assisted process was optimized using RSM and CCD was conducted using Design-Expert Statistical package version 7.0 (Statease, Inc Minneapolis, Minn, USA). Two independent variables studied were the amplitude (X_1, %) and sonication time (X_2, min) as evaluated at five different levels ($-\alpha$, -1, 0, $+1$, $+\alpha$). The actual and coded forms of each independent variable are given in Table 1. The dependent variables included %yield, TPC, DPPH and ABTS radical scavenging activities and FRAP. Experimental data were fitted to a first-order polynomial model (1) and second-order model (2) as follows:

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ij} X_i X_j \quad (1)$$

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_i X_i^2 + \beta_j X_j^2 + \beta_{ij} X_i X_j \quad (2)$$

where Y are the dependent variables. X_i and X_j are the independent variables. The regression coefficients are designated as β_0 for model constant, β_i for linear and β_{ij} for interaction coefficients. The fitted polynomial equations were then illustrated in the form of three-dimensional response surface graphs.

Analyses

Extraction yield

Extraction yield was calculated and reported as percentage (Chotphruethipong et al. 2017).

Total phenolic content (TPC)

TPC was examined as described by Sato et al. (1996) and expressed as mg gallic acid equivalent (GAE)/g dry extract.

Table 1 Central composite design used to optimize the extraction of antioxidative phenolics from cashew leaves using ultrasound-assisted process and experimental results

Run order	Independent variables		Dependent variables				
	Amplitude (X_1)	Time (X_2)	Yield (%)	TPC (mg GAE/g dry extract)	DPPH radical scavenging activity (mmol TE/g dry extract)	ABTS radical scavenging activity (mmol TE/g dry extract)	FRAP (mmol TE/g dry extract)
1	30 (− 1)	7 (− 1)	20.15 ± 0.84	516.15 ± 18.09	10.39 ± 0.14	4.62 ± 0.08	8.87 ± 0.10
2	77 (1)	7 (− 1)	20.58 ± 0.40	558.87 ± 14.99	11.72 ± 0.07	5.67 ± 0.08	10.40 ± 0.04
3	30 (− 1)	31 (1)	22.92 ± 2.29	545.69 ± 3.53	11.10 ± 0.13	5.30 ± 0.07	10.07 ± 0.24
4	77 (1)	31 (1)	23.19 ± 0.13	579.00 ± 8.91	12.14 ± 0.01	6.29 ± 0.03	10.28 ± 0.21
5	20.27 (− 1.41)	19 (0)	21.51 ± 1.86	525.73 ± 3.97	10.96 ± 0.21	4.73 ± 0.08	9.22 ± 0.02
6	86.73 (1.41)	19 (0)	22.59 ± 0.25	570.47 ± 16.84	11.65 ± 0.07	6.21 ± 0.14	10.57 ± 0.02
7	53.50 (0)	2.03 (− 1.41)	20.91 ± 2.76	527.07 ± 11.19	11.08 ± 0.27	4.83 ± 0.08	8.95 ± 0.23
8	53.50 (0)	35.97 (1.41)	22.78 ± 0.62	571.94 ± 15.71	12.06 ± 0.14	6.11 ± 0.07	10.63 ± 0.04
9	53.50 (0)	19 (0)	21.84 ± 0.01	543.14 ± 3.10	11.21 ± 0.01	5.27 ± 0.04	10.20 ± 0.23
10	53.50 (0)	19 (0)	22.21 ± 1.40	545.85 ± 8.30	11.39 ± 0.03	5.18 ± 0.07	9.89 ± 0.05
11	53.50 (0)	19 (0)	22.41 ± 2.26	550.55 ± 1.17	11.32 ± 0.06	5.14 ± 0.02	9.97 ± 0.06
12	53.50 (0)	19 (0)	22.40 ± 2.97	548.73 ± 1.99	11.58 ± 0.43	5.11 ± 0.10	10.10 ± 0.22
13	53.50 (0)	19 (0)	22.32 ± 3.37	550.13 ± 3.44	11.26 ± 0.13	5.25 ± 0.09	10.10 ± 0.04

Values are mean ± standard error (SE) (n = 3)

Antioxidative activities

Firstly, all the samples were appropriately diluted using the distilled water as diluent. DPPH and ABTS radical scavenging activities and FRAP were determined following the procedures of Wu et al. (2003), Arnao et al. (2001) and Benzie and Strain (1996), respectively.

Verification of the optimum condition

The experimental errors of model were calculated by comparing between experimental and predicted values as follows:

$$\text{Error}\,(\%) = [(\text{Experimental value} - \text{predicted value})/\text{Experimental value}] \times 100$$

LC/DAD/MSD analysis of phenolic compounds

Phenolics in the selected extract prepared using the optimized condition were analysed using LC/DAD/MSD as described by Chotphruethipong et al. (2017). A diode array detector together with a scan mode (100–700 *m/z*) MS detector were equipped.

Effect of cashew leaf extract on the protection of AAPH-induced plasmid DNA damage

Activity of the extract against AAPH-induced plasmid DNA damage was determined as tailored by Yarnpakdee et al. (2015) with some modification. The reaction system included 4 µL of 0.025 µg/µL pUC 18 supercoiled plasmid dissolved in buffer solution (Tris–HCl (10 mM) buffer comprising 1 mM EDTA (1 mM), pH 7.0), 4 mL of 10 mM AAPH and 2 µL of cashew leaf extract at different levels (75, 100 and 200 µg/mL). Thereafter, the incubation of mixture was conducted at 37 °C for 30 min in dark. The mixtures were subsequently stained with SYBR gold and loaded onto 1% agarose gel electrophoresis using a horizontal gel electrophoresis system (Mini-Sub® cell GT, Biorad, Hercules, CA, USA) at 100 V for 40 min equipped with PowerPac™ basic power supply (Biorad, Hercules, CA, USA). The DNA bands were visualized under transillumination of UV light with the aid of Uvitec chemiluminescence Documentation System (Uvitec, Cambridge, UK). The supercoiled DNA band retained was calculated and reported as % relative to the control (without AAPH).

Statistical analysis

UAE was optimized using CCD according to the above mentioned procedure.

Results and discussions

Optimization of extraction of antioxidative phenolic compounds by ultrasound-assisted process using RSM

Based on CCD experiment, 13 treatments derived from two independent variables, named amplitude (X_1) and sonication time (X_2), are used (Table 1). All the experimental data, fitted well with the first order polynomial equations, except ABTS radical scavenging activity, which fitted with the second order polynomial equation. Variation in the response variables as indicated by F value and P value ($P < 0.05$) (Table 2) could be attributed to at least one of the model's parameters as shown in the analysis of variance (ANOVA) result (Table 2). The non-significant value of lack of fit ($P > 0.05$) revealed the validation of the obtained models.

Impact of independent variables on extraction yield

Among all dependent variables studied, the extraction yield was greatly affected by linear term of time (X_2) ($P < 0.0001$). Although the interaction between two parameters showed no influence on the yield, the linear term of amplitude (X_1) was still considered as a significant parameter in this model ($P < 0.05$). The yield was in the range of 20.15–23.19% (Table 1). The response surface plot of extraction yield is illustrated in Fig. 1. The result indicated that both amplitude and sonication time had significantly positive effect on yield. An increase in variable values resulted in higher extraction yield. The highest extraction yield was 23.61% when the optimal extraction condition (77% amplitude for 31 min) was implemented. This was approximately threefold higher, compared to that of typical solvent extraction method (Chotphruethipong et al. 2017). The increased extraction yield was mostly owing to the cavitation effect. Ultrasonic induced cavitation caused by violent collapse of bubbles in the system (Pang et al. 2011). The increased cavitation bubble collision promoted the diffusion of solvent into the plant material and provided the enhanced rate of mass transfer (Avhad and Rathod 2015; Soria and Villamiel 2010). This led to the increased extraction efficacy as indicated by increased yield. The coefficient of determination ($R^2 = 0.8406$) indicates that 84.06% of the response variability was reasonably explained by the model. Furthermore, the non-significant value of lack of fit (0.0987) and the model's P value (< 0.001) (Table 2) reflected that the model was in line with the prediction. Influence of independent variables, amplitude (X_1) and time (X_2), on the yield of extracts is given as follows:

$$Y = 22.01 + 0.32X_1 + 0.97X_2 \quad (3)$$

Table 2 Analysis of variance (ANOVA) for the fitted linear and quadratic polynomial models for optimization of extraction parameters

Source	DF[a]	SS[b]	MS[c]	F value	P value
% Yield					
Model	2	8.26	4.13	26.36	0.0001[s]
Lack of fit	6	1.34	0.22	4.04	0.0987[ns]
Pure error	4	0.22	0.055		
Total error	12	9.82			
R^2	0.8406				
TPC					
Model	2	4187.70	2093.85	71.75	< 0.0001[s]
Lack of fit	6	252.52	42.09	4.28	0.0903[ns]
Pure error	4	39.30	9.83		
Total error	12	4479.52			
R^2	0.9713				
DPPH radical scavenging activity					
Model	2	2.19	1.10	24.37	< 0.0001[s]
Lack of fit	6	0.37	0.061	2.94	0.1580[ns]
Pure error	4	0.083	0.021		
Total error	12	2.64			
R^2	0.8298				
ABTS radical scavenging activity					
Model	5	3.59	0.72	96.77	< 0.0001[s]
Lack of fit	3	0.033	0.011	2.31	0.2182[ns]
Pure error	4	0.019	0.0005		
Total error	12	3.64			
R^2	0.9857				
FRAP					
Model	3	3.59	1.20	27.67	< 0.0001[s]
Lack of fit	5	0.33	0.066	4.44	0.0869[ns]
Pure error	4	0.059	0.015		
Total error	12				
R^2	0.9022				

[a]Degree of freedom
[b]Sum of squares
[c]Mean square
[s]Significant
[ns]Non-significant

Impact of independent variables on total phenolic contents (TPC)

Various extraction conditions showed different TPC of resulting extracts. TPC obtained varied from 516.15 to 579 mg GAE/g dry extract (Table 1). TPC were markedly affected by linear term of amplitude (X_1) and time (X_2) ($P < 0.0001$). Impact of independent variables on TPC of the extracts is demonstrated below.

$$Y = 548.72 + 17.41X_1 + 14.14X_2 \quad (4)$$

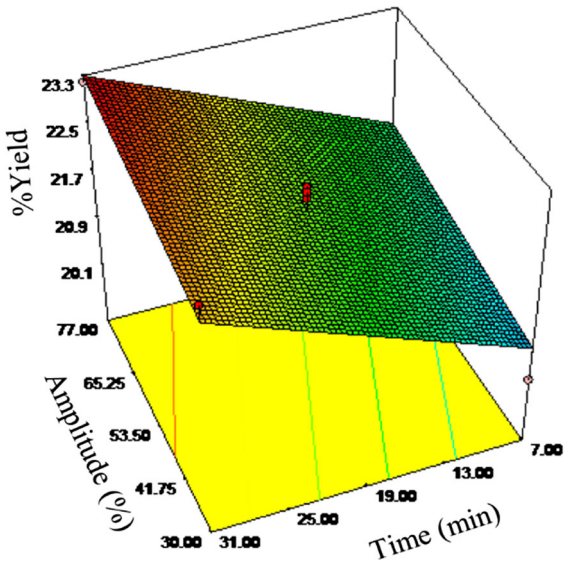

Fig. 1 Response surface plot of extraction yield of cashew leaf extracts as affected by the selected independent variables

Relationship between different variables is shown in Fig. 2a. The increases in amplitude and time tended to increase TPC of the extract, more likely due to the enhanced acoustic cavitation in conjunction with heat generated, which caused plant cell disruption, particle reduction and intensification of mass transfer (Sharmila et al. 2016). Soluble constituents were transferred or extracted into the solvents increasingly when ultrasonic process is applied (Shirsath et al. 2012). The highest TPC was observed at amplitude of 77% and time of 31 min. Increasing amplitude above 77% had no effect on TPC ($P > 0.05$). The R^2 value of model was 0.9713, which showed high correlation between predicted and experimental values (97.13% of data matching). Moreover, the *P*-value of the model (< 0.0001) and non-significant lack of fit (0.0903) (Table 2) also proved that the data obtained from experiment was adequately good with the model.

Impact of independent variables on antioxidant activities

Most of linear term of variables (X_1 and X_2) had significant impact on all the antioxidative activities examined ($P < 0.05$). However, linear terms (X_1 and X_2) showed the most significance toward ABTS radical scavenging activity ($P < 0.0001$). Quadratic terms (X_1^2 and X_2^2) were shown to be significant for ABTS radical scavenging activity ($P < 0.01$). When considering interactive terms between variables, the significance was found only for FRAP ($P < 0.05$). Effect of amplitude (X_1) and time (X_2) on DPPH and ABTS radical scavenging activities and FRAP of the extracts are expressed as follows:

$$Y_{DPPH} = 11.37 + 0.42X_1 + 0.31X_2 \quad (5)$$

$$Y_{ABTS} = 5.19 + 0.52X_1 + 0.39X_2 - 0.015X_1X_2 + 0.14X_1^2 + 0.14X_2^2 \quad (6)$$

$$Y_{FRAP} = 9.94 + 0.46X_1 + 0.43X_2 - 0.33X_1X_2 \quad (7)$$

Relationship between variables in term of response surface plots is shown in Fig. 2b–d. Overall, antioxidative activities increased with increasing amplitude and sonication time. According to Eqs. (5–7), the amplitude was the higher significant variable on activities ($P < 0.001$) than sonication time ($P < 0.05$). The highest activities of extract were found at amplitude 77% and time 31 min. It was noted that antioxidative activities were in accordance with TPC and extraction yield. The increase in amplitude mostly caused the augmented decomposition of plant cell. As a result, the phenolics with antioxidative activity were more released. However, the higher amplitude might lead to the decrease in activities of phenolics. Pyrolysis during cavitational collapse and the formation of hydroxyl radicals (·OH) were caused by cavitational thermolysis. This resulted in the chemical decomposition by opening ring of phenolic compounds (Alighourchi et al. 2013; Tiwari et al. 2009). The R^2 value of the predicted models for DPPH and ABTS radical scavenging activities and FRAP were 0.8298, 0.9857 and 0.9022, respectively. Moreover, *P*-value of the model was found to be significant (< 0.0001), while lack of fit was insignificant (0.1580, 0.2182 and 0.0869, respectively) (Table 2).

Verification of the optimum condition

The regression equation and model of optimum condition (77%, and 31 min) were verified. The predicted values under optimized condition were: extraction yield (23.29%), TPC (581.50 mg GAE/g dry extract), DPPH, ABTS radical scavenging activities and FRAP (12.11, 6.36 and 10.50 mmol TE/g dry extract, respectively). The observed experimental value of TPC was 579.55 ± 6.82 mg GAE/g dry extract (error = 0.34%). DPPH, ABTS radical scavenging activities and FRAP were 11.85 ± 0.18 (error = 2.15%), 6.04 ± 0.13 (error = 5.30%), 10.28 ± 0.42 mmol TE/g dry extract (error = 2.14%), respectively. The yield was 23.61 ± 0.06% (error = 1.36%). It was noted that predicted value was similar to the observed values, indicating the validation of the model for the optimized condition for the extraction of phenolics from cashew leaves using ultrasound-assisted process. Therefore, ultrasound assisted process was the potential tool for manufacturing of nutraceutical and functional foods from indigenous plant.

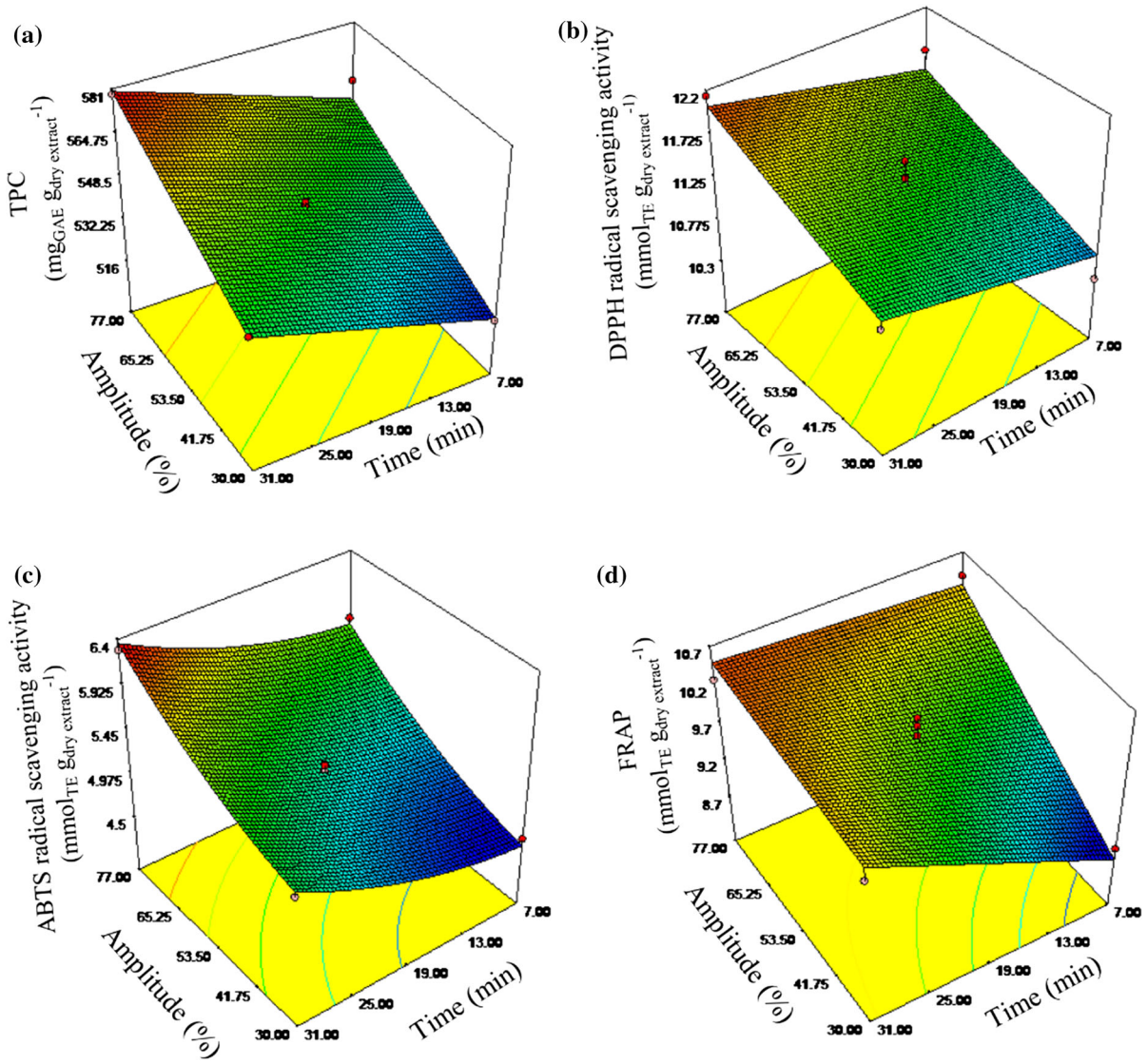

Fig. 2 Response surface plots of phenolic compounds and antioxidative activities of cashew leaf extracts as affected by the selected independent variables. TPC **a**, DPPH radical scavenging activity **b**, ABTS radical scavenging activity **c** and FRAP **d**

LC/DAD/MSD analysis of phenolic compounds

The extract contained nine phenolic compounds. Isoquercetin (7002 mg/kg) and catechin (4946 mg/kg) constituted as the major compounds. Gallic acid (2692 mg/kg), quercetin (2660 mg/kg) and hydroquinin (1291 mg/kg) were also present at high amount (Table 3). Tannic acid, eriodictyol, apigenin and rutin were the minor components. Recently, Chotphruethipong et al. (2017) reported six phenolic compounds including isoquecetin, tannic acid, catechin, gallic acid, rutin and hydroquinin in the extract from cashew leaves using 80% ethanol as the solvent. It was noted that catechin and isoquercetin were the major phenolic compounds in both ethanolic extracts of cashew leaves, regardless of extraction methods used. However, some additional phenolics including quercetin, apigenin

Table 3 Phenolic compounds in cashew leaf extract prepared under the optimum extraction condition analyzed by LC/DAD/MSD

Phenolic compounds	Retention time (min)	Content of phenolic compounds[a] (mg/kg dry extract)
Gallic acid	6.70–7.20	2692 ± 56
Catechin	12.40–12.60	4946 ± 69
Tannic acid	12.70–13.00	281 ± 19
Rutin	15.20–15.40	499 ± 50
Isoquercetin	16.40–16.50	7002 ± 87
Hydroquinin	24.00–24.30	1291 ± 33
Eriodictyol	31.20–31.30	5.9 ± 0.5
Quercetin	33.90–34.00	2660 ± 18
Apigenin	41.90–42.00	6.6 ± 0.3

[a]Values are mean ± standard error (SE) (n = 3)

and eriodictyol were found in the ethanolic extract when ultrasound-assisted process was used. Coincidentally the extraction yield, TPC and antioxidative activities obtained from ultrasound-assisted process under the optimal extraction condition were higher than those from solvent extraction as reported by Chotphruethipong et al. (2017). Similar result was documented by Singh et al. (2017) who found that the use of ultrasound-assisted process could increase TPC of the extracts from whole mung bean, hull and cotyledon more effectively, compared to conventional solvent extraction using acetone, ethanol, methanol and water. Ultrasounds have been used to induce the plant cell destruction, liberating cell contents (Mason et al. 1996). This directly contributed to the increased efficiency in extraction of phenolics as shown by the presence of additional compounds as well as increased yield. Thus, ultrasound-assisted process was able to enhance the effectiveness in extraction of phenolics from cashew leaves.

Protective effect of cashew leaf extract against AAPH-induced plasmid DNA damage

The ability of cashew leaf extract in preventing AAPH-induced oxidative DNA damage is shown in Fig. 3. No damage of supercoiled DNA was found in the control sample, while the supercoiled DNA was changed to the relaxed and linear forms when exposed to AAPH. The result indicated that DNA damage was indicated by peroxyl radicals (ROO·) generated by AAPH. The degree of DNA damage was in the following order linear DNA > relaxed DNA > supercoiled DNA (Strick et al. 1998). Reactive oxygen species (ROS) are generally formed by cellular metabolisms (Yoshioka et al. 2017). An excessive production of ROS results in their accumulation, causing oxidative stress in the cells. ROS caused the DNA modifications, including sister chromatid exchange, base modification, strand scission, DNA–DNA or DNA–protein cross-links (Yarnpakdee et al. 2015). When DNA was treated with cashew leaf extract, the rates of DNA nicking by AAPH decreased, especially when the concentration of cashew leaf extract increased. The supercoiled DNA band intensity was retained by 60.38, 90.05%, and 94.81% when cashew leaf extract at the concentration of 75, 100 and 200 μg/mL were present, respectively (lane 3, 4 and 5, respectively). Conversely, supercoiled DNA was not found in the sample treated with AAPH (lane 2). This result indicated that the cashew leaf extract could inhibit the DNA strand scission as evidenced by the remaining supercoiled DNA bands. The inhibitory effect toward DNA strand scission of cashew leaf extract was more likely because of their ability in scavenging peroxyl radicals. Recently, Chotphruethipong et al. (2017) reported that the

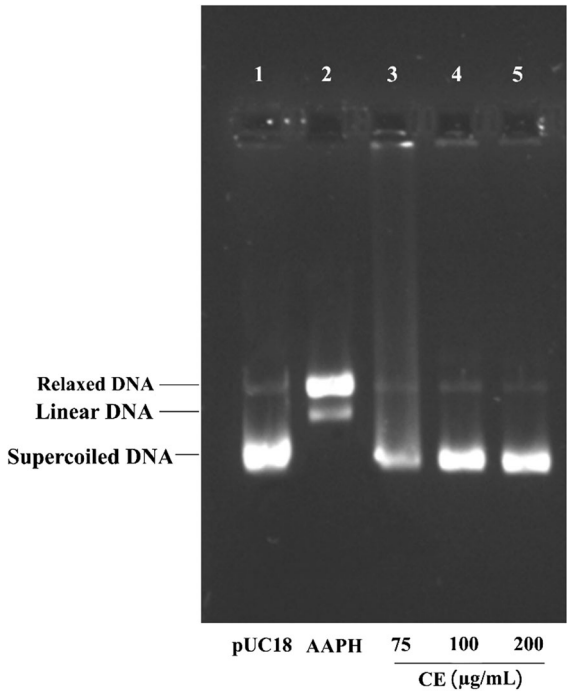

Fig. 3 Agarose gel electrophoresis of pUC 18 plasmid DNA treated with AAPH in the present of cashew leaf extract at different concentrations. Lane 1: DNA alone; lane 2: DNA + AAPH; lanes 3–5: DNA + AAPH + cashew leaf extract at the concentration of 75, 100, and 200 μg/mL, respectively

ethanolic cashew leaf extract had ability in scavenging both peroxyl and hydroxyl radicals. Moreover, the predominant phenolics in cashew leaf extract were isoquercetin, catechin and quercetin. These phenolic compounds were reported to have capability of scavenging both hydroxyl and peroxyl radicals as well as can protect the oxidative damage of DNA (Min and Ebeler 2009; Treml and Šmejkal 2016; Yang et al. 2008). Therefore, cashew leaf extract rich in polyphenols could prevent DNA damage induced by peroxyl radical.

Conclusion

Ultrasound-assisted process was successfully optimized using RSM to extract antioxidative phenolics from cashew leaves. Optimal conditions for extraction were: 77% of amplitude and time of 31 min. Isoquercetin was the most predominant in the extract. Cashew leaf extract exhibited the inhibitory effect toward DNA damage induced by peroxyl radical. Cashew leaf extract could serve as alternative natural antioxidant as food additive and nutraceutical.

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