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Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts

[1]Chew, K. K., [1]Ng, S. Y., [1]Thoo, Y. Y., [1]Khoo, M. Z., [1]Wan Aida, W. M. and [1,*]Ho, C. W.

[1]*School of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, Cheras 56000 Kuala Lumpur, Malaysia*
[2]*School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia 43600 Bangi, Selangor Darul Ehsan, Malaysia*

Abstract: The present study was to optimize the phenolic recovery from *Centella asiatica* by investigating the effects of ethanol concentration (0-100%, v/v), extraction time (60-300 min) and extraction temperature (25-65°C) on phenolic extraction using single-factor experiments. Total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin content (CTC) were used for determination of phenolic content while 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging capacity and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity were used for measuring the antioxidant capacity of *C. asiatica* extract. All extraction conditions had significant effect (p<0.05) on the phenolic contents and antioxidant capacities of *C. asiatica* extract. The optimal conditions for phenolic recovery were 40% ethanol for 60 min at 65°C, with values of 1203.49 mg GAE/ 100 g DW for TPC, 561.92 mg CE/ 100 g DW for TFC, 181.25 mg CE/ 100 g DW for CTC, 730.37 µmol TEAC/ 100 g DW for ABTS and 1948.30 µmol TEAC/ 100 g DW for DPPH. TFC was found to be positive correlated significantly (0.902, p<0.05) with DPPH under influence of ethanol concentration. However, all antioxidant compound assays (TPC, TFC and CTC) were negatively correlated significantly with ABTS under the effect of extraction temperature.

Keywords: *Centella asiatica*, Total phenolic content (TPC), Total flavonoid content (TFC), Condensed tannin content (CTC), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging capacity, 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity

Introduction

"Pegaga" (*Centella asiatica*) is one of the famous Malaysian herbal remedies that had been used widely as folk medicine to treat a wide range of illness (Brinkhaus *et al*., 2000). Further experimental studies showed that several biological properties of *C. asiatica* extract have antioxidant activity (Randriamampionona *et al*., 2007). According to Zainol *et al*. (2003), phenolic compounds, such as phenolic acid, flavonoids and tannins are the major contributors to the antioxidant property of *C. asiatica*. Phenolic compounds are especially important antioxidants because their redox potentials can allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Tsao and Deng, 2004).

Extraction process is widely used as a process of separation to obtain a crude extract of phytochemicals from the plant materials (Chirinos *et al*., 2007; Tabart *et al*., 2007). However, owing to each plant material has its unique properties in term of phenolic extraction, different plants may require different extraction conditions to achieve maximum recovery of phenolic compounds (Chirinos *et al*., 2007). There are few factors would contribute in influencing the rate of extraction and quality of extracted bioactive phenolic compounds, including type of extraction solvent, solvent concentration, particle size of medicinal plants, temperature and pH of extraction and extraction time (Liyana-Patthirana and Shahidi, 2004; Nobre *et al*., 2005).

Optimization of extraction process can be achieved by either empirical or statistical methods and it is essential for commercial application of the phenolic extraction process (Liyana-Pathirana and Shahidi, 2004; Rodrigues *et al*., 2008). In present study, single factor experiment was employed to optimize the extraction of phenolic compounds from *C. asiatica*. Although this method is time-consuming and has several drawbacks, such as lack of information on the interaction of dependent response (Zhang *et al*., 2007), results from present study which apply the empirical method can be used as screening or preliminary test for purification of phenolic compounds in future studies. Besides that, the results

*Corresponding author.
Email: *cwho@ucsi.edu.my*
Tel: +603 91018880; Fax: +603 91023606

from this study can also be used in determination of the lower, middle and upper levels of independent variables employed in response surface methodology (RSM) (Liyana-Pathirana and Shahidi, 2005).

To the best of our knowledge, there is no optimal extraction protocol has been developed for phenolic extraction from *C. asiatica* using mixture of ethanol and water as a extraction solvent. Thus, it is important to develop an optimal and specific extraction condition using binary solvent of ethanol and water for extraction of *C. asiatica* which would maximize the recovery yield of phenolic compounds and process efficiency. The aim of this study was to investigate the effects of ethanol concentration, extraction time and extraction temperature on the extraction of phenolic compounds (total phenolic content, TPC; total flavonoid content, TFC; and condensed tannin content, CTC) and the free radical-scavenging capacity of extracts from *C. asiatica* for radicals generated by 2,2'-azinobis(3-ehtylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Materials and Methods

Chemicals

Concentrated hydrochloric acid (37% purity), sodium carbonate (≥99.9% purity) and Folin-Ciocalteu phenol reagent were purchased from Merck (Darmstadt, Germany). Gallic acid (98% purity), vanillin (99%) and trolox were purchased from Acros Organics (New Jersey, USA). Sodium nitrute, (+)-catechin hydrate (98% purity), potassium persulphate (≥98% purity), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS, ≈98 purity) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH, 95% purity) were purchased from Sigma-Aldrich (Steinheim, Germany). Absolute ethanol (≥99.4% v/v), methanol (99% v/v) denatured ethanol, aluminium chloride-6-hydrate (>99% purity) and sodium hydroxide were purchased from Fisher Scientific (Leicestershire, UK). All chemicals used throughout the experiment were analytical grade and all stock solutions were prepared using purified deionized water (MilliQ purification system, Millipore, France).

Plant material

Powdered form of "Pegaga" (*Centella asiatica*) was purchased from local herb supplier, Ethno Resources Sdn. Bhd. (Selangor, Malaysia). Upon arrival of the herb samples, they were packaged into a nylon-linear-low-density polyethylene film and stored in dark environment at an ambient temperature for further experiment.

Preparation of extracts

2 g of powdered *C. asiatica* were extracted with 20 mL of extraction solvent in a 100 mL glass conical flask. The conical flask was sealed with parafilm and wrapped with aluminum foil to prevent solvent loss and exposure of light. The mixture was then shaken in a shaking machine (Model Green SSeriker, Vision, Korea) or a temperature-controlled water bath shaker (Model WNB 7–45, Memmert, Germany) at a constant speed throughout the extraction process at the required temperature. After completing the extraction process, the *C. asiatica* extract was filtered through Whatman No.1 filter paper (Whatman International Ltd., England) in order to obtain a clear crude extract solution. Subsequently, this crude extract was subjected to antioxidant assays for analysis purpose without storage overnight. All the extraction processes were done in replicate and all the analyses on each sample were done in triplicate.

Experimental design

In this study, the effects of extraction parameters (ethanol concentration, extraction time and extraction temperature) on extracting of phenolic compounds from *C. asiatica* were determined based on single factor experiment. After solvent extraction, the *C. asiatica* extracts were subjected to antioxidant compound assays (total phenolic content (TPC) assay, total flavonoid content (TFC) assay and condensed tannins content (CTC) assay) and antioxidant capacity assays (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging activity assay and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay). However, the optimum conditions for solvent extraction of *C. asiatica* were mainly determined based on the profile of TPC assay as this assay represents an important parameter in evaluation the efficiency of extraction process. TFC and CTC assay were also conducted in this study for determination of concentration of flavonoids and tannins, respectively responsible in the TPC assay.

(1) Ethanol and water were selected as extraction solvent in present study. *C. asiatica* were extracted with ethanol concentration ranging from 0 to 100% (0, 20, 40, 60, 80 and 100% ethanol) at fixed extraction time (180 min) and extraction temperature (25^{o}C). The best ethanol concentration was selected based on the values of TPC assay.

(2) *C. asiatica* was extracted with the best ethanol concentration as determined in the first step at fixed extraction temperature (25^{o}C) with varying extraction

time, ranging from 60 to 300 min (60, 120, 180, 240 and 300 min). The best extraction time was selected based on the value of TPC assay.

(3) *C. asiatica* was extracted with the best ethanol concentration as determined in the first step and extraction time as determined in the second step at vary extraction temperature, ranging from 25 to 65°C (25, 35, 45, 55 and 65°C). The best extraction temperature was selected based on the value of TPC assay.

Determination of total phenolic content

TPC of *C. asiatica* extracts was determined with Folin-Ciocalteu reagent, according to the method of Li *et al*. (2008) with slight modifications. Crude extracts were diluted to 50 times with deionized water prior to analysis. 1 mL of diluted extract was mixed with 1 mL of diluted Folin-Ciocalteu reagent (10 times diluted with deionized water). After incubating the mixture at room temperature for 4 min, 0.8 mL of 7.5% (w/v) sodium carbonate anhydrous solution was added into the mixture. The mixture was then immediately vortexed for 10 s and incubated in dark environment at room temperature for 2 h. Blank was prepared by replacing 1 mL of *C. asiatica* extract with 1 mL of deionized water. The absorbance of the mixture was measured against blank at 765 nm by using UVi light spectrophotometer (Model XTD 5, Secomam, France). Gallic acid was used to calibrate the standard curve and the calibration equation for gallic acid was $y = 0.0396x$ ($R^2 = 0.9975$). Each crude extract was analyzed in triplicate and the results were expressed in milligrams of gallic acid equivalents per 100 gram of dry weight (mg GAE / 100 g DW).

Determination of total flavonoid content (TFC)

TFC of *C. asiatica* extract was determined by using a method described by Ozsoy *et al*. (2008) with slight modifications. 0.25 mL of *C. asiatica* extract was firstly mixed with 1.25 mL of deionized water, followed by addition of 75 µL of 5% (w/v) sodium nitrite solution. After 6 min, 150 µL of 10% (w/v) aluminium chloride solution was added and the mixture was allowed to stand at room temperature for 5 min. Subsequently, 0.5 mL of 1 M sodium hydroxide was added into mixture and followed by addition of 275 µL of deionized water. The mixture was then vortexed for 10 s and its absorbance was immediately measured at 510 nm by using UVi light spectrophotometer (Model XTD 5, Secomam, France). Blank was prepared by replacing 0.25 mL of *C. asiatica* extract with 0.25 mL of deionized water. (+)-catechin was used to calibrate the standard curve and the calibration equation for (+)-catechin was $y = 0.0033x$ ($R^2 = 0.9991$). Each crude extract was analyzed in triplicate and the results were expressed as milligrams of (+)-catechin equivalent per 100 g dried weight (mg CE / 100 g DW).

Determination of condensed tannin content (CTC)

CTC of crude extracts was estimated by using vanillin-HCl method according to the method of Makkar and Becker (1993) with slight modifications. 3 mL of 4% (w/v) vanillin reagent were added into 0.5 mL of *C. asiatica* extract, followed by addition of 1.5 mL of concentrated hydrochloride acid. The mixture was vortexed for 10s and then allowed to stand at room temperature for 15 min. Blank was prepared by replacing 0.5 mL of *C. asiatica* extract with 0.5 mL of deionized water. The absorbance of mixture was measured against blank at 500 nm by using UVi light spectrophotometer (Model XTD 5, Seconman, France). (+)-catechin was used to calibrate the standard curve and the calibration equation for (+)-catechin was $y = 0.002x$ ($R^2 = 0.9922$). Each crude extract was analyzed in triplicate and the results were expressed as milligrams of (+)-catechin equivalent per 100 g dried weight (mg CE / 100 g DW).

ABTS radical-scavenging capacity

The radical scavenging capacity of *C. asiatica* extract against ABTS radical cation was measured by using the method of Wetwitakyaklung *et al*. (2006) and Li *et al*. (2008) with slight modifications. An ABTS radical solution was prepared by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate solution at a ratio 1:1 (v/v) in a 250 mL amber volumetric flask. The mixture was vortexed for 10 s and then allowed to stand in dark condition at room temperature for 12-16 hrs to give a dark blue solution and it was used for analysis within 2 days. The absorbance of ABTS radical solution was equilibrated to an absorbance of 0.7 ± 0.02 at 734 nm by diluting with ethanol before used. 0.1 mL of *C. asiatica* extract was mixed with 3.9 mL of ABTS radical solution. Blank was also prepared by replacing 0.1 mL deionized water with *C. asiatica* extract. The absorbance of mixture was read immediately at 734 nm against blank after incubation at room temperature for 6 min.

Simultaneously, absorbance of negative control (3.9 mL of ABTS radical solution and 0.1 mL of ethanol) was also measured at 734 nm by using UVi spectrophotometer (Model XTD5, Secomam, France). Absorbance of negative control was measured at each level of treatments and the values were averaged and used as a constant for calculation. The radical-scavenging capacity of ABTS (%) was calculated as

[1- (A_s / A_c)] x 100% (A_s = Absorbance of sample at 734 nm; A_c = Absorbance of negative control at 734 nm). Trolox solution was used to calibrate the standard curve and the calibration equation for trolox was y = 120.1142x (R^2 = 0.9984). Each crude extract was analyzed in triplicate and the results were expressed as µmol trolox equivalent per 100 g dried weight (µmol TEAC / 100 g DW).

DPPH radical-scavenging capacity

DPPH assay was carried out as described by Saha *et al*. (2004) and Tenpe *et al*. (2008) with slight modification. 100 µL of *C. asiatica* extract was mixed with 3.9 mL of ethanolic DPPH (60 µM). Subsequently, the mixture was immediately vortexed for 1 min and it was allowed to stand at room temperature for 30 min. Blank was prepared by replacing 100 µL of *C. asiatica* extract with 100 µL deionized water. The absorbance of mixture was measured against blank at 517 nm by using UVi light spectrophotometer (Model XTD 5, Secomam, France).

Simultaneously, the absorbance of negative control (100 µL of ethanol and 3.9 mL of ethanolic DPPH) was measured at 517 nm. Absorbance of negative control was measured three times and the values were averaged and used as a constant for calculation. The DPPH solution scavenging activity (%) was calculated as [1- (A_s / A_c)] x 100% (A_s = Absorbance of sample at 517 nm; A_c = Absorbance of negative control at 517 nm). Trolox solution was used to calibrate the standard curve and the calibration equation for trolox was y = 37.284x (R^2 = 0.9997). Each crude extract was analyzed in triplicate and the results were expressed as µmol trolox equivalent per 100 g dried weight (µmol TEAC / 100 g DW).

Statistical analysis

Experiment results were reported as mean ± standard deviation of replicate solvent extraction and the triplicate of assays. All the results were analyzed by using Minitab software (Minitab Version 15.1.1.0) for One-way analysis of variance (ANOVA) with Tukey's test which was used to determine the significant differences between the means at the 5% level. Pearson correlations between variables were also established using MINITAB software (Minitab Version 15.1.1.0) to access the correlation among the antioxidant compound assays and antioxidant capacity assays.

Results and Discussion

Extraction solvent concentration evaluation

The selection of ethanol and water as extraction solvent in this study because they are safer and less toxic as compared to acetone, methanol and other organic solvent. FIGURE 1a-e show the effects of ethanol concentration on phenolic contents and antioxidant capacities of *C. asiatica*'s crude extract. As observed in FIGUREs 1a-e, ethanol concentration had significant effect ($p<0.05$) on both phenolic contents (TPC, TFC and CTC) and antioxidant capacities (ABTS and DPPH) of crude extract. The effect of ethanol concentration on TPC, TFC, ABTS and DPPH followed parabola shape, in which the highest value for all assays were at 60% ethanol except ABTS at 80% ethanol. In contrast, the CTC was increased as the ethanol concentration was increased, reaching a maximum CTC at 100% ethanol concentration.

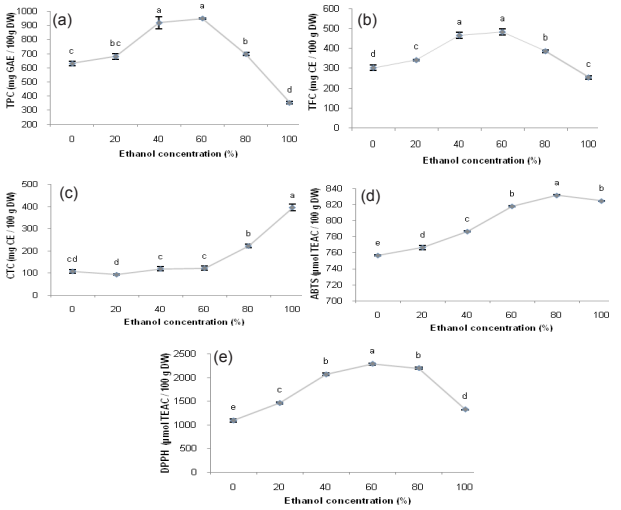

Figure 1. Effect of ethanol concentration on (a) TPC, (b) TFC, (c) CTC, (d) ABTS and (e) DPPH assays from C. asiatica (n = 2)*. Values are presented as means ± standard deviation of six measurements. Values marked by different lower case letters (a-e) are significantly different ($p<0.05$). *Replication of solvent extractions. Note: Error bars represent the standard deviation

Binary-solvent system demonstrated higher yield of phenolic compounds and flavonoids as compared to mono-solvent system in present study and this finding was reported by some of the previous studies (Chirinos *et al*., 2007; Silva *et al*., 2007; Spigno *et al*., 2007; Wang *et al*., 2008). However, the highest recovery of tannins was exhibited at 100% ethanol concentration (mono-solvent). This result could be due to most of tannins presented in bounded or polymerized forms were more soluble in moderate polar/ week polar extraction medium and this finding was supported by Tian *et al*. (2009).

A general principle in solvent extraction is "like dissolves like", which means that solvents only extracts those phytochemicals which have similar polarity with the solvents (Zhang *et al*., 2007). Based on the FIGUREs 1a-e, there was no single ethanol concentration could give the highest value

for all these antioxidant compounds assays. Thus, we suggested that *C. asiatica* contained diverse phenolic compounds with different polarity. However, based on the FIGURE 1a, the highest TPC was achieved at 60% ethanol concentration. Hence, we proposed that most of the phenolic compounds in *C. asiatica* had a moderately polar characteristic.

It was also observed that the antioxidant capacities of *C. asiatica* crude extract were sensitive to ethanol concentration. The highest ABTS and DPPH were achieved at 80% and 60% of ethanol concentration, respectively. This result indicate that *C. asiatica* extract had the highest antioxidant capacity at 60% and 80% ethanol concentration despite of CTC was optimized at 100% ethanol concentration. This circumstance could be due to 100% ethanol was unable to extract polar phenolic compounds that had high antioxidant capacity (Chirinos *et al*., 2007). Previous studies stated that antioxidant capacities of phenolic compounds were associated with the availability of the phenolic compound acting as hydrogen-donating radical scavengers (Yokozawa *et al*., 1998; Karadeniz *et al*., 2005; Khamash *et al*., 2006). Thus, it could be expected that there was a high availability of phenolic compounds which can act as hydrogen-donating radical scavengers in *C. asiatica* extract at treatment of 60% and 80% ethanol concentration.

From the economical point of view, 40% ethanol was selected instead of 60% ethanol for the determination of optimum ethanol concentration for phenolic extraction from *C. asiatica*. It was because there was no significant difference (p>0.05) in TPC between these two ethanol concentration as depicted in FIGURE 1a.

Extraction time evaluation

Extraction time is crucial in minimizing energy and cost of the extraction process. FIGURE 2a-e show the effects of extraction time on antioxidant compounds assays (TPC, TFC and CTC) and antioxidant capacities (ABTS and DPPH) of crude extract. Overall, extraction time had significant effect (p<0.05) on TPC, CTC, ABTS and DPPH except TFC.

In general, the maximum concentration of phenolic compounds was achieved at extraction time of 120 min. After this point, the TPC and CTC were decreased. It was believed that prolonged extraction time would lead to exposure of more oxygen and thus increase the chances for occurrence of oxidation on phenolic compounds (Naczk and Shahidi, 2004; Chirinos *et al*., 2007). Apart from environmental factor, reduction of phenolic content with longer extraction time could also be due to the endogenous enzymes in plant tissues destroyed the phenolic compounds in *C. asiatica* extract (Kuljarachanan *et al*., 2009).

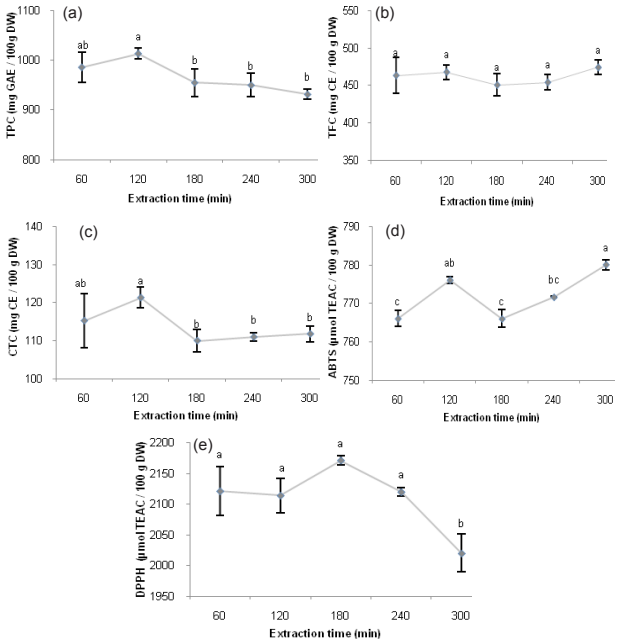

Figure 2. Effect of extraction time on (a) TPC, (b) TFC, (c) CTC, (d) ABTS and (e) DPPH assays from *C. asiatica* (n = 2)*. Values are presented as means ± standard deviation of six measurements. Values marked by different lower case letters (a-c) are significantly different (p<0.05). *Replication of solvent extractions. Note: Error bars represent the standard deviation

On the other hand, the phenolic contents were observed to be no significant change (p<0.05) after 180 min extraction time. This phenomenon could be explained by Fick's second law of diffusion revealing that final equilibrium will be attained between the solution concentrations in the solid matrix and solvent after a particular duration (Pinelo *et al*., 2006).

In term of antioxidant capacity, it was observed that the DPPH was decreased after reaching a maximum value at 180 min. As stated by Naczk and Shahidi (2004), prolonged extraction would increase the chance for occurrence of oxidation of phenolic compounds, thus, the DPPH free radical scavenging ability of *C. asiatica* was decreased after extraction time of 180 min. On the contrary, the highest ABTS was achieved at extraction time of 300 min. This phenomenon could be attributed to an error introduced in ABTS assay which was an end point assay, one could be measuring the antioxidant capacity of the reaction by-products, rather than the phenolic compounds presented in original sample (Huang *et al*., 2005; Wong *et al*., 2006).

Based on the FIGURE 2a-e, it was observed that there was no significant difference (p>0.05) existed in TPC, TFC, CTC and DPPH at extraction time of 60 min and their optimum extraction time (120 min for TPC, TFC and CTC; 180 min for DPPH).

Hence, it was recommended to shorten the range of extraction time for *C. asiatica* to less than 60 min in future investigation.

From the economic point of view, extraction time of 60 min was chosen as the optimum extraction time for next parameter (extraction temperature) although the highest TPC was achieved at extraction time of 120 min. It was because there was no significant difference (p>0.05) in TPC existed between extraction time of 60 and 120 min.

Extraction temperature evaluation

The effects of extraction temperature on phenolic contents (TPC, TFC and CTC) and antioxidant capacities (ABTS and DPPH) of crude extract are showed in FIGURE 3a-e. Based on the FIGURE 3a-e, the yield of all phenolic compounds was increased proportionally with the increasing of extraction temperature, reaching maximum values at 65°C. However, antioxidant capacity showed different tendency with phenolic content with increasing of extraction temperature. The highest ABTS and DPPH were achieved at extraction temperatures of 35 and 45°C, respectively. Beyond these extraction temperatures, both ABTS and DPPH scavenging capacity were decreased.

Based on the FIGURE 3a-c, heat was found to enhance the recovery of phenolic compounds from *C. asiatica*. This finding was supported by Lim and Murtijaya (2007) and Silva *et al*. (2007), who reported that higher temperature could result in a significant higher TPC in extracting the Indian medicinal plant and mashua, respectively. Al-Farsi and Lee (2008) reported that increased temperature could promote the phenolic extraction by increasing both diffusion coefficient and solubility of phenolic compounds in extraction solvent. Besides that, intense heat from solvent was also able to release the cell wall phenolics and bounded phenolics by breaking down of cellular constituents (Wang *et al*., 2007) and hence increases the phenolic yield in extract.

Although the recovery of phenolic compounds was linearly increasing with increasing of extraction temperature, the antioxidant capacities of *C. asiatica* extract were started to decrease when the extraction temperature beyond 45°C. Similarly, Abdul Hamid *et al*. (2002) also revealed that the antioxidant capacity of *C. asiatica* extract was stable up to 50°C. According to Chan *et al*. (2009) and Liyana-Pathirana and Shahidi (2005), the loss in antioxidant capacities of plant extracts at high extraction temperature was likely due to degradation of phenolic compounds which were previously mobilized at low temperature. Thus, we believed that the phenolic compounds which were extracted under high temperature had lower antioxidant capacity as compared to those which were extracted under low temperature. A high phenolic content not necessary accompanies with high antioxidant capacity. The antioxidant capacity also depends on the structure and interaction between extracted phenolic compounds (Huang *et al*., 2005). Hence, further study on identification of phenolic compounds in *C. asiatica* extracts which are extracted at different temperature with respect to their antioxidant mechanism and synergistic effects should be carried out.

Based on the FIGURE 3a, the highest value was achieved at 65°C. Hence, the extraction temperature of 65°C was selected as the optimized temperature for the recovery of phenolic compounds from *C. asiatica* in this study.

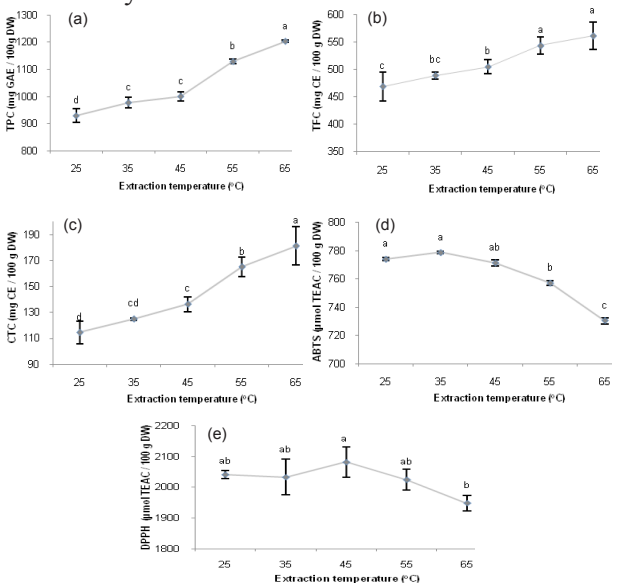

Figure 3. Effect of extraction temperature on (a) TPC, (b) TFC, (c) CTC, (d) ABTS and (e) DPPH assays from C.asiatica (n=2)*. Values are presented as means ± standard deviation of six measurements. Value marked by different lower case letters (a-d) are significantly different (p<0.05). *Replication of solvent extractions. Note: Error bars represent the standard deviation

Person correlation analysis

In order to make a further understanding of the interrelationship between antioxidant capacity and their phenolic compounds content, correlations among antioxidant assays under different extraction conditions were analyzed and presented in TABLE 1. Under the parameter of extraction solvent concentration, TFC was observed to be positive correlated with DPPH significantly (0.902, p<0.05). It could be concluded that flavonoids were the main contributor for the DPPH free radical scavenging capacity of *C. asiatica* extract. Pittela *et al*. (2009) reported that flavonoids are highly polar compounds with low molecular weight. These compounds favour in reacting readily with DPPH radicals, as DPPH radicals preferentially react with low molecular

Table 1. Correlation coefficients between assays under the influence of ethnol concentration

Assay	TPC	TFC	CTC	ABTS
TFC	0.962**			
CTC	-0.758	-0.557		
ABTS	-0.084	0.169	0.648	
DPPH	0.771	0.902*	-0.229	0.544

*p<0.05 and **p<0.01.

Table 2. Correlation coefficients between assays under the influence of extraction time

Assay	TPC	TFC	CTC	ABTS
TFC	0.287			
CTC	0.948*	0.539		
ABTS	-0.245	0.314	-0.033	
DPPH	0.261	-0.846	-0.035	-0.485

*p<0.05.

Table 3. Correlation coefficients between assays under the influence of extraction temperature

Assay	TPC	TFC	CTC	ABTS
TFC	0.991**			
CTC	0.997**	0.997**		
ABTS	-0.930*	-0.889*	-0.918*	
DPPH	-0.730	-0.638	-0.681	0.874

*p<0.05 and **p<0.01.

weight phenolic compounds. (Paixão *et al*., 2007).

With respect to the parameter of extraction time, all phenolic compound assays were found to be either weakly positive correlated or weakly negatively correlated with antioxidant capacity (DPPH and ABTS), except for TFC with DPPH (-0.846, p>0.05). Previous study investigated that the radical scavenging capacity of flavonoids were highly controlled by the number and configuration of phenolic hydroxyl groups in molecules (Yokozawa *et al*., 1998; Cai *et al*., 2006). Thus, we believed that this negative relationship was due to the flavonoids which were extracted with different time exhibited different capacity of DPPH in scavenging free radical.

All antioxidant compound assays were found to be negative correlated significantly (p<0.05) with ABTS except DPPH under the influence of extraction temperature. In other word, *C. asiatica* extract which was extracted at high temperature had high phenolic content but exhibited low antioxidant capacity and vice versa for the extract which was extracted at low temperature. From this correlation, we believed that the phenolic compounds in *C. asiatica* had low stability at elevated temperature. As the extraction temperature was increased, these phenolic compounds would be degraded and resulted in the loss of antioxidant capacity of *C. asiatica* extract. Similarly, Mueller-Harvey (2001) reported that some phenolic compounds decomposed rapidly under high temperature and thus caused a reduction in the antioxidant capacity of plant sample.

Conclusion

The extraction parameter of ethanol concentration, extraction time and extraction temperature had significant (p<0.05) effect on the phenolic contents and antioxidant capacities of *C. asiatica* extract. The optimal extraction conditions for optimized phenolic recovery from *C. asiatica* were 40% ethanol for 60 min at 65°C, with values of 1203.49 mg GAE/ 100 g DW for TPC, 561.92 mg CE/ 100 g DW for TFC and 181.25 mg CE/ 100 g DW for CTC. The antioxidant capacities for the *C. asiatica* extract under optimized conditions were 730.37 µmol TEAC/ 100 g DW for ABTS and 1948.30 µmol TEAC/ 100 g DW for DPPH. As a function of ethanol concentration, TFC showed strong positive correlation with DPPH (0.902, p<0.05). TPC, TFC and CTC were all negative correlated significantly (p<0.05) with ABTS under the effect of extraction temperature with the correlation coefficients thereof were -0.930, -0.889 and -0.918.

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