International Food Research Journal 20(3): 1103-1111 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

A binary solvent extraction system for phenolic antioxidants and its application to the estimation of antioxidant capacity in *Andrographis paniculata* extracts

[1]Thoo, Y. Y., [1]Ng, S. Y., [1]Khoo, M. Z., [2]Wan Aida, W. M. and [1]Ho, C. W.

[1]Department 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 UKM Bangi, Selangor Darul Ehsan, Malaysia

Article history

Received: 15 July 2012
Received in revised form: 14 December 2012
Accepted:14 December 2012

Keywords

Andrographis paniculata 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

Abstract

The effects of ethanol concentration (0-100%, v/v), extraction time (60-300 min) and extraction temperature (25-65°C) on the extraction of phenolic antioxidants from *Andrographis paniculata* was evaluated using single-factor experiments. The following complementary assays were used to screen the antioxidant properties of the crude extracts: 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 and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity. The extraction conditions chosen had significant effects ($p < 0.05$) on the extraction of phenolic compounds and antioxidant capacity. The optimal conditions were 60% ethanol for 60 min at 65°C for phenolic compounds and at 25°C for antioxidant capacity. Strong negative significant ($p < 0.05$) correlations were observed between the phenolic compounds (TPC, TFC and CTC) and antioxidant capacity comprising ABTS (-0.924, -0.909, -0.887, respectively) and DPPH radical-scavenging capacities (-0.992, -0.938, -0.928, respectively) were determined under the influence of extraction temperature.

Introduction

Andrographis paniculata is a local plant in the family of Acanthaceae. It has been used in folk medicines to treat diabetes and hypertension (Kumoro and Hasan, 2007; Mishra *et al.*, 2007). These properties were linked to the fact that the aerial parts of *A. paniculata* are rich in diterpenoids and 2'-oxygenated flavonoids (Jarukamjorn and Nemoto, 2008). However, systematic research on the recovery of phenolic compounds from *A. paniculata* in literature has been limited. These phenolic compounds, especially phenolic acids and flavonoids are the interest of recent researches on natural products as they possess potent antioxidant activity that capable in the prevention of the onset and/or progression of many human diseases by counteracting reactive oxygen species (ROS) (Palasuwan *et al.*, 2005; Cai *et al.*, 2006; Bouayed *et al.*, 2007; Liu *et al.*, 2007). Thus, a systematic study on the extraction variables that potentially influence the recovery of these potent phenolic compounds from *A. paniculata* is indeed important.

Taking into consideration the compositions of phenolic compounds, as well as the structure and physicochemical properties of these compounds, a definite extraction procedure must be designed and optimized for each polyphenol source (Silva *et al.*, 2007; Contini *et al.*, 2008). A binary solvent of ethanol and water was introduced into the solvent extraction of phenolic compounds in *A. paniculata* from the considerations of safety and handling (Shouqin *et al.*, 2007). Many factors contribute to the efficiency of the solvent extraction process and the recovery of antioxidant phytochemicals from natural materials, namely, the type of solvent composition, pH, the extraction time, the extraction temperature, the number of extraction steps, the ratio of solvent to solid material and the particle size of the solid matrix (Liyana-Pathirana and Shahidi, 2005; Chirinos *et al.*, 2007; Wijngaard and Brunton, 2010).

In the present study, the availability of potent phenolic compounds in *A. paniculata* as an antioxidant source was ensured. Classical optimization with a one-factor-at-a-time approach was used, in which each factor varies while all others are kept constant

*Corresponding author.
Email: *cwho@ucsi.edu.my*
Tel: 03 91018880; Fax: 03 91023606

(Liyana-Pathirana and Shahidi, 2005; Silva *et al*., 2007; Contini *et al*., 2008). The drawbacks of this approach are time consuming and does not allow the study on the interaction of dependent responses (Liyana-Pathirana and Shahidi, 2005; Chirinos *et al*., 2007; Hernández *et al*., 2009; Wijngaard and Brunton, 2010). However, this approach is crucial in present study to determine the ranges of the factors that show significant effects on the yields of phenolic compounds and their antioxidant capacities prior to statistical optimization via the response surface methodology (RSM).

The aim of this study was to investigate the effects of ethanol concentration, extraction time and temperature on the phenolic antioxidant (total phenolic content, TPC; total flavonoid content, TFC; and condensed tannin content, CTC) and the free radical-scavenging capacity of extracts from *A. paniculata* for radicals generated by 2,2'-azinobis(3-ehtylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Materials and Methods

Chemicals

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS, ≈98 purity), (+)-catechin hydrate (≥98% purity), 2,2'-diphenyl-1-picrylhydrazyl (DPPH, 95% purity), potassium persulphate (≥98% purity) and sodium nitrite were purchased from Sigma-Adrich (Steinheim, Germany). Concentrated hydrochloric acid (32% purity), Folin-Ciocalteu's phenol reagent, sodium carbonate (≥99.9% purity) and a sodium hydroxide solution (1 mol/L, 1N) were purchased from Merck (Darmstadt, Germany). Gallic acid (98% purity), trolox (97% purity) and vanillin (99% purity) were purchased from Acros Organics (New Jersey, USA). Absolute ethanol (≥99.4% v/v), aluminum chloride-6-hydrate (>99 purity), denatured ethanol and methanol (99%) were purchased from Fisher Scientific Co. (Leicestershire, UK). All other chemicals used were analytical grade, and all stock solutions were prepared using purified deionized water (MilliQ purification system, Millipore, France).

Plant material

Powdered aerial parts of *A. paniculata* were purchased from a local supplier, Ethno Resources Sdn. Bhd (Selangor, Malaysia). *A. paniculata* powder was then vacuum-packaged in nylon-linear low-density polyethylene pouches and stored in the dark at an ambient temperature before it was analyzed.

Preparation of extracts

The *A. paniculata* powder was extracted with aqueous ethanol solvent at a ratio of 1:10 in an agitated 100 mL conical flask. The solvent extraction was performed with a shaking machine (Model Green SSeriker, Vision, Korea) or a temperature-controlled water bath shaker (Model WNB 7-45, Memmert, Germany). The shaking speed was kept constant throughout the experiments to avoid a possible effect on the extraction process. The conical flask was covered with parafilm and aluminum foil to prevent solvent loss and the degradation of bioactive compounds by light during the extraction process. The crude extracts were immediately filtered and the filtrate obtained was used directly for further analysis. Each extraction was carried out in replicate.

Experimental design

Single factor experiments were used to investigate the feasibility of solvent extraction for phenolic compounds from *A. paniculata*. Three independent variables were studied, namely ethanol concentration, extraction time and extraction time. The level for each independent variables were chosen based on the process responses, TPC, TFC, CTC, ABTS radical-scavenging capacity and DPPH radical-scavenging capacity.

Initially, the effect of ethanol concentration (0, 20, 40, 60, 80 and 100% ethanol) on the phenolic yields and antioxidant capacity from *A. paniculata* was investigated. At this step, the extraction time and extraction temperature were kept constant at 25°C for 180 min. Subsequently, the effect of extraction time was investigated by varying the extraction time from 60 to 300 min using the best ethanol concentration chosen in the initial step and kept the extraction temperature constant at 25°C. Lastly, the effect of extraction temperature was investigated using the best ethanol concentration and extraction time determined in the earlier part of present study with extraction temperature ranged from 25 to 65°C.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of crude extracts was evaluated by usinge Folin-Ciocalteu (FC) procedure with slight modifications (Li *et al*., 2008). Approximately 1 mL of diluted crude extract was mixed with 1 mL of FC reagent (diluted 10-fold). Deionized water was used for dilution and blank. After incubation for 3 min at room temperature, 800 µL of sodium carbonate anhydrous solution (7.5%, w/v) was added to mixture. The mixture was then immediately vortexed and incubated for 2 h in the dark at room temperature. The absorbance was read at

765 nm using an Uvi light spectrophotometer (Model XTD 5, Secomam, France). Measurements were recorded in triplicate and calibrated to a standard curve of prepared gallic acid solution (10-70 mg/L) with $y = 0.0396x$ ($R^2 = 0.9975$). The results were expressed in milligrams of gallic acid equivalents (GAE) per 100 gram of dry weight (DW).

Determination of total flavonoid content (TFC)

The estimation of the TFC in crude extracts was conducted using the protocols explained by Karadeniz *et al.* (2005) and Ozsoy *et al.* (2008) with slight modifications. Briefly, 0.25 mL of crude extract was mixed with 1.25 mL of deionized water and 75 µL of 5% sodium nitrite. After 6 min, 150 µL of 10% aluminum chloride-6-hydrate was added. In the next 5 min, 0.5 mL of 1 M sodium hydroxide solution and 275 µL deionized water were added and mixed. The reaction mixture absorbance was immediately recorded at 510 nm on a Uvi light spectrophotometer (Model XTD 5, Secomam, France). The TFC measurements were determined in triplicate and expressed as catechin equivalents (CE), in milligrams of CE per 100 grams of DW by using catechin (50 – 800 µg/ mL) as standard ($y = 0.0033x$; $R^2 = 0.9991$).

Determination of condensed tannin content (CTC)

Condensed tannin content (CTC) in crude extracts was estimated using vanillin-HCl method with slight modifications (Makkar and Becker, 1993). Briefly, 0.5 mL of crude extract was mixed with 3 mL of vanillin reagent (4% w/v in methanol) and 1.5 mL of concentrated HCl (37%) and then immediately mixed and incubated at room temperature for 15 min. The absorbance was measured against the blank at 500 nm using an Uvi light spectrophotometer (Model XTD 5; Secomam, France). Each crude extract was analyzed in triplicate, and CTC was expressed as a catechin equivalent in milligrams per 100 grams of DW. A calibration curve prepared from catechin (50 – 800 µg/mL) was constructed with $y = 0.002x$ ($R^2 = 0.9922$).

Evaluation of antioxidant capacity

ABTS radical-scavenging capacity

The ABTS radical-scavenging capacity assay was carried out according to Guimaraes *et al.* (2007) and Surveswaran *et al*. (2007) with some modifications. ABTS radical solution was prepared by mixing a 7 mM ABTS solution and a 2.45 mM potassium persulphate solution at a ratio of 1:1 before keeping it in the dark at room temperature for 12-16 hours. The ABTS radical solution adjusted to an absorbance of 0.7 (±0.02) at 734 nm before its usage by using ethanol. Then, 100 microliters of crude extract was added to 3.9 mL of the ABTS radical solution. Ethanol was used as blank and control. The absorbance was read immediately at 734 nm after incubation at room temperature for 6 min. The percentage of the radical-scavenging capacity of ABTS was calculated as [1-(A_e - A_c)] x 100% ($A_e = A_{517}$ in the presence of crude extract; $A_c = A_{734}$ of negative control solution). Trolox was used as standard, and ABTS was then expressed as micromoles of trolox equivalent antioxidant capacity (TEAC) using the equation obtained from the standard curve of prepared trolox (0.1-0.8 mM) $y = 120.1142x$, $R^2 = 0.9984$.

DPPH radical-scavenging capacity

The DPPH radical-scavenging capacity assay was performed using the modified method developed by Cai *et al*. (2006), Miliauskas *et al*. (2004) and Saha *et al.* (2004). Crude extract or absolute ethanol (as control) (100 µL) was mixed with 3.9 mL of ethanolic DPPH (60 µM). The reaction mixture was then immediately mixed for 1 min and incubated in the dark for 30 min before the absorbance was measured at 517 nm. The DPPH radical-scavenging capacity (%) was calculated as [1- (A_e - A_c)] x 100% ($A_e = A_{517}$ in the presence of crude extract; $A_c = A_{517}$ of negative control solution). Measurements were recorded in triplicate. Trolox (0-2.5 mM) was used as the standard, and the measurements were expressed as micromoles of TEAC per 100 gram DW.

Statistical analysis

Data were expressed as the mean ± standard deviation of replicate solvent extractions and the triplicate of assays and analyzed by MINITAB (version 14). A one-way analysis of variance (ANOVA) with Tukey test was used to determine the significant differences between means at the 5% level. Pearson correlations between variables were established using MINITAB (version 14).

Results and Discussion

Effect of different ethanol concentrations on phenolics yield and antioxidant capacity recovery

A. paniculata was found rich in diterpenoids and phenolics (Reddy *et al*., 2003; Koteswara Rao *et al*., 2004). The diverse structure of these bioactive compounds has complicated the extraction process. Thus, solvent extraction composed of binary mixture of ethanol and water is essential to extract bioactive compounds of different solubility and polarity. Amongst the organic solvents (methanol, ethanol, propanol, acetone and ethyl acetate) used, ethanol was

used throughout this study from the considerations on the safety for human consumption and compatibility with food systems (Soong and Barlow, 2004; Othman *et al*., 2007; Spigno *et al*., 2007).

Different ethanol concentrations were needed for the maximum recovery of different phenolic compounds and antioxidant capacities (Figures 1 and 2). The yield of TPC and TFC showed a parabolic shape with maximum yields at ethanol levels of 60% and 80%, respectively. Similarly, the recovery of the DPPH radical-scavenging capacity and ABTS-radical scavenging capacity was also optimal at ethanol levels of 60% and 80%, respectively. On the contrary, CTC yields decreased as ethanol concentration increased, reaching a minimum at 100% ethanol. Similarly, ethanol was proposed by (Mohsen and Ammar, 2009); in that study, ethanol exhibited the highest extraction ability for phenolic compounds, followed by methanol and water, acetone, petroleum ether, butanol, choloroform, methylene chloride and hexane.

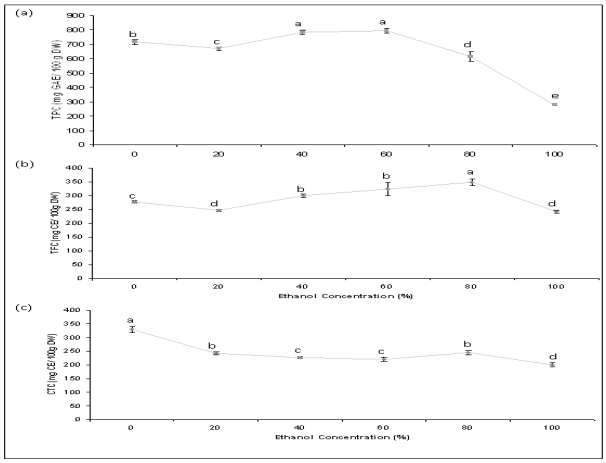

Figure 1. Effect of ethanol concentration on (a) TPC, (b) TFC and (c) CTC assays from *A. paniculata* (n=2)[x]

Values are presented as means ± SD of six measurements. Values marked with the different lower case letters (a-e) are significantly ($p < 0.05$) different.

[x] Replication of solvent extractions

Note: The error bars represent the standard deviation

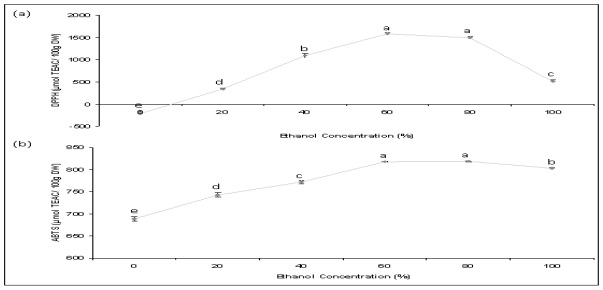

Figure 2. Effect of ethanol concentration on (a) DPPH and (b) ABTS radical-scavenging capacity assays from *A. paniculata* (n=2)[x]

Values are presented as means ± SD of six measurements. Values marked with the different lower case letters (a-e) are significantly ($p < 0.05$) different.

[x] Replication of solvent extractions

Note: The error bars represent the standard deviation

Binary solvent system was found superior to the mono-solvent system (water or pure ethanol) in the extraction of phenolic compounds, indicating the positive effect of the solvent relative polarity on the extractability of the phenolic compounds. From current study, it is noticed that water favors the extraction of condensed tannins; however it had a negative impact on DPPH radical-scavenging capacity. The low antioxidant capacity determined for DPPH was also proposed due to the absence or the presence of low amount total soluble phenolic compounds or bounded phenolic compounds in the extracts (Vasco *et al*., 2008). Similarly, Afolayan *et al*. (2008) reported negative value for DPPH radical-scavenging capacity when *M. parviflora* extract below 200 ppm was tested for DPPH radical-scavenging capacity, but this value increased along with its concentration.

The extraction of phenolic compounds from the sample is directly related to the compatibility of the compounds with the ethanol according to the "like dissolve like" principle (Zhang *et al.*, 2007). This principle is also observed in the present study, where the increment of TFC at 80% was found to accompany a decrement in TFC (Figure 1). Thus, there is no general ethanol concentration that is able to extract all phenolic compounds from the same sample or from different samples. Although TPC decreases significantly ($p < 0.05$) from 60 to 80%, but it does not show a strong impact on the antioxidant capacity of the crude extracts in scavenging DPPH and ABTS cation free radicals (Figures 1 and 2). This indicates that the solvent polarity determined the type of phenolic compounds being extracted and thus exhibits different degrees of antioxidant capacity. A similar study has been reported by Lapornik *et al.* (2005); in that study, more phenolic compounds and antioxidant activity were obtained in ethanol extracts as compared to those obtained in water extracts. In addition, it also been emphasized that ethanol of its non-polar property is more efficient in degrading cell walls that aid in the release of phenolic compounds from cells to the extraction solvent (Lapornik *et al*., 2005).

Zhang *et al.* (2007) and Chan *et al.* (2009) reveal ethanol concentrations of 70% and 60%, respectively, as optimal ethanol concentrations in combination with their respective optimal solvent extraction conditions to extract phenolic compounds from plant materials. Similar to these studies, 60% ethanol was chosen in present study based on its being economically and environmentally optimal. Even so, 80% ethanol may also be used in order to produce high-quality crude extract with a higher yield of TFC and antioxidant

capacity recoveries. However, the selection of a higher proportion of ethanol may limit the extraction temperature applied at the later stage.

Effect of extraction time on phenolics yield and antioxidant capacity recovery

Extraction time is another important parameter in optimizing the recovery of phenolic compounds and antioxidant capacity. From literature, extraction time varies from a few min to 24 h depending on the phenolic compounds present in the sample (Naczk and Shahidi, 2004; Liyana-Pathirana and Shahidi, 2005; Chan *et al*., 2009). In the present study, the maximum yields of TPC and CTC and the maximum recovery of DPPH radical-scavenging capacity occurred at 60 min (Figure 3). However, these yields and capacity decreased gradually with the further increment in extraction time after the optimum recoveries. Meanwhile, TFC and ABTS radical-scavenging capacity showed a parabolic shape with an optimum point at 120 min (Figures 3 and 4).

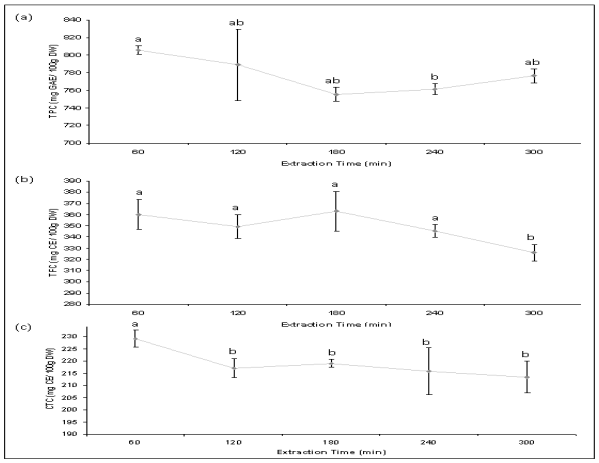

Figure 3. Effect of extraction time on (a) TPC, (b) TFC and (c) CTC assays from *A. paniculata* (n=2)[x]

Values are presented as means ± SD of six measurements. Values marked with the different lower case letters (a-b) are significantly ($p < 0.05$) different.

[x] Replication of solvent extractions

Note: The error bars represent the standard deviation

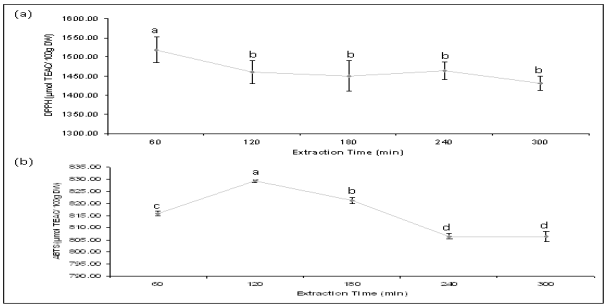

Figure 4. Effect of extraction time on (a) DPPH and (b) ABTS radical-scavenging capacity assays from *A. paniculata* (n=2)[x]

Values are presented as means ± SD of six measurements. Values marked with the different lower case letters (a-d) are significantly ($p< 0.05$) different.

[x] Replication of solvent extractions

Note: The error bars represent the standard deviation

Plant material known to contain diverse structure of bioactive compounds and these criteria had led to different optimum times when different complementary assays were used although other solvent extraction parameters were kept constant. Figure 3 shows that the increment of TPC after 180 min leads to the decrement of the other groups of phenolic compounds (TFC and CTC) present in the sample. This postulated that the increment or decrement of any group of compounds may potentially act as an interfering agent in quantification assays. This finding is in accordance with Soong and Barlow (2004), who reported the present of equilibrium point for the extraction of diverse group of phenolic compounds present in the sample reached before their corresponding apparent reduction in a solvent extraction system.

In addition, prolonged extraction time was also suspected to lead to an unfavorable decomposition of phenolic compounds with the prolonged exposure of polyphenols to temperature, light and oxygen (Lafka *et al*., 2007). This decomposition is also manifested to a decline in antioxidant activity (Lafka *et al*., 2007). This decomposition process was observed in the present study, where DPPH radical-scavenging at 120 min with its decrease in TEAC values. A similar phenomenon has been reported by Liyana-Pathrina and Shahidi (2005); in that study prolonged extraction time led to the decomposition of active compounds in wheat.

Effect of extraction temperature on phenolics yield and antioxidant capacity recovery

Heat has been found to enhance the recovery of the phenolic compounds by enhancing both diffusion coefficients and the solubility of phenolic compounds (Al-Farsi and Lee, 2008). Generally, a high extraction temperature had positive effect on the yield of phenolic compounds but these increments are not consistent. In the present study, the yields of phenolic compounds increased linearly with the increasing extraction temperature. However, the recovery of antioxidant capacity showed an opposite tendency with phenolic compounds with the increasing temperature. This finding has been reported by Zhang *et al*. (2007), where very high extraction temperatures might denature phenolic compounds, thereby leading to a decrease in the antioxidant capacity exhibited by these phenolic compounds.

From the equilibrium view point, an elevated temperature could increase the extraction rate and thus reduce the extraction time needed to reach the maximum recovery of phenolic compounds (Ho *et al*., 2007). However, elevated temperature may not

be suitable for all types of phenolic compounds. For instance, the yields of TPC, TFC and CTC reached a maximum at 65°C, while the recovery of ABTS and DPPH radical-scavenging capacities were optimized at 25°C (Figures 5 and 6). Therefore, further study on the interaction effect of extraction time and extraction temperature is indeed important to produce economical and practical extraction parameters to generate maximum yields of phenolic compounds and antioxidant capacity.

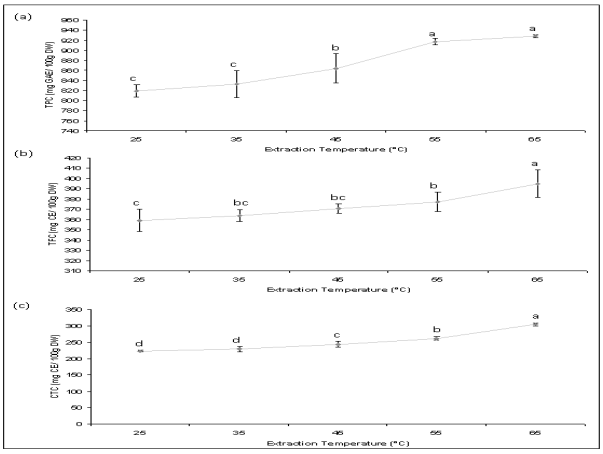

Figure 5. Effect of extraction temperature on (a) TPC (b) TFC and (c) CTC assays from *A. paniculata* (n=2)[x]

Values are presented as means ± SD of six measurements. Values marked with the different lower case letters (a-d) are significantly ($p < 0.05$) different.

[x] Replication of solvent extractions

Note: The error bars represent the standard deviation

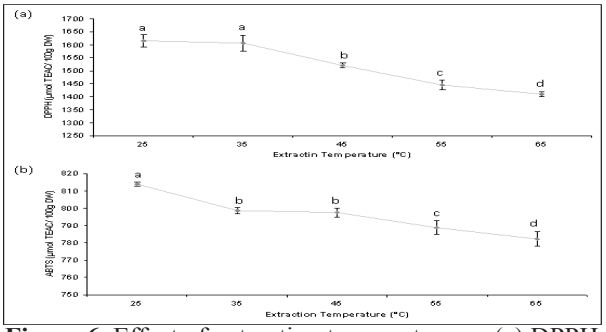

Figure 6. Effect of extraction temperature on (a) DPPH and (b) ABTS radical-scavenging capacity assays from *A. paniculata* (n=2)[x]

Values are presented as means ± SD of six measurements. Values marked with the different lower case letters (a-d) are significantly ($p < 0.05$) different.

[x] Replication of solvent extractions

Note: The error bars represent the standard deviation

Pearson correlation analysis

Correlations were observed between phenolic compounds and antioxidant capacities in ethanolic extracts from *A. paniculata* under the influence of different extraction parameters. For the effect of ethanol concentration, a moderately strong positive correlation was observed between TFC and DPPH ($r = 0.776$). From this experimental result, we proposed that the presence of impurities (non-flavonoid) compounds that are able to absorb the same wavelength at 510 nm may have caused the overestimation of TFC values as shown in Figure 1. This is likely explained by the negative value for the DPPH radical-scavenging capacity obtained for crude extract obtained at 0% ethanol (Figure 2). Similarly, the negative value obtained for DPPH also indicates the presence of impurities that can absorb at 517 nm.

A high and significant negative correlation was observed between CTC and ABTS ($r = -0.827$) under the influence of ethanol concentration, indicating the present of different predictive capacity for crude extract from *A. paniculata* extracted at different ethanol concentrations. However, this negative correlation is suspected due to the presence of interfering agents in crude extract that eventually influence the quantification by using ABTS assay. As shown in Figure 1c, condensed tannins, which were optimized at 0%, may be water soluble secondary metabolites that preferably dissolve in water. However, ABTS and DPPH radical-scavenging capacity showed parabolic shapes with optimum points at 60%. Similar literatures were reported by Turkmen *et al*. (2006) and Zhang *et al.* (2008) where increased ethanol concentration could modify solvent polarity and lead to alteration in the ability of the solvent to dissolve from plant tissues a selected group of antioxidant compounds that may further influence estimates of antioxidant capacity.

Correlations between phenolic compounds and antioxidant capacity assays under the influence of extraction temperature were found to vary with ethanol concentration and extraction time, as observed in Table 1. All of the phenolic compounds (TPC, TFC and CTC) had negative correlations with the antioxidant capacity assays. The highest negative correlation was found between TPC and DPPH (-0.992) and the lowest was between ABTS and TFC (-0.909). From these causative correlations, we suggest that phenolic compounds from *A. paniculata* are not stable, as they easily degraded, which results in the loss of antioxidant capacity with the increasing temperature. Similar finding was reported on dried sage, where an increase in extraction temperature degrades polyphenols that responsible for the decrease in antioxidant capacity (Durling *et al*., 2007).

Meanwhile, positive correlations were found between ABTS and DPPH radical-scavenging capacities under the influence of ethanol concentration and extraction temperature (Table 1). These positive correlations indicate that the ethanol concentration and extraction temperature had the same effect on the

Table 1. Correlation between assays[x] under influence of different extraction conditions (n=2)[y]

r [z]	Ethanol Concentration				Extraction Time				Extraction temperature			
	TPC	TFC	CTC	ABTS	TPC	TFC	CTC	ABTS	TPC	TFC	CTC	ABTS
TFC	0.501				0.072				0.925*			
CTC	0.381	0.025			0.639	0.704			0.917*	0.997***		
ABTS	-0.259	0.470	-0.827*		0.245	0.574	0.231		-0.924*	-0.909*	-0.887*	
DPPH	0.238	0.776	-0.600	0.869*	0.685	0.606	0.939*	0.141	-0.992**	-0.938*	-0.928*	0.901*

[x] TPC, total phenolic content; TFC, total flavonoid content; CTC, condensed tannin content; ABTS, ABTS radical-scavenging capacity; DPPH, DPPH radical-scavenging capacity.
[y] Replication.
[z] r, correlation coefficient.
* Significant level at $p < 0.05$.
** Significant level at $p < 0.01$.
*** Significant level at $p < 0.001$

recoveries of antioxidant capacity when assessed by ABTS and DPPH radical-scavenging capacities. On contrary, no significant correlation was found between ABTS and DPPH under the influence of extraction time (0.141), indicating the extraction duration has contradict effect on the extraction of bioactive compounds of different molecular weights. In present study, bioactive compounds of low-molecular-weights (assessed by DPPH) was optimized at 60 min, however high-molecular-weights compounds as assessed by ABTS reached maximum recovery at 120 min. This finding is in accordance to Herodež *et al.* (2003), where the two extraction stages found in solvent extraction, involving with the initial fast step corresponding to the recovery of solutes from the superficial sites of plant material and second lower step corresponding to the molecular diffusion of solutes from the internal sites through porous medium is the determinative factor for the optimal extraction time. Thus, extraction time play an important role for equilibrium recoveries of bioactive compounds of different molecular weight from *A. paniculata* when extraction temperature and ethanol concentration were kept constant. Similarly, the effect of different degree of phenolics polymerization, solubility and interaction of phenolics with other food constituents on the extractability of phenolics from date seeds by Al-Farsi and and Lee (2008); in that study, 1 h extraction time was optimal for phenolic extraction date seeds.

Conclusions

A. paniculata plant showed to be an ideal food antioxidant in food industry. Conclusively, extraction parameters (ethanol concentration, extraction time, and extraction temperature) had a significant effect ($p < 0.05$) on the extraction yield of phenolic compounds and antioxidant capacity from dried aerial parts of *A. paniculata*. The yield of TPC, TFC and CTC were optimized by using 60% ethanol for 60 min at 25°C, while antioxidant capacity assessed by ABTS and DPPH radical-scavenging capacities were optimized by using 60% ethanol for 60 min at 65°C. From the Pearson correlation analysis, it is noted that the antioxidant capacity is represented by the phenolic present in the extract with the strong positive correlation between TFC and DPPH (0.776) under the influence of ethanol concentration. The controversial effect of extraction temperature on the yields of phenolic compounds and recoveries antioxidant capacity was reinforced by the significant negative correlations obtained between phenolic compounds (TPC, TFC and CTC) with ABTS and DPPH radical-scavenging capacities. This finding indicates the possible degradation of antioxidant capacity of the phenolics obtained at high extraction temperature.

Acknowledgement

The authors would like to thank UCSI University for their laboratory facilities.

References

Afolayan, A. J., Aboyade, O. M. and Sofidiya, M. O. 2008. Total phenolic content and free radical scavenging activity of *Malva parviflora* L. (Malvaceae). Journal of Biological Sciences 8: 945-949.

Al-Farsi, M. A. and Lee, C. Y. 2008. Optimization of phenolics and dietary fibre extraction from date seeds. Food Chemistry 108 (3): 977-985.

Bouayed, J., Piri, K., Rammal, H., Dicko, A., Desor, F., Younos, C. and Soulimani, R. 2007. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. Food Chemistry 104 (1): 364-368.

Cai, Y.-Z., Mei, S., Jie, X., Luo, Q. and Corke, H. 2006. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sciences 78 (25): 2872-2888.

Chan, E. W. C., Lim, Y. Y., Wong, S. K., Lim, K. K., Tan, S. P., Lianto, F. S. and Yong, M. Y. 2009. Effects of different drying methods on the antioxidant properties

of leaves and tea of ginger species. Food Chemistry 113 (1): 166-172.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R. and Larondelle, Y. 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers. Separation and Purification Technology 55 (2): 217-225.

Contini, M., Baccelloni, S., Massantini, R. and Anelli, G. 2008. Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. Food Chemistry 110 (3): 659-669.

Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y. and Perry, N. B. 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures. Food Chemistry 101 (4):1417-1424.

Guimarães, C. M., Gião, M. S., Martinez, S. S., Pintado, A. I., Pintado, M. E., Bento, L. S. and Malcata, F. X. 2007. Antioxidant Activity of Sugar Molasses, Including Protective Effect Against DNA Oxidative Damage. Journal of Food Science 72 (1): C039-C043.

Hernández, Y., Lobo, M. G. and González, M. 2009. Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. Food Chemistry 114 (2): 734-741.

Herodež, Š. S., Hadolin, M., Škerget, M. and Knez, Ž. 2003. Solvent extraction study of antioxidants from Balm (*Melissa officinalis* L.) leaves. Food Chemistry 80 (2): 275-282.

Ho, C. H. L., Cacace, J. E. and Mazza, G. 2007. Extraction of lignans, proteins and carbohydrates from flaxseed meal with pressurized low polarity water. LWT - Food Science and Technology 40 (9): 1637-1647.

Jarukamjorn, K. and Nemoto, N. 2008. Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent andrographolide. Journal of Health Science 54 (4): 370-381.

Karadeniz, F., Burdurlu, H. S., Koca, N. and Soyer, Y. 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. Turkish Journal of Agriculture and Forestry 29: 297-303.

Koteswara Rao, Y., Vimalamma, G., Venkata Rao, C. and Tzeng, Y.-M. 2004. Flavonoids and andrographolides from Andrographis paniculata. Phytochemistry 65 (16): 2317-2321.

Kumoro, A. C. and Hasan, M. 2007. Supercritical carbon dioxide extraction of andrographolide from Andrographis paniculata: Effect of the solvent flow rate, pressure, and temperature. Chinese Journal of Chemical Engineering 15 (6): 877-883.

Lafka, T.-I., Sinanoglou, V. and Lazos, E. S. 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chemistry 104 (3): 1206-1214.

Lapornik, B., Prošek, M. and Golc Wondra, A. 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering 71 (2): 214-222.

Li, H.-B., Wong, C.-C., Cheng, K.-W. and Chen, F. 2008. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT - Food Science and Technology 41 (3): 385-390.

Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X. and Yan, G. 2007. Antioxidant activity and phenolics of an endophytic *Xylaria* sp. from Ginkgo biloba. Food Chemistry 105 (2): 548-554.

Liyana-Pathirana, C. and Shahidi, F. 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry 93 (1): 47-56.

Makkar, H. P. S. and Becker, K. 1993. Vanillin-HCl method for condensed tannins: Effect of organic solvents used for extraction of tannins. Journal of Chemical Ecology 19 (4): 613-621.

Miliauskas, G., Venskutonis, P. R. and van Beek, T. A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 85 (2): 231-237.

Mishra, S. K., Sangwan, N. S. and Sangwan, R. S. 2007. Andrographis paniculata (Kalmegh): A review. Pharmacognosy Reviews 1 (2): 283-298.

Mohsen, S. M. and Ammar, A. S. M. 2009. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chemistry 112 (3): 595-598.

Naczk, M. and Shahidi, F. 2004. Extraction and analysis of phenolics in food. Journal of Chromatography A 1054 (1-2): 95-111.

Othman, A., Ismail, A., Abdul Ghani, N. and Adenan, I. 2007. Antioxidant capacity and phenolic content of cocoa beans. Food Chemistry 100(4): 1523-1530.

Ozsoy, N., Can, A., Yanardag, R. and Akev, N. 2008. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chemistry 110 (3): 571-583.

Palasuwan, A., Soogarun, S., Lertlum, T., Pradniwat, P. and Wiwanitkit, V. 2005. Inhibition of Heinz body induction in an in vitro model and total antioxidant activity of medicinal Thai plants. Asian Pacific Journal of Cancer Prevention 6: 458-463.

Reddy, M. K., Reddy, M. V. B., Gunasekar, D., Murthy, M. M., Caux, C. and Bodo, B. 2003. A flavone and an unusual 23-carbon terpenoid from Andrographis paniculata. Phytochemistry 62 (8): 1271-1275.

Saha, K., Lajis, N. H., Israf, D. A., Hamzah, A. S., Khozirah, S., Khamis, S. and Syahida, A. 2004. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. Journal of Ethnopharmacology 92 (2-3): 263-267.

Shouqin, Z., Ruizhan, C. and Changzheng, W. 2007. Experiment study on ultrahigh pressure extraction of Ginsenosides. Journal of Food Engineering 79 (1): 1-5.

Silva, E. M., Rogez, H. and Larondelle, Y. 2007. Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. Separation and Purification Technology 55 (3): 381-387.

Soong, Y.-Y. and Barlow, P. J. 2004. Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry 88 (3): 411-417.

Spigno, G., Tramelli, L. and De Faveri, D. M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering 81 (1): 200-208.

Surveswaran, S., Cai, Y.-Z., Corke, H. and Sun, M. 2007. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry 102 (3): 938-953.

Turkmen, N., Sari, F. and Velioglu, Y. S. 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chemistry 99 (4): 835-841.

Vasco, C., Ruales, J. and Kamal-Eldin, A. 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chemistry 111 (4): 816-823.

Wijngaard, H. H. and Brunton, N. 2010. The optimisation of solid-liquid extraction of antioxidants from apple pomace by response surface methodology. Journal of Food Engineering 96 (1): 134-140.

Zhang, Y., Li, S.-F. and Wu, X.-W. 2008. Pressurized liquid extraction of flavonoids from *Houttuynia cordata* Thunb. Separation and Purification Technology 58 (3): 305-310.

Zhang, Z.-S., Li, D., Wang, L.-J., Ozkan, N., Chen, X. D., Mao, Z.-H. and Yang, H.-Z. 2007. Optimization of ethanol-water extraction of lignans from flaxseed. Separation and Purification Technology 57 (1): 17-24.