In vitro antioxidant activities and anti-proliferative properties of the functional herb Abrus cantoniensis and its main alkaloid abrine

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Phytochemical profiles, antioxidant activities of functional herb *Abrus cantoniensis* and *Abrus mollis* ☆

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ABSTRACT

It has been claimed that consumptions of *Abrus cantoniensis* (AC) and *Abrus mollis* (AM) as folk beverages and soups are good to cleanse liver toxicants and prevent liver diseases. There is scant information on the phytochemical profiles and antioxidant activities of these two varieties. Five major phytochemicals in these two cultivars were qualitatively and quantitatively compared using UPLC-PDA. A high level of total phenolic content (TPC) and total flavonoid content (TFC) was found in AC and AM. AC, in general, showed some antioxidant activities comparable to that of BHT, and stronger radical scavenging activities and higher reducing power than that of AM ($p < 0.05$). When principal component analysis (PCA) was applied, high correlation between TPC, TFC and their antioxidant activities was found. Hence, this study proved that, both AC and AM could serve as antioxidant-rich component in foods or beverages to promote health function.

1. Introduction

Abrus cantoniensis Hance (Leguminosae family), which is a native plant in Southern China and some countries of Southeast Asia, is a well-known vegetative food added to beverage, soup and folk medicine (Zheng, Li, & Mu, 2011). This plant material could be used alone or with other food ingredients, such as egg, pork, chicken, ginger, glace date, etc. Decoction of the plant has been claimed effective to clear liver toxicants, relieve heat in the liver, prevent hepatitis and other chronic liver diseases (National Pharmacopoeia Committee, 2010). It is also an important ingredient in a precious traditional functional food tortoise jelly (GuiLing-Gao), which is good to boost immunity, alleviate damp-heat, nourish yin and improve skin disorder after consumption (Zhang, Zhang, & Cheung, 2009).

Previous studies have revealed that, the water or methanol/ethanol extracts of AC showed hepatoprotective (Qin, Huang, He, & Lin, 2006), anti-proliferative (Yang, Al Zaharna, Chen, Li, & Cheung, 2014) and antibacterial activities (Cheng et al., 2006). It has also been revealed that the phenolic compounds, alkaloids, terpenoids, sapogenols and saponins, were the components responsible for these activities (Miyao, Sakai, Takeshita, Ito, Kinjo, & Nohara, 1996; Miyao, Sakai, Takeshita, Kinjo, & Nohara, 1996; Wong, 1979; Yang et al., 2014). Among the alkaloid, abrine which is the dominant (National Pharmacopoeia Committee, 2010; Qiu, Xiao, & Li, 2011), while chrysophanol and physcion which are the main hydroxyanthraquinones (Wong, Chiang, & Chang, 1982) and ursolic acid, soyasaponin I, kaikasaponin III, which are the major triterpenoids, were identified in the herbal extract (Miyao, Sakai, Takeshita, & Ito et al., 1996; Miyao, Sakai, Takeshita, & Kinjo et al., 1996; Zhou & Zhou, 2010).

Abrus mollis (AM) is an alike species that belongs to the same *Abrus* genus. It is widely cultivated in Guangdong and Guangxi province of China as the growth rate of AC is relative low. Besides, it is used in health care soups and Chinese patent medicine as an alternative species of AC in folk beverage manufacturing. In the latest National Pharmacopeia of China (2010), only AC is recommended. It is necessary to perform a systematic study of their biological functions. Although a few works on biological activities of phytochemicals and extracts of AC or AM have been recorded in literature, there is a lack of a systematic comparative study on the phytochemicals profile and bioactive functions of AC and AM. *Abrus* herbs are well known for the liver protection capacity (Miyao, Arao, Udayama, Kinjo, & Nohara, 1998; National Pharmacopoeia Committee, 2010). Numerous studies revealed the positive correlation between hepatoprotective effects and antioxidant activities of the corresponding compounds (Dey & Lakshmanan, 2013; Saleem et al., 2010; Yuan, Wu, Liu, & Zhang, 2013). Thus, a comprehensive comparative study on the phytochemicals profile and antioxidant activities of the two species

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may give a better understanding of the species selection and the potential beneficial effects on human health.

In this study, five major phytochemicals of 20 AC and AM batches were quantified using UPLC-PDA. The TPC and TFC as well as antioxidant activities of the two species were comprehensively determined. Pearson's correlation was performed to analyze the correlations between phytochemical content and antioxidant activities, while PCA gave the overview of the inter-relationships among TPC, TFC and antioxidant activity of AC and AM.

2. Materials and methods

2.1. Materials and chemicals

A. cantoniensis and *A. mollis*, 10 batches of each, were collected from different sources. Each lot of herbal sample collected from one area at a particular season is considered as a batch. Table 1 shows the detail information of samples. AC and AM were collected from various origins in China, or from a country in Southeast Asia. The botanical identification of the plants was performed by Dr. Zhi-Feng Zhang in the Ethnic Pharmaceutical Institute of the Southwest University for Nationalities (Chengdu, Sichuan Province, China). All the samples were kept in a storage room in the Department of Biomedical Sciences, City University of Hong Kong. All herbal samples were stored in a tightly closed containers in cool, dark, dry conditions below 20 °C and humidity not more than 60%. 2, 2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), β-nicotinamide adenine dinucleotide (NADH), sodium nitroprusside (SNP), Griess reagent, linoleic acid, Tween-20 and butylated hydroxytoluene (BHT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents used were of HPLC grade. Water was purified using a Milli-Q system from Millipore (Billerica, MA, USA).

2.2. Sample preparation

All air dried herbal samples were cut into thin pieces and ground into powder using a blender to avoid sample bias. The powder obtained by the blender were passed through a 50 mesh filter and the particle size of powder was around 355 ± 15 μm. Powder of herb (5 g) was extracted twice with 500 mL methanol by sonication on ice for 30 min. Power output of the sonicator was 100 kHz. After filtration, the methanol extracts (ME) were combined, concentrated under vacuum, and subjected to lyophilization. For antioxidative assays, ME samples were prepared at a concentration of 1 mg/mL in methanol and stored at −20 °C until further use. The commercial antioxidant, BHT, was set as positive control and prepared at a stock concentration of 1 mg/mL in methanol. For UPLC analysis, the stock ME samples were diluted with 50% methanol to a concentration of 0.1 mg/mL.

2.3. UPLC-PDA

UPLC analysis was performed on Waters ACQUITY UPLC® system with a sample manager, binary solvent manager and PDA eλ detector. A Halo Fused-Core® C18 UPLC column (2.1 × 150 mm, 5 μm) was used, and the column temperature was maintained at 25 °C. The mobile phase included H_2O (A) and acetonitrile (B) with a gradient program was shown as follows: 0–8 min, 5–20% B; 8–13 min, 20–60% B, 13–23 min, 60–100% B; 23–25 min, 100–100% B, 25–27 min, 100–5% B. The flow rate was 0.3 mL/min. Monitoring was performed at 205 nm, and the injection volume was 2 μL. For quantitative analysis, the marker abrine, catechin hydrate, emodin, chrysophanol and ursolic acid were dissolved and diluted with 50% methanol. All sample solution and markers were passed through 0.20 μm nylon membrane before subjected to UPLC-PDA analysis.

To ensure accurate quantitative analyses of the content of five phytochemicals in AC and AM batches, method validation, including linearity, limit of detection (LOD), limit of quantification (LOQ), precisions and recovery was conducted (Corradini, 2012; Snyder, Kirkland, & Glajch, 2012).

2.4. Total phenolic content (TPC)

TPC was tested using the Folin–Ciocalteu method (Müller, Fröhlich, & Böhm, 2011), with minor modifications. Briefly, in a 96-well microplate, an aliquot of 50 μL of the extracts was mixed by 50 μL of Folin–Ciocalteu reagent, followed by addition of 50 μL of sodium carbonate solution (7.5%, w/v). The mixture was stirred and measured at 765 nm after remaining in the dark for

Table 1
Sample information, and concentration (mg/kg DW) of major phytochemicals detected in AC and AM batches using UPLC-PDA.

Batch No.[a]	Production/collection Area[b]	Abrine	Catechin hydrate	Emodin	Chrysophanol	Ursolic acid
AC01	Hengxian, GD	599.95 ± 9.35	150.53 ± 0.10	156.15 ± 0.26	222.34 ± 2.91	122.21 ± 7.48
AC02	Baise, GX	499.18 ± 13.00	266.86 ± 8.06	189.81 ± 5.58	247.72 ± 3.38	136.96 ± 2.91
AC03	Nanning, GX	256.76 ± 2.49	149.96 ± 0.01	127.85 ± 0.85	202.43 ± 0.54	126.35 ± 2.11
AC04	Heyuan, GD	418.04 ± 6.70	293.34 ± 5.25	225.63 ± 2.01	280.31 ± 2.06	171.54 ± 1.84
AC05	Shaoguan, GD	483.62 ± 15.09	317.24 ± 8.76	202.34 ± 2.43	297.78 ± 1.53	198.73 ± 11.89
AC06	Wuzhou, GX	551.28 ± 1.91	337.42 ± 0.55	199.90 ± 5.30	321.19 ± 0.06	196.64 ± 3.42
AC07	GX	533.46 ± 5.34	149.90 ± 0.05	169.79 ± 4.29	203.06 ± 2.62	114.56 ± 1.30
AC08	Wuzhou, GX	365.03 ± 1.20	150.09 ± 0.05	151.97 ± 0.80	250.52 ± 8.09	88.59 ± 2.47
AC09	Thailand	315.92 ± 4.17	257.57 ± 11.07	188.75 ± 1.32	280.57 ± 5.35	127.30 ± 10.10
AC10	Thailand	553.35 ± 6.93	285.75 ± 10.18	203.37 ± 9.45	284.88 ± 0.26	193.43 ± 8.92
AM01	Yingde, GD	492.12 ± 9.28	229.95 ± 5.17	69.74 ± 3.82	140.61 ± 1.22	30.04 ± 3.76
AM02	Meizhou, GD	279.44 ± 2.84	267.01 ± 4.32	52.16 ± 1.05	86.21 ± 2.37	9.29 ± 2.84
AM03	Meizhou, GD	260.75 ± 7.39	150.36 ± 0.01	45.72 ± 0.96	78.49 ± 3.58	10.11 ± 0.95
AM04	GX	273.38 ± 0.70	154.59 ± 0.38	66.74 ± 5.18	92.62 ± 0.06	24.52 ± 0.88
AM05	Qingyuan, GD	475.46 ± 5.11	268.94 ± 10.27	105.16 ± 1.34	102.54 ± 7.81	46.56 ± 2.66
AM06	Meizhou, GD	335.89 ± 0.67	232.47 ± 1.31	66.90 ± 2.78	113.13 ± 2.39	23.56 ± 4.07
AM07	Yunfu, GD	356.31 ± 1.71	259.53 ± 14.45	103.23 ± 0.10	106.50 ± 0.45	63.65 ± 2.11
AM08	Jiexi, GD	277.01 ± 1.00	162.70 ± 0.30	50.65 ± 0.23	90.55 ± 0.09	46.66 ± 6.08
AM09	Yulin, GX	535.56 ± 10.25	243.97 ± 14.85	94.49 ± 1.35	138.47 ± 1.33	49.76 ± 3.05
AM10	Heyuan, GD	301.39 ± 0.93	151.33 ± 0.04	40.10 ± 1.02	98.70 ± 0.94	27.96 ± 2.26

Results were expressed as mean ± SD from two independent experiments.
[a] AC and AM indicated *Abrus cantoniensis* and *Abrus mollis*, respectively.
[b] GD and GX indicated Guangdong and Guangxi province of China, respectively.

30 min. A blank sample consisting of water and reagents was used as a reference. A sample colour blank was examined using the sample and water to exclude the colour interference of the sample extract. The results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW), utilizing a calibration curve of gallic acid in a concentration range of 10–60 μg/mL.

2.5. Total flavonoids content (TFC)

The $AlCl_3$ method was adapted for the determination of total flavonoids (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004), with minor changes. An aliquot (50 μL) of each sample was mixed with an equal volume of a solution of 2% $AlCl_3.6H_2O$ (2 g in 100 mL of methanol). The absorbance of the mixture was measured in a quartz well at 367 nm after 10 min of incubation. Results were expressed as mg of rutin equivalents per 100 g of dry weight (mg RE/100 g DW), with a calibration curve of rutin within the concentration range of 10–120 μg/mL.

2.6. Antioxidant activity

2.6.1. FRAP assay

The FRAP assay was determined as described by Müller et al. (2011), with some modifications. The working FRAP solution was freshly prepared by mixing acetate buffer (300 mM, pH3.6), 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution in 40 mM HCl, and 20 mM ferric chloride solution at a ratio of 10:1:1. The working solution was warmed at 37 °C before usage. An aliquot of 100 μL sample was mixed with 3 mL FRAP solution at 1 min intervals. After 4 min of incubation at 37 °C, the absorbance was read at 593 nm. A calibration curve was made by ferrous sulphate and results were expressed as mM Fe^{2+} per g dried weight (mM Fe^{2+}/g DW) from three determinations.

2.6.2. DPPH radical scavenging activities

DPPH radical scavenging activity was conducted according to the method described by Gülçin (2006), with some modifications. In a 96-well microplate, an aliquot of 100 μL DPPH radical (0.1 mM, dissolved in ethanol) solution was mixed with 50 μL sample extract at different concentrations or methanol as negative control. The mixture was reacted at dark for 30 min and then read at 517 nm with a blank contain only DPPH solution and methanol. The sample colour blank was examined containing sample and methanol to exclude the colour interference of sample extract. The DPPH radical-scavenging activity (%) was calculated with Eq. (1).

$$\text{Scavenging activity } (\%) = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100 \quad (1)$$

where A_{sample} is the absorbance in the presence of extracts, and A_{control} is the absorbance of the control (Gülçin, 2006).

2.6.3. ABTS radical cation scavenging activities

ABTS radical cation scavenging activity was assayed according to method of Re et al. (1999), with minor variation. The $ABTS^+$ solution was generated by reacting 7 mM ABTS solution and 2.45 mM potassium persulfate (final concentration) in the dark for at least 16 h at room temperature. The solution was then diluted with phosphate buffer (pH 7.4) to an absorbance of 0.750 at 734 nm as the working solution. An aliquot of 1 mL working solution was mixed with 10 μL of extracts at different concentrations and allowed to react at 30 °C for 20 min. A blank containing $ABTS^+$ working solution and ethanol was detected. The absorbance was measured at 734 nm. The radical scavenging activity of the samples was calculated with Eq. (1).

2.6.4. Superoxide anion radical scavenging activities

The superoxide anion scavenging activity was determined as described by Zhang et al. (2009), with minor modifications. Briefly, 150 μM nitroblue tetrazolium (NBT), 60 μM phenazine methosulphate (PMS), and 468 μM β-nicotinamide adenine dinucleotide (NADH) were prepared in 16 mM Tris–HCl buffer (pH 8.0). An equal volume (50 μL) of NBT, NADH, sample extract at different concentrations, and PMS were mixed and incubated for 5 min at room temperature. A blank containing the NBT, PMS, and NADH solution and water was detected. The absorbance was measured at 560 nm using a microplate reader. The radical scavenging activity of samples was calculated using Eq. (1).

2.6.5. Nitric oxide radical scavenging activity

Nitric oxide radical scavenging assay was conducted as described by Ebrahimzadeh, Nabavi, Nabavi, and Pourmorad (2013). NO generated from sodium nitroprusside (SNP) was detected by Griess reagent. The reaction mixtures containing 0.2 mL 100 mM SNP in PBS (pH 7.4) and 1.8 mL samples at varied concentrations were incubated at 25 °C for 180 min in front of a visible polychromatic light source. After incubation, 1 mL reaction mixture was added with 1 mL Griess reagent (1% sulphanilamide and 0.1% naphthyletylenediamine dihydrochloride in 2% phosphoric acid). The absorbance of the chromophore, which is formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene-diamine, was determined at 540 nm. The mixture contained only SNP and PBS was set as blank. The nitric oxide radical scavenging activity of the samples was calculated using Eq. (1).

2.6.6. Reducing power

The reducing power of extracts was determined by the method of Zhang et al. (2009), with minor modifications. Briefly, an aliquot of 50 μL samples at various concentrations was mixed with 50 μL potassium ferricyanide (1%, w/v) in PBS (0.2 M, pH 6.6). The mixture was incubated at 50 °C for 20 min and then added to 50 μL of trichloroacetic acid (10%, w/v), followed by centrifugation at 3000*g* for 10 min. The upper layer of the solution (50 μL) was mixed with distilled water (50 μL) and ferric chloride (10 μL, 0.1%, w/v), and the absorbance was measured at 700 nm after 30 min. The blank contained all reagents except the sample extract.

2.6.7. Linoleic acid peroxidation

The linoleic acid peroxidation assay was performed using the thiocyanate method (Yen & Hsieh, 1998), with some modifications. The linoleic acid emulsion was prepared by homogenizing 0.0701 g of linoleic acid, 0.0701 g of Tween-20 and 12.5 mL of PBS (0.2 M, pH 7.0). A mixture of 0.1 mL test samples (400 μg/mL; BHT in ethanol, others in water), 0.5 mL linoleic acid emulsion and 0.4 mL PBS was incubated at 37 °C for 144 h. The control included the mixture without test samples. An aliquot of 0.05 mL solution was taken from the incubation mixture every 24 h and added to 2.35 mL 75% ethanol, 0.05 mL 30% ammonium thiocyanate, and 0.05 mL 20 mM ferrous chloride in 3.5% HCl. Then the solution was incubated at room temperature for 3 min and measured the absorbance at 500 nm.

2.7. Statistical analysis

All data were presented as mean ± standard deviation (SD) from three independent experiments. One-way ANOVA and differences between mean vales were assessed by Duncan's test with a 95% confidence level. The correlations between phytochemical contents and antioxidant activities were statistically evaluated by Two-tailed Bivariate Correlate analysis, and were indicated by Pearson's coefficient indexes. To gain an overview of the inter-relationships

among TPC, TFC, antioxidant activities, and to understand the similarities and differences between AC and AM batches, Principal Comment Analysis (PCA) was conducted. PCA based on the correlation matrix was performed using the IBM SPSS Statistics version 19.0 software package for Windows.

3. Results and discussion

3.1. Quantitative analysis of phytochemicals in AC and AM batches

UPLC-PDA analysis was employed for quality control to identify and quantify the major phytochemicals present in *Abrus* herbs. Abrine, the dominant alkaloid in *A. cantoniensis*, has been suggested as the marker for quality control of *A. cantoniensis* (National Pharmacopoeia Committee, 2010; Qiu et al., 2011). Hydroxyanthraquinones chrysophanol and triterpenoid ursolic acid were also indicated as additional markers in the quality control of the herbal extract (Wong et al., 1982; Zhou & Zhou, 2010). Fig. 1A illustrates the HPLC chromatogram of mixed standard of 5 compounds, i.e., abrine, catechin hydrate, emodin, chrysophanol and ursolic acid. Fig. 1B and C show the representative chromatogram of phytochemicals in methanol extracts of AC and AM, respectively. By comparing the migration time and UV spectra of the standards, peak 1, 2 + 3, 4, 5 and 6 in the chromatogram were identified as abrine, catechin hydrate, emodin, chrysophanol and ursolic acid, respectively (Fig. 1B and C).

The methanol extracts of AC and AM, 10 batches of each sample, were detected by UPLC for their fingerprinting. AC and AM showed similar chromatogram profile but varied in phytochemical contents. Table 1 lists the concentration of the dominant five phytochemicals in these 20 batch samples. The contents of abrine varied from 256.76 to 599.95 mg/kg DW, while those of catechin hydrate were in the range of 150.36–337.42 mg/kg DW. Contents of anthraquinones such as emodin and chrysophanol were higher in AC than in AM. The highest content of chrysophanol was 321.19 ± 0.06 mg/kg DW in AC06 sample. The levels of ursolic acid in AC were up to 198.73 ± 11.89 mg/kg DW, while those in AM were all lower than 63.65 mg/kg DW. The content of abrine in AC and AM was in the range of other reports (Huang, Mo, Ma, & Wei, 2009; Huang, Wen, & Ou, 2007; Huang et al., 2011). However, the content of ursolic acid in AC were lower than a study by Zhou and Zhou (2010) at around 300–500 mg/kg DW, which might be due to different extraction method and time.

A statistic AVNON analysis was performed to indicate the significant difference between the levels of each phytochemicals in AC and AM batches. Results indicated no significant difference in the content of abrine and catechin hydrate between AC and AM samples ($p > 0.05$). However, contents of emodin, chrysophanol and ursolic acid were detected significantly higher in AC than in AM ($p < 0.01$). Thus, AC and AM species might be distinguished from the level of phytochemicals, including emodin, chrysophanol and ursolic acid.

3.2. TPC and TFC of AC and AM batches

TPC and TFC are the two key indicators widely used to represent overall antioxidant capacity in a sample (Velioglu, Mazza, Gao, & Oomah, 1998). The TPC of AC and AM batches was tested by Folin–Ciocalteu method with gallic acid as standard. As shown in Table 2, all 10 batches of AC exhibited a generally higher TPC over those of AM ($p < 0.01$). The highest TPC was obtained in AC06, followed by AC05 and AC04, all of which were over 2500 mg GAE/100 g DW. The TPC of AM samples was only around 787–1421 mg GAE/100 g DW. According to Vasco, Ruales, and Kamal-Eldin (2008) and Rufino et al. (2010), the TPC over 500 mg GAE/100 g was classified as a high category. All tested samples in our research fitted in the high category.

Flavonoids are common secondary metabolites presented in plants (Cai, Luo, Sun, & Corke, 2004). TFC of *Abrus* samples was analyzed by $AlCl_3$ method with rutin as standard. In this study, TFC varied in AC and AM batches. For AC, TFC ranged from 1474.60 to 3162.04 mg RE/00 g DW, while those of AM were in the range of 423.13–1372.84 mg RE/100 g DW. It is obvious that, AC showed general higher content of total flavonoid than AM ($p < 0.01$) (Table 2).

3.3. Antioxidant activities of AC and AM batches

3.3.1. FRAP scavenging activities

FRAP result suggests an antioxidant in capable to reduce the Fe^{3+}/tripyridyl-*s*-triazine complex ((Müller et al., 2011)). As shown in Table 2, all the AC methanol extracts had FRAP values of over 278 mM Fe^{2+}/g DW. In this study, the highest value was obtained for AC06 (817.30 ± 3.87 mM Fe^{2+}/g DW), followed by AC04 (757.41 ± 4.46 mM Fe^{2+}/g DW) and AC10 (597.51 ± 3.04 mM Fe^{2+}/g DW). All the AM methanol extracts showed relatively lower FRAP values, ranging from 73.45 to 281.24 mM Fe^{2+}/g DW. It is therefore shown that methanol extracts of AC have a stronger antioxidant capacity to reduce the Fe^{3+}-TPTZ complex ($p < 0.01$).

3.3.2. DPPH radical scavenging activities

DPPH is a stable free radical that can be reduced markedly in the presence of proton radical scavengers (Gülçin, 2006). The IC_{50} value is defined as the concentration of the sample at which the inhibition rate reaches 50%. Table 2 shows a comparison of the IC_{50} values. All AC extracts fell within the range of 14.19–65.06 μg/mL, while AM extracts were within the range of 72.41 to over 800 μg/mL. Lower IC_{50} values indicated a stronger antioxidant activity for the AC extracts than for the AM samples ($p < 0.05$). Some AC extracts even showed comparable DPPH radical scavenging activities to the commercial antioxidants BHT, the IC_{50} values of which was at 28.13 ± 2.69 μg/mL. The strongest DPPH radical scavenging activity was recorded for AC06 (14.19 ± 2.73 μg/mL), followed by AC05 (18.81 ± 0.79 μg/mL) and AC04 (19.97 ± 1.37 μg/mL). The DPPH radical scavenging activity of AC was much higher than some tested herb extracts, such as *Khaya grandifoliola* (Welw) CDC (302.8 ± 0.08 μg/mL), *Tanacetum chiliophyllum* (114.64 ± 2.47 μg/mL), *Tanacetum budjunurdense* (133.32 ± 3.72 μg/mL), *Tanacetum sonboli* (132.30 ± 2.17 μg/mL) and *Tanacetum tabrisianum* (157.24 ± 1.62 μg/mL), etc. (Cai et al., 2004; Vasco et al., 2008).

3.3.3. ABTS radical cation scavenging activities

The ABTS radical cation is generated by oxidation of ABTS with potassium persulfate, and can be reduced when exposed to antioxidants (Re et al., 1999). Table 2 shows the corresponding IC_{50} values of the AC and AM batches against ABTS cations. The IC_{50} values of BHT was recorded at 63.25 ± 0.24 μg/mL. As can be observed, the AC05 and AC06 exhibited the most potent ABTS radical cation scavenging with low IC_{50} value at 33.17 ± 0.82 and 33.49 ± 2.87 μg/mL, respectively. The AC batches had IC_{50} values in the range of 33.17–60.99 μg/mL, while AM had those ranged from 68.06 to 192.88 μg/mL. That is, AC had more potent ABTS radical cation scavenging activities than AM ($p < 0.01$). The antioxidant activity of the AC extract was higher when compared with that of the functional food ingredient *Rhizoma Smilacis* Glabrae (130 ± 8 μg/mL IC_{50} value) (Zhang et al., 2009), further supporting the antioxidative potential of this plant.

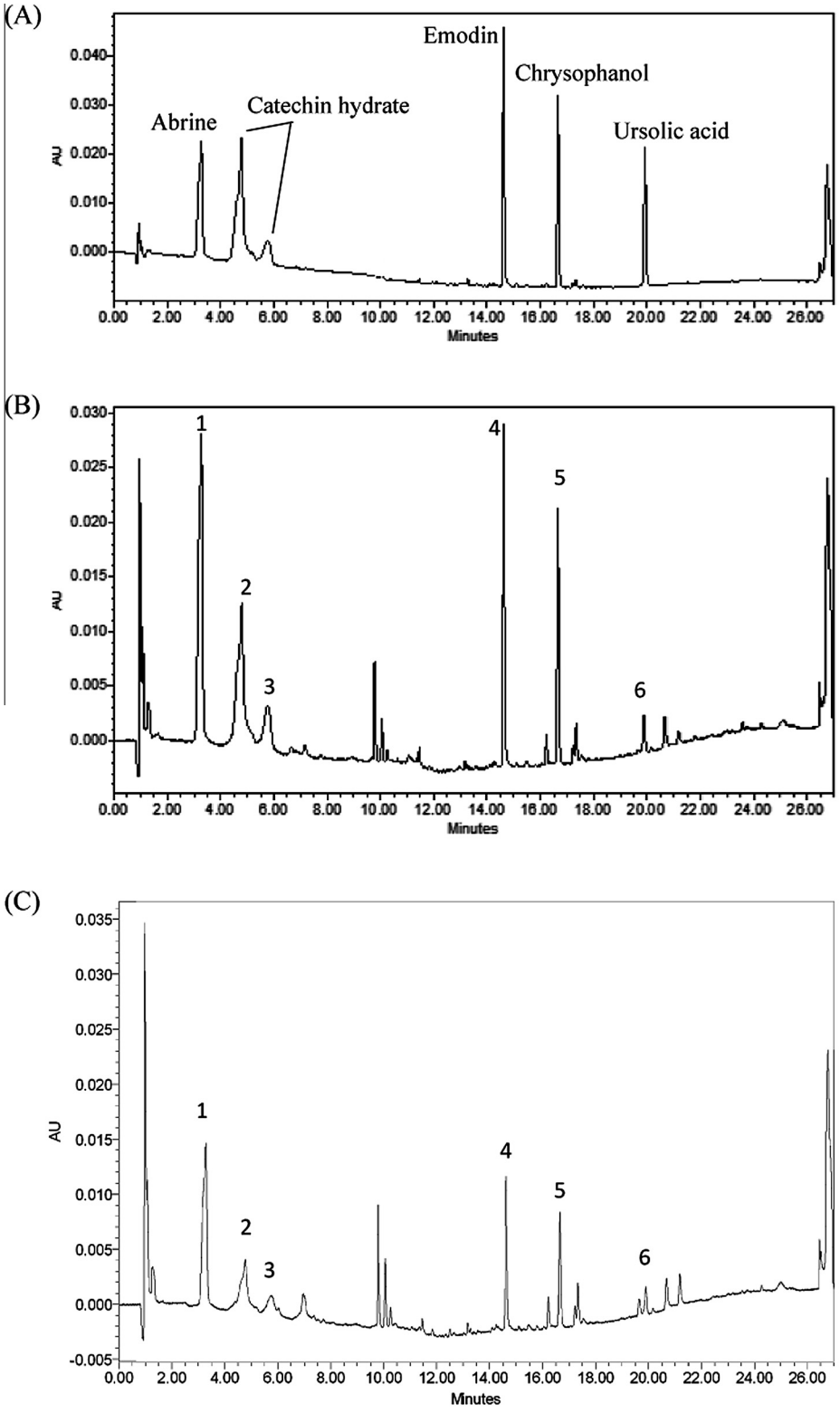

Fig. 1. Representative UPLC-PAD chromatogram of (A) mix five standards, phytochemicals in (B) AC06 extract and (C) AM06 extract as detected at 205 nm.

3.3.4. Superoxide anion radical scavenging activities

In the PMS/NADH-NBT system, superoxide anion generated by the oxidation of NADH reduces NBT. Antioxidant consumes superoxide anion and decreases the absorbance of reduced NBT at 560 nm (Zhang et al., 2009). As shown in Table 2, IC_{50} values revealed higher antioxidant activity against superoxide anion radicals of the AC samples than of the AM samples ($p < 0.01$). In comparison with the commercial antioxidant BHT (79.36 ± 1.32 μg/mL), AC06 showed the strongest superoxide anion radical scavenging activity with the lowest IC_{50} value (41.84 ± 1.28 μg/mL), while AC04 ranked second highest with an IC_{50} value of 59.62 ± 2.03 μg/mL. The antioxidant activities of AM batches varied greatly and the IC_{50} vales were in the range of 117.86 to over 800 μg/mL. The AC showed much more potent antioxidant

Table 2
TPC, TFC, FRAP vale and IC_{50} values of DPPH, ABTS, superoxide anion, nitric oxide radical of AC and AM batches.

Batch No.[a]	TPC (mg GAE/ 100 g DW[b])	TFC (mg RE/ 100 g DW[c])	FRAP (mM Fe^{2+}/ g DW[d])	DPPH IC_{50} (μg/ mL)	ABTS IC_{50} (μg/ mL)	Superoxide anion IC_{50} (μg/mL)	Nitric oxide radical IC_{50} (μg/mL)
AC01	1769.62 ± 4.32	1517.00 ± 38.86	278.14 ± 3.58	65.06 ± 0.12	55.33 ± 1.38	104.85 ± 3.58	140.57 ± 9.57
AC02	2190.11 ± 2.16	1533.96 ± 25.44	550.73 ± 4.70	33.57 ± 5.29	49.16 ± 0.12	81.07 ± 2.50	75.10 ± 0.54
AC03	1676.35 ± 15.14	1474.60 ± 14.69	286.74 ± 3.03	48.65 ± 4.95	54.49 ± 0.60	96.72 ± 3.41	101.61 ± 0.41
AC04	2586.14 ± 95.15	2203.84 ± 38.86	757.41 ± 4.46	19.97 ± 1.37	39.27 ± 0.88	59.62 ± 2.03	77.44 ± 7.73
AC05	2740.57 ± 71.36	2271.68 ± 116.57	461.55 ± 3.04	18.81 ± 0.79	33.17 ± 0.82	82.23 ± 3.86	98.99 ± 1.08
AC06	3132.01 ± 75.68	1686.59 ± 25.44	817.30 ± 3.87	14.19 ± 2.73	33.49 ± 2.87	41.84 ± 1.28	54.93 ± 2.26
AC07	1687.05 ± 86.50	3162.04 ± 25.44	323.45 ± 1.15	58.71 ± 2.24	60.99 ± 0.17	104.12 ± 3.06	173.93 ± 9.12
AC08	1954.64 ± 15.14	1745.95 ± 144.65	379.68 ± 4.70	38.89 ± 1.83	44.32 ± 0.95	87.52 ± 4.39	136.45 ± 2.91
AC09	2388.89 ± 105.96	1864.66 ± 44.06	462.52 ± 0.84	36.36 ± 0.13	35.57 ± 0.43	77.73 ± 1.53	137.40 ± 3.26
AC10	2407.24 ± 110.28	1822.26 ± 52.95	597.51 ± 3.04	33.60 ± 1.42	43.83 ± 0.54	75.76 ± 4.36	135.92 ± 3.55
AM01	1313.97 ± 43.25	1194.77 ± 29.37	118.47 ± 2.92	77.28 ± 2.00	76.01 ± 1.52	153.22 ± 3.35	263.98 ± 1.79
AM02	884.30 ± 28.11	1118.46 ± 73.44	99.90 ± 1.52	198.66 ± 28.23	136.60 ± 2.19	179.19 ± 1.12	257.19 ± 5.16
AM03	787.97 ± 0.00	1398.28 ± 14.69	73.45 ± 1.52	>800	185.45 ± 7.75	777.81 ± 7.42	318.68 ± 7.05
AM04	838.43 ± 41.09	1288.05 ± 117.50	281.24 ± 3.04	72.41 ± 4.95	79.23 ± 1.41	347.35 ± 5.93	270.54 ± 9.86
AM05	1323.14 ± 95.15	423.13 ± 38.86	243.09 ± 2.07	458.54 ± 0.12	192.88 ± 9.84	>800	508.29 ± 14.43
AM06	1266.56 ± 19.46	1423.72 ± 102.81	266.90 ± 2.29	82.05 ± 4.23	83.26 ± 0.95	263.36 ± 2.43	371.39 ± 8.96
AM07	1199.29 ± 80.01	1381.32 ± 25.44	196.13 ± 3.76	86.95 ± 4.70	79.03 ± 1.44	135.97 ± 2.74	154.18 ± 10.85
AM08	839.96 ± 17.30	1109.98 ± 14.69	98.25 ± 3.76	361.07 ± 14.45	133.57 ± 1.60	190.45 ± 3.72	189.23 ± 7.19
AM09	1421.00 ± 12.97	1372.84 ± 125.49	273.93 ± 4.47	73.43 ± 2.24	68.06 ± 0.62	117.86 ± 1.70	179.13 ± 0.83
AM10	995.92 ± 17.30	1262.61 ± 117.50	138.59 ± 0.57	198.15 ± 15.11	129.17 ± 4.24	488.91 ± 2.90	209.97 ± 1.78
BHT	N.A.[e]	N.A.	1299.97 ± 3.58	28.13 ± 2.69	63.25 ± 0.24	79.36 ± 1.32	318.48 ± 5.88

Results were expressed as mean ± SD from three independent experiments.
[a] AC and AM indicated *Abrus cantoniensis* and *Abrus mollis*, respectively.
[b] TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of dry weight material.
[c] TFC was expressed as mg rutin equivalents (RE) per 100 g of dry weight material.
[d] FRAP value was expressed as mM Fe^{2+} per g of dry weight material.
[e] N.A. indicates not available.

activities than other functional foods, such as the polysaccharide isolated from *Corbicula fluminea*, the IC_{50} values of which was 600 μg/mL (Liao et al., 2013).

3.3.5. Nitric oxide radical scavenging activities

Besides reactive oxygen species, nitric oxide plays a vital role in inflammation, cancer and other pathological conditions (Moncada, Palmer, & Higgs, 1991). As shown in Table 2, AC samples exhibited a potent nitric oxide radical scavenging capacity with all IC_{50} values lower than 174 μg/mL. The AC06 showed the strongest antioxidant activity (54.93 ± 2.26 μg/mL), followed by AC02 (75.10 ± 0.54 μg/mL) and AC04 (77.44 ± 7.73 μg/mL). The commercial antioxidant BHT had an IC_{50} value of 318 μg/mL. That means AC extracts exhibited a stronger antioxidant activity than the synthetic antioxidant BHT. For AM extracts, the IC_{50} values ranged from 179 to 508 μg/mL, showing a weaker antioxidant activity than that of AC extracts ($p < 0.01$).

3.3.6. Reducing power

The total reduction capability is served as a significant indicator of the potential antioxidant activity, and is expressed as the increased absorbance of the reaction mixture at 700 nm (Zhang et al., 2009). Fig. 2A shows the reduction of Fe^{3+} to Fe^{2+} in the presence of plant samples. The IC_{50} value is defined as the effective concentration at which the absorbance is 0.5. As shown in the figure, the absorbance of the BHT dramatically increased in a dose dependent-manner. The AC06 extract exhibited a potent reducing power, followed by AC10 and AC03. The IC_{50} value of AC06 was approximately 300 μg/mL, while that of AC10 and AC03 was around 400 μg/mL. The absorbance of AC samples at 700 nm was higher than most of the AM samples, showing a stronger reducing power than AM samples ($p < 0.01$). It has been reported that, herbal extract possesses reducing power is potent in reducing the toxic iron level and attenuating oxidative stress and fibrosis status in the liver of mice (Sarkar, Hazra, & Mandal, 2012). The reducing power of AC and AM extracts might also contribute to their hepatoprotective effects.

3.3.7. Linoleic acid system antioxidant activities

Peroxidation of linoleic acid can lead to severe oxidative DNA damage (De Kok, Ten Vaarwerk, Zwingman, Van Maanen, & Kleinjans, 1994). Fig. 2B shows the time course of the peroxidation prevented by antioxidants. For the control sample without antioxidants, oxidation of linoleic acid generates corresponding hydroperoxides then decomposes to various secondary oxidation products. Antioxidants prevent the peroxidation reaction and hinder the appearance of the blood-red colour arising from ferric thiocyanate (Yen & Hsieh, 1998). In this study, the absorbance of the control steadily increased up to 3.89 after 72 h, subsequently decreasing slightly and reaching a plateau. All AC samples showed potent inhibition effects on the peroxidation, as the optical density values were lower than 0.75 until the end of incubation. The antioxidant activity varied in AM batches. Sample AM08, AM10 and AM06 showed weak antioxidant activities over the entire incubation time. After 144 h, AC batches showed a stronger antioxidant capacity than AM ($p < 0.05$). The antioxidant activities of AC extracts were more potent in preventing linoleic acid peroxidation, compared to other natural product extracts such as *Rhizoma Smilacis* Glabrae and grape seeds (*Vitis vinifera*) (Jayaprakasha, Singh, & Sakariah, 2001; Zhang et al., 2009).

3.4. Correlations between phytochemical contents and antioxidant activities

The TPC and TFC of plant materials have been reported positively correlated to their antioxidant activities (Cai et al., 2004; Velioglu et al., 1998). Table 3 lists the Pearson's coefficients between TPC, TFC and various antioxidant capacities. The TPC showed a high positive correlation with FRAP, DPPH and ABTS cation scavenging activities ($r = 0.919$, 0.957 and 0.948, respectively) ($p < 0.01$). The TPC had mild correlation with superoxide anion

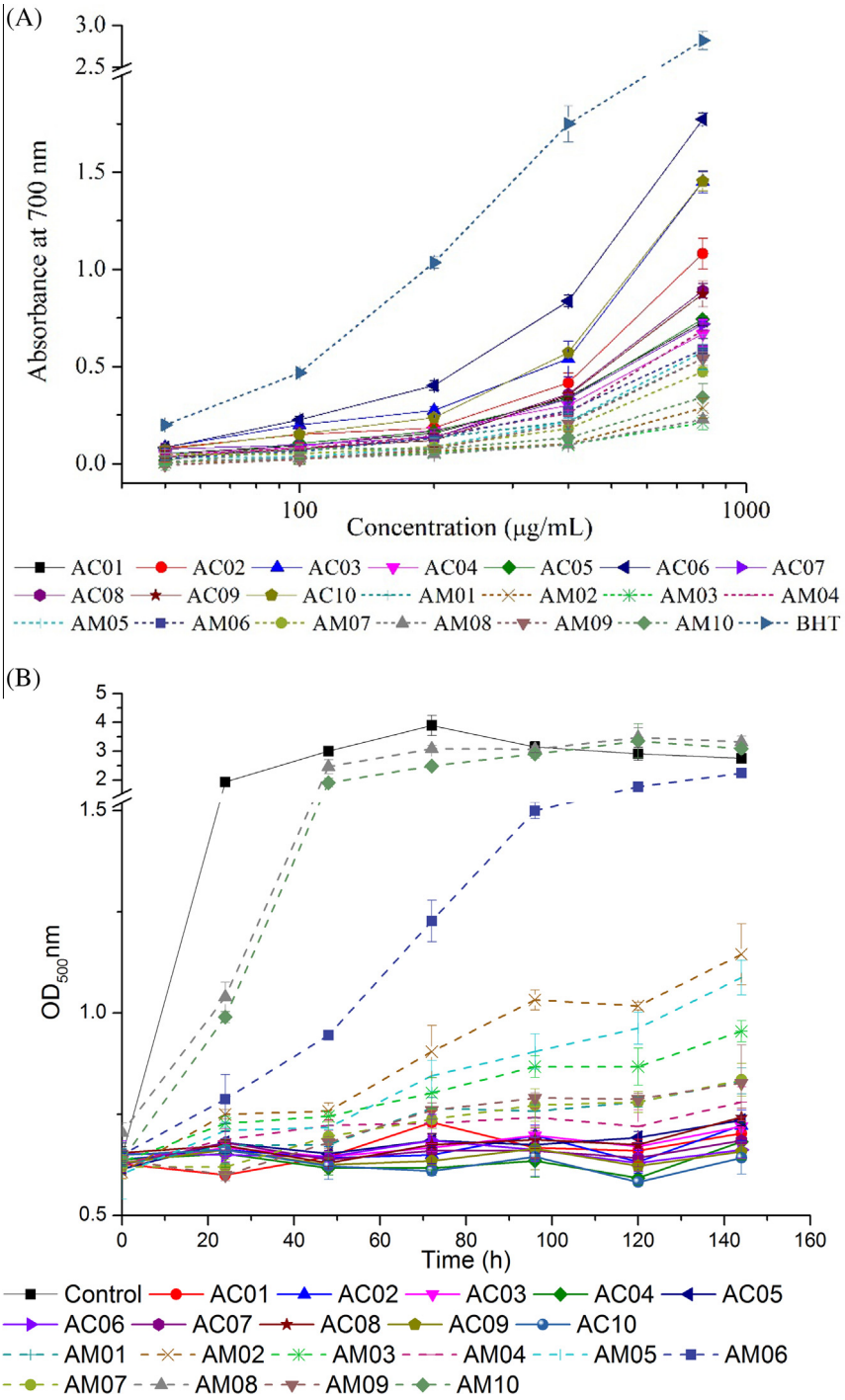

Fig. 2. Reducing power, linoleic acid system antioxidant activities of AC and AM batches.

and nitric oxide radical scavenging capacities as well as reducing power, showing coefficients at 0.812 and 0.771 and 0.751 ($p < 0.01$), respectively. The correlation coefficients of the TFC to the FRAP value and DPPH reducing capacities were 0.864 and 0.860, respectively ($p < 0.01$). Mild correlations were observed between TFC and the ABTS cation, superoxide anion, nitric oxide scavenging activities and reducing power, as the correlation coefficients were all over 0.708.

Different detection methods were used to comprehensively evaluate the antioxidant activities of herbal samples. Primary antioxidants have been revealed to react against the oxidation involving the breakage of chain reaction or scavenging of free radicals. Secondary antioxidants function by deactivating metal, inhibiting the breakdown of lipid hydroperoxides, regenerating primary antioxidants, and quenching singlet oxygen (Gordon, 1990). Thus, several chemical-based assay methods that have their own focuses have been developed and adjusted for the detection of antioxidant activities during the past decades (Moein, Moein, & Ahmadizadeh, 2008; Re et al., 1999; Sánchez-Moreno, 2002). The DPPH and ABTS method focused on the non-specific radicals existed in the reaction system. The IC_{50} values of DPPH and ABTS radical scavenging activities were similar for all herbal samples, and they showed high correlation with each other ($r = 0.920$, $p < 0.01$). The IC_{50} values of superoxide anion and nitric oxide radical scavenging activities

Table 3
Pearson's correlation coefficients of TPC, TFC and antioxidant activities of AC and AM batches.

Trait	TPC	TFC	FRAP	DPPH	ABTS	Superoxide	Nitric oxide	Reducing power	Linoleic acid
TPC	1.000	.854**	.919**	.957**	.948**	.812**	.771**	.751**	−.504*
TFC		1.000	.864**	.860**	.777**	.747**	.806**	.708**	−.355
FRAP			1.000	.939**	.876**	.842**	.736**	.752**	−.460*
DPPH				1.000	.920**	.825**	.757**	.757**	−.556*
ABTS					1.000	.836**	.787**	.738**	−.516*
Superoxide						1.000	.755**	.795**	−.486*
Nitric oxide							1.000	.653**	−.314
Reducing power								1.000	−.484*
Linoleic acid									1.000

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

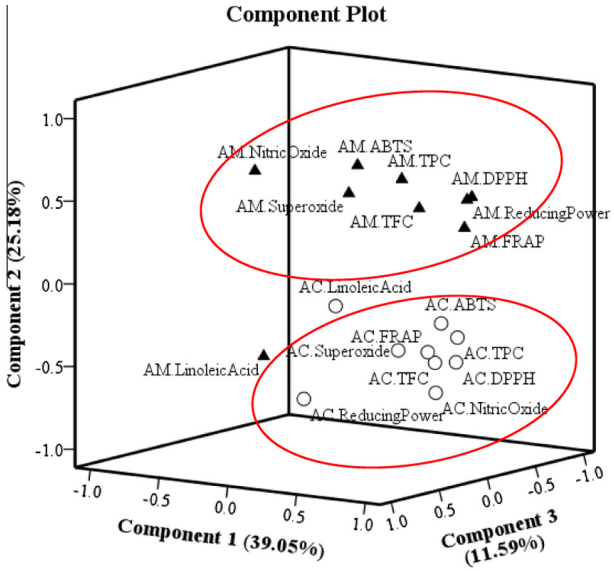

Fig. 3. Principal component analysis plot of TPC, TFC and antioxidant activities of C and AM batches.

were higher than those of DPPH and ABTS radical scavenging activities of most herbal samples, indicating stronger antioxidant capacities against non-specific radicals. Although both FRAP and reducing power evaluated the reducing capacity of sample against Fe^{3+} in the system, their correlation index (0.736) was lower than that between FRAP and DPPH or ABTS (0.939 and 0.876, respectively). Peroxidation of linoleic acid evaluates the antioxidant capacities in a non-polar system. In our study, the linoleic acid peroxidation inhibitory assay only shows mild or weak negative correlation with other detection methods. This might be attributed that, the linoleic acid peroxidation inhibitory activity was mostly controlled by some non-polar metabolites present in extracts, while these hydrophilic antioxidants and total chelation activity were mainly attributed to polar secondary metabolites such as phenolics and flavonoids.

The PCA was used to gain an overview of the inter-relationships among TPC, TFC, antioxidant activities, and to understand the similarities and differences between AC and AM batches. Fig. 3 shows the loading plot of the first, second and third principal components (PC1, PC2 and PC3) accounted for 39.05%, 25.18% and 11.59% of the variance, respectively. The distribution of the parameters implied two clusters of the AC and AM. That means the two species were significantly different from each other. TPC, FRAP, DPPH and ABTS of AC were heavily loaded on the PC1 with squared cosine value of 0.931, 0.852, 0.845 and 0.851, respectively; whereas, TPC, ABTS and nitric oxide of AM were loaded positively on the PC2 with squared cosine value of 0.609, 0.734 and 0.705, respectively. It is indicated herein that, except linoleic acid peroxidation, antioxidant properties and phenolic and flavonoid contents were highly correlated with each other. The linoleic acid peroxidation inhibitory activity was mostly controlled by some non-polar metabolites in extracts, while these hydrophilic antioxidants and total chelation activity were mainly attributed to polar secondary metabolites such as phenolics and flavonoids. The results obtained by PCA were consistent with those from Pearson's correlation analysis (Table 3). Feng, Luo, Zhang, Zhong, and Lu (2014) reported a similar cluster of TPC, TFC, DPPH and FRAP loaded heavily on PC1 when evaluating the sugarcane (*Saccharum officinarum* L.). Ghosal and Mandal (2012) performed PCA on the antioxidant attributes of two fruits *Solanum anguivi* and *Solanum incanum*. Results also demonstrated that, TPC, TFC, DPPH, reducing power and nitric oxide scavenging were positively loaded on PC1.

4. Conclusions

Ten batches of each of AC and AM collected from various origins were subjected to qualitative and quantitative analysis by means of UPLC-PAD technology using five dominant phytochemicals, namely abrine, catechin hydrate, emodin, chrysophanol and ursolic acid as markers. Methanol extract of the samples gave individual fingerprint pattern with significant difference. Although these two species exhibited high antioxidant capacities as evaluated by different assays, results revealed that, the antioxidative capacity of AC samples were stronger than AM samples in most tested systems. PCA study convinced that, the profound antioxidative effect of the AC and AM is attributed to the presence of phenolics and flavonoids. Hence, daily consumption of AC and AM as beverages and soups may be effective in preventing diseases implicated oxidants and free radicals. Moreover, from the point of quality control and authentication, it is recommended to choose AC rather than AM for the guarantee of health promoting function.

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