Sains Malaysiana 47(4)(2018): 725-730
http://dx.doi.org/10.17576/jsm-2018-4704-10

Antioxidant and Mutagenic Activity of Herbal Tea Prepared from *Cosmos caudatus* Leaves at Different Maturity Stages

(Aktiviti Antioksida dan Mutagen di dalam Teh Herba daripada Daun *Cosmos caudatus* pada Tahap Kematangan Berbeza)

Dian-Nashiela Fatanah, Noriham Abdullah*, Nooraain Hashim & Azizah Abd. Hamid

ABSTRACT

Different maturity stages of Cosmos caudatus *leaves have been used to prepare herbal tea were investigated for their effect on antioxidant activity and mutagenic activity. The analyses carried out were total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), 2-2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, β-carotene bleaching assay, oxygen radical absorbance capacity (ORAC) and* Ames Salmonella *mutagenicity. The results demonstrated that,* C. caudatus *herbal tea prepared from young leaves showed significantly highest antioxidant activity for all assays tested, followed by mixed leaves, mature leaves, old leaves and the lowest was in* C. caudatus *herbal tea from a commercial brand. Pearson's correlation coefficient also demonstrated that TPC and TFC displayed a strong correlation with all antioxidant activity assays, showing that these compounds were the major contributors to the antioxidant activity in* C. caudatus *herbal tea. However, all studied* C. caudatus *herbal tea showed no mutagenic effects against* Salmonella typhimurium *tester strain TA98 and TA100 with and without S9 metabolic activation. Hence, it can be concluded that, different maturity stages could affect the antioxidant activity in* C. caudatus *herbal tea as it reduced the antioxidant activity as maturity increased, but did not give any effect on the mutagenic activity.*

Keywords: Antioxidant activity; Cosmos caudatus*; herbal tea; maturity; mutagenic activity*

ABSTRAK

Kesan aktiviti antioksida dan mutagen ke atas teh herba yang dihasilkan daripada daun Cosmos caudatus *pada tahap kematangan yang berbeza telah dikaji. Analisis yang dijalankan ialah jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC), ujian penurunan ferik (FRAP), pemerangkapan radikal bebas 2-2-Difenil-1-pikrilhidrazil (DPPH), pelunturan β-karotena, kapasiti penyerapan radikal oksigen (ORAC) dan mutagenik* Ames Salmonella*. Keputusan menunjukkan bahawa, teh herba* C. caudatus *dihasilkan daripada daun muda mempunyai aktiviti antioksida yang tertinggi bagi kesemua asai yang diuji, diikuti oleh daun campuran, daun matang, daun tua dan nilai terendah diperoleh dari teh herba* C. caudatus *daripada jenama komersial. Pekali kolerasi Pearson juga memaparkan kolerasi yang kuat bagi TPC dan TFC dengan kesemua asai aktiviti antioksida yang dijalankan. Ini menunjukkan bahawa sebatian tersebut merupakan penyumbang utama kepada aktiviti antioksida di dalam teh herba* C. caudatus*. Walau bagaimanapun, kesemua teh herba* C. caudatus *yang dikaji tidak menunjukkan kesan mutagen terhadap strain penguji* Salmonella typhimurium *TA98 dan TA100 dengan dan tanpa pengaktifan metabolic S9. Sebagai kesimpulannya, peringkat kematangan daun yang berbeza boleh memberi kesan ke atas aktiviti antioksida di dalam teh herba* C. caudatus*, semakin meningkat kematangan daun yang digunakan, semakin rendah aktiviti antioksida, tetapi tiada kesan ke atas aktiviti mutagen.*

Kata kunci: Aktiviti antioksida; aktiviti mutagen; Cosmos caudatus*; kematangan; teh herba*

Introduction

Herbal tea is a tea brewed from the leaves, flowers, seeds, fruits and roots of plant species other than *Camellia sinensis* (Kara 2009). Nowadays, many countries especially Asia have varieties of herbal teas. In Malaysia, there are abundant of herbal teas produced by Small Medium Enterprise (SME) industry such as *misai kucing (Orthosiphon stamineus)* tea, *kacip fatimah (Labisa pumila)* tea, *kaca beling (Strobilanthes cripa)* tea, ginger *(Zingiber officinale)* tea and lemongrass tea. *Cosmos caudatus* or known as *ulam raja* is amongst of the herbs that are rich in potential health properties and has functionality to the consumer health and recently it is getting attention by the Malaysian herbal industries to be developed in tea form (Dian-Nashiela et al. 2015). Traditionally, *C. caudatus* has been used to reduce body health, improving blood circulation, as anti-aging agent, strengthening bone marrow (because of high calcium content), to treat infection associated with pathogenic microorganisms and to promote fresh breath (Amna et al. 2013). It has been reported that, these beneficial health properties have been attributed to the higher antioxidant content in *C. caudatus* plant predominantly, a number of

proanthocyanidins that exist as dimers through hexamers, quercetin, chlorogenic acid, catechin, epicatechin, myricetin and naringenin (Shui et al. 2005). Nevertheless, usage of *C. caudatus* leaves at different maturity stages as raw material in herbal tea preparation could affect their antioxidant activity as well as their tolerance towards the mutagenicity level. Some current studies showed that, maturity stages could influences the antioxidant activity, biochemical compositions and physicochemical properties of the plants (Fawole & Opara 2013). In fact, there have been reports on increased global demands for herbal products that acts as energy boosters, detoxifiers, immune boosters and aphrodisiacs, thus, the evaluation of bacterial mutagenicity is important as an initial test for complex mixtures because of the possibility that, one or more of their components can be mutagenic (Ndhlala et al. 2010). Therefore, this present study was conducted to investigate the effects of antioxidant activity and mutagenic activity of herbal tea prepared from *C. caudatus* leaves at different maturity stages.

Materials and Methods

Chemicals

All chemicals and standards were purchased from Sigma-Aldrich Chemie, Germany.

Raw Materials Collections and Selection

The fresh leaves of *C. caudatus* plant were planted and harvested in Durian Tunggal, Malacca, Malaysia at 8-week-old. The leaves were divided into 3 groups and classified as young leaves, mature leaves and old leaves. As method described by Dian-Nashiela et al. (2015), young leaves were selected from the first four tiers where the leaves are still tender, newly emerged and not attaining full expansion. Mature leaves are located at the middle part of the plant where the leaves are fully developed while old leaves are situated at the lower part of the plant and the leaves had showed initial sign of senescence. Mature leaves were selected between the fifth to eighth tiers and old leaves were selected starting from ninth tiers and above.

Sample Preparation

Each group of *C. caudatus* leaves were prepared according to the normal procedure as being conducted for herbal tea preparation by Small Medium Enterprise (SME) industry in Malaysia. The leaves at different maturity stages were dried at 50°C for 8 h in cabinet dryer until constant weight. Then, the dried leaves of *C. caudatus* were processed in powder form based on Giảo et al. (2009). The dried leaves were milled using ultra centrifugal mill at 8000 rpm and the milled leaves were then screened through different sieves sized ranged from 1 to 2 mm. After that, 2 g of the sieved leaves were collected and packed in a tea bag. Then, all the *C. caudatus* herbal teas were infused in 200 mL boiling distilled water for 3 min according to method suggested by Horžić et al. (2009). The infused *C. caudatus* herbal tea filtered through Whatman filter paper No. 41 prior to further analyses.

Determination of Antioxidant Activity

Total Phenolic Content (TPC)

The total phenolic content in *C. caudatus* herbal tea samples were determined by using the Folin-Ciocalteu assay (Harbourne et al. 2009). Accurately, 0.5 mL Folin-Ciocalteu reagent, 1.5 mL 7.5% sodium carbonate and 7.8 mL distilled water were introduced in a test tube containing 0.1 mL sample/standard. The solution was mixed thoroughly and allowed to stand for 2 h in a dark place. The absorbance was read at 765 nm and the results were expressed as mg gallic acid equivalent (mg GAE)/mL of herbal tea.

Total Flavonoid Content (TFC)

The total flavonoid content was analysed according to method as described by Singh et al. (2012). One mL of *C. caudatus* herbal tea sample/standard was dilute with 4 mL distilled water, then 0.3 mL 5% sodium nitrate solution and 0.3 mL 10% aluminium chloride were added. The mixture of solution was kept for 5 min. After that, 2 mL of 1 M sodium hydroxide were added to the mixture and the mixture was vortexed thoroughly. The absorbance was measured at 510 nm using UV-VIS spectrophotometer. This was calculated as mg of quercetin (mg QE)/mL of herbal tea.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay for *C. caudatus* herbal tea samples was carried according to method of Deetae et al. (2012). The FRAP reagent was freshly prepared by mixing 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ was made to 40 mM hydrochloride at a ratio of 10:1:1. Briefly, 0.1 mL sample/standard was mixed with 3 mL FRAP reagent and 3 mL distilled water. The mixture was incubated in the dark place at 37°C for 8 min and the absorbance was then read at 595 nm. The total antioxidant activity of *C. caudatus* herbal tea samples were determined against a standard of known FRAP value and was expressed as μM of trolox equivalent (μM TE)/mL of herbal tea.

DPPH (2-2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Assay

The DPPH assay was performed according to procedure as described by Nuengchamnong and Ingkaninan (2010). Accurately, 0.1 mL of sample/standard was mixed with 2.9 mL 0.05 mM DPPH in methanol and incubated in the dark at room temperature for 30 min. The radical scavenging activity of *C. caudatus* herbal tea/standard was measured as a decrease in the absorbance of DPPH using UV-VIS spectrophotometer where methanol was used as blank.

B-CAROTENE BLEACHING ASSAY

The antioxidant activity of all *C. caudatus* herbal tea samples is based on the *β*-carotene bleaching assay method developed by Velioglu et al. (1998). The *β*-carotene (0.2 mg in 1 mL chloroform), linoleic acid (0.02 mL) and Tween 20 (0.2 mL) were transferred into a round bottom flask. Chloroform was removed at room temperature under vacuum at reduced pressure using rotary evaporator. Following evaporation, 50 mL of distilled water was added to the mixture and then shaken vigorously to form emulsion. About 2 mL aliquots of the emulsion were pipette into test tubes containing 0.2 mL of the ethanol/combination of BHA/BHT standard/*C. caudatus* herbal tea samples and immediately placed in water bath at 50°C for 120 min and was read at 470 nm. Antioxidant activity (AA) was expressed as percent of inhibition relative to the control.

OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) ASSAY

For ORAC assay, the antioxidant activity of *C. caudatus* herbal tea samples were analysed according to Khairusy et al. (2012) method. About 25 µL of sodium phosphate buffer (as a blank)/standard/samples were added into 96-well plate containing 150 µL of fluorescein working solution. The 96-well plate was then placed in the multi-mode microplate reader and incubated for 5 min at 37°C. After the incubation period, 25 µL of AAPH solution was added to all of the experimental wells. The excitation and emission were set at 495 and 528 nm, respectively. The relative fluorescein intensity was monitored and recorded every 2 h. Then, the final ORAC value was calculated using the net area under the decay curves and was expressed as µmol of trolox equivalent (µmol TE)/mL of herbal tea.

DETERMINATION OF MUTAGENIC ACTIVITY

AMES *SALMONELLA* ASSAY

The Ames *Salmonella* assay was performed using method suggested by Mortelmans and Zieger (2000). The procedure involves adding the buffer or S9 liver metabolic activation, the histidine dependent bacteria (about 10^8) and test chemical to 2 mL of top agar containing biotin and trace amount of histidine (0.05 mM each). The mixture was then gently mixed and poured on glucose minimal (GM) agar plates. When the top agar solidified, the plates were incubated in an inverted position at 37°C inside an incubator for 48 h at time which the histidine revertant colonies were counted. The colonies were counted using automatic colony counter.

STATISTICAL ANALYSIS

All experiments were run in triplicates and results are expressed as the means ± standard deviation. The data were statistically analysed in one-way ANOVA using the Statistical Analysis System (SAS) 9.1.3 software package. Significant differences between means were determined by Duncan's multiple range tests ($p<0.05$).

RESULTS AND DISCUSSION

ANTIOXIDANT CONTENT AND ANTIOXIDANT ACTIVITY

All the *C. caudatus* herbal tea prepared from leaves at different maturity stages were analysed for their antioxidant content and antioxidant activity (Table 1). Among five *C. caudatus* herbal teas under studied, *C. caudatus* herbal tea prepared from young leaves was recognised as the richest source of both, TPC and TFC, whereas *C. caudatus* herbal tea from commercial brand exerted the lowest value. The trend for the declining of TPC and TFC in *C. caudatus* herbal tea as maturity increased did not vary markedly from their antioxidant activity when screened through FRAP, *β*-carotene and ORAC assay. However, for DPPH scavenging assay, the IC_{50} values increased as the maturity of the leaves used to prepare *C. caudatus* herbal tea increased, which indicates that young leaves had the strongest antioxidant activity compared to the other *C. caudatus* herbal teas.

This finding is consistent with those obtained by Mediani et al. (2007), who found the similar trend,

TABLE 1. Antioxidant content and antioxidant activity of *C. caudatus* herbal tea prepared at different maturity stages

Herbal tea sample	Assay					
	TPC (mg GAE/mL herbal tea)	TFC (mg QE/mL herbal tea)	FRAP (µM TE/mL herbal tea)	DPPH (µg/mL)	*β*-carotene (Antioxidant activity, %)	ORAC (µmol TE/mL of herbal tea
Young leaves	66.30 ± 5.20^{a}	203.22 ± 15.90^{a}	502.21 ± 21.18^{a}	1055.37 ± 42.38^{a}	77.89 ± 6.38^{a}	3.65 ± 0.15^{a}
Mature leaves	36.55 ± 2.77^{b}	124.55 ± 6.22^{b}	332.00 ± 8.81^{b}	1409.99 ± 103.17^{b}	65.89 ± 3.00^{b}	2.18 ± 0.18^{c}
Old leaves	18.38 ± 2.46^{c}	72.24 ± 3.04^{c}	239.18 ± 19.19^{c}	2408.84 ± 365.36^{c}	56.01 ± 3.76^{c}	0.92 ± 0.09^{d}
Mixed leaves	46.79 ± 1.32^{b}	149.32 ± 2.84^{b}	373.36 ± 12.30^{b}	1362.19 ± 217.39^{d}	62.72 ± 4.38^{b,c}	3.20 ± 0.02^{b}
Commercial brand	7.39 ± 0.91^{e}	17.20 ± 1.07^{e}	84.17 ± 5.84^{e}	7033.00 ± 612.25^{a}	44.12 ± 2.66^{d}	0.62 ± 0.06^{e}

Values are expressed as mean and standard deviation. Means with different letter within a column are significantly different ($p<0.05$)

TABLE 2. Pearson's correlation coefficient (R) between antioxidant activity of *C. caudatus* herbal tea prepared from leaves at different maturity stages

	FRAP	DPPH	*β-carotene*	ORAC
TPC	0.9967 ± 0.002[a]	–0.9224 ± 0.058[a]	0.978 ± 0.030[a]	0.984±0.015[a]
TFC	0.9980 ± 0.002[a]	–0.9288 ± 0.060[a]	0.986 ± 0.017[a]	0.993±0.008[a]

Values are expressed as mean and standard deviation. Means with different letter within a column are significantly different ($p<0.05$)

in which the 8-week-old *C. caudatus* plant give most powerful antioxidant activity compared to 10-week-old and 12-week-old of this plant. This is because the better sunlight exposure on young leaves than on mature and old leaves (Fernando et al. 2013) might aids most of new biosynthesis, simultaneously produce higher antioxidant in young leaves (Menichini et al. 2011). Due to strong antioxidant activity, it leads to the formation of macromolecular compounds with the stronger radical scavenging power which may be attributable to the increased resonance delocalisation and higher stability of the aryloxyl radicals incurred by hydrogen bonding (Farhoosh et al. 2007). For these reasons, Müller et al. (2013) believed that, total phenolics and flavonoids that prevailed during the early maturity stages possess a great ability to scavenge light-induced reactive oxygen species (ROS). Other than that, Barros et al. (2007) believed that, the reduction of antioxidant activity is due to the aging process, stimulates the formation of ROS and cause extensive production of ROS, which are then neutralised by the phenolic compounds, resulting in the lowering of their content and antioxidant activity. Since the mature and old leaves possess inadequate antioxidant activity and/or owing overproduction of ROS, this equilibrium is hampered favouring the ROS upsurge that culminates in oxidative stress, eventually senescence of plant tissues (Sreelatha & Padma 2009).

Nevertheless, at a physiological level, the combination of many phytochemicals even at low concentrations, provides the molecules that display additive and very often synergistic effects in their antioxidant properties (Blasa et al. 2010). This is proven from the combination of *C. caudatus* leaves from three different maturity stages, where *C. caudatus* herbal tea prepared from mixed leaves showed highest antioxidant activity than mature and old leaves singly. This effect most probably due to depolymerisation and intermolecular of H-bonding reaction as well as might be due to the easier accessibility of the phenolic-OH (Celik et al. 2010). On the other hand, *C. caudatus* herbal tea from commercial brand recorded the lowest antioxidant activity than other *C. caudatus* herbal teas. This high variation in the antioxidant activity is due to the manufacturing conditions employed by the company, the differences in the composition of teas, that is, the company uses different leaf to stem ratios (Zielinski et al. 2014) and also because of the geographical origin as well as agronomic situation (Shahidi & Naczk 2004).

PEARSON'S CORRELATION COEFFICIENT

The correlation analyses by using Pearson's correlation coefficient were conducted to determine the interrelationship between antioxidant content (TPC and TFC) with antioxidant activity of four independent assays (FRAP, DPPH, *β*-carotene and ORAC) of *C. caudatus* herbal tea. It was observed that (Table 2), FRAP, *β*-carotene and ORAC assay had positively strong correlation with TPC and TFC while DPPH scavenging assay showed negative correlation with TPC and TFC. This is in agreement with study by Barros et al. (2007) who reported that, samples with higher antioxidant content showed higher antioxidant activity and lower IC_{50} values while the sample with lowest antioxidant content exhibited lower antioxidant activity and IC_{50}, thus produced negative correlation between DPPH with TPC and TFC. Therefore, from these correlations, it indicated that, phenolic and flavonoid compounds might be the major contributor in *C. caudatus* herbal tea samples. It has been highlighted that the contribution of phenolic and flavonoid compounds to *in vitro* antioxidant activity of herbs (Andarwulan et al. 2010).

MUTAGENIC ACTIVITY

In terms of mutagenicity, all *C. caudatus* herbal tea samples were investigated by using *Salmonella typhimurium* tester strains TA98 and TA100 in the absence and presence of the S9 metabolic activation. Tables 3 and 4 tabulated the number of revertants/plate in *S. typhimurium* strains and the mutagenic index (MI) respectively, after treatment with all *C. caudatus* herbal tea samples. From the results obtained, none of these *C. caudatus* herbal teas were detected any sign of mutagenicity towards the *S. typhimurium* strain TA98 and TA100 for the assay with and without S9 metabolic activation since the average revertants numbers did not satisfy the criteria for mutagenicity. According to Ndhlala et al. (2010), if there were no notable increase in the number of revertants and the number of revertants were not equal or not greater than two times of the positive control or there were no reduction in the number of revertant colonies to levels far below the negative control, then, the samples can be classified as non-toxic. On the other hand, the mutagenic index (MI) also displayed the values lower than that of potential mutagenicity. Santos et al. (2011) remarked that, sample is considered mutagenic potential when the MI is equal to or greater than two for at least one of the tested doses.

It should be noted that, the high antioxidant activity in all *C. caudatus* herbal tea samples could give protection against the mutagenic effect. This is because in general, the antioxidant activity of plant extracts is associated with group of compounds such as phenolic acids and flavonoids which could acts as stabilising agent of scavenging radicals (Fernando et al. 2013). This had been proven by few studies, for examples, Marnewick et al. (2000) reported

TABLE 3. Mutagenic activity in bacterial strains TA98 and TA100 treated with *C. caudatus* herbal tea prepared from leaves at different maturity stages, with (+ S9) and without (– S9) metabolic activation

Treatment/*C. caudatus* herbal tea	Number of revertants/ plate in *S. typhimurium* strain			
	TA98		TA100	
	–S9	+S9	–S9	+S9
Positive control	27.2 ± 2.0[a]	43.0 ± 3.0[a]	152.0 ± 4.0[a]	100.0 ± 8.0[a]
Negative control	11.3 ± 1.0[d]	15.7 ± 2.0[c]	93.3 ± 4.0[b]	58.0 ± 2.0[d]
Young leaves	15.0 ± 1.0[c]	19.7 ± 1.0[b]	86.7 ± 2.0[c]	77.0 ± 2.0[b]
Mature leaves	15.7 ± 1.0[c]	19.3 ± 1.0[b]	94.7 ± 2.0[b]	64.3 ± 2.0[c]
Old leaves	21.0 ± 1.0[b]	14.0 ± 2.0[c]	81.7 ± 3.0[c]	57.7 ± 3.0[d]
Mixed leaves	16.0 ± 2.0[c]	15.7 ± 2.0[c]	87.3 ± 2.0[c]	58.0 ± 2.0[d]
Commercial brand	20.3 ± 3.0[b]	14.3 ± 2.0[c]	96.7 ± 2.0[b]	61.3 ± 3.0[d]

Values are expressed as mean and standard deviation. Means with different letter within a column are significantly different ($p<0.05$)
If the number of revertants are not ≥ than 2 times of the positive control or there are no reduction in the number of revertant colonies to levels far below the negative control, the sample is considered as non-toxic

TABLE 4. Mutagenic index of *C. caudatus* herbal tea prepared from leaves at different maturity stages, with (+ S9) and without (– S9) metabolic activation

Treatment/ *C. caudatus* herbal tea	Mutagenic index (MI)			
	TA98		TA100	
	With S9	Without S9	With S9	Without S9
Young leaves	1.33	1.25	0.92	1.32
Mature leaves	1.39	1.22	1.02	1.10
Old leaves	1.86	0.89	0.88	0.99
Mixed leaves	1.42	1.00	0.94	1.00
Commercial brand	1.80	0.91	1.04	1.06

If Mutagenic Index (MI) is not ≥ than 2 for at least one of tested doses, the sample is considered as non-toxic

that, phenolic compounds from water extracts of herbal teas dramatically decreased the mutagenicity of a variety of genetoxic and carcinogens while Saraç and Şen (2014) found that, extract of *Liquidambar orientalis* Mil var. *orientalis* which possessed higher antioxidant activity did not exhibit any mutagenic effect in the mutagenicity assay performed with *S. typhimurium* TA98 and TA100.

Conclusion

Hence, it can be concluded that, as the maturity of *C. caudatus* leaves used to prepare herbal tea increased, the antioxidant activity decreased significantly with the *C. caudatus* herbal tea prepared from young leaves possessed highest antioxidant activity. The strong Pearson's correlation coefficient showed that, phenolic and flavonoid compounds are the major contribution to the antioxidant activity in *C. caudatus* herbal tea. Nevertheless, none of *C. caudatus* herbal teas studied induced any increased in the number of revertants, demonstrating the absence of mutagenic activity.

Acknowledgements

The authors would like to thank Universiti Teknologi MARA (UiTM) for their technical and financial support.

References

Amna, O.F., Nooraain, H., Noriham, A., Azizah, A.H. & Husna, R.N. 2013. Acute and oral subacute toxicity study of ethanolic extract of *Cosmos caudatus* leaf in *Sprague Dawley* rats. *International Journal of Bioscience, Biochemistry and Bioinformatics* 3: 301-305.

Andarwulan, N., Batari, R., Sandrasari, D.A., Bolling, B. & Wijaya, H. 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chemistry* 121: 1231-1235.

Barros, L., Queiro, B., Ferreira, I.C.F.R. & Baptista, P. 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry* 103: 413-419.

Blasa, M., Gennari, L., Angelino, D. & Ninfali, P. 2010. Bioactive foods in promoting health. In *Fruit and Vegetable Antioxidants in Health: Fruits and Vegetables,* edited by Watson, R.S. & Preedy, V.R. Burlington: Academic Press. pp. 37-58.

Celik, S.E., Ozyürek, M., Güçlü, K. & Apak, R. 2010. Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/ persulphate and FRAP methods. *Talanta* 81: 1300-1309.

Deetae, A., Parichanon, P., Trakunleewatthana, P., Chanseetis, C. & Lertsiri, S. 2012. Antioxidant and anti-glycation properties of Thai herbal teas in comparison with conventional teas. *Food Chemistry* 133: 953-959.

Dian-Nashiela, F., Noriham, A., Nooraain, H. & Azizah, A.H. 2015. Quantification of phenolic compounds and sensorial properties of *Cosmos caudatus* herbal tea at different maturity stages. *Advances in Environmental Biology* 9: 15-20.

Farhoosh, R., Golmovahhed, G.A. & Khodaparast, M.H.H. 2007. Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). *Food Chemistry* 100: 231-236.

Fawole, O.A. & Opara, U.L. 2013. Changes in physical properties, chemical and elemental composition and antioxidant capacity of pomegranate (cv. Ruby) fruit at five maturity stages. *Scientia Horticulturae* 150: 37-46.

Fernando, I.D.N.S., Abeysinghe, D.C. & Dharmadasa, R.M. 2013. Determination of phenolic contents and antioxidant capacity of different parts of *Withania somnifera* (L.) Dunal from three different growth stages. *Industrial Crops Products* 50: 537-536.

Gião, M.S., Pereira, C.I., Fonseca, S.C., Pintado, M.E. & Malcata, F.X. 2009. Effect of particle size upon the extent of extraction of antioxidant power from the plants Agrimonia eupatoria, Salvia sp. and Satureja montana. *Food Chemistry* 117(3): 412-416.

Harbourne, N., Marete, E., Jacquier, J.C. & O'Riordan, D. 2009. Effect of drying methods on the phenolic constituents of meadowsweet *(Filipendula ulmaria)* and willow *(Salix alba)*. *LWT - Food Science and Technology* 42: 1468-1473.

Horžić, D., Komes, D., Belščak, A., Ganić, K.K., Iveković, D. & Karlović, D. 2009. The composition of polyphenols and methylxanthines in teas and herbal infusions. *Food Chemistry* 115: 441-448.

Kara, D. 2009. Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis. *Food Chemistry* 119: 376-412.

Khairusy, S.Z., Noriham, A., Aminah, A., Nurain, A. & Wan, S.S.W.K. 2012. Phytochemical screening and activities of hydrophilic and lipophilic antioxidant of some fruit peels. *Malaysia Journal of Analytical Sciences* 16: 309-317.

Marnewick, J.L., Gelderblom, W.C.A. & Joubert, E. 2000. An investigation on the antimutagenic properties of South African herbal teas. *Mutation Research* 471: 157-166.

Mediani, A., Abas, F., Ping, T.C., Khatib, A. & Lajis, N.H. 2012. Influence of growth stage and season on the antioxidant constituents of *Cosmos caudatus*. *Plant Foods for Human Nutrition* 67: 344-350.

Menichini, F., Loizzo, M.R., Bonesi, M., Conforti, F., Luca, D.D., Statti, G.A. & Tundis, R. 2011. Phytochemical profile, antioxidant, anti-inflammatory and hypoglycemic potential of hydroalcoholic extracts from *Citrus medica* L. cv Diamante flowers, leaves and fruits at two maturity stages. *Food and Chemical Toxicology* 49: 1549-1555.

Mortelmans, K. & Zeiger, E. 2000. The Ames *Salmonella*/ microsome mutagenicity assay. *Mutation Research* 117: 660-667.

Müller, V., Albert, A., Winkler, J.B., Lankes, C., Noga, G. & Hunsche, M. 2013. Ecologically relevant UV-B dose combined with high PAR intensity distinctly affect plant growth and accumulation of secondary metabolites in leaves of *Centella asiatica* L. Urban. *Journal of Photochemistry and Photobiology B: Biology* 127: 161-169.

Ndhlala, A.R., Anthonissen, R., Stafford, G.I., Finnie, J.F., Verschaeve, L. & Staden, J.V. 2010. *In vitro* cytotoxic and mutagenic evaluation of thirteen commercial herbal mixtures sold in KwaZulu-Natal , South Africa. *South African Journal of Botany* 76: 132-138.

Nuengchamnong, N. & Ingkaninan, K. 2010. On-line HPLC–MS–DPPH assay for the analysis of phenolic antioxidant compounds in fruit wine: *Antidesma Thwaitesianum* Muell. *Food Chemistry* 118: 147-152.

Santos, J.L., Bosquesi, P.L., Almeida, A.E., Chin, C.M. & Varanda, E.A. 2011. Mutagenic and genotoxic effect of hydroxyurea. *International Journal of Biomedical Science* 7: 263-267.

Saraç, N. & Şen, B. 2014. Antioxidant, mutagenic, antimutagenic activities, and phenolic compounds of *Liquidambar orientalis* Mill. var. *orientalis*. *Industrial Crops and Products* 53: 60-64.

Shahidi, F. & Naczk, M. 2004. *Phenolic in Food and Nutraceuticals.* New Jersey: CRC Press. pp. 242-443.

Shui, G., Leong, L.P. & Wong, S.P. 2005. Rapid screening and characterisation of antioxidants of *Cosmos caudatus* using liquid chromatography coupled with mass spectrometry. *Journal Chromatography B. Analytical Technology Biomedical Life Science* 827: 127-138.

Singh, V., Guizani, N., Essa, M.M., Hakkim, F.L. & Rahman, M.S. 2012. Comparative analysis of total phenolics, flavonoid content and antioxidant profile of different date varieties (*Phoenix dactylifera* L.) from Sultanate of Oman. *International Food Research Journal* 9: 1063-1070.

Sreelatha, S. & Padma, P.R. 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition* 64: 303-311.

Velioglu, Y.S., Mazza, G., Gao, L. & Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agriculture and Food Chemistry* 46: 4113-4117.

Zielinski, A.A.F., Haminiuk, C.W.I., Alberti, A., Nogueira, A., Demiate, I.M. & Granato, D. 2014. A comparative study of the phenolic compounds and the *in vitro* antioxidant activity of different Brazilian teas using multivariate statistical techniques. *Food Research International* 60: 246-254.

Dian-Nashiela Fatanah, Noriham Abdullah* & Nooraain Hashim
Faculty of Applied Sciences
Universiti Teknologi MARA
40450 Shah Alam, Selangor Darul Ehsan
Malaysia

Noriham Abdullah*
Malaysia Institute of Transport (MITRANS)
Universiti Teknologi MARA
40450 Shah Alam, Selangor Darul Ehsan
Malaysia

Abd. Hamid, Azizah
Department of Food Sciences
Faculty of Food Science and Technology
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: noriham985@salam.uitm.edu.my

Received: 27 March 2017
Accepted: 26 October 2017