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Antioxidant activity of herbal tea prepared from *Cosmos caudatus* leaves at different maturity stages

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Abstract

Herbal tea is widely consumed around the world because people believed that it contained high amount of antioxidant. However, usage of different maturity of the plants as raw materials could affect the antioxidant capacities in herbal tea. Hence, this paper reports the antioxidant activity of herbal tea prepared from *Cosmos caudatus* at different maturity namely young, mature and old leaves. The analyses carried out were total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) and DPPH (2-2-diphenyl-1-picrylhydrazyl) radical scavenging assays. Based on the results obtained, herbal tea prepared from young leaves had significantly strong ($p<0.05$) antioxidant activity for all assays tested than herbal tea prepared from mature and old leaves. In fact, TPC and TFC exhibited a strong positively correlation with reducing power but inversely correlated with DPPH scavenging activity indicating that these compounds are major contributors to the antioxidant activity. Thus, this shows that antioxidant activity in *C. caudatus* leaves reduced with increase maturity and hence it is recommended to use young leaves for herbal tea preparation since it possessed good antioxidant activity which benefits human health.

Introduction

Tea is second widely consumed beverage after water worldwide and because a combination factors are believed to play a role such as refreshing taste, attractive aroma and potential positive health effect (Sari and Velioglu, 2011). According to Quispe *et al*. (2012) increasing consumption of herbal teas is a worldwide trend because supplementation of human diet with herbal provides high antioxidant compounds that may have beneficial effects. Additionally, due to the advance development of technology and time constraint, people have started to seek for convenient herbal products. Herbal tea has been used for health care and diseases prevention for thousands of years in many countries (Zhao *et al.*, 2013) because according to Tschiggerl and Bucar (2012) herbal teas are convenience to take, easy to prepare, mild in action and in most cases with negligible side effects besides, cheap in price and rich in resource. *Cosmos caudatus,* commonly known as ulam raja by the Malay society, is getting attention by Malaysian herbal industries to be developed in tea form. It bear purple, pink or white ray florets, grows up to about 1-8 feet tall, hairless or sparsely hairy, leaves are finely dissected, 10-20 cm long and having 20-26 species worldwide (Rasdi *et al*., 2010). *C. caudatus i*s used traditionally to reduce body heat, improving blood circulation, as anti-ageing 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). In addition, some of literature reported, *C. caudatus* is amongst herbs that have high antioxidant activity. According to Shui *et al*. (2005), the major antioxidants in *C. caudatus* could be due to a number of proanthocyanidins that exists as dimers through hexamers, quercetin glycosides, chlorogenic, neo-chlorogenic, crypto-chlorogenic acid and (+)-catechin. With the extremely high antioxidant capacity of about 2,400 mg L-ascorbic acid equivalent antioxidant activity (AEAC) per 100 g of fresh sample, *C. caudatus* is believed to reduce oxidative stress. It is well reported that the total amount of phenolic compounds may have a direct contribution in the defence against oxidative stress and could be considered to be active metabolites involved in the antioxidant activity of herbs (Mediani *et al*., 2012). However, a few literatures had found a declining trend of antioxidant activities with advancing maturity. Sreelatha and Padma (2009) demonstrated, this may due to old plants possesses inadequate antioxidant defence and/or owing overproduction of reactive oxygen species (ROS), this equilibrium is hampered favouring the ROS upsurge that culminates in oxidative stress. Compared to young plant, Menichine *et al.* (2011)

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found highest flavanones contents in young plants, with most synthesis taking place during the early stages of plant growth, is adequate enough to react with ROS produced. This study therefore designed to determine antioxidant activity of herbal tea prepared from *C. caudatus* leaves at different maturity since to our knowledge there is no literatures exists on antioxidant activity of these types of tea.

Materials and Method

Chemicals

Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), ferric chloride ($FeCl_3$) and sodium hydroxide (NaOH) were purchased from Merck, Germany. Gallic acid, quercetin, trolox, 2-4-6-tripyridyl-s-triazine (TPTZ), 2-2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were supplied by Sigma-Aldrich Chemie, Germany. Sodium acetate was purchased from R & M Chemicals, U.K while glacial acetic acid was purchased from Friendemann Schmidt Chemicals, U.K. All samples were measured using Helios Zeta UV-VIS Spectrophotometer from Thermo Fisher Scientific, USA.

Plant sample collection and selection

The fresh leaves of 8-week-old *Cosmos caudatus* plant were collected from Durian Tunggal, Malacca, Malaysia. The 8-week-old *C. caudatus* plant was selected based on the findings from Mediani *et al.* (2012) where they reported that 8-week-old plant consist higher antioxidant activity compared to 10 and 12-week-old *C. caudatus* plant. The leaves were divided into 3 groups, classified as young, mature and old leaves. 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 *C. caudatus* plant where the leaves are fully developed while old leaves are located 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 tier and above.

Sample preparation

Each group of *Cosmos 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 hours in cabinet dryer until constant weight. *C. caudatus* herbal tea powder was prepared according to method described by Giảo *et al*. (2009) with some modifications. The dried leaves were milled using centrifugal mill, which were then screened through different sized sieves ranged from 2 mm to 1mm to separate the milled leaves. Then, 2 g of milled leaves were collected and packed in a tea bag. The *C. caudatus* herbal teas were infused in 200 ml boiling distilled water for 3 minutes according to method suggested by Horžić *et al.* (2009) with some modifications. The infused teas were filtered through a Whatman filter paper No. 41 prior for further analyses.

Total phenolic content (TPC)

The total phenolic content in herbal tea sample was determined by using the Folin-Ciocalteu method (Harbourne *et al.*, 2009) with minor modification. Accurately, 0.5 ml Folin-Ciocalteu reagent, 1.5 ml 7.5% sodium carbonate and 7.9 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 hours in a dark place. The absorbance at 765 nm was read by UV-VIS Spectrophotometer. The TPC of herbal tea sample was expressed as mg of gallic acid equivalents (mg GAE)/ml of tea sample.

Total flavonoid content (TFC)

The total flavonoids content was analysed according to method as described by Singh *et al.* (2012). One ml of sample/standard was diluted with 4 ml distilled water then 0.3 ml 5% sodium nitrate solution and 0.3 ml 10% aluminium chloride were added. The mixture was kept for 5 minutes. Then, 2 ml of 1M 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 equivalents (mg QE)/ ml of tea sample.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out by the method of Deetae *et al.* (2012). 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 HCl 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 minutes and the absorbance at 595 nm was then read. The total antioxidant capacity of samples were determined against a standard of known FRAP value and was expressed as µM of trolox equivalent (mg

TE)/ml of tea sample.

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 sample/ standard was mixed with 2.9 ml of 0.05 mM DPPH in methanol and incubated in the dark at room temperature for 30 minutes. The absorbance of the sample/standard was measured using UV-VIS Spectrophotometer at 515 nm where methanol was used as blank.

Statistical analysis

All experiments were run in triplicates. Statistical analyses were conducted with the Statistical Analysis System (SAS) 9.1.3 software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($P<0.05$) were determined by least square means comparison.

Results and Discussion

Total phenolic content (TPC)

The total phenolic content in *C. caudatus* herbal tea samples was determined using Folin-Ciocalteu reagent where this reagent is used to estimate the amount of TPC present in the herbal tea samples. Total amount of phenolic compounds in sample would be oxidised by phosphotungstic and phosphomolybdic acids present in the reagent (Wong et al., 2006) which then develop a blue colour solution. The deeper blue colour solution indicated the higher total phenolic compounds present in the sample. The TPC of herbal tea prepared from *C. caudatus* leaves at different maturity stages are summarized in Table 1. From the table, herbal tea prepared from young leaves had significantly higher TPC value than the other two samples. According to Mediani *et al.* (2012), this may due to the energy required to synthesize the metabolites is spent for other activities such as flowering while in mature and old plants most of these phenolic compounds are enzymatically convert to other metabolites such as sugar. This is supported by another study where they found the phenol content of *C. chinenses cv. Habanero* samples decreased with increase of maturity (Siddiqui *et al*., 2012). Barros *et al.* (2007) believed the aging process stimulates the formations of ROS, which are neutralized by the polyphenol compounds, resulting in the lowering of their content and antioxidant capacities. Thus, only small amount of phenolic compounds would be undergoing complex redox reaction with the Folin-Ciocalteu reagent.

Total flavonoid content (TFC)

Flavonoids constitute a special class of phenolic compounds which exhibit higher antioxidant activities than phenolic acids. Catechin, epicatechin and quercetin are most well known members in flavonoid family (Oh *et al*., 2013). As stated by Shui *et al*. (2005), ulam raja is rich in quercetin and its derivatives which might be partly responsible for its medicinal function to decrease blood pressure. However, as the leaves aged, flavonoids may loss due to metabolic conversion to other secondary phenolic compounds or degradation via enzyme action (Siddiqui *et al.*, 2012) which lowers the level of flavonoid compounds. Differ from young leaves, the secondary metabolites act as plant's defence mechanism, in which it provide protection to the younger plant tissues against pests and disease (Shuib *et al*., 2011). The results obtained in Table 1 were in agreement with previous studies that the TFC of *C. caudatus* herbal tea samples significantly decreased ($p<0.005$) as the maturity of the leaves used increased. The values ranged between 203.22 to 72.24 mg QE/ml of tea sample. Müller *et al*. (2013) supports the view, where they found younger leaves contain higher amounts of flavonoids and saponins than older leaves where these compounds possess a greater ability to scavenge light-induced ROS. The lower level of flavonoids in mature and old plant produced extensive ROS that reduced antioxidant activity, eventually cause the senescence of plant tissues. Sreelatha and Padma (2009) reported that the components of both the enzymatic and the non-enzymatic antioxidant system correlate well with oxidative stress during senescence and plant development.

Ferric reducing antioxidant power (FRAP)

Studies on FRAP for plants at different maturity had been published in several papers. Declined in TPC and TFC corresponded with significant ($p<0.05$) decrease in FRAP as maturity of *C. caudatus* leaves used to prepare herbal tea progressed. Table 1 shows herbal tea prepared from young leaves had higher FRAP value followed by mature leaves and old leaves with the value of 502.21, 332.00, 239.18 µM TE/ml of tea sample respectively. FRAP is used to measure the reductive ability of antioxidant, and it is evaluated by the transformation of ferric-TPTZ (Fe(III)-TPTZ) complex to ferrous-TPTZ (Fe(II)-TPTZ) complex where, the ability of sample samples to reduce Fe(III)-TPTZ may be attributed from hydrogen donation from phenolic compounds which

Table 1. Summary results for antioxidant activity in herbal tea prepared from leaves at different maturity stages

Herbal tea sample	Assay			
	TPC (mg GA/ml of tea extract)	TFC (mg QE/ml of tea extract)	FRAP (µM TE/ml of tea extract)	DPPH (µg/ml of tea extract)
Young leaves	66.2986 ± 5.1997[a]	203.2171 ± 15.8990[a]	502.2083 ± 21.1779[a]	1055.3655 ± 42.3797[a]
Mature leaves	36.5487 ± 2.7742[b]	124.5505 ± 6.2166[b]	332.0000 ± 8.8114[b]	1409.9965 ± 103.1731[b]
Old leaves	18.3778 ± 2.4597[c]	72.2387 ± 3.0426[c]	239.1759 ± 19.1863[c]	2408.8439 ± 365.3596[c]

Values are expressed as mean ± standard deviation. Means with different letters are significantly different ($p<0.05$)

is also related to presence of reducing agent (Huda-Faujan *et al.*, 2009). Barros *et al.* (2007) demonstrated that the reducing properties are generally associated with the presence of reductones, which had been shown to exert antioxidant action by breaking the free radical chain by donating the hydrogen atom. Higher level of flavonoids compounds in young leaves than other two leaves could act as reductone where these compounds could react with free radicals by converting them to more stable products and terminating the radical chain reaction (Oh *et al.*, 2013). Siddiqui *et al.* (2012) claimed antioxidants chelate and disengage transition metals, thereby preventing such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal catalyzed reaction. They also revealed that higher metal chelating activity in green fruit is higher than fully ripe *Dennettia tripetala* fruits.

DPPH (2-2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit samples and food materials (Wong *et al.*, 2006). *C. caudatus* herbal tea prepared from young leaves showed significantly higher ability in scavenging free radicals compared to mature leaves and old leaves. The difference in the antioxidant capacity between samples is summarized in Table 1 which indicates the increase in the IC_{50} value reflects a decrease in the antioxidant power. This is in agreement with Fawole and Opara (2013) where they reported total phenolics prevailed during the early maturity but their contents and antioxidant activity decreased with advancing maturation. In young leaves, the accumulation of TPC and TFC may contribute to high antioxidant power. As reported by some previous studies, phenols are responsible for quenching free radicals that cause oxidative stress in plants (Mediani *et al* 2012), while quercetin are attributed to the presence of two antioxidant pharmacophores within the molecule that have the optimal configuration for free radical scavenging (Menichini *et al.*, 2011). Rodríguez *et al.* (2011) added, flavonoids had been devoted to their antioxidant activity, due to their ability to reduce in vitro free radical formation and to scavenge free radicals. According to Siddiqui *et al.* (2012), anti-radical scavenging activity or namely antioxidant activity, is related to substitution of hydroxyl groups in the aromatic phenols, thus contributing to their hydrogen-denoting ability which is show by the degree of discolouration points of antioxidant sample.

Pearson's correlation coefficient

Table 2 demonstrate the interrelationships between antioxidant activity (FRAP and DPPH) with TPC and TFC of *C. caudatus* herbal tea prepared from young, mature and old leaves by using Pearson's correlation coefficient. From the table, it was observed that FRAP had strong correlation with TPC and TFC. The values for TPC and TFC were $R = 0.9967$ and $R = 0.9980$ respectively. These results shows that phenolic and flavonoid compounds in *C. caudatus* herbal tea might be major contributor to the ferric reducing capacity. In agreement with Andarwulan *et al.* (2010), they highlighted the contribution of phenolics to in vitro antioxidant activity of vegetables. Natural antioxidants that are present in herbs are responsible for inhibiting or preventing the deleterious consequences of oxidative stress (Sreelatha and Padma, 2009). In contradicted to FRAP correlation with TPC and TFC, DPPH was inversely correlated with TPC and TFC with the values $R = -0.9224$ and $R = -0.9288$ respectively. This complies with study by Barros *et al.* (2007) where they reported that DPPH was negatively correlated with TPC and TFC which indicates the sample with highest antioxidant content shows higher antioxidant activity and lower IC_{50} values while the sample with lowest antioxidant content exhibited lower antioxidant activity and higher IC_{50} values. The correlations between antioxidant activities with TPC and TFC are mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers

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

	FRAP	DPPH
TPC	0.9967 ± 0.0018[a]	-0.9224 ± 0.0580[a]
TFC	0.9980 ± 0.0024[a]	-0.9288 ± 0.0596[a]

(Sreelatha and Padma, 2009).

Conclusion

This study concluded that, maturation could decrease antioxidant activity in *C. caudatus* leaves. *C. caudatus* herbal tea prepared from young leaves exhibited significantly high in TPC, TFC, FRAP as well as DPPH inhibition as compared to mature and old leaves. It is supported by the Pearson's correlation coefficient which shows strong positive correlation between reducing power with TPC and TFC but negatively correlated between DPPH scavenging activity with TPC and TFC. Based on these findings, it is recommended to use young leaves for herbal tea preparation since it possessed high antioxidant activity which is beneficial for human health.

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