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Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah Tea Plantation in Sabah, Malaysia

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Abstract

This study was conducted to determine the total phenolic (TPC) and total flavonoid content (TFC) as well as the antioxidant activity of 50% ethanolic extracts from different parts of *Camellia sinensis* (shoot, young and matured leaves). Comparison was also made between black (fermented) and green (unfermented) tea. For green tea, the results showed that the shoot contained significantly higher total phenolic content, followed by the young and matured leaves ($p<0.05$). The same trend was also observed for antioxidant activity as assessed using FRAP (ferric reducing/antioxidant power), DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and ABTS (2,2'-azinobis(3-ethylbenzthiazoline)-6-sulphonic) radical scavenging assays. As for black tea, the highest total phenolic and total flavonoid content were observed in the shoot, followed by the young and old leaves. The same trend of antioxidant activity with green tea was also observed in black tea extracts. In addition, black tea compost showed comparable high total phenolic and flavonoid content as well as antioxidant activities as assessed using different antioxidant assays. High antioxidant activity of tea leaves grown in Sabah might be contributed by phenolic phytochemicals that presence in the extracts.

Introduction

Tea is one of the most widely consumed beverages worldwide due to its health benefit for human consumption. Black and green teas are the two main types. Green tea is a non-fermented tea used as the main beverage in China and Japan, while black tea is more popular in North America and Europe. Oolong tea is an intermediate variant between green and black tea. Most commercially prepared tea is obtained from the leaf of the plant *Camellia sinensis*. There are two varieties of tea plant. *Camellia sinensis* var *sinensis* (China tea) is grown extensively used in China and Japan, while *C. sinensis* var *assamica* (Assam tea) predominates in South and South-East Asia.

Amongst the benefits of tea is that it can prevent tumour cells growth, reduce cardiovascular disease, reduce cholesterol and induce body weight loss. As consequences, a range of tea products or tea food additive was introduced into the market, such as confectionery, instant noodles and titbits (Wang *et al*., 2000). One of the advantages of tea is that it has high antioxidant activities due to the presence of polyphenols that enable it to scavenge free radicals. The term green tea refers to the product manufactured from fresh *C. sinensis* leaves in which significant oxidation of the major leaf polyphenols known as catechins is prevented. Green tea extract has strong antioxidant due to the presence of (+)catechin, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG). Catechin is a compound which does not evaporate and it contained about 8-15% from the dry weight of plant (Farhoosh *et al*., 2007). Catechin solution is colourless, however it tastes bitter (Wang *et al*., 2000). Moreover, production of black tea leaves involved extensive enzymatic oxidation of the leaf polyphenols to dark products such as theaflavins and thearubigens. The major theaflavins in black tea are theaflavin (TF1), theaflavin monogallate A (TF2A), theaflavin monogallate B (TF2B) and theaflavin digallate (TF3). Oolong tea is partially oxidized and retains a considerable amount of the original catechins (Ho *et al*., 1994).

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Young leaves are discovered to have higher antioxidant activity than mature leaves. From previous studies, green tea was found to contain higher antioxidant activity than black tea. Total phenolic content, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, ferric reducing antioxidant power (FRAP) and ABTS [2,2'-azinobis-(3-ethylbenzothiazoneline-6-sulfonic acid)] decolorization assays conducted showed that green tea has higher antioxidant properties than black tea (Gadow *et al*., 1997; Chan *et al*., 2007; Pilar *et al*., 2008). Other than that, Farhoosh *et al*. (2007) reported that black tea compost had higher antioxidant properties than mature leaves.

A number of *in vitro* and *in vivo* studies showed that catechins in tea function as anticancer, antibacterial, antiviral, antitoxin and antifungal. Besides, it was proven that drinking too much tea that contains catechins would not affect human's life (Duda-Chodak *et al*., 2008). Leung *et al*. (2001) reported that drinking black tea showed similar benefit as in green tea from the perspective of antioxidant capacity. This could be explained by the presence of theaflavin in black tea having the similar amount of catechins as present in green tea. Every tea differs from the perspective of composition and concentration of antioxidant compound. Black tea has low amount of theaflavin (2-6%) and high thearubigin (20%), while green tea has higher catechins (30-42%), especially EGCG which has the highest amount of catechins (Leung *et al*., 2001).

Recently, antioxidant activity of tea leaves has been studied intensively. However, the study on antioxidant activity of tea leaves in Sabah is rarely reported. Therefore, the objective of this study is to determine the antioxidant activity as well as total phenolic content and total flavonoid content of Sabah tea leaves in different ages and fermentation stages, namely green tea leaves (shoots, young and mature), black tea leaves (shoots, young and mature) and black tea compost. Antioxidant activities were evaluated by DPPH free radical scavenging activity, FRAP (ferric reducing/antioxidant power) and ABTS decolourization assay.

Materials and Methods

Sample collection

Fresh shoots (leaf bud and two youngest leaves; yellowish green), young leaves (third to fifth leaves from the top; light green) and mature leaves (sixth to eighth leaves; dark green) of *C. sinensis* were collected from Sabah Tea Farm, Ranau, Sabah (Chan *et al*., 2007). Three individual plants were sampled. These three maturity levels of fresh leaves were collected and undergone different states of fermentation process. They were made into green tea, black tea and tea compost.

Sample preparation

Green tea: Freshly picked leaves were steam-blanched for 10 min to deactivate enzymes in the leaves. Then, the leaves were dried and ground into smaller particles (Lena *et al*., 2003).

Black tea: For fermentation procedure, blended tea leaves were put into an air tight container and steamed at 40^0C for 5 h. The leaves were dried in oven for 10 min at 100^0C, followed by 10 min at 900C, 10 min at 600C and finally 10 min at 40^0C until the moisture content was reduced to approximately 5% (Templer and Boctel, 2000). Tea compost was derived from black tea which they went through the fermentation process but at the end of the grading process, it cannot be graded with other tea leaves and then separated to be tea waste. These tea wastes were then grinded into smaller parts and become tea compost.

Sample extraction

Blended samples of green tea, black tea and tea compost (1 g each) were extracted with 50 ml of 50% ethanol for 1 h on an orbital shaker. The mixture was centrifuged at 8500 g for 10 min. The pellets were re-extracted at identical conditions. The supernatants was combined and stored at -18°C until further analysis (Turkmen *et al*., 2006).

Determination of total phenolics content (TPC)

Total phenolics was determined using Folin-Ciocalteu's reagent as adapted from Velioglu *et al*. (1998). One hundred microliters of extract was mixed with 0.75 mL of Folin-Ciocalteu's reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 0.75 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 90 min at 22 °C, absorbance was measured at 725 nm. Result was expressed as gallic acid equivalent (mg GAE/g).

Determination of total flavonoid content (TFC)

Total flavonoid was measured according to Zhishen *et al*. (1999). One ml aliquot of extract and appropriately diluted standard solution of quercetin (20, 40, 60, 80 and 100 mg/l) was added into a 10 ml volumetric flask containing 4 ml deionized water. At zero time, 0.3 ml of 10% $AlCl_3$ was added. At 6 minutes, 2 ml of 1M NaOH was added to the mixture. Immediately, the reaction flask was diluted to the volume with the addition of 2.4 ml of deionized

water and thoroughly mixed. Absorbance of the mixture, pink in colour was determined at 510 nm versus prepared water blank. Total flavonoid of the samples was expressed on a dried weight as quercetin equivalent (mg QE/g).

DPPH (2, 2 -diphenyl-1-pycryl-hydrazyl) free radical scavenging activity

According to Mensor *et al*. (2001), 1 mL from 0.3 mM methanol solution of 2, 2 -diphenyl-1-pycrylhydrazyl (DPPH) was added into 2.5 mL sample or standards. The solution was mixed vigorously and left to stand at room temperature for 30 min in the dark. The mixture was measured spectrophotometrically at 518 nm. The percentage inhibition was calculated against a control and compared to BHT standard curve (0-1000µm). The antioxidant activity (AA) was calculated as below:

$$AA\% = 100 - [(Abs_{sample} - Abs_{empty\ sample})/Abs_{control} \times 100$$

where Abs is absorbance

Empty sample= 1 mL methanol + 2.5 mL extract

Control sample= 1 mL 0.3 mM DPPH + 2.5 mL methanol

IC_{50}, the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial DPPH concentration, was derived from the % disappearance versus concentration plot (at this point concentration means mg of sample extracted into 1.0 mL solution).

FRAP (Ferric reducing/antioxidant power) assay

This procedure was carried out according to Benzie and Strain (1996) with slight modification. The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio prior to use and heated to 37°C in water bath. A total of 3.0 mL FRAP reagent was added to a cuvette and blank reading was then taken at 593 nm using spectrophotometer. A total of 100 µL selected plant extracts and 300 µL distilled water was added to the cuvette, and a second reading at 593 nm was performed after 4 min. The changes in absorbance after 4 min from initial blank reading were then compared with standard curve. A standard of known Fe (II) concentrations was carried out using several concentrations from 100 to 1000 µM. A standard curve was plotted by plotting the FRAP value of each standard versus its concentration. The FRAP values for the samples were determined using this standard curve. The final result was expressed as the concentration of antioxidant having a ferric reducing ability.

$ABTS^{\bullet+}$ decolourization assay

2,2'-azinobis(3-ethylbenzthiazoline)-6-sulphonic acid or ABTS free radical decolourization assay was done according to Re *et al*. (1999) with some modification. Briefly, the pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. The aliquot of 200 µl of each sample was added to 2000 µl of ABTS free radical cation solution. The absorbance, monitored for 5 min was measured spectrophotometrically at 734 nm using a spectrophotometer. Appropriate solvent blanks were run in each assay. The percentage inhibition was calculated against a control and compared to a Trolox standard curve (10-100 mM). The radical-scavenging activity was expressed in IC_{50}, the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial ABTS concentration.

Statistical analysis

All experiments were carried out in triplicate and presented as mean ± standard deviation of mean (SD) using SPSS version 15.0. The data were statistically analysed by one-way ANOVA and Duncan's test. Correlations among data obtained were analysed using Pearson's coefficient. A significance difference was considered at the level of $p < 0.05$.

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Results

TPC, TFC and antioxidant activities of tea leaves at different maturity stages

From the results (Table 1), the ranking of the TPC and TFC (were similar to the trend of three antioxidant assessments (Table 2): Green tea (shoots)> Black tea (shoots)> Green tea (young)> Black tea (young)> Black tea compost> Green tea (mature)> Black tea (mature) which means they are highly correlated between the TPC, TFC and their antioxidant activity. Moreover, the trend of TPC and TFC as well as antioxidant analysis in the shoots parts were the highest followed by young leaf and mature leaf. Green tea (shoots) showed the highest value for TPC, TFC and antioxidant activity compared to other samples. Green tea (young), black tea (young) and black tea compost did not show any significant difference at $p<0.05$ when compared with each other. The results also revealed that green tea (mature) and black tea (mature) did not differ significantly in TPC, TFC and their antioxidant activities.

Table 1. Total phenolic and flavonoid content for green tea, black tea (three different maturity levels) and black tea compost

	Total phenolic content (mg GAE / g dry weight)	Total flavonoid content (mg QE/ g dry weight)
Green tea (Shoots)	80.27 ± 0.61^{a}	35.17 ± 0.91^{a}
Green tea (Young)	72.70 ± 0.46^{b}	31.83 ± 0.80^{b}
Green tea (Mature)	63.87 ± 1.36^{c}	20.90 ± 0.36^{c}
Black tea (Shoots)	76.93 ± 1.72^{b}	$33.70 \pm 1.34^{a,b}$
Black tea (Young)	71.13 ± 1.02^{b}	31.07 ± 1.46^{b}
Black tea (Mature)	56.63 ± 1.56^{c}	19.07 ± 1.46^{c}
Black tea compost	70.70 ± 0.66^{b}	$32.33 \pm 0.50^{a,b}$

Data are expressed in mean ± SD (n=3) in which different letters for each column (a-c) are significantly different at $p<0.05$

Table 2. Antioxidant properties of *C. sinensis* with different maturity and fermentation stages

	DPPH (IC_{50})	FRAP ($\mu mol\ Fe_2SO_4 \cdot 7H_2O/ml$)	ABTS (IC_{50})
Green tea (Shoots)	0.03 ± 0.03^{a}	14.83 ± 0.21^{a}	0.17 ± 0.02^{a}
Green tea (Young)	$0.03 \pm 0.00^{a,b}$	$14.03 \pm 0.21^{b,c}$	$0.18 \pm 0.02^{b,c}$
Green tea (Mature)	0.04 ± 0.00^{c}	13.03 ± 0.21^{d}	$0.18 \pm 0.00^{d,e}$
Black tea (Shoots)	$0.03 \pm 0.00^{a,b}$	14.33 ± 0.15^{b}	$0.18 \pm 0.00^{a,b}$
Black tea (Young)	$0.03 \pm 0.00^{b,c}$	$14.00 \pm 0.10^{b,c}$	$0.18 \pm 0.00^{c,d}$
Black tea (Mature)	0.04 ± 0.00^{d}	12.40 ± 0.10^{e}	0.19 ± 0.00^{e}
Black tea compost	$0.03 \pm 0.00^{a,b}$	13.67 ± 0.21^{c}	$0.18 \pm 0.00^{d,e}$

Results are expressed in mean ± SD (n=3) in which different letters for each column (a-e) are significantly different at $p<0.05$

Discussion

Phenolic is a kind of polyphenols that can be divided into tannin, propanoid and flavonoid. Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi and Wanasundara, 1992), which may contribute directly to antioxidative action (Duh *et al*., 1999). These compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups (Hatano *et al*., 1989). Although the mechanism of action of DPPH, ABTS and FRAP are different, i.e. scavenging of DPPH and ABTS radicals in the DPPH and ABTS assays and reduction of ferric ion in the FRAP assay, the ranking of the three antioxidant assessments (Table 2) conducted were similar to the trend of TPC and TFC (Table 1). Phenolic compound have been reported to protect plants against microorganisms and herbivores (Hada *et al*., 2001). This might explain the importance of high phenolic compound in the leaves. Moreover, high correlations were observed in antioxidant capacities as well as total phenolic and flavonoid content of *C. sinensis*. This finding was in agreement with Abu Bakar *et al*. (2009) and Ling *et al*. (2010) in which high correlations were observed between antioxidant activities and polyphenol phytochemicals content. Thus, the antioxidant activities most probably might be contributed by polyphenols contents in the plant extracts.

Wang *et al*. (2000) reported that flavonoid was believed to be responsible for antioxidant activity, anticarcinogenic and anti-arteriosclerosis. Flavonoid in tea involved catechins, quercetin, kaempferol and myricetin. Flavonoid in tea has high antioxidant activities and radical scavenging.

Result showed that green tea has higher phenolic content than black tea (Table 1). In this study, Sabah tea exhibited significant better results in green tea (shoots) especially antioxidant activities and polyphenols compound (TPC and TFC). This showed that green tea has a great antioxidant potential. Moreover, the TPC of green tea was much higher than black tea and this current study was in the agreement of the findings of Gadow *et al*. (1997) and Pilar *et al*. (2008). According to Gadow *et al*. (1997) DPPH free radical scavenging activity of green tea was higher than black tea, with scavenging activity of 90.8% and 81.7%; respectively. Moreover, Pilar *et al*. (2008) revealed that TPC in green tea was higher than black tea with the values of 2083 mg gallic acid/ litre and 1844 mg gallic acid/litre; respectively. The polyphenolic components and antioxidant values decreased with the increment of maturity stage of tea leaves. These trend might be contributed by the morphological changes of leaf with age and the unique chemical compounds transportation within the plant (Farhoosh *et al*., 2007). Chen *et al*. (2003) discovered that young tea leaves were richer in EGCG and ECG than mature leaves, whereas Lin *et al*. (2003) claimed that old leaves contained more EGCG, EGC, EC and catechin than young leaves. These different claims and arguments will be further determined with chemical profiling of the catechins in future studies.

In this study, 50% aqueous ethanol was used as solvent to extract tea. Turkmen *et al*. (2006) reported that extraction with aqueous-methanol contributed to higher antioxidant activity if compared to methanol and hot water extraction. Using 50% ethanol and 80% ethanol will show 68.7% and 49.6% antioxidant activity, respectively. Other than that, Koffi *et al*. (2010) revealed that ethanol is an efficient solvent to extract polyphenols. According to Turkmen *et al*. (2006), solvent that has higher polarity is more efficient to scavenge free radicals than less polar solvent. Different solvents with different polarities will definitely contribute to the efficiency of determining antioxidant activities.

Conclusion

In conclusion, polyphenols in tea contributed significantly to the antioxidant activities of tea extracts. Green tea especially made from shoot showed the most promising result as antioxidant agent. The potential medicinal uses of these teas from Sabah Tea Plantation are supported by the presence of above mentioned antioxidants and polyphenolic

compounds. Hence, the need to exploit the potentials of *C. sinensis* especially in pharmaceutical industries arises.

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