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Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia

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Abstract

Methanol extracts of fresh tea leaves from a lowland plantation in Malaysia were screened for total phenolic content (TPC) and antioxidant activity (AOA). AOA evaluation included 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging ability, ferric-reducing antioxidant power (FRAP), and ferrous-ion chelating (FIC) ability. Ranking, based on TPC and AOA, was as follows: shoots > young leaves > mature leaves. TPC and AOA of lowland leaves were comparable to those of highland plants. A green tea produced by drying young leaves in a household microwave oven for 4 min showed significantly higher TPC and AOA than did four commercial brands of green and black tea.

Keywords: *Camellia sinensis*; Fresh leaves; Tea; Lowland; Highland; Total phenolic content; Antioxidant activity

1. Introduction

The tea plant *Camellia sinensis* (L.) Kuntze (family Theaceae) is grown in about 30 countries worldwide (Graham, 1992). It grows best in tropical and subtropical areas with adequate rainfall, good drainage, and slightly acidic soil (Graham, 1999).

There are two varieties of tea. *C. sinensis* var. *sinensis* (China tea) is grown extensively in China, Japan, and Taiwan, while *C. sinensis* var. *assamica* (Assam tea) predominates in south and southeast Asia, including Malaysia (Adiwinata, Martosupono, & Schoorel, 1989) and, more recently, Australia (Caffin, D'Arcy, Yao, & Rintoul, 2004).

Tea is often planted in the highlands. In India and Sri Lanka, it is cultivated at elevations up to 2000 m asl (Graham, 1999). In plantations, tea is planted at a density of 5000–10,000 plants per hectare and maintained as low shrubs of 1–1.5 m in height through regular pruning during harvesting. Manual plucking of the terminal bud and two youngest leaves yields the finest quality of tea, but the high cost of labour in some countries makes mechanical harvesting an economic necessity (Caffin et al., 2004).

Fresh tea leaves are very rich in catechins, which may constitute up to 30% of dry weight (Graham, 1992). Principal catechins of young tea leaves are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), epicatechin (EC) and catechin. Content of catechins varies with climate, season, horticultural practices, leaf age and variety.

Chen et al. (2003) reported that young tea leaves were richer in caffeine, EGCG and ECG than were mature leaves. Old leaves had higher levels of theanine, EGC and EC. However, Lin, Tsai, Tsay, and Lin (2003) observed that old leaves contained less caffeine, but more EGCG, EGC, EC and catechin than did young leaves. Yao et al. (2004) reported that EGCG was the main flavanol in fresh tea shoots in Australia, constituting up to 115 mg/g dry weight of tea shoots. Bhatia and Ullah (1968) had earlier reported that the leaf bud and first leaf were richest in EGCG. Wild tea plants contained more EGCG, EGC, ECG, and total catechins than did cultivated plants (Lin et al., 2003).

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Tea is the most widely consumed beverage in the world, second only to water (Muktar & Ahmad, 2000). Of the total amount of teas produced and consumed in the world, 78% are black, 20% are green, and 2% are oolong tea. In green tea manufacture, catechin oxidation by polyphenol oxidase is prevented by steaming (Japan) or by panning (China) (Graham, 1999). The leaves retain their green colour and almost all of their original polyphenol content. Oolong tea is allowed to ferment to a limited extent and contains a mixture of catechins, theaflavins and thearubigens (Wheeler & Wheeler, 2004). Black tea is produced from fully fermented leaves and has a characteristic colour and taste.

The chemical composition of green tea is similar to that of fresh tea leaves (Chen et al., 2003). The amount of EGCG and total catechins was in the order: green tea > oolong tea > fresh tea leaves > black tea (Lin et al., 2003). Yen and Chen (1995) found the greatest amount of catechins in green tea (26.7%), followed by oolong tea (23.2%) and black tea (4.3%). Similarly, Cabrera, Gimenez, and Lopez (2003) found higher content of catechins in green tea than in oolong and black tea. Of teas sold in Australian supermarkets, the polyphenol content of green tea (25%) was much higher than that of black tea (18%) (Yao et al., 2006). Green and black teas produced from var. *assamica* had higher polyphenol contents (30%) than those from var. *sinensis* (20%) (Harbowy & Balentine, 1997).

Catechins and other polyphenols in tea exhibit powerful antioxidant activities (Dufresne & Farnworth, 2001). They act as antioxidants *in vitro* by sequestering metal ions and by scavenging reactive oxygen and nitrogen species (Frei & Higdon, 2003; Wiseman, Balentine, & Frei, 1997). They may also function indirectly as antioxidants through their effects on transcription factors and enzyme activities (Higdon & Frei, 2003).

During the processing of tea, fermentation results in the production of theaflavins and thearubigins (Lee, Lee, & Lee, 2002). Black tea comprises 2–6% of theaflavins and more than 20% of thearubigens, whereas green tea has 30–42% of catechins.

Leung et al. (2001) reported that the conversion of catechins to theaflavins during tea fermentation does not significantly alter its free-radical scavenging activity. They argued that theaflavins in black tea and catechins in green tea are equally effective antioxidants. In response to Leung et al. (2001), Lee et al. (2002) argued that green tea has a higher antioxidant capacity than black tea, regardless of whether or not fermentation affects the antioxidants in tea. This means that green tea has more antioxidant compounds than has black tea. This is in agreement with findings by Atoui, Mansouri, Boskou, and Kefalas (2005) and Yokozawa et al. (1998) that TPC of green tea was higher than that of black tea. These studies showed that the reduction of catechins during the fermentation process of tea manufacturing affects the radical-scavenging activity of tea.

Studies on the antioxidant activity of fresh leaves and tea of *C. sinensis* were carried out primarily on tea from highland plantations (Chen et al., 2003; Gulati, Rawat, Singh, & Ravindranath, 2003; Lin et al., 2003). This is the first report on TPC and AOA of *C. sinensis* var. *assamica* from a lowland tea plantation in Malaysia. Our findings would have significant implications for the quality of tea planted in the lowlands in comparison with highland tea and on the feasibility of establishing tea plantations in the lowlands. This study also investigated the possibility of using microwave drying as a rapid method for producing green tea of a quality comparable to that of commercial teas.

2. Materials and methods

2.1. Samples

Fresh shoots (leaf bud and two youngest leaves; yellowish green), young leaves (third to fifth leaves; light green) and mature leaves (sixth to eighth leaves; dark green) of *C. sinensis* var. *assamica* were collected from a lowland tea plantation in Bukit Cheeding, Selangor (altitude ~20 m asl). Fresh young leaves were also collected from a highland tea plantation in the Cameron Highlands, Pahang (altitude ~1400 m asl), for comparison. From each location, three individual plants were sampled.

Four brands of commercial *C. sinensis* tea were studied. Sea Dyke green tea, Lipton Yellow Label black tea, and Boh Cameron Highlands black tea were highland teas, while Boh Bukit Cheeding No. 53 black tea was a lowland tea. The two brands of Boh tea were produced from plantations in Malaysia. All the commercial teas were purchased from the supermarket. For each brand of commercial tea, three tea bags were sampled.

2.2. Chemicals and reagents

Chemicals used were as follows: total phenolic content (TPC) determination: Folin–Ciocalteu's phenol reagent (Fluka, 2 N), gallic acid (Fluka, 98%), anhydrous sodium carbonate (Fluka, 99%). DPPH assay: 1,1-diphenyl-2-picrylhydrazyl (Sigma, 90%), methanol (Mallinckrodt, 100%). FRAP assay: ferric chloride hexa-hydrate (Fisher, 100%), potassium ferricyanide (Unilab, 99%), trichloroacetic acid (Fisher, 99.8%), potassium dihydrogen phosphate (Bendosen, 99.5%), dipotassium hydrogen phosphate (Merck, 99%). FIC assay: ferrous sulphate hepta-hydrate (Hamburg), ferrozine iron reagent (Acros Organics, 98%). Water was purified by Elga deionizer. Absorbance was measured with an Anthelie Advanced 5 Secoman UV–vis spectrophotometer. pH was measured with a Hanna pH211 meter. Altitude of plantations was measured using a Casio altimeter (Model PRG-70-1VDR).

2.3. Microwave drying of tea leaves

Microwaved green tea was produced by drying fresh tea leaves for 4 min using a household microwave oven (Sharp Model R-248E; 800 W; 230–240 V; 50 Hz). Drying was

done in batches, of 2 g each, of leaves cut into 1 cm^2 pieces. The leaves were put into a beaker and placed in the middle of the turntable of the microwave oven. After drying, dry weights were recorded.

2.4. Preparation of extracts

2.4.1. Methanol extraction of fresh leaves

Fresh leaves (1 g) were powdered with liquid nitrogen in a mortar and extracted using 50 ml of methanol, with continuous swirling for 1 h at room temperature. Extracts were filtered and stored at −20 °C for further use.

To test the efficiency of methanol extraction, second and third extractions were conducted on some samples. After filtration, residues, along with the filter paper, were transferred back into the extraction vessel and extracted again each time with 50 ml of methanol.

2.4.2. Hot-water extraction of tea

Microwaved green tea (0.3 g dry weight, which is equivalent to 1 g fresh weight) was ground in a mortar and extracted with 50 ml of boiling water with continuous swirling for 1 h. The boiling water was allowed to cool throughout the extraction to mimic tea brewing. The same amount of microwaved green tea was extracted with 50 ml of methanol to serve as a control. Extracts were filtered and stored at 4 °C. Commercial teas were extracted in a similar manner.

2.5. Determination of total phenolic content

The amount of total phenolic content (TPC) in extracts was determined according to the Folin–Ciocalteu procedure used by Kahkonen et al. (1999). Samples (300 μl in triplicate) were introduced into test tubes, followed by 1.5 ml of Folin–Ciocalteu's reagent (diluted 10 times) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before absorbance at 765 nm was measured. TPC was expressed as gallic acid equivalents (GAE) in mg/100 g material. The calibration equation for gallic acid was $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$).

2.6. Determination of antioxidant activity

2.6.1. DPPH free-radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay was carried out in triplicate, based on the method used by Leong and Shui (2002) and Miliauskas, Venskutonis, and van Beek (2004) with slight modifications. Different dilutions of the extract, amounting to 1 ml, were added to 2 ml of DPPH (5.9 mg/100 ml methanol). The DPPH solution was then allowed to stand for 30 min before absorbance was measured at 517 nm. Spectrophotometric measurements were made using methanol as blank. An appropriate dilution of the DPPH solution was used as negative control, i.e., methanol in place of the sample. AOA was expressed as IC_{50} (inhibitory concentration in mg/ml of plant material necessary to reduce the absorbance of DPPH by 50%). The lower the IC_{50} the higher is the antioxidant activity. Results were also expressed as AEAC (ascorbic acid equivalent antioxidant capacity) in mg/100 g and calculated as follows:

$$\text{AEAC (mg AA/100 g)} = \text{IC}_{50(\text{ascorbate})}/\text{IC}_{50(\text{sample})} \times 100{,}000$$

The IC_{50} of ascorbate used for calculation of AEAC was 0.00387 mg/ml.

2.6.2. FRAP assay

The ferric-reducing antioxidant power (FRAP) of extracts was determined, following the method of Chu, Chang, and Hsu (2000) with modifications. Samples often have to be diluted because precipitation occurs upon colour development when the reducing power is too high. Different dilutions of extracts, amounting to 1 ml, were added to 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50 °C for 20 min. A total of 2.5 ml trichloroacetic acid solution (10% w/v) was added to the mixture to stop the reaction. The mixture was then separated into aliquots of 2.5 ml and each was diluted with 2.5 ml of water. To each diluted aliquot, a total of 500 μl of ferric chloride solution (0.1% w/v) was added and they were allowed to stand for 30 min for colour development. Absorbance measured at 700 nm in triplicate was used to calculate the gallic acid equivalents. Results of the FRAP assay were expressed as mg GAE/g. The calibration equation for gallic acid was $y = 16.767x$ ($R^2 = 0.9974$).

2.6.3. FIC assay

The ferrous-ion chelating (FIC) assay was adapted from Singh and Rajini (2004). Solutions of 2 mM $FeSO_4$ and 5 mM ferrozine were prepared. Each solution was diluted 20 times. Diluted $FeSO_4$ (1 ml) was mixed with 1 ml of sample, followed by 1 ml of diluted ferrozine. Assay mixtures were allowed to equilibrate for 10 min before measuring the absorbance at 562 nm. As the FIC assay is very concentration-dependent, different dilutions of each sample were assayed in triplicate. Measurements were compared with a negative control, comprising solvent in place of sample. As the sample volumes were quite large, the absorbance inherent to the sample may interfere with measurements. Furthermore, it was noted that both leaves and tea samples form a dark blue complex with ferrous ions. To correct for this occurrence, blanks containing the appropriate dilution of each sample with $FeSO_4$ were used. The ability of the sample to chelate ferrous ions was calculated relative to a negative control using the formula:

$$\text{Chelating effect } \% = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

3. Results and discussion

3.1. Fresh tea leaves

3.1.1. Methanol extraction efficiency

Based on TPC, methanol showed a high extraction efficiency for young lowland tea leaves. The first extraction resulted in a yield 92.6 ± 1.4%, the second and third extractions yielding only 6.0 ± 1.4% and 1.4 ± 0.1%, respectively. Waterman and Mole (1994) had recommended methanol for the extraction of phenolic compounds from fresh plant tissues. Methanol had been reported to be the most suitable solvent for extracting phenolic compounds from fresh young shoots of tea, compared with chloroform, ethyl acetate and water (Yao et al., 2004).

3.1.2. TPC and AOA of lowland tea leaves of different ages

Phenolic compounds in tea have been found to be efficient free-radical scavengers, partly due to their one-electron reduction potential, i.e., the ability to act as hydrogen or electron donors (Higdon & Frei, 2003). A lower reduction potential indicates that less energy is required for hydrogen or electron donation that would lead to higher antioxidant activity. FRAP measures the ability of compounds to act as an electron donor while DPPH measures their ability to act as hydrogen donors.

There appear to be some discrepancies in the phenolic content of tea leaves. Chen et al. (2003) found that young tea leaves were richer in EGCG and ECG than were mature leaves, whereas Lin et al. (2003) observed that old leaves contained more EGCG, EGC, EC and catechin than did young leaves.

From this study, TPC and FRAP of shoots (7666 ± 448 mg GAE/100 g and 55.6 ± 1.8 mg GAE/g) and young leaves (7280 ± 126 mg GAE/100 g and 54.5 ± 2.8 mg GAE/g) were significantly higher than those of mature leaves (5836 ± 294 mg GAE/100 g and 21.3 ± 3.5 mg GAE/g) (Table 1). AEAC of shoots (14,470 ± 577 mg AA/100 g), young leaves (12,817 ± 537 mg AA/100 g), and mature leaves (10,219 ± 674 mg AA/100 g) were significantly different from each other. FIC ability was in the order: shoots > young leaves > mature leaves (Fig. 1).

This is the first study on FRAP and FIC ability on fresh tea leaves of different ages. No studies were made on FIC ability of tea and tea leaves. The few studies on FRAP of tea were based on evaporated extracts of old leaves (Farhoosh, Golmovahhed, & Khodaparast, 2007) and dry weights of different commercial teas (Benzie & Szeto, 1999).

Findings of significantly higher TPC, AEAC and FRAP in shoots and young leaves than mature leaves in this study support those of Bhatia and Ullah (1968) and Chen et al. (2003). EGCG and ECG, found abundantly in young leaves, lead to the higher AEAC and FRAP values observed in shoots and young leaves, compared with mature leaves, but contradict Lin et al. (2003), who had found that old leaves are rich in EGCG, EGC, EC and catechin.

The high FIC ability of shoots and young leaves (Fig. 1) suggests that they contain greater amounts of ligands that compete very well with ferrozine in chelating ferrous metal ions. This high secondary antioxidant activity acts by preventing the generation of OH radicals via the Fenton reaction. Metal ions are largely sequestered *in vivo* but high FIC ability would prevent compounds with high FRAP from aggravating certain metal overload diseases (Cao, Sofic, & Prior, 1997). Recently, Kostyuk, Potapovich, Strigunova, Kostyuk, and Afanas (2004) reported that flavonoids, bound to metal ions, were much less subject to oxidation than were the free compounds in the presence of superoxide. Flavonoids in a complex gain an additional active centre, namely, the metal ion $[M^{(n+1)+}]$ via the following reaction:

$$M^{(n+1)+} + O_2^{-\cdot} \rightarrow M^{n+} + O_2$$

$$M^{n+} + O_2^{-\cdot} + 2H^+ \rightarrow M^{(n+1)+} + H_2O_2$$

3.1.3. TPC and AOA of lowland and highland young tea leaves

Young leaves sampled from lowland and highland plants showed comparable TPC and AOA. ANOVA was insignificant at $P < 0.05$ for TPC, AEAC and FRAP (Table 2). Based on the three separate samplings and each sampling done in triplicate, highland tea leaves showed greater variability than did lowland tea leaves. In terms of FIC ability, lowland leaves were slightly better than highland leaves (Fig. 1). This would imply that lowland leaves are slightly more effective than highland leaves in sequestering 'free' metal ions, rendering them inactive in generating free radicals.

In most countries, tea has traditionally been planted in the highlands in the belief that tea quality is improved at

Table 1
Total phenolic content (TPC) and antioxidant activity (DPPH free-radical scavenging and FRAP) of lowland tea leaves of different ages (fresh weight)

Leaf age	TPC (mg GAE/100 g)	Antioxidant activity (AOA)		
		DPPH free radical scavenging		FRAP (mg GAE/g)
		IC_{50} (mg/ml)	AEAC (mg AA/100 g)	
Shoots	7666 ± 448a	0.026 ± 0.001a	14,470 ± 577a	55.6 ± 1.8a
Young leaves	7280 ± 126a	0.030 ± 0.001a	12,817 ± 537b	54.5 ± 2.8a
Mature leaves	5836 ± 294b	0.037 ± 0.002b	10,219 ± 674c	21.3 ± 3.5b

Results are means ± SD ($n = 3$). For each column, values followed by the same letter (a–c) are not statistically different at $P < 0.05$ as measured by the Tukey HSD test.

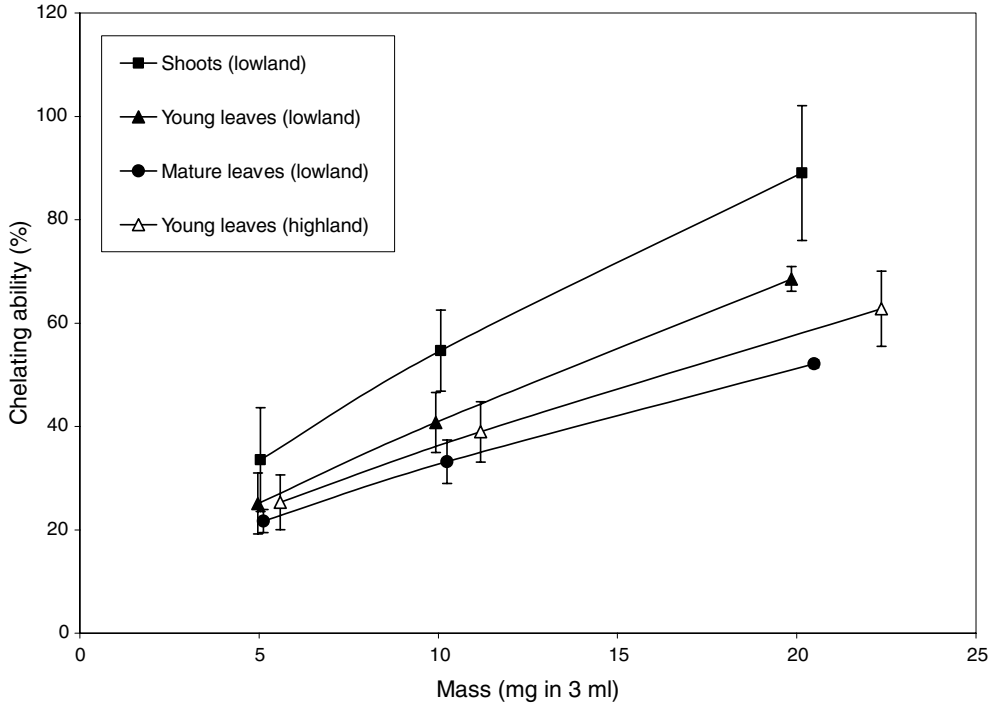

Fig. 1. Ferrous-ion chelating (FIC) ability of lowland tea leaves of different ages in comparison with highland leaves (fresh weight).

Table 2
Total phenolic content (TPC) and antioxidant activity (DPPH free radical scavenging and FRAP) of lowland and highland young tea leaves (fresh weight)

Location	TPC (mg GAE/100 g)	Antioxidant activity (AOA)		
		DPPH free-radical scavenging		FRAP (mg GAE/g)
		IC_{50} (mg/ml)	AEAC (mg AA/100 g)	
Lowland	7280 ± 126	0.030 ± 0.001	12,817 ± 537	54.5 ± 2.8
Highland	7586 ± 1995	0.035 ± 0.010	11,382 ± 3355	50.4 ± 12.9

Results are means ± SD ($n = 3$).

higher altitudes (Graham, 1999). Results from this study show that tea planted in the lowlands is comparable to highland tea in terms of TPC and AOA.

Growing tea in the lowlands has a number of advantages over tea grown in the highlands. In terms of growth and yield, tea plants in the highlands have more shoots, but lower yield in terms of dry weight, than have those in the lowlands (Balasuriya, 1999). It has also been reported that leaves are smaller in the highlands and that lowland shoots develop faster. This would mean higher tea production per unit area in lowland plantations. In terms of physical features, lowland plantations with more gentle terrains are easier to manage and harvesting can be mechanized without encountering environmental problems of soil erosion and slope failure.

3.2. Microwaved green tea and commercial teas

3.2.1. Microwave drying of tea leaves

Tea leaves microwaved for 4 min shrivelled, but remained green with a faint fragrance. When ground, the green-coloured tea produced a mild-tasting yellowish infusion similar to that of commercial green tea.

This study used a one-step process of polyphenol oxidase inactivation by heating and drying using microwave energy. Batches of leaves of 2 g each were completely dry after microwaving for 4 min. Heating and drying are caused by excitation of water molecules in the leaves due to microwave absorption (Pokorný & Schmidt, 2001). Heating is reduced once the leaves are dry.

Microwave heating, using household ovens, can lead to heterogeneous heating patterns within samples (Regier & Schubert, 2001). This does not apply when microwaving leaves which were cut into 1 cm^2 pieces and placed at the centre of the oven turntable. Leaves were rapidly and evenly dried.

Gulati et al. (2003) used a two-step process, i.e., inactivation and drying. Up to 2 kg of leaves were exposed to microwave energy from 2 to 6 min, followed by a separate drying step. Drying treatments used included microwave, conventional oven, and sun drying. Although the duration of drying was not mentioned, oven and sun drying may

take hours and days, respectively. Furthermore, because microwave energy is directed from a magnetron tube as a beam in household ovens (Regier & Schubert, 2001), it would be difficult to achieve homogeneous heating and drying of 2 kg of leaves using a household microwave oven (Gulati et al., 2003).

The microwave technique used in this study can be scaled-up for industrial application. In terms of commercial feasibility, microwave ovens are more energy-efficient than are conventional ovens (Pokorný & Schmidt, 2001). Water boils much faster in a microwave oven because of efficient heat transfer. In industrial microwave ovens, even application of microwave energy allows for homogeneous heating (Regier & Schubert, 2001).

3.2.2. Water and methanol extraction of microwaved green tea

Hot-water extraction of microwaved green tea resulted in a significantly lower TPC and DPPH free-radical scavenging than did methanol extraction (Table 3). However, FRAP (Table 3) and FIC abilities (Fig. 2) were similar for both methods of extraction. Methanol appears to be a more efficient solvent than is hot water. Yao et al. (2004) also reported that hot water extracted less catechins from tea than methanol. However, after repeated extraction, both solvents yielded similar amounts of polyphenols.

The water content of fresh young leaves from Bukit Cheeding was found to be 67.0 ± 2.9%. Expressed in terms of fresh weight equivalent, TPC of methanol extract of microwaved green tea was 6784 ± 69 mg GAE/100 g. This was significantly lower ($P < 0.05$) than fresh leaves with TPC of 7280 ± 126 mg GAE/100 g, representing a 6.8% reduction.

3.2.3. TPC and AOA of commercial teas and microwaved green tea

Of the commercial highland teas, TPC, AEAC and FRAP of Sea Dyke green tea were significantly higher than Lipton Yellow Label and Boh Cameron Highlands black teas (Table 4). Lipton Yellow Label black tea had significantly higher TPC, AEAC and FRAP than had Boh Cameron Highlands black tea. However, the black teas

Table 3
Total phenolic content (TPC) and antioxidant activity (DPPH free radical scavenging and FRAP) of microwaved green tea based on methanol and hot-water extraction (dry weight)

Solvent	TPC (mg GAE/100 g)	Antioxidant activity (AOA)		
		DPPH free radical scavenging		FRAP (mg GAE/g)
		IC_{50} (mg/ml)	AEAC (mg AA/100 g)	
Methanol	20,556 ± 211a	0.013 ± 0.001a	30,000 ± 778a	126 ± 4.5a
Hot water	19,126 ± 365b	0.015 ± 0.001a	26,213 ± 923b	123 ± 10.8a

Results are means ± SD ($n = 3$). For each column, values followed by the same letter (a–b) are not statistically different at $P < 0.05$ as measured by the Tukey HSD test.

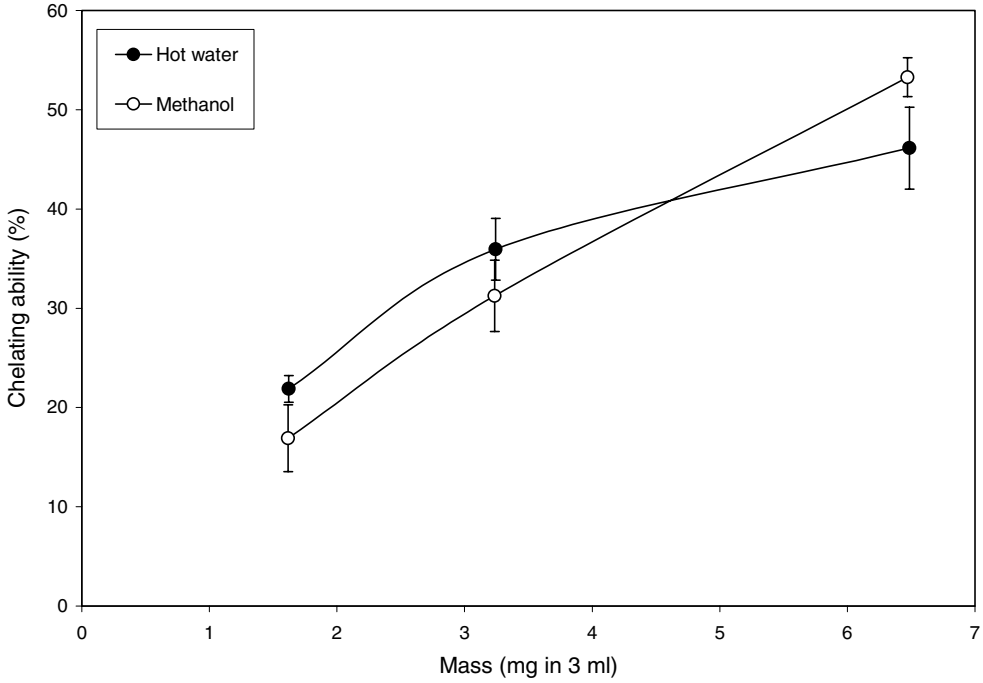

Fig. 2. Ferrous-ion chelating (FIC) ability of microwaved green tea extracted using water and methanol (dry weight).

Table 4
Total phenolic content (TPC) and antioxidant activity (DPPH free radical scavenging and FRAP) of microwaved green tea and four brands of commercial green and black tea (dry weight)

Type and brand of tea	TPC (mg GAE/100 g)	Antioxidant activity (AOA)		
		DPPH free-radical scavenging		FRAP (mg GAE/g)
		IC_{50} (mg/ml)	AEAC (mg AA/100 g)	
Lowland tea				
Microwaved green tea	19,126 ± 365a	0.015 ± 0.001a	26,213 ± 923a	123 ± 10.8a
Boh Bukit Cheeding No. 53 black tea	7409 ± 120bd	0.037 ± 0.002b	10,299 ± 563bd	44.3 ± 1.4b
Highland tea				
Sea Dyke green tea	11,367 ± 1475c	0.021 ± 0.002c	18,457 ± 1737c	83.8 ± 10.9c
Lipton Yellow Label black tea	8494 ± 803b	0.033 ± 0.003b	11,546 ± 1149b	52.5 ± 3.0b
Boh Cameron Highlands black tea	6061 ± 543d	0.051 ± 0.008d	7507 ± 1256d	36.4 ± 2.4b

Results are means ± SD ($n = 3$). For each column, values followed by the same letter (a–d) are not statistically different at $P < 0.05$ as measured by the Tukey HSD test.

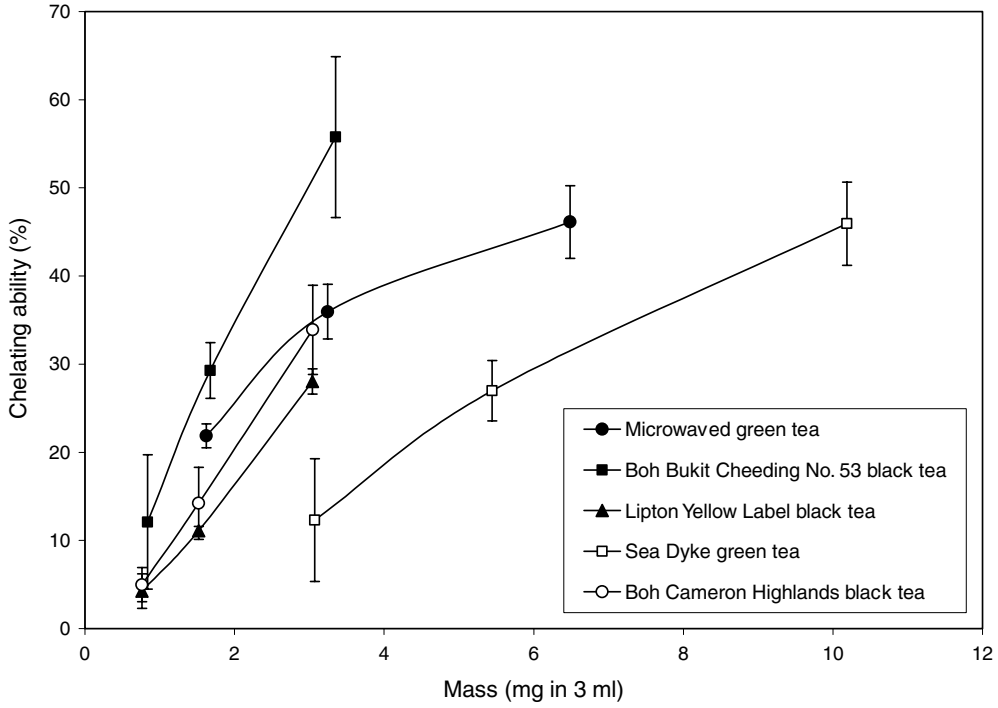

Fig. 3. Ferrous-ion chelating (FIC) ability of microwaved green tea in comparison with commercial teas (dry weight).

outperformed Sea Dyke green tea in terms of FIC ability (Fig. 3).

Comparing between the commercial lowland Boh Bukit Cheeding No. 53 black tea and the highland teas, TPC, AEAC, and FRAP values were significantly lower than those of Sea Dyke green tea (Table 4). The differences were not significant compared to Lipton Yellow Label black tea and Boh Cameron Highlands black tea. As with fresh lowland and highland leaves (Table 2), values of Boh Cameron Highlands black tea were more variable than those of Boh Bukit Cheeding No. 53 black tea.

In terms of sensory quality, there are subtle differences between the highland and lowland Boh teas. Boh Cameron Highlands black tea is characterized by its rich and invigorating aromatic flavour, and Boh Bukit Cheeding No. 53 black tea has a robust and full-bodied flavour.

In terms of FIC ability, the commercial lowland Boh Bukit Cheeding No. 53 black tea ranked the highest (Fig. 3). Ranking in FIC ability was as follows: Boh Bukit Cheeding No. 53 black tea (lowland) > microwaved green tea (lowland) ≈ Boh Cameron Highlands black tea (highland) ≈ Lipton Yellow Label black tea (highland) > Sea Dyke green tea (highland).

The microwaved green tea showed outstanding TPC, AEAC, and FRAP values (Table 4). Its values were significantly the highest compared to the four commercial brands of green and black tea. In terms of FIC ability, the microwaved green tea was better than Sea Dyke green tea (Fig. 3).

Gulati et al. (2003) dried leaf shoots using various treatments to produce green teas with TPCs ranging from 11% to 13% GAE (dry weight). This amounts to 11,000–13,000 mg GAE/100 g, which is similar to the Sea Dyke

green tea (11,367 ± 1475 mg GAE/100 g) (Table 4). The microwaved green tea, produced in this study, with TPC of 19,126 ± 365 mg GAE/100 g, was far superior. Furthermore, the 50% acetone used by Gulati et al. (2003) for extraction could have led to an over-estimation, as acetone was found to reduce the Folin–Ciocalteu reagent. Hot-water extraction yielded only 4000 mg GAE/100 g (Gulati et al., 2003).

The outstanding TPC, AEAC, and FRAP of the microwaved green tea might be caused by the release of bound phenolic compounds (Gulati et al., 2003). Microwave energy could have prevented the binding of polyphenols, including catechins, to the leaf matrix, thereby increasing their solubility. In addition, heat generated during microwaving may release additional bound phenolic compounds, brought about by the breakdown of cellular constituents (Dewanto, Wu, & Liu, 2002).

4. Conclusion

Methanol showed high extraction efficiency for fresh tea leaves. Between leaves of different ages, shoots and young leaves showed significantly higher TPC and FRAP than did mature leaves. AEAC of shoots, young leaves, and mature leaves were significantly different from each other.

TPC, AEAC and FRAP of lowland tea leaves were comparable to those of highland plants with the latter showing greater variability. In terms of FIC ability, lowland leaves were slightly better than highland leaves.

Sea Dyke green tea had significantly higher TPC, AEAC, and FRAP than had black teas of Lipton Yellow Label, Boh Cameron Highlands and Boh Bukit Cheeding No. 53 with the exception of FIC ability. The microwaved green tea had significantly higher TPC and AOA than had all the four brands of commercial green and black teas studied. Boh Bukit Cheeding No. 53 black tea showed outstanding FIC ability, surpassing that of the microwaved green tea. This study showed that tea planted in lowlands is comparable to those planted in highlands in terms of TPC and AOA.

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