ANTIOXIDANT ACTIVITY OF *THUNBERGIA LAURIFOLIA* TEA

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CHAN, E. W. C. & LIM, Y. Y. 2006. **Antioxidant activity of *Thunbergia laurifolia* tea.** Different drying treatments were tested on *Thunbergia laurifolia* leaves to produce a tea with total phenolic content (TPC) and antioxidant activity (AOA) comparable to fresh leaves and with acceptable aroma. TPC was measured using the Folin-Ciocalteu method while AOA was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and ferrous ion chelating (FIC) assays. Using a household microwave oven for drying, *Thunbergia* tea with superior antioxidant activity could be produced. Unlike oven-dried and sun-dried leaves, TPC and AOA of microwave-dried *Thunbergia* leaves were higher than fresh leaves. Leaves microwave-dried for 4 min remained green with a faint fragrance and when ground, the aromatic green coloured tea produced a mild tasting green infusion. The efficiency of hot water extraction as part of normal tea brewing was assessed, and TPC and AOA of the microwave-dried tea were compared with other commercial teas. The microwave-dried *Thunbergia* tea is far superior in terms of TPC and AOA compared with all the commercial herbal teas tested (Java, rooibos and rang jeud). Green and black tea of *Camellia sinensis,* however, showed higher TPC and AOA. Nonetheless, the microwave-dried *Thunbergia* tea had the best chelating ability among all the commercial teas studied, including those of green and black tea.

Keywords: Total phenolic content, half-leaf test, microwave-dried *Thunbergia* tea, commercial teas

CHAN, E. W. C. & LIM, Y. Y. 2006. **Aktiviti bahan antioksida dalam teh *Thunbergia laurifolia*.** Berbagai-bagai rawatan pengeringan telah diuji ke atas daun *Thunbergia laurifolia* untuk menghasilkan teh dengan kandungan jumlah fenol (TPC) dan aktiviti bahan antioksida (AOA) yang boleh dibandingkan dengan kandungan daun segar serta mempunyai aroma yang sesuai. TPC disukat menggunakan kaedah Folin-Ciocalteu. AOA pula disukat dengan menggunakan 1,1-difenil-2-pikrilhidrazil (DPPH), kuasa penurunan ferik (FRAP) dan pengkelat ion ferus. Teh *Thunbergia* dengan aktiviti antioksida yang sangat baik dapat dihasilkan dengan menggunakan ketuhar mikrogelombang. Berbeza dengan daun yang dikeringkan dengan ketuhar dan di bawah sinar matahari, TPC dan AOA bagi daun *Thunbergia* yang dikeringkan dengan mikrogelombang lebih tinggi daripada daun segar. Daun ini yang dikeringkan selama 4 min dengan mikrogelombang masih hijau dan mempunyai sedikit bau wangi. Apabila dikisar, teh hijau beraroma ini menghasilkan seduhan hijau yang berasa tidak begitu pekat. Kecekapan pengekstrakan menggunakan air panas dinilai dan seterusnya TPC dan AOA bagi teh yang dikeringkan dengan mikrogelombang telah dibandingkan dengan teh-teh komersial lain. Dari segi TPC dan AOA, teh *Thunbergia* yang dikeringkan dengan mikrogelombang jauh lebih baik berbanding dengan teh herba komersial (Java, rooibos dan rang jeud) yang diuji. Bagaimanapun teh hijau dan teh hitam daripada *Camellia sinensis* menunjukkan nilai TPC dan AOA yang lebih tinggi. Teh *Thunbergia* yang dikeringkan dengan mikrogelombang mempunyai kebolehan pengkelat yang paling baik berbanding semua teh komersial, termasuk teh hijau dan teh hitam.

INTRODUCTION

Thunbergia laurifolia (family Thunbergiaceae) or the blue trumpet vine is a popular ornamental plant in tropical gardens. The species occurs from Indo-China to Malaysia. Its leaves are opposite, heart-shaped with serrated leaf margin and taper to a pointed tip. Flowers are borne on pendulous inflorescences. The hermaphrodite flower is trumpet-shaped with a short broad tube, white on the outside and yellowish, inside. The corolla is pale blue in colour with five to seven petals, one larger than the others. The plant flowers almost continuously throughout the year with flowers opening early in the morning and aborting in the evening of the same day. Flowers are not scented. Carpenter bees are frequent visitors, creeping into the flowers for pollen and

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nectar while black ants are present probably as nectar scavengers.

In Malaysia, the leaves of *T. laurifolia* or 'akar tuau' are known to have some ethno-medicinal uses, e.g. juice of leaves taken for menorrhagia or put into the ear for deafness, and applied for poulticing cuts and boils (Burkill 1966). In Thailand, *T. laurifolia* or locally known as 'rang jeud' leaves are used in traditional medicine as an antipyretic, as well as an antidote for detoxification of poisons (Kanchanapoom *et al.* 2002). In recent years, several herbal companies in Thailand have started producing and exporting rang jeud tea. The tea has been claimed to be able to detoxify the harmful effects of drugs, alcohol and cigarettes. Many of these therapeutic properties have little or no biochemical basis while the antioxidant properties and phytochemistry of this plant are poorly studied.

Kanchanapoom *et al.* (2002) isolated two novel iridoid glucosides, 8-*epi*-grandifloric acid and 3'-*O*-β-glucopyranosyl-stilbericoside from *T. laurifolia* along with seven known compounds. Thongsaard & Marsden (2002) investigated the effects of *T. laurifolia* on dopaminergic neurotransmission in comparison with amphetamine. They reported that *T. laurifolia* might stimulate dopamine release in the same manner as amphetamine. However, in a study involving ethanolic extracts of nine Thai medicinal plants, leaf extracts of *T. laurifolia* did not show effective antiproliferative activity against SKBR3 human breast adenocarcinoma cells (Moongkarndi *et al.* 2004).

In this study, different drying treatments were tested on the leaves of *T. laurifolia* to produce a tea with total phenolic content (TPC) and antioxidant activity (AOA) comparable with fresh leaves and with acceptable aroma. The efficiency of hot water extraction as part of normal tea brewing was assessed based on its TPC and AOA. The water extract of the tea was compared with other commercial teas.

MATERIALS AND METHODS

Materials

Leaves of *T. laurifolia* were freshly collected from the first author's home garden at Bukit Maluri in Kepong. Identification was verified using pictorial illustrations in Anonymous (2002). The leaves (developing stage, several nodes from the bud) were packed into polythene bags, sealed with labels and brought to the laboratory the day before extraction for analysis.

Commercial teas studied included three brands of tea (*Camellia sinensis*), namely, Sea Dyke green tea, Lipton Yellow Label black tea and Boh Superior black tea, and two brands of herbal tea, namely, Pure Herb Java tea (*Orthosiphon stamineus*) and Dr. Nortier's rooibos tea (*Aspalathus linearis*). They were purchased from a local supermarket. TriSiam rang jeud tea was imported from Thailand.

Drying treatments

About 2 g of *T. laurifolia* leaves were each subjected to three different drying methods, namely, sun drying, oven drying and microwaving. Oven drying involved drying for 5.5 hours in an oven set at 50 °C. Leaves were sun dried in a glasshouse for two days. This was equivalent to 16 hours of drying in the sun. For microwave drying, 2 g of leaves were put in a household microwave oven (Sharp Model R-248E, 230-240V, ~50Hz) for 4 min. After drying, dry weights of the leaves in each treatment were recorded to determine their moisture contents.

Methanol extraction

Dried leaves were ground into powder with liquid nitrogen in a mortar. They were then extracted using 100 ml of methanol at room temperature with continuous swirling for one hour. Extracts were filtered and stored at -20 °C for further use. To determine the effectiveness of each treatment, fresh leaves (2 g) were used for control and subjected to methanol extraction.

Hot water extraction

Microwaved-dried *T. laurifolia* leaves were ground in a mortar and separated into two 0.3 g portions (equivalent to 2 g fresh weight). One portion was extracted for one hour with 100 ml boiling ultra-pure water, while the other with 100 ml methanol to serve as a control. In the hot water extraction, the boiling water was allowed to cool throughout extraction period to mimic tea brewing. Extracts were filtered and stored at 4 °C for further analysis.

Half-leaf test

The half-leaf test was specifically designed to verify observations as to whether microwave treatment does indeed increase antioxidant content. Fresh leaves were cut in half along the central vein. One half was microwaved-dried for 4 min while the other was retained as control. Both halves were ground separately in liquid nitrogen prior to extraction in 50 ml methanol for one hour. This would effectively rule out inter-leaf variation.

Extraction of commercial teas

Commercial teas were removed from tea bags and 1 g portions from each bag were extracted in 100 ml boiling water similar to that of microwaved *T. laurifolia* leaves. For each type of commercial tea, samples from three different tea bags were used.

Total phenolic content

The amount of TPC in extracts was determined according to the Folin-Ciocalteu procedure used by Kahkonen *et al.* (1999). Samples (300 µl in triplicates) were dispensed into test tubes followed by 1.5 ml Folin-Ciocalteu's reagent (10× dilution) and 1.2 ml sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before absorption was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in mg/100 g material.

DPPH assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was carried out in triplicates based on the method used by Leong and Shui (2002) and Miliauskas *et al.* (2004) with slight modifications. Defined amounts (10 to 100 µl) of the extract were added to 3 ml of DPPH (3.9 mg/100 ml methanol). The DPPH solution was left to stand for 30 min before absorbance was measured at 517 nm. Spectrometric 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 the antioxidant activity. Results were also expressed as AEAC (ascorbic acid, AA, equivalent antioxidant capacity) in mg/100 g and calculated as follows:

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

FRAP assay

The ferric reducing antioxidant power (FRAP) of extracts was determined following the methods of Chu *et al.* (2000) with modifications. Samples often had to be diluted because precipitation occurred upon colour development if the reducing power was 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 diluted with 2.5 ml water. A total of 500 µl ferric chloride solution (0.1% w/v) was added and allowed to stand for 30 min to allow colour development. Absorbance measured at 700 nm in triplicates was used to calculate the amount of GAE from a gallic acid standard curve. Results of the FRAP assay were expressed as mg GAE/g.

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 and 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 later 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 triplicates. 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. Therefore, blanks containing the appropriate dilution of each sample in ultra-pure water were used. The ability of the sample to chelate ferrous ions was

calculated relative to a negative control using the formula:

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

RESULTS AND DISCUSSION

Drying treatments

Oven drying and sun drying of leaves resulted in a drastic loss of TPC while microwaving resulted in an increase of TPC when compared with fresh leaves as control. TPC values of fresh, oven-dried, sun-dried and microwave-dried leaves were 477, 102, 95 and 624 mg GAE/100 g respectively. Thus TPC loss of oven-dried and sun-dried leaves was 78.6 and 80.1% respectively while TPC gain of microwave-dried leaves was 30.8% compared with fresh leaves.

From the half-leaf test, we observed that microwave-dried leaf 1 and 2 showed an increase of 69.3 and 62.3% respectively in TPC compared with fresh half leaves (Table 1). The increase was accompanied by a decrease of 49.5 and 50.5% in IC_{50} and an increase of 98.4 and 102.1% in AEAC based on DPPH radical scavenging.

Heating during microwave treatment of *T. laurifolia* leaves is a likely cause for the increase in TPC and AOA. It is probably due to the production of additional antioxidant phenolic compounds from precursors already present in the samples. This postulation is consistent with findings of Lianto (2004) whereby heat treatment of *T. laurifolia* leaves through boiling in water for 3, 5 and 10 min gave higher TPC values compared with fresh samples.

In terms of efficiency, the microwave treatment is a good drying method. Microwave heating, brought about by absorption of microwave energy by water molecules, is more energy efficient than conventional heating (Pokorny & Schmidt 2001). Heat transfer is very efficient as it is generated within the product itself.

Thunbergia tea

Unlike oven-dried and sun-dried leaves which turned brown in colour, microwave-dried leaves remained green with a faint fragrance. When ground, the aromatic green coloured tea produced a mild tasting green tea infusion.

In microwave-dried *Thunbergia* tea, hot water extracted almost double the amount of phenols compared with methanol. Based on the two samples studied, hot water extraction gave a higher TPC value of 4940 mg GAE/100 g as compared with methanol with a TPC value of 2720 mg GAE/100 g. Likewise, hot water extracts yielded lower IC_{50} (0.08 mg/ml), and higher AEAC (4840 AA/100 g). Methanol extracts, on the other hand, yielded higher IC_{50} (0.16 mg/ml) and lower AEAC (2380 mg AA/100 g). Hot water was also shown to be a better extraction solvent for the commercial rang jeud tea. TPC values of hot water extraction (819 mg GAE/100 g) were much higher than methanol extraction (199 mg GAE/100 g). These values were far lower than that of microwave-dried *Thunbergia* tea.

Table 1 TPC and AOA (fresh weight) of fresh and microwave-dried *Thunbergia laurifolia* leaves

Material	Half leaf	Treatment	TPC (mg GAE/100 g)	AOA (DPPH) IC_{50} (mg/ml)	AOA (DPPH) AEAC (mg AA/100 g)
Leaf 1	First	Fresh (control)	518 ± 5	0.99 ± 0.03	384 ± 12
	Second	Microwave	877 ± 4	0.50 ± 0.01	762 ± 12
Leaf 2	First	Fresh (control)	562 ± 18	0.99 ± 0.02	384 ± 7
	Second	Microwave	912 ± 16	0.49 ± 0.00	776 ± 7

Commercial teas

The results for the comparative analysis of commercial teas with the microwave-dried *Thunbergia* tea based on hot water extraction are presented in Table 2. The teas are ranked according to their TPC values.

The three brands of commercial *C. sinensis* teas generally performed better than the microwave-dried *Thunbergia* and the rest of the herbal teas tested. Sea Dyke green tea had the highest TPC (11 400 mg GAE/100 g) and AOA (IC_{50} = 0.02 mg/ml, AEAC = 18 500 mg AA/100 g and FRAP = 83.8 mg GAE/g). Ranking second and third were Lipton and Boh teas with TPC values of 8490 and 6060 mg GAE/100 g respectively.

The TPC value of the microwave-dried *Thunbergia* tea (5170 mg GAE/100 g) was not significantly different from that of Java tea (4320 mg GAE/100 g) because of large variation in the TPC of the latter. The microwave-dried *Thunbergia* tea performed better than the other two commercial herbal teas in which rang jeud tea was the poorest followed by rooibos tea with TPC values of 805 and 3750 mg GAE/100 g respectively. Our results are in accordance with Du Toit *et al.* (2001) who reported that green and black teas have higher AOA than herbal teas including rooibos tea. Lipton Yellow Label tea was reported to have an AEAC of 138 mg AA/g compared with 115 mg AA/g from the present study.

Results of FIC assays showed that the teas studied could be categorized into good chelating teas (able to function at low concentrations of 0.7 to 3.0 mg in 3 ml of water) or poor chelating teas (only able to function at higher concentrations of 2.5 to 10.0 mg in 3 ml of water). Among the good chelators, the microwave-dried *Thunbergia* tea had the best FIC ability followed by Boh and Lipton teas (Figure 1a). The poor

Table 2 Comparing the TPC and AOA (dry weight) of microwave-dried *Thunbergia* tea with commercial *Camellia sinensis* and herbal teas using hot water extraction (n = 3)

Type of tea	TPC (mg GAE/100 g)	Antioxidant activity (AOA)		
		DPPH		FRAP (mg GAE/g)
		IC_{50} (mg/ml)	AEAC (mg AA/100 g)	
Camellia sinensis tea				
Green tea (Sea Dyke)	11 400 ± 1480	0.02 ± 0.00	18 500 ± 1740	83.8 ± 10.9
Black tea (Lipton Yellow Label)	8490 ± 803	0.03 ± 0.00	11 500 ± 1150	52.5 ± 3.0
Black tea (Boh Superior)	6060 ± 543	0.05 ± 0.01	7510 ± 1260	36.4 ± 2.4
Herbal tea				
Microwave-dried *Thunbergia* tea	5170 ± 398	0.07 ± 0.01	5150 ± 544	39.4 ± 8.5
Java tea (Pure Herb)	4320 ± 1170	0.08 ± 0.03	4910 ± 1480	30.0 ± 10.1
Rooibos tea (Dr. Nortier's)	3750 ± 235	0.13 ± 0.02	3020 ± 456	19.1 ± 0.4
Rang jeud tea (TriSiam)	805 ± 50	0.64 ± 0.03	591 ± 29	4.3 ± 0.5

chelators included green, Java, rang jeud and rooibos teas (Figure 1b). This has very important implications as metal ions chelated to flavonoids can have their pro-oxidant activity reduced while the antioxidant activity of flavonoids enhanced as a result of complex formation (Afanas'ev *et al.* 2001).

The antioxidant capacity of tea largely depends on the degree of fermentation during processing (Muktar & Ahmad 2000). In black tea, the fermentation process destroys some of the phenolic compounds resulting in lower AOA. Green tea has no fermentation because its leaves are steamed or pan-fried to inactivate enzymes soon after harvest. Therefore, the phenolic compounds of green tea are conserved resulting in higher AOA and the tea has a green appearance.

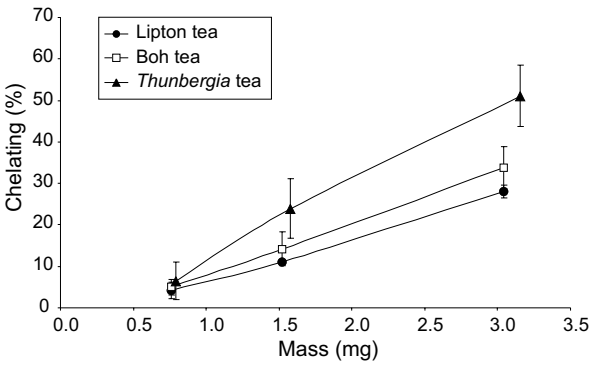

Figure 1(a) The ferrous ion chelating (FIC) ability of microwave-dried *Thunbergia*, Boh and Lipton teas (0.7–3.0 mg in 3 ml of water)

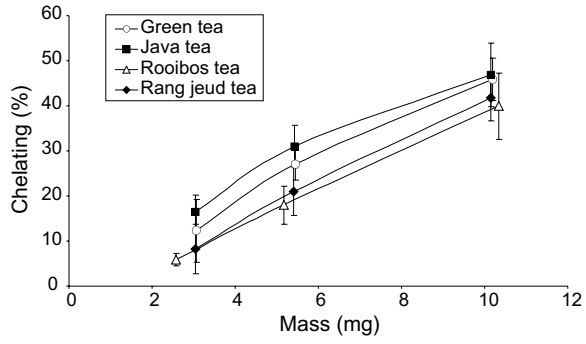

Figure 1(b) The ferrous ion chelating (FIC) ability of green, Java, rang jeud and rooibos teas (2.5–10.0 mg in 3 ml of water)

The microwave-dried *Thunbergia* tea is similar to green tea in that there is hardly any fermentation. High heat and rapid drying of the microwave treatment of fresh leaves effectively inactivated enzymes, thus stopping fermentation. Furthermore, results showed that the microwave treatment increased TPC and AOA. These are possible reasons why the microwave-dried tea is superior to all the herbal teas studied.

CONCLUSIONS

The present study showed that green and black tea of *C. sinensis* showed superior TPC and AOA over all the herbal teas studied (Java, rooibos and rang jeud). Nevertheless, leaves of *T. laurifolia* have great potential to be processed into herbal tea. In terms of TPC and AOA, the microwave-dried *Thunbergia* tea is comparable with Java tea, which is commercially manufactured in Malaysia. The microwave-dried *Thunbergia* tea has the best chelating ability among all the six commercial teas studied.

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