

## Review article

# Antioxidant properties of herbs with enhancement effects of drying treatments: A synopsis

Eric Wei Chiang Chan\*, Phui Yan Lye, Suit Ying Eng, Yuen Ping Tan

Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia

## ARTICLE INFO

## Article history:

Received 25 December 2012

Accepted 1 February 2013

Available online 29 March 2013

## Keywords:

Herbs

Fresh and dried

Phenolic content

Antioxidant activity

Enhancement

## ABSTRACT

Our recent work on the antioxidant properties (AOP) of herbs showed that three species (*Etligeria elatior*, *Morus alba*, and *Thunbergia laurifolia*) displayed enhancement effects of microwave-, oven-, and freeze-drying. AOP analysed were total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), ferric reducing power (FRP), and chelating efficiency concentration (CEC<sub>50</sub>). Microwave- and oven-drying led to drastic decline in AOP but freeze-drying resulted in significantly increase for leaves of *E. elatior*. Leaves of *M. alba* responded positively to all three drying treatments. TPC and FRP following oven-drying remained unchanged but AEAC and CEC<sub>50</sub> increased by 27% and 22%, respectively. Freeze-drying resulted in increase in TPC (16%), AEAC (26%), FRP (20%), and CEC<sub>50</sub> (44%). Microwave-drying increased TPC, AEAC, and FRP by 24%, 91%, and 30%, respectively. Microwave-drying enhanced AOP of *T. laurifolia* leaves. TPC and AEAC increased by 34% and 67%, respectively. Results indicated that different drying treatments have variable effects on AOP of herbs. Effects include little or no change, significant losses or enhancement in phenolic content and antioxidant activity. Decline in AOP following drying treatments has been attributed to thermal degradation of phytochemicals, enzymatic degradation of phenolic compounds and loss of antioxidant enzyme activities. Reasons for the increase in AOP following drying treatments include the release of bound phenolic compounds by the breakdown of cellular constituents and the formation of new compounds with enhanced antioxidant properties.

Copyright © 2013, SciBioMed.Org and Phcog.Net, Published by Reed Elsevier India Pvt. Ltd. All rights reserved.

## 1. Introduction

*Etligeria elatior* (Jack) Smith (Zingiberaceae) or torch ginger grows up to 5–6 m tall forming clumps.<sup>1–3</sup> Rhizomes are stout (3–4 cm in diameter), strongly aromatic and found just below ground level. Leaves are entirely green (80 × 18 cm) with young leaves sometimes flushed pink. Petioles are 2.5–3.5 cm in length. Borne on stalks protruding from the ground, inflorescences are large and attractive with showy bracts, which are pink to red and sometimes white. Young inflorescences have a spear-like head. Crushed leaves and inflorescences emit a distinctive pleasant sour fragrance. Native to Malaysia and Indonesia, *E. elatior* is widely cultivated in Southeast Asia. Farms in Australia and Hawaii are cultivating the species for inflorescences, which are sold as cut flowers.<sup>4</sup> Young inflorescences are a key ingredient of sour curries and other spicy dishes. The hearts of young shoots, inflorescences, and fruits are consumed by indigenous communities as condiment,

eaten raw or cooked as vegetable.<sup>5</sup> A decoction of fruits is used to treat earache while a decoction of leaves is applied for cleaning wounds.<sup>6</sup> Leaves are also used by post-partum women and mixed with other aromatic herbs in water for bathing to remove body odour.

*Morus alba* L. (Moraceae) or mulberry is a shrub or tree that grows in the tropics, sub-tropics and temperate.<sup>7</sup> Native to China, the species has spread throughout Europe and USA, and is widely cultivated in Japan, Korea, and India.<sup>8</sup> Plants of *M. alba* can grow up to 20 m in height.<sup>9</sup> Under cultivation, the tree is pollarded or pruned to a low-growing bush to facilitate the harvesting of fruit or leaves. Leaves of *M. alba* are simple, alternate, stipulate, light green, and cordate at the base.<sup>10</sup> Having a long petiole and a serrated margin, leaves are very variable in form, even on the same tree. Some leaves are unlobed while others are almost palmate. In temperate and sub-tropical climates, mulberry trees are deciduous, but trees grown in the tropics are evergreen.<sup>9</sup> Mulberry trees are often dioecious (monosexual with separate male and female plants) but may be monoecious (bisexual with male and female flowers on the same plant). Flowers are borne on short, green, and pendulous catkins. Male catkins are generally longer and loosely

\* Corresponding author.

E-mail address: [chanwc@ucsiuniversity.edu.my](mailto:chanwc@ucsiuniversity.edu.my) (E.W.C. Chan).

arranged. Mulberry fruits (1–2 cm long) are rich in anthocyanin and red when ripe but can vary from light purple to white.<sup>11,12</sup> Mulberry has been cultivated for centuries in China and Japan as feed for silkworms.<sup>13</sup> Its leaves are rich in protein and amino acids with high correlation between leaf protein level and silk production efficiency. The leaves of *M. alba*, when fed to dairy animals, improves milk production.<sup>14</sup> In traditional Chinese medicine, mulberry tea is consumed as a health-promoting drink.<sup>15</sup> The root bark of *M. alba* is used to treat diabetes, arthritis, rheumatism, and high blood pressure.<sup>13,16</sup> In Turkey and Greece, *M. alba* fruits are processed into canned mulberries, and mulberry juice, jam, and wine.<sup>17</sup>

*Thunbergia laurifolia* Lindl. (Thunbergiaceae) or blue trumpet vine or laurel clock vine is native to India.<sup>18,19</sup> The species is grown as an ornamental plant and being a fast-growing vine, it has become an exotic weed in some countries. Its leaves are green, opposite, heart-shaped, with a pointed tip and slightly serrated leaf margin. The leaf blade (20 × 16 cm) has a petiole of up to 6 cm in length. Leaves are thin and bright green when young, and dark green, thicker and slightly variegated when mature. Borne on pendulous inflorescences, flowers are attractive, trumpet-shaped, with 5–7 round pale purplish-blue petals and a yellow throat. The flower is up to 8 cm long and 6–8 cm across. The plant produces round green stems and a tuberous root system. Propagation is from stem or root cuttings. The plant flowers 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 the pollen and nectar while black ants are present probably as nectar scavengers. In Thai traditional medicine, leaves of *T. laurifolia* are used as an antidote for poisons and drugs including the treatment of drug addiction.<sup>20,21</sup> The plant has also been reported to have anti-inflammatory, anti-diabetic, and antipyretic properties.<sup>22–24</sup> Local herbal companies in Thailand are producing and marketing herbal teas and capsules of *T. laurifolia*.

Our recent work on the antioxidant properties (AOP) of selected herbs showed that three species displayed enhancement effects of drying treatments. AOP analysed were total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), ferric reducing power (FRP), and chelating efficiency concentration (CEC<sub>50</sub>). Drying treatments were microwave-, oven-, and freeze-drying. Freeze-drying enhanced the AOP of leaves of *E. elatior*. Leaves of *M. alba* responded positively to all three drying treatment. Microwave-drying enhanced the AOP of *T. laurifolia* leaves. Their AOP and the enhancement effects of drying treatments are reviewed.

## 2. *E. elatior*

### 2.1. Antioxidant properties

There are several publications on the AOP of leaves of *Etingera* with emphasis on *E. elatior*. Of leaves of five *Etingera* species screened, *E. elatior* had the highest TPC (3550 GAE/100 g), AEAC (3750 mg AA/100 g), and FRP (2000 GAE/100 g).<sup>25</sup> Screening of AOP of leaves of 26 ginger species belonging to nine genera and three tribes showed that *Etingera* had the highest values followed by *Alpinia* and *Hedychium*.<sup>26</sup> *Zingiber*, *Curcuma*, *Boesenbergia*, *Elettariopsis*, *Kaempferia*, and *Scaphochlamys* had significantly lower values. The outstanding AOP of *Etingera* species were attributed to their size and growth habitat. Plants of *Etingera* are the largest among the gingers and can grow up to 6 m in height. They grow in gaps of disturbed forest and are continually exposed to direct sunlight. The other genera are small- to medium-sized herbs.

**Table 1**

Percentage gain (+) or loss (–) in total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric reducing power (FRP) of leaves of *Etingera elatior* following drying compared to fresh leaves (fresh weight).<sup>31</sup>

Drying treatment	Water loss (%)	Gain/loss (%) compared to fresh leaves		
		TPC	AEAC	FRP
Microwave-drying	75	–40	–59	–44
Oven-drying	68	–42	–58	–43
Freeze-drying	76	+26	+45	+36

TPC (mg GAE/100 g), AEAC (mg AA/100 g), and FRP (mg GAE/100 g) are means ± standard deviation ( $n = 3$ ). Abbreviations: GAE = gallic acid equivalent and AA = ascorbic acid. Drying protocols: fresh leaves (1 g) were microwave-dried for 4 min, oven-dried at 50 °C for 5 h, and freeze-dried overnight. Extraction: leaves (1 g) were powdered with liquid nitrogen in a mortar and extracted with 50 ml of methanol.

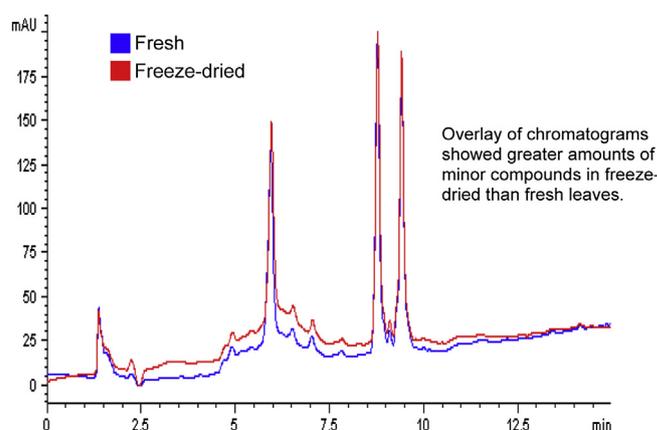
Larger ginger plants growing in exposed forest sites have greater AOP than smaller plants growing in shaded sites.

TPC and AEAC of leaves of *E. elatior* were eight times higher than rhizomes.<sup>26</sup> Leaves had significantly higher TPC, AEAC, and FRP than inflorescences and rhizomes.<sup>25</sup> Ranking was leaves > inflorescences > rhizomes. It has been reported much greater concentrations of flavones and flavonols in leaves which are exposed to sunlight.<sup>27</sup> Only trace amounts were found in unexposed parts below the soil surface, which include roots and rhizomes.

Leaves of highland populations of *Etingera* species were found to have higher TPC and AEAC than lowland counterparts.<sup>25</sup> Values of *E. elatior* in the highland were 3550 mg GAE/100 g and 3750 mg AA/100 g compared to 2390 mg GAE/100 g and 2280 mg AA/100 g in the lowland. Higher altitudes seem to trigger an adaptive response in leaves of *Etingera* species. The stronger AOP of highland over lowland plant populations might be due to environmental factors such as higher UV-B radiation and lower air temperature.<sup>28–30</sup>

### 2.2. Effects of drying treatments

The effects of different drying methods on the AOP of leaves of *E. elatior* have been reported.<sup>31</sup> Thermal drying treatments i.e. microwave- and oven-drying of leaves resulted in drastic declines in TPC, AEAC, and FRP (Table 1). Declines were ~41% for TPC, ~59% for AEAC, and ~44% for FRP. Leaves of other Zingiberaceae species such as *Alpinia zerumbet*, *Curcuma longa*, and *Kaempferia galanga*



**Fig. 1.** Overlay of chromatograms (254 nm) of fresh and freeze-dried leaves of *Etingera elatior*.<sup>31</sup>

**Table 2**

Total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), ferric reducing power (FRP), and chelating efficiency concentration (CEC<sub>50</sub>) of leaves and fruits of *Morus alba* (fresh weight).<sup>39</sup>

Plant part	TPC	AEAC	FRP	CEC <sub>50</sub>
Young leaves	587 ± 32 <sup>b</sup>	427 ± 22 <sup>b</sup>	343 ± 19 <sup>a</sup>	2.3 ± 0.3 <sup>a</sup>
Developing leaves	688 ± 23 <sup>a</sup>	508 ± 5.6 <sup>a</sup>	335 ± 11 <sup>a</sup>	2.7 ± 0.2 <sup>a</sup>
Mature leaves	607 ± 37 <sup>b</sup>	446 ± 33 <sup>b</sup>	338 ± 18 <sup>a</sup>	2.5 ± 0.2 <sup>a</sup>
Mature fruits	391 ± 10 <sup>c</sup>	81 ± 2.6 <sup>c</sup>	57 ± 0.3 <sup>b</sup>	ND

TPC (mg GAE/100 g), AEAC (mg AA/100 g), FRP (mg GAE/100 g), and CEC<sub>50</sub> (mg/ml) are means ± standard deviation ( $n = 3$ ). 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. Abbreviations: GAE = gallic acid equivalent, AA = ascorbic acid, and ND = not detected.

showed similar declines. Many studies have reported losses in AOP of plant samples following thermal treatments. Loss in AOP of heat-treated samples has been attributed to thermal degradation of phenolic compounds, to loss of antioxidant enzyme activities and to degradative enzymes.<sup>32,33</sup> Declines are often accompanied by loss of other bioactive properties.<sup>34</sup>

On the contrary, freeze-drying of *E. elatior* leaves resulted in significant gains in TPC (26%), AEAC (45%), and FRP (36%) (Table 1). After one-week storage, AOP of freeze-dried leaves remained significantly higher than those of fresh leaves as control. Phenolic content and antioxidant activity of freeze-dried inflorescences of *E. elatior* have been reported to be nine and eight times that of fresh samples.<sup>35</sup>

There is no thermal degradation in freeze-drying and neither does the process allow degradative enzymes to function.<sup>31</sup> Furthermore, freeze-drying is known to have high extraction efficiency because ice crystals formed within the plant matrix can rupture cell structure.<sup>36</sup> This allows the exit of cellular components and access of solvent, and consequently better extraction. The HPLC chromatogram of leaves of *E. elatior*, which showed greater amounts of minor compounds following freeze-drying, supported this inference (Fig. 1). Freeze-drying remains the best method of drying foods as the quality of freeze-dried products is comparable to fresh products.<sup>37</sup>

Chlorogenic acid (CGA) in leaves of *E. elatior* (294 mg CGA/100 g) was significantly higher in content than flowers of *Lonicera japonica* or Japanese honeysuckle (173 mg CGA/100 g), the commercial source. A protocol to produce a standardised extract of CGA with 40% w/w purity from freeze-dried leaves of *E. elatior* has been developed.<sup>38</sup> Freeze-drying of *E. elatior* leaves followed by extraction with 30% ethanol, and sequential fractionation using Diaion HP-20 and Sephadex LH-20 yielded a CGA extract with 40% w/w purity. CGA fractions had antioxidant, antibacterial and anti-tyrosinase properties. The entire fractionation process took only 6.5 h, using gravity flow. From 50 g of leaves, the final yield of CGA extract was 0.2 g (0.4%). CGA content of the standardised extract from leaves (40%) of *E. elatior* is 1.6 times that of commercial extracts from honeysuckle flowers (25%).

**Table 3**

Antioxidant properties of fresh, microwave-, oven-, and freeze-dried, and percentage gain (+) or loss (–) of *Morus alba* leaves with comparisons to fresh leaves (fresh weight).<sup>39</sup>

Drying treatment	Moisture loss (%)	TPC	AEAC	FRP	CEC <sub>50</sub>
Fresh leaves		688 ± 22 <sup>c</sup>	508 ± 6 <sup>c</sup>	335 ± 11 <sup>c</sup>	2.7 ± 0.2 <sup>c</sup>
Microwave-drying	61.3	855 ± 11 <sup>a</sup> (+24%)	970 ± 31 <sup>a</sup> (+91%)	437 ± 7 <sup>a</sup> (+30%)	2.7 ± 0.2 <sup>c</sup> (NC)
Oven-drying	63.3	711 ± 21 <sup>c</sup> (+3%)	644 ± 19 <sup>b</sup> (+27%)	336 ± 21 <sup>c</sup> (NC)	2.1 ± 0.1 <sup>b</sup> (+22%)
Freeze-drying	63.3	799 ± 8 <sup>b</sup> (+16%)	639 ± 18 <sup>b</sup> (+26%)	403 ± 8 <sup>b</sup> (+20%)	1.5 ± 0.1 <sup>a</sup> (+44%)

TPC (mg GAE/100 g), AEAC (mg AA/100 g), FRP (mg GAE/100 g), and CEC<sub>50</sub> (mg/ml) are means ± standard deviation ( $n = 3$ ). 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. Abbreviations: GAE = gallic acid equivalent, AA = ascorbic acid, and NC = no change. Drying protocols: fresh leaves (15 g) were shredded, and microwave-dried for 1.5 min, oven-dried at 50 °C for 15 min and freeze-dried overnight. Extraction: leaves (1 g) were powdered with liquid nitrogen in a mortar and extracted with 50 ml of 50% methanol.

### 3. *M. alba*

#### 3.1. Antioxidant properties

Based on TPC of first, second, and third extractions of fresh leaves of *M. alba*, extraction efficiency values of 50% aqueous methanol were 82%, 13%, and 4%, respectively.<sup>39</sup> Comparing different age groups of leaves, developing leaves of *M. alba* had the highest TPC and AEAC, followed by mature leaves and young leaves (Table 2). Although mature leaves had slightly higher values than young leaves, their values were comparable. In terms of FRP and CEC<sub>50</sub>, there were no significant differences between leaves of different age groups. AOP of mature mulberry fruits were significantly lower than those of leaves. Overall ranking of AOP was developing leaves > young leaves ~ mature leaves > mature fruits.

#### 3.2. Effects of drying treatments

AOP of microwave-, oven-, and freeze-dried leaves of *M. alba* are shown in Table 3. Results are expressed in term of percentage gain (+) and loss (–) in comparison to values of fresh leaves. All the three drying methods resulted in the enhancement of AOP.

Microwave-drying resulted in a significant increase in the AOP of leaves of *M. alba* with the exception for CEC<sub>50</sub> (no change). TPC, AEAC, and FRP of microwave-dried leaves were 855 mg GAE/100 g, 970 mg AA/100 g, and 437 mg GAE/100 g; these values were significantly higher than 688 mg GAE/100 g, 508 mg AA/100 g, and 335 mg GAE/100 g of fresh leaves. The TPC, AEAC, and FRP of microwave-dried leaves were 24%, 91%, and 30% higher than those of fresh leaves, respectively.

The higher AOP of microwave-dried *M. alba* leaves can be explained by the release of bound phenolic compounds, brought about by the breakdown of cellular constituents.<sup>31</sup> Microwave energy could have increased the solubility of polyphenols by preventing them from binding to the leaf matrix.<sup>25,40</sup> Other contributing factors included short drying time and rapid inactivation of degradative enzymes.

Oven-drying of *M. alba* leaves showed little or no change in AOP with the exception of AEAC and CEC<sub>50</sub> which showed significant increase. The increase from 688 to 711 mg GAE/100 g (3.3%) for TPC and from 335 to 336 mg GAE/100 g (0.3%) for FRP was not significant. However, AEAC and CEC<sub>50</sub> of oven-dried leaves increased significantly by 27% and 22%, respectively.

Freeze-drying resulted in enhanced AOP of *M. alba* leaves. Freeze-dried leaves showed significantly higher values of TPC, AEAC, FRP, and CEC<sub>50</sub> compared to those of fresh leaves. Values of freeze-dried leaves were 799 mg GAE/100 g, 639 mg AA/100 g, 403 mg GAE/100 g, and 1.5 mg/ml while those of fresh leaves were 688 mg GAE/100 g, 508 mg AA/100 g, 335 mg GAE/100 g, and 2.7 mg/ml. This amounted to gains of 16%, 26%, 20%, and 44%, respectively.

Results showed that microwave-dried tea infusions of *M. alba* had the highest phenolic content and antioxidant activity among the three drying methods (Table 4). TPC, AEAC, and FRP of the microwave-dried tea were 1873 mg GAE/100 g, 1917 mg AA/100 g, and 865 mg GAE/100 g, respectively. FIC ability of the microwave-dried tea was also observed to be the highest with CEC<sub>50</sub> of 0.3 mg/ml. Values of the commercial tea were the lowest. Based on AOP of tea infusions, the overall ranking was microwave-dried > freeze-dried > oven-dried > commercial.

#### 4. *T. laurifolia*

##### 4.1. Antioxidant properties

A study on the optimum time and efficiency of methanol extraction for leaves of *T. laurifolia* demonstrated that 1 h was the optimum extraction time.<sup>19</sup> TPC values for 0.5, 1.0, and 2.0 h were 418, 721, and 636 mg GAE/100 g, respectively. Based on TPC, the first extraction extracted about 88% of the phenolic compounds. Yields of the second and third extractions were only 10% and 2%, respectively, suggesting that methanol is efficient in extracting leaves of *T. laurifolia*.

Variations in TPC between *T. laurifolia* leaves of different ages, collection times, and locations were also reported.<sup>19</sup> Developing leaves had the highest TPC of 513 mg GAE/100 g, followed by young and mature leaves with values of 407 and 298 mg GAE/100 g, respectively. TPC values varied from 532 to 795 mg GAE/100 g for four batches of leaves collected from the same source in April and May 2004. Leaves collected from plants located in three different locations on the same day had significantly different TPC values of 543, 734, and 892 mg GAE/100 g, suggesting variation between plants. Within plants, leaves and flowers had comparable phenolic content and free radical scavenging ability.

##### 4.2. Effects of drying treatments

The effects of different drying methods on the AOP of *T. laurifolia* leaves have been reported.<sup>18,19</sup> For oven-drying, TPC and AEAC declined 73% and 80%, respectively. For microwave-drying, TPC and AEAC gained 38–41% and 50–51%, respectively. Microwave-dried leaves remained green with a faint fragrance and when ground, the aromatic green-coloured tea produced a mild tasting green infusion. For the microwave-dried tea, hot water extraction yielded TPC and AEAC values that were 1.7–1.9 and 2.0–2.1 times higher than those of methanol extraction. When compared to other commercial teas, TPC, AEAC, and FRP values of the microwave-dried tea were 6.4, 8.7, and 9.3 times those of the commercial *T. laurifolia* tea.

**Table 4**

Total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), ferric reducing power (FRP), and chelating efficiency concentration (CEC<sub>50</sub>) of tea infusions of *Morus alba* in comparison with the commercial tea (dry weight).<sup>39</sup>

Tea infusion	TPC	AEAC	FRP	CEC <sub>50</sub>
Microwave-dried	1873 ± 44 <sup>a</sup>	1917 ± 23 <sup>a</sup>	865 ± 24 <sup>a</sup>	0.3 ± 0.0 <sup>a</sup>
Oven-dried	1643 ± 130 <sup>b</sup>	1322 ± 90 <sup>c</sup>	695 ± 49 <sup>c</sup>	0.3 ± 0.0 <sup>a</sup>
Freeze-dried	1845 ± 68 <sup>ab</sup>	1560 ± 66 <sup>b</sup>	807 ± 26 <sup>ab</sup>	0.4 ± 0.0 <sup>ab</sup>
Commercial tea	1205 ± 35 <sup>c</sup>	1020 ± 80 <sup>d</sup>	732 ± 22 <sup>bc</sup>	0.8 ± 0.0 <sup>b</sup>

TPC (mg GAE/100 g), AEAC (mg AA/100 g), FRP (mg GAE/100 g), and CEC<sub>50</sub> (mg/ml) are means ± standard deviation ( $n = 3$ ). 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. Abbreviations: GAE = gallic acid equivalent and AA = ascorbic acid. Drying protocols: fresh leaves (15 g) were microwave-dried for 1.5 min, oven-dried at 50 °C for 15 min, and freeze-dried overnight. Extraction: tea infusions were produced by steeping 0.3 g of dried leaves in 50 ml of boiling water for 1 h.

**Table 5**

Percentage gain (+) or loss (–) in total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of oven-, freeze-, and microwave-dried leaves of *Thunbergia laurifolia* in comparison with fresh leaves (fresh weight).<sup>19</sup>

Drying treatment	Water loss (%)	Gain/loss (%) compared to fresh leaves	
		TPC	AEAC
Oven-drying	78	–36	–25
Freeze-drying	80	+0.4	–0.7
Microwave-drying	79	+38	+84

TPC (mg GAE/100 g) and AEAC (mg AA/100 g) are means ± standard deviation ( $n = 3$ ). Abbreviations: GAE = gallic acid equivalent and AA = ascorbic acid. Drying protocols: fresh leaves (15 g) were microwave-dried for 1.5 min, oven-dried for 3 h, and freeze-dried overnight. Extraction: leaves (2 g) were ground in a mortar with liquid nitrogen before extraction with 100 ml of 50% methanol.

A study on the effects of various thermal and non-thermal drying methods on the AOP of leaves and teas of *T. laurifolia* showed remarkable differences.<sup>19</sup> Leaves of *T. laurifolia* (15 g) were shredded, and oven-dried (3 h), microwave-dried (1.5 min), and freeze-dried (overnight). Dried leaves were extracted by steeping in hot water (1 h) to obtain the tea infusions. Oven-drying led to declines in TPC (36%) and AEAC (25%), respectively, compared to fresh leaves (Table 5). Values of freeze-dried leaves remained unchanged i.e. comparable to those of fresh leaves. Interestingly, values of microwave-dried leaves were 38% and 84% higher than those of fresh leaves, respectively.

An earlier study on the effects of microwave-drying on leaves of *T. laurifolia* using the half-leaf test has been reported.<sup>18</sup> The half-leaf test was specifically designed to verify whether microwave treatment does indeed increase phenolic content and antioxidant activity. Fresh leaves were cut in half along the central vein. One half was microwave-dried for 4 min while the other half was retained as control. This would effectively rule out inter-leaf variation. Results based on two leaves showed an increase of 41% and 50% in TPC and AEAC for the first leaf, and an increase of 38% and 51% for the second leaf, respectively.

A likely cause for the increase in antioxidant activity following microwave-drying is the production of additional phenolic compounds from precursors already present in the samples.<sup>18</sup> Another possible explanation is the rapid inactivation of polyphenol oxidase (PPO) activity in samples due to microwave irradiation.<sup>41</sup> The enzyme is therefore unable to generate oxidation products that would reduce the phenolic content.

AOP of all *T. laurifolia* teas produced were significantly higher than those of the commercial tea.<sup>19,42</sup> Freeze-, microwave-, and oven-dried teas had TPC values (6.7, 5.3, and 3.1 times) and AEAC values (11.4, 8.7, and 4.0 times) that of the commercial tea (Table 6).

**Table 6**

Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of tea infusions of *Thunbergia laurifolia* in comparison with the commercial tea (dry weight).<sup>19,42</sup>

Tea infusion	TPC	AEAC
Freeze-dried	3850 ± 127 <sup>a</sup>	4520 ± 100 <sup>a</sup>
Microwave-dried	3080 ± 202 <sup>b</sup>	3450 ± 273 <sup>b</sup>
Oven-dried	1800 ± 57 <sup>c</sup>	1590 ± 55 <sup>c</sup>
Commercial tea	577 ± 39 <sup>d</sup>	398 ± 22 <sup>d</sup>

TPC (mg GAE/100 g) and AEAC (mg AA/100 g) are means ± standard deviation ( $n = 3$ ). 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. Abbreviations: GAE = gallic acid equivalent and AA = ascorbic acid. Drying protocols: fresh leaves (15 g) were microwave-dried for 1.5 min, oven-dried at 50 °C for 3 h, and freeze-dried overnight. Extraction: tea infusions were produced by steeping 0.6 g of dried leaves in 100 ml of boiling water for 1 h.

Ranking of the *T. laurifolia* teas based on AOP was freeze-dried > microwave-dried > oven-dried > commercial.

TPC and AEAC values of 577 mg GAE/100 g and 398 mg AA/100 g of the commercial *T. laurifolia* tea were significantly lower than values of 805 mg GAE/100 g and 591 mg AA/100 g reported earlier,<sup>43</sup> suggesting variation in antioxidant values between different batches of the commercial tea produced.

## 5. Conclusion

Freeze-drying enhanced the AOP of leaves of *E. elatior*. TPC, AEAC, and FRP increased by 26%, 45%, and 36%, respectively. The HPLC chromatogram of freeze-dried leaves of *E. elatior* showed greater amounts of minor compounds than fresh leaves. Leaves of *M. alba* responded positively to all three drying treatments. TPC and FRP following oven-drying remained unchanged but AEAC and CEC<sub>50</sub> increased by 27% and 22%, respectively. Freeze-drying resulted in increase in TPC (16%), AEAC (26%), FRP (20%), and CEC<sub>50</sub> (44%). Microwave-drying enhanced TPC, AEAC, and FRP by 24%, 91%, and 30%, respectively. Microwave-drying enhanced the AOP of *T. laurifolia* leaves with 34% increase in TPC and 67% increase in AEAC as confirmed by the half-leaf test. With the enhancement effects of drying treatments on AOP, these three species of herbs have the potential to be developed into nutraceutical and pharmaceutical products for wellness.

## Conflicts of interest

All authors have none to declare.

## Acknowledgements

This study formed part of the research project on the effects of different drying methods on the antioxidant properties of fresh and dried herbs. The support of the Faculty of Applied Sciences, UCSI University is gratefully acknowledged.

## References

- Khaw SH. The genus *Etingera* (Zingiberaceae) in Peninsular Malaysia including a new species. *Gard Bull Singapore*. 2001;53:191–239.
- Lim CK. Taxonomic notes on *Etingera* (Zingiberaceae) in Peninsular Malaysia: the “*Nicolaia*” taxa. *Folia Malaysiana*. 2000;1:1–12.
- Lim CK. Taxonomic notes on *Etingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the “*Achasma*” taxa and supplementary notes on the “*Nicolaia*” taxa. *Folia Malaysiana*. 2001;2:41–78.
- Larsen K, Ibrahim H, Khaw SH, Saw LG. *Gingers of Peninsular Malaysia and Singapore*. Kota Kinabalu: Natural History Publications (Borneo); 1999.
- Noweg T, Abdullah AR, Nidang D. Forest plants as vegetables for communities bordering the Crocker Range National Park. *ASEAN Rev Biodiv Environ Conser*; Jan–Mar 2003:1–18.
- Ibrahim H, Setyowati FM. *Etingera*. In: De Guzman CC, Siemonsma JS, eds. Leiden, Netherlands: Backhuys Publisher; 1999:123–126. *Plant Resources of South-east Asia*. vol. 13.
- Srivastava S, Kapoor R, Thathola A, Srivastava RP. Mulberry (*Morus alba*) leaves as human food: a new dimension of sericulture. *Int J Food Sci Nutr*. 2003;54:411–416.
- Dat NT, Xuan Binh PT, Phuong Quynh LT, Minh CV, Huong HT, Lee JJ. Cytotoxic prenylated flavonoids from *Morus alba*. *Fitoterapia*. 2010;81:1224–1227.
- Suttie JM. *Morus alba* L. [Online]. In: *Grassland Species Profiles*. Food and Agriculture Organization (FAO) [updated 2005; cited 2012 Dec]. Available from: [www.fao.org/ag/AGP/AGPC/doc/Gbase/data/pf000542.htm](http://www.fao.org/ag/AGP/AGPC/doc/Gbase/data/pf000542.htm); 2005.
- Huo Y. Mulberry cultivation and utilization in China. [Online]. Food and Agriculture Organization (FAO) [updated 2000; Cited 2012 Dec]. Available from: [www.fao.org/ag/AGA/AGAP/FRG/Mulberry/Papers/HTML/YONGKANG.htm](http://www.fao.org/ag/AGA/AGAP/FRG/Mulberry/Papers/HTML/YONGKANG.htm); 2000.
- Ercisli S, Orhan E. Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. *Food Chem*. 2007;103:1380–1384.
- Du Q, Zheng J, Xu Y. Composition of anthocyanins in mulberry and their antioxidant activity. *J Food Compos Anal*. 2008;21:390–395.
- Machii H, Koyama A, Yamanouchi H. *Mulberry Breeding, Cultivation and Utilization in Japan* [Online]. Food and Agriculture Organization (FAO) [updated 2000; cited 2012 Dec]. Available from: [www.fao.org/ag/AGA/AGAP/FRG/Mulberry/Papers/HTML/machii2.htm](http://www.fao.org/ag/AGA/AGAP/FRG/Mulberry/Papers/HTML/machii2.htm); 2000.
- Arabshahi-Delouee S, Urooj A. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chem*. 2007;102:1233–1240.
- Ma X, Iwanaka N, Masuda S, et al. *Morus alba* leaf extract stimulates 5'-AMP-activated protein kinase in isolated rat skeletal muscle. *J Ethnopharmacol*. 2009;122:54–59.
- Yang Y, Zhang T, Xiao L, Yang LX, Chen RY. Two new chalcones from leaves of *Morus alba* L. *Fitoterapia*. 2010;81:614–616.
- Butt MS, Nazir A, Sultan MT, Schroën K. *Morus alba* L. nature's functional tonic. *Trends Food Sci Technol*. 2008;19:505–512.
- Chan EWC, Lim YY. Antioxidant activity of *Thunbergia laurifolia* tea. *J Trop For Sci*. 2006;18:130–136.
- Chan EWC, Eng SY, Tan YP, Wong ZC. Phytochemistry and pharmacological properties of *Thunbergia laurifolia*: a review. *Pharmacogenomics J*. 2011;3:1–6.
- Thongsard W, Marsden CA. A herbal medicine used in the treatment of addiction mimics the action of amphetamine on in vitro rat striatal dopamine release. *Neurosci Lett*. 2002;329:129–132.
- Thongsard W, Marsden CA, Morris P, Prior M, Shah YB. Effect of *Thunbergia laurifolia*, a Thai natural product used to treat drug addiction, on cerebral activity detected by functional magnetic resonance imaging in the rat. *Psychopharmacology*. 2005;180:752–760.
- Kanchanapoom T, Kasai R, Yamasaki K. Iridoid glucosides from *Thunbergia laurifolia*. *Phytochemistry*. 2002;60:769–771.
- Aritajat S, Wutteeapol S, Saenphet K. Anti-diabetic effect of *Thunbergia laurifolia* Linn. aqueous extract. *SE Asian J Trop Med Public Health*. 2004;35:53–58.
- Chivapat S, Chavalittumrong P, Attawish A, Bansiddhi J, Padungpat S. Chronic toxicity of *Thunbergia laurifolia* Lindl. extract. *J Thai Tradit Altern Med*. 2009;7:17–24.
- Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of *Etingera* species (Zingiberaceae) in Peninsular Malaysia. *Food Chem*. 2007;104:1586–1593.
- Chan EWC, Lim YY, Wong LF, et al. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chem*. 2008;109:477–483.
- Herrmann K. On the occurrence of flavonol and flavone glycoside in vegetables. *Eur Food Res Technol A*. 1988;186:1–5.
- Jansen MAK, Gaba V, Greenberg BM. Higher plants and UV-B radiation: balancing damage, repair and acclimation. *Trends Plant Sci*. 1998;3:131–135.
- Bassman JH. Ecosystem consequences of enhanced solar ultra-violet radiation: secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities. *Photochem Photobiol*. 2004;79:382–398.
- Chalker-Scott L, Scott JD. Elevated ultraviolet-B radiation induces cross-protection to cold in leaves of *Rhododendron* under field conditions. *Photochem Photobiol*. 2004;79:199–204.
- Chan EWC, Lim YY, Wong SK, et al. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chem*. 2009;113:166–172.
- Larrauri JA, Rupérez P, Saura-Calixto F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *J Agric Food Chem*. 1997;45:1390–1393.
- Lim YY, Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT – Food Sci Technol*. 2007;40:1664–1669.
- Roy MK, Takenaka M, Isobe S, Tsushida T. Antioxidant potential, anti-proliferative activities and phenolic content in water-soluble fractions of some commonly consumed vegetables: effects of thermal treatment. *Food Chem*. 2007;103:106–114.
- Yan SW, Asmah R. Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. *Int Food Res J*. 2010;17:417–423.
- Asami DK, Hong YJ, Barrett DM, Mitchell AE. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *J Agric Food Chem*. 2003;51:1237–1241.
- Ratti C. Hot air- and freeze-drying of high-value foods: a review. *J Food Eng*. 2001;49:311–319.
- Chan EWC, Lim YY, Tan SP. Standardised herbal extract of chlorogenic acid from leaves of *Etingera elatior* (Zingiberaceae). *Pharm Res*. 2011;3:177–183.
- Lye PY. *Antioxidant Properties of Thai Herbal Teas and Effects of Microwave, Oven and Freeze Drying on Antioxidant Properties of Morus Alba Tea*. B.Sc. thesis. Kuala Lumpur, Malaysia: Faculty of Applied Sciences, UCSI University; 2012.
- Gulati A, Rawat R, Singh B, Ravindranath SD. Application of microwave energy in the manufacture of enhanced-quality green tea. *J Agric Food Chem*. 2003;51:4764–4768.
- Rodriguez-Lopez JN, Fenoll LG, Tudela J, et al. Thermal inactivation of mushroom polyphenol oxidase employing 2450 MHz microwave radiation. *J Agric Food Chem*. 1999;47:3028–3035.
- Chan EWC, Eng SY, Tan YP, Wong ZC, Lye PY, Tan LN. Antioxidant and sensory properties of Thai herbal teas with emphasis on *Thunbergia laurifolia* Lindl. *Chiang Mai J Sci*. 2012;39:599–609.
- Chan EWC, Lim YY, Chong KL, Tan JBL, Wong SK. Antioxidant properties of tropical and temperate herbal teas. *J Food Compos Anal*. 2010;23:185–189.