



Antioxidant activity and oxalate content of selected Thai herbal teas

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Abstract

The extracts of 20 Thai herbal teas were evaluated for antioxidant activity by ferric reducing antioxidant power (FRAP) assay, total phenolic compounds using a modified Folin-Ciocalteu method, and calcium and oxalate contents using atomic absorption spectrophotometry. The results showed that the highest level of antioxidant activity was found in the tea extract of *Phyllanthus emblica* Linn., followed by *Momordica charantia* Linn. and *Pluchea indica* (L.) Less., respectively. The herbal extract that had the highest phenolic compounds was also *Phyllanthus emblica* Linn. A positive linear relationship between the antioxidant activity and total phenolic content of tea extracts (r value = 0.92) was found, indicating that phenolic compounds were the dominant antioxidant components in the studied herbal teas. Although the strong antioxidant effects present in herbal teas, high levels of calcium and oxalate can increase the risk of renal stone formation, leading to kidney stone disease. Therefore, from this present study, the recommended tea to consume is *Phyllanthus emblica* Linn. because of its low levels in calcium and oxalate and high levels of natural antioxidants.

1. Introduction

Tea has been consumed worldwide, especially in Asian countries. Numbers of scientific evidence indicated that tea extracts have antioxidant properties and health benefits including reducing risks of coronary heart disease, hypertension, cancers, and arthritis (1-4). Most of the beneficial effects come from antioxidants present in a variety of teas. Antioxidants can suppress free radicals and protect cells against oxidative stress, therefore preventing cell damages and diseases (5). For

instance, a powerful antioxidant found in green tea, epigallocatechin-3-gallate, can reduce risks of coronary heart disease and cancers including those of prostate, breast and gastric as showed in several studies (6-9). It was also found that green tea can reduce body weight in obese Thais (10). Besides green tea and other Chinese teas, a wide variety of Thai herbal tea products were increasingly distributed all over the country. Consumers have increased interest in drinking herbal teas due to the belief that they have less side effects comparing to

synthetic medicine. Few studies reported the antioxidant activity and the effects of Thai herbal tea extracts (11-13). However, there has been some articles provided information on Thai medicinal plant such as *Phyllanthus emblica* Linn. and *Phyllanthus amarus* Schum. & Thonn. which were recently shown to have a protective effects against ethanol induced liver injury in rats (14, 15). Curcumin had also preventive and curative effects on the development of gastric inflammatory diseases in rats (16). The extract of *Garcinia hanburyi* Hook.f. could inhibit growth of cholangiocarcinoma cell lines in a dose-dependent manner which was due to apoptosis (17). In addition, it has been demonstrated that Rhinacanthone isolated from *Rhinacanthus nasutus* Kurz. roots induced apoptosis in human cervical carcinoma cells (18).

Nevertheless, there are studies showed that a number of plants, vegetables and nuts contain high levels of oxalate which is a primary risk factor for calcium oxalate kidney stones (19-21). Restriction of dietary oxalate and calcium can decrease the urinary excretion of oxalate, which in turn reduces risk of kidney stone formation. Moreover, it has been reported that tea is a main source of oxalate (22, 23). Thus, consuming teas with high levels of oxalate may increase urinary oxalate concentration, a significant factor of renal stone. However, the studies on the antioxidant activity and calcium oxalate content of Thai herbal tea extracts have not yet been reported. This present work is aimed to measure the levels of phenolic compounds, antioxidant activity and the levels of calcium and oxalate presented in currently available Thai herbal teas. We are also aimed to determine whether what kind of tea has good antioxidant commaeffects and low content of oxalate, which could be an alternative drink for people who tend to form calcium oxalate stones and for patients at risk for recurrent stone formation.

2. Materials and methods

Sample preparation and extraction

A total of 20 commercially available herbal teas were manufactured by Pathom Asoke Community, Nakhon Pathom Province, Thailand. The information on their scientific, family, English common and Thai vernacular names and their traditional uses is presented in Table 1. One gram of each tea was allowed to infuse in 20 mL of deionized hot water (98°C) for 5 minutes, under the same conditions of preparing drinking tea in everyday life. The extracts were filtered through a Whatman number 4 filter paper and adjusted to a final volume of 20 mL with deionized water.

Determination of total phenolic content

The total phenolic content of the tea extracts were determined according to a modified spectrometric method of Folin-Ciocalteu (24). One hundred microliters of the tea extract was added to 500 μ L of 0.2 M Folin-Ciocalteu reagent. After incubation at room temperature in the dark for 30 minutes, 400 μ L of 7% sodium carbonate was then added to the mixture. The absorbance of each sample was measured at 750 nm using a Spectronic® 20 genesys TM spectrophotometer. Gallic acid solution was used as a standard. Results are expressed as milligrams 100 g of gallic acid equivalents (GAE) per of dry weight (mg% GAE/ g dry wt.). All data are reported as the average of three measurements.

Determination of total antioxidant activity

The total antioxidant activity assay (Ferric reducing antioxidant power, FRAP assay) was performed according to a modified method of Benzie and Strain (25). The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, acetate 6-tri2-pyridyl-s-triazine (TPTZ) in 40 mM 2,4,6,-trl 2-pyridyld-s-triazinc (TPT2) in 40 mM

ngdrochloric acid HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in a 10:1:1 ratio. Nine hundred microliters of working FRAP reagent and ninety microliters of deionized water were mixed in a test tube and allowed to warm at 37 °C. Thirty microliters of each tea extract was added to the mixture and allowed to incubate at 37 °C for exactly 4 minutes. Due to the formation of blue colored Fe (II)-tripyridyltriazine complex, the change in absorbance was measured at 593 nm using a spectronic®20genesys TM spectrophotometer. Standard curve was prepared using different concentrations (100-1500 μM) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. All solutions were used on the day of preparation. The results are expressed in μM Fe (II)/g of dry weight. Presented data are average of three replications.

Determination of oxalate and calcium contents

The oxalate and calcium contents in teas were determined by the method of Sriboonlue *et al.* (26). Teas were diluted with 5% lanthanum. Oxalate was precipitated from prepared tea extract with excess calcium ions. Calcium oxalate precipitation was collected by mailliliters centrifugation at 1500g for 10 minutes. Two

mailliliters of 1 M hydrochloric acid was added into the precipitate, followed by addition of deionized water. The calcium content was determined using an atomic absorption spectrophotometer (AVANTA GBC Model Q442). The oxalate content was indirectly calculated from the calcium content of the calcium oxalate precipitate. All data are average of duplication.

3. Results and discussion

Total antioxidant activity and phenolic content in the studied teas are showed in Figure 1. Among 20 of Thai herbal teas, *Phyllanthus emblica* Linn. exhibited the strongest total antioxidant activity (116.67 mmol Fe(II)/g dry wt.), followed by *Phyllanthus amarus* Schum. & Thonn. (46.39 mmol Fe(II)/g dry wt.), and *Pluchea indica* (L.) Less. (35.14 mmol Fe(II)/g dry wt.), respectively. The highest level of phenolic content is found in the extract of *Phyllanthus emblica* Linn. (426.96 mg% GAE/ g dry wt.), followed by *Stevia rebaudiana* Bertoni. (259.10 mg% GAE/ g dry wt.), *Phyllanthus amarus* Schum. & Thonn. (257.43 mg% GAE/ g dry wt.), respectively. A positive correlation between the level of antioxidant activity and the phenolic

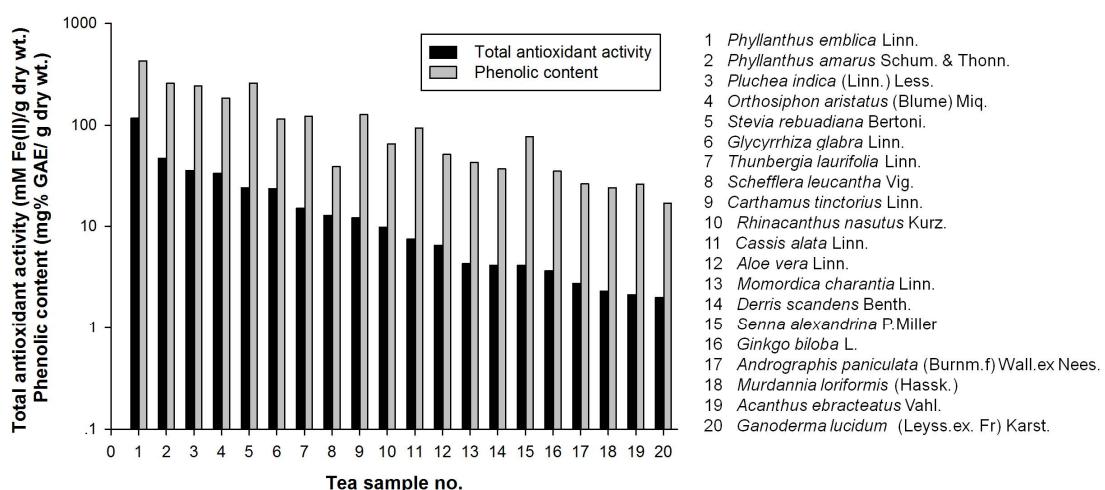


Figure 1. Total antioxidant activity and phenolic content of 20 Thai herbal teas. The numbers marked on the x-axis correspond to the scientific names indicated on the right. The y-axis has a logarithmic scale.

- 1 *Phyllanthus emblica* Linn.
- 2 *Phyllanthus amarus* Schum. & Thonn.
- 3 *Pluchea indica* (Linn.) Less.
- 4 *Orthosiphon aristatus* (Blume) Miq.
- 5 *Stevia rebaudiana* Bertoni.
- 6 *Glycyrrhiza glabra* Linn.
- 7 *Thunbergia laurifolia* Linn.
- 8 *Schefflera leucantha* Vig.
- 9 *Carthamus tinctorius* Linn.
- 10 *Rhinacanthus nasutus* Kurz.
- 11 *Cassis alata* Linn.
- 12 *Aloe vera* Linn.
- 13 *Momordica charantia* Linn.
- 14 *Derris scandens* Benth.
- 15 *Senna alexandrina* P.Miller
- 16 *Ginkgo biloba* L.
- 17 *Andrographis paniculata* (Burm.f) Wall.ex Nees.
- 18 *Murdannia loriformis* (Hassk.)
- 19 *Acanthus ebracteatus* Vahl.
- 20 *Ganoderma lucidum* (Leyss.ex Fr) Karst.

Table 1. Calcium and oxalate contents in Thai herbal teas used for this study with the information on their scientific, family, English common and Thai vernacular names, and their traditional uses.

No.	Scientific name	Family	Common names Thai (English)	Traditional uses	Oxalate (ppm/ g tea)	Calcium (ppm/ g tea)
1	<i>Murdannia loriformis</i> (Hassk.)	COMMELINACEAE	Yar-Pak-Khing (Rolla Rao et Kammathy)	Cures lymphadenoph- aty	89.50	54.67
2	<i>Andrographis paniculata</i> (Burnm.f.) Wall.ex Nees.	ACANTHACEAE	Fah-Ta-Lai-Joan (Chiretta)	Antipyretics	72.50	70.67
3	<i>Thunbergia laurifolia</i> Linn.	ACANTHACEAE	Rhang-Jeud	Antipyretics	66.00	23.33
4	<i>Momordica charantia</i> Linn.	CUCURBITACEAE	Ma-Ra-Khi-Nok (Bitter cucumber)	Antipyretics, appetizer	59.50	105.67
5	<i>Orthosiphon aristatus</i> (Blume) Miq.	LABIATAE	Yar-Nuod-Maew (Cat's whisker)	Diuretics	45.50	70.33
6	<i>Senna alexandrina</i> P.Miller	LEGUMINOSAE	Ma-Kham-Khak (Senna)	Laxatives	39.00	641.67
7	<i>Aloe vera</i> Linn.	ALOACEAE	Whan-Khang-Jor- Ra- Kae (Aloe vera)	Lowers blood sugar and used as laxatives	29.50	979.33
8	<i>Schefflera leucantha</i> Vig.	ARALIACEAE	HaNuMan-Pra- Sarn- Kay	Relieves cough and nausea	27.0	162.0
9	<i>Pluchea indica</i> (Linn.) Less.	COMPOSITAE	Kluu (Indian march fleabane)	Diuretics	24.50	455.67
10	<i>Cassis alata</i> Linn.	LEGUMINOSAE	Chum-Hed-Thed (Ringworm bush)	Laxatives	19.00	103.67
11	<i>Ginkgo biloba</i> L.	GINKGOACEAE	Pae-Gouy (Ginkgo)	Increases and improve blood flow, improving memory and thought	14.00	94.00
12	<i>Carthamus tinctorius</i> Linn.	COMPOSITAE	Dok-Kum-Foy (Safflower)	Blood tonic	13.00	56.00
13	<i>Rhinacanthus nasutus</i> Kurz.	ACANTHACEAE	Thong-Pan- Chung	Lowers blood pressure	13.00	122.00
14	<i>Acanthus ebracteatus</i> Vahl.	ACANTHACEAE	Ngeug-Pla-Mor (Sea holly)	Cures lymphadenoph- aty and relieves body aches, fever.	13.00	32.07
25	<i>Ganoderma lucidum</i> (Leyss.ex. Fr) Karst.	GANODERMA	Hed-Lhinn-Jeu (Linzhi or reishi)	Tonic	12.50	10.37
16	<i>Phyllanthus emblica</i> Linn.	EUPHORBIACEAE	Ma-Kham-Pom (Indian gooseberry)	Expectorant	12.00	28.53
17	<i>Stevia rebuadiana</i> Bertoni.	COMPOSITAE	Yar-Wharn (Stevia)	Sugar substitute in diabetic or hyperlipidemic patients without inducing weight gain	10.00	69.00

Table 1. Calcium and oxalate contents in Thai herbal teas used for this study with the information on their scientific, family, English common and Thai vernacular names, and their traditional uses. (cont.)

18	<i>Glycyrrhiza glabra</i> Linn.	LEGUMINOSAE	Cha-Aim-Thep (Licorice)	Expectorant, soothes dry throat	4.00	20.97
19	<i>Phyllanthus amarus</i> Schum. & Thonn.	EUPHORBIACEAE	Look-Tai-Bai	Antipyretics	1.50	136.33
20	<i>Derris scandens</i> Benth.	LEGUMINOSAE	Kra-Sai-Toa-Wan-Prieng (Jewel vine)	Relieves fatigue and malaise	1.50	17.33

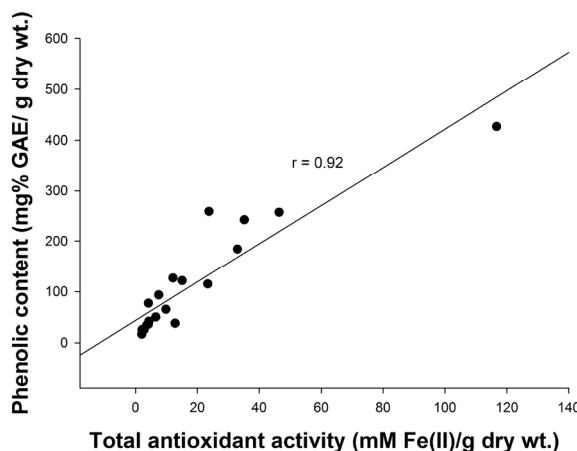


Figure 2. The correlation between total antioxidant activity and phenolic content of 20 Thai herbal teas.

content were observed (r value = 0.92) (Figure 2). Thus, it was concluded that phenolic compounds were the major antioxidant compositions in the studied herbal tea extracts. This result is consistent with earlier research which reported such positive correlation between total phenolic content and antioxidant activity (27, 28).

As shown in Table 1, *Murdannia loriformis* (Hassk.) had the highest level of oxalate (89.50 ppm/g dry wt.) among 20 kinds of tea. The top five low oxalate teas (1.50-12.00 ppm/g dry wt.) were *Derris scandens* Benth. (1.50 ppm/g dry wt.), *Phyllanthus amarus* Schum. & Thonn. (1.50 ppm/g dry wt.), *Glycyrrhiza glabra* Linn. (4.00 ppm/g dry wt.), *Stevia rebaudiana* Bertoni. (10.00 ppm/g dry wt.), and *Phyllanthus emblica* Linn. (12.00 ppm/g dry wt.), respectively. Tea extracts that had low calcium levels (10.37-28.53 ppm/g dry wt.) were *Ganoderma lucidum* (Leyss.ex. Fr) Karst. (10.37

dry wt.), *Glycyrrhiza glabra* Linn. (20.97 ppm/g dry wt.), *Thunbergia laurifolia* Linn. (23.33 ppm/g dry wt.), *Phyllanthus emblica* Linn. (28.53 ppm/g dry wt.), respectively. Nonetheless, the results showed that *Phyllanthus emblica* Linn. had low levels of both calcium and oxalate.

Conclusion

This study showed the different levels of antioxidant activity, phenolic compounds, and calcium and oxalate content of Thai herbal tea extracts. A high total antioxidant activity is related to the presence of phenolic compounds in the studied teas. *Phyllanthus emblica* Linn. which has a high level of antioxidant activity and a low content of calcium and oxalate is probably an alternative drink for people who tend to form calcium oxalate stones. Other biological effects of this set of herbal teas will be investigated further.

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