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 comama effects 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 µL of 0.2 M Folin-Ciocalteu reagent. After incubation at room temperature in the dark for 30 minutes, 400 µL 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 of the tea extracts 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-pyridyd-s-triazing (TPT2) in 40 mM
ngdrochioric acid HCl and 20 mM FeCl₃•6H₂O 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) -tripryidyltriazine complex, the change in absorbance was measured at 593 nm using a spectronic®20genesy TM spectrophotometer. Standard curve was prepared using different concentrations (100-1500 µM) of FeSO₄•7H₂O. 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.
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.

<table>
<thead>
<tr>
<th>No.</th>
<th>Scientific name</th>
<th>Family</th>
<th>Common names Thai (English)</th>
<th>Traditional uses</th>
<th>Oxalate (ppm/tea)</th>
<th>Calcium (ppm/tea)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Murdannia loriformis</em> (Hassk.)</td>
<td>COMMELINACEAE</td>
<td>Yar-Pak-Khing (Rolla Rao et Kammathy)</td>
<td>Cures lymphadenopathy</td>
<td>89.50</td>
<td>54.67</td>
</tr>
<tr>
<td>2</td>
<td><em>Andrographis paniculata</em> (Burnm.f) Wall.ex Nees.</td>
<td>ACANTHACEAE</td>
<td>Fah-Ta-Lai-Joan (Chiretta)</td>
<td>Antipyretics</td>
<td>72.50</td>
<td>70.67</td>
</tr>
<tr>
<td>3</td>
<td><em>Thunbergia laurifolia</em> Linn.</td>
<td>ACANTHACEAE</td>
<td>Rhang-Jeud</td>
<td>Antipyretics</td>
<td>66.00</td>
<td>23.33</td>
</tr>
<tr>
<td>4</td>
<td><em>Momordica charantia</em> Linn.</td>
<td>CUCURBITACEAE</td>
<td>Ma-Ra-Khi-Nok (Bitter cucumber)</td>
<td>Antipyretics, appetizer</td>
<td>59.50</td>
<td>105.67</td>
</tr>
<tr>
<td>5</td>
<td><em>Orthosiphon aristatus</em> (Blume) Miq.</td>
<td>LABIATAE</td>
<td>Yar-Nuod-Maew (Cat’s whisker)</td>
<td>Diuretics</td>
<td>45.50</td>
<td>70.33</td>
</tr>
<tr>
<td>6</td>
<td><em>Senna alexandrina</em> P.Miller</td>
<td>LEGUMINOSAE</td>
<td>Ma-Kham-Khak (Senna)</td>
<td>Laxatives</td>
<td>39.00</td>
<td>641.67</td>
</tr>
<tr>
<td>7</td>
<td><em>Aloe vera</em> Linn.</td>
<td>ALOACEAE</td>
<td>Whan-Khang-Jor-Ra-Kae (Aloe vera)</td>
<td>Lowers blood sugar and used as laxatives</td>
<td>29.50</td>
<td>979.33</td>
</tr>
<tr>
<td>8</td>
<td><em>Schefflera leucantha</em> Vig.</td>
<td>ARALIACEAE</td>
<td>HaNuMan-Pra-Sarn-Kay</td>
<td>Relieves cough and nausea</td>
<td>27.00</td>
<td>162.00</td>
</tr>
<tr>
<td>9</td>
<td><em>Pluchea indica</em> (Linn.) Less.</td>
<td>COMPOSITAE</td>
<td>Kluu (Indian march fleabane)</td>
<td>Diuretics</td>
<td>24.50</td>
<td>455.67</td>
</tr>
<tr>
<td>10</td>
<td><em>Cassis alata</em> Linn.</td>
<td>LEGUMINOSAE</td>
<td>Chum-Hed-Thed (Ringworm bush)</td>
<td>Laxatives</td>
<td>19.00</td>
<td>103.67</td>
</tr>
<tr>
<td>11</td>
<td><em>Ginkgo biloba</em> L.</td>
<td>GINKGOACEAE</td>
<td>Pae-Gouy (Ginkgo)</td>
<td>Increases and improve blood flow, improving memory and thought</td>
<td>14.00</td>
<td>94.00</td>
</tr>
<tr>
<td>12</td>
<td><em>Carthamus tinctorius</em> Linn.</td>
<td>COMPOSITAE</td>
<td>Dok-Kum-Foy (Safflower)</td>
<td>Blood tonic</td>
<td>13.00</td>
<td>56.00</td>
</tr>
<tr>
<td>13</td>
<td><em>Rhinacanthus nasutus</em> Kurz.</td>
<td>ACANTHACEAE</td>
<td>Thong-Pan-Chung</td>
<td>Lowers blood pressure</td>
<td>13.00</td>
<td>122.00</td>
</tr>
<tr>
<td>14</td>
<td><em>Acanthus ebracteatus</em> Vahl.</td>
<td>ACANTHACEAE</td>
<td>Ngeug-Pla-Mor (Sea holly)</td>
<td>Cures lymphadenophaty and relieves body aches, fever.</td>
<td>13.00</td>
<td>32.07</td>
</tr>
<tr>
<td>15</td>
<td><em>Ganoderma lucidum</em> (Leyss.ex. Fr) Karst.</td>
<td>GANODERMA</td>
<td>Hed-Lhinn-Jeu (Linzhi or reishi)</td>
<td>Tonic</td>
<td>12.50</td>
<td>10.37</td>
</tr>
<tr>
<td>16</td>
<td><em>Phyllanthus emblica</em> Linn.</td>
<td>EUPHORBIACEAE</td>
<td>Ma-Kham-Pom (Indian gooseberry)</td>
<td>Expectorant</td>
<td>12.00</td>
<td>28.53</td>
</tr>
<tr>
<td>17</td>
<td><em>Stevia rebaudiana</em> Bertoni.</td>
<td>COMPOSITAE</td>
<td>Yar-Wharn (Stevia)</td>
<td>Sugar substitute in diabetic or hyperlipidemic patients wintout inducing weight gain</td>
<td>10.00</td>
<td>69.00</td>
</tr>
</tbody>
</table>
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.

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.)

<table>
<thead>
<tr>
<th>#</th>
<th>Scientific Name</th>
<th>Family</th>
<th>English Common Name</th>
<th>Thai Vernacular Name</th>
<th>Traditional Use</th>
<th>Calcium (ppm/g dry wt.)</th>
<th>Oxalate (ppm/g dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Glycyrrhiza glabra Linn.</td>
<td>LEGUMINOSAE</td>
<td>Cha-Aim-Thed (Lorice)</td>
<td></td>
<td></td>
<td>4.00</td>
<td>20.97</td>
</tr>
<tr>
<td>19</td>
<td>Phyllanthus amarus Schum. &amp; Thonn.</td>
<td>EUPHORBIACEAE</td>
<td>Look-Tai-Bai</td>
<td></td>
<td></td>
<td>1.50</td>
<td>136.33</td>
</tr>
<tr>
<td>20</td>
<td>Derris scandens Benth.</td>
<td>LEGUMINOSAE</td>
<td>Kra-Sai-Toa-Wan-Prieng (Jewel vine)</td>
<td></td>
<td></td>
<td>1.50</td>
<td>17.33</td>
</tr>
</tbody>
</table>

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

Figure 2. The correlation between total antioxidant activity and phenolic content of 20 Thai herbal teas.
16. Mahattanadul S, Reammongkol W, Yano S,


