Antioxidant activities of *Pluchea indica* Less tea after *in vitro* digestion

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**ABSTRACT**

In this study, antioxidant activities of *Pluchea indica* Less. tea both before and after *in vitro* digestion were investigated. Such activities were performed using DPPH, reducing power assay, total phenolic and total flavonoid contents. Before the digestion, the result showed the greatest inhibition on DPPH with IC\textsubscript{50} = 0.05 ± 0.005 mg/ml. However, after digestions, the remarkable decrease of DPPH scavenging activity was observed in all stages (IC\textsubscript{50} = 0.34 ± 0.05 (oral), 0.44 ± 0.01 (gastric) and 0.42 ± 0.02 mg/ml (intestinal stage), respectively). A little difference of reducing power was found between pre-digestion (143.46 ± 0.04 mg quercetin equivalent (QE)/g sample) and post-digestion (154.67 ± 0.01 mg QE/g sample for oral stage, 240.08 ± 0.008 mg QE/g sample for gastric stage and 146.04 ± 0.001 mg QE/g sample for intestinal stage, respectively) at the highest concentration of the experiment. In addition, total phenolic and total flavonoid contents of samples in post-digestion have significantly increased (*p* < 0.05) when compared to the pre-digestion condition. Conclusively, the digestive process in GI tract possibly influenced to the antioxidant activity and bioactive compounds of *Pluchea indica* Less. tea.

**Keywords:** *Pluchea indica* Less. tea; *in vitro* digestion, DPPH, Reducing power, Total phenolic content, Total flavonoid content
1. INTRODUCTION

Pluchea indica Less. (Asteraceae) or locally known as Khlu is a widespread medicinal plant of Asia, especially India, Thailand, Malaysia and Philippines. It is a perennial shrub plant with medicinal properties and antioxidant activities which have many beneficial effects [1]. A decoction of the leaves has been used to combat fever. The sap expressed from leaves is used to treat dysentery. A poultice of leaves is applied externally to treat ulcers and soothe sores [2]. However, its antioxidant activity after digestion has not been studied. The aim of this study is to investigate the antioxidant activity of *P. indica* tea both before and after digestion that simulate the human gastrointestinal (GI) system. Such activities were performed using DPPH assay, reducing power, total phenolic and total flavonoid contents. *P. indica* tea may provide a potential natural source of bioactive compounds and may be beneficial to the human health.

2. MATERIALS AND METHODS

Preparation of extract

*P. indica* leaves were obtained from Chantaburi province, Thailand. Samples were cleaned, washed with water, cut into small pieces, dried overnight in a hot air oven at 60°C and rendered to smaller size particles using a grinder. *P. indica* (dried leaves) was ground into a fine powder. Powder (100g) was added into boiling distilled water (10L) and further heated for 30 min. Water extract was filtered by vacuum pump (GAST, USA) via Whatman filter paper No.1 (Whatman, UK) at room temperature. The remaining residues were re-extracted with boiling water as described above. Then, extract was centrifuged at 3,000 rpm for 5 minutes (K240R, Centurion Scientific, UK). The collected filtrate was evaporated by rotary evaporator and dried in a freeze-dryer (GAST, U.S.A). All extracts were weighed and stored at -20°C in an airtight container until use.

DPPH assay

The scavenging activity of *P. indica* tea was evaluated on the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals according to the previous procedure described [3]. Stock solution of *P. indica* tea. tea was prepared as sample in methanol. For each concentration, 50 µl of the test fraction was mixed with 100 µl of 0.2 mM DPPH in methanol in a 96-well plate, incubated at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm by a microplate reader. Percentage of inhibition was calculated using the following formula: OD(DPPH) – OD(DPPH + sample)/OD(DPPH)× 100 [4]. The IC_{50} value denotes the concentration of the sample that inhibited DPPH by 50%. All tests were employed in triplicate. Quercetin was used as positive control while methanol was the negative control [5].

Reducing power assay

1 mL of *P. indica* tea was mixed with 0.2mM phosphate buffer, pH 6.6 (2.5 ml) and 1% potassium ferricyanide (2.5 ml) and incubated at 50°C for 30 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3,000 rpm for 10 min whenever necessary [6]. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared 0.01% ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Quercetin at various concentrations was used for standard curve [7].

Total phenolic content

The total phenolic content of each sample was determined using Folin-Ciocalteu method [8] with slight modification. In this method, quercetin was used as a standard. Briefly, 125 µl of sample or the standard were mixed with 500 µl distilled water. 125 µl Folin-Ciocalteu reagent was added and then incubated for 6 minutes 1.25 ml of 7%(w/v) sodium bicarbonate was added then adjusted volume to 3 ml with distilled water and the incubation was allowed for 90 min. At the end, the absorption of each concentration was measured at 765 nm.

Total flavonoid content

The total flavonoid content was determined using a colorimetric method [9]. The sample was diluted with distilled water or methanol. The sample 0.5 ml was mixed with distilled water 2 ml. The next step adding 150 µl 5% NaNO_{2} and mixed. Then, 150 µl of 10% AlCl_{3} was added, the volume was adjusted to5 ml with distilled water and the incubation was allowed for 90 min. At the end, the absorption of each concentration was measured at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents/g extract.

In vitro digestion

An understanding of the basic physicochemical and physiological processes that occur as a food passes through the human gastrointestinal (GI) tract is required to develop effective *in vitro* models that accurately simulate digestion. After ingestion, food experience a complex series of physical and chemical changes as they pass through the mouth, stomach, small intestine, and large intestine, which affect their ability to be digested and/or absorbed.
However, this method was adapted from previous study [10-12]. Briefly, *P. indica* Less. tea were digested with a mixture of α-amylase (pH 6.8) for 1h for oral digestion, followed by 1h gastric digestion with pepsin (pH 3-4) and then 2h intestinal digestion with pancreatin and bile salts (pH 7-8). All steps of the digestion were incubated in shaking water bath at 37°C (55 oscillation/min) [13]. All digestive products were collected and stored at -80°C in an airtight container until analysis.

### 3. RESULTS

The antioxidant activity was investigated. The results of the assay were expressed as % inhibition of DPPH radical both pre and post-digestion in oral, gastric and in intestinal phases (Figures 1 and 2). Pre-digestion showed the great inhibition of DPPH by 84.77% at 0.1 mg/ml. After digestion, % inhibition of DPPH radical markedly decreased nearly tenfold. When considered the IC₅₀ to indicate the potential of this activity, the value in pre-digestion had lower than post-digestion of *P. indica* Less. The structural modification of bioactive compounds in sample due to the pH change during the GI system may influence to this scavenging activity [14].

![Figure 1. DPPH scavenging activity of *Pluchea indica* Less. tea prior to digestion.](image1)

![Figure 2. DPPH scavenging activity of *Pluchea indica* Less. tea after digestion.](image2)
The results of the reducing power, total phenolic contents and the total flavonoid content are shown in Table 1. All experiments were tested at a concentration of 1 mg/ml, and used quercetin as a reference for standard curve. No difference of reducing ability was observed both pre-digestion (143.46 ± 0.04 mg QE/g sample) and after the intestinal digestion (146.04 ± 0.001 mg QE/g sample). However, *P. indica* Less. tea was still effective for this activity. Total phenolic and total flavonoid contents of *P. indica* Less. tea displayed with high amounts at pre-digestion (196.70 ± 0.01 mg QE/g sample) while the post-digestion (230.41 ± 0.01 mg QE/g sample) significantly increased their phenolic and flavonoid contents. Especially, total flavonoid contents had the increasing trend in the intestinal stage. It may be due to some enzymes in the digestive system react with any bioactive compounds that present in *P. indica* Less. tea and thus free aglycone flavonoids were released [14-16].

Table 1. Antioxidant activities of *Pluchea indica* Less. tea at 1 mg/ml, which was used quercetin as a standard antioxidant.

<table>
<thead>
<tr>
<th>Method</th>
<th>Pre-digestion</th>
<th>Mouth</th>
<th>Stomach</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing power assay</td>
<td>143.46±0.04</td>
<td>154.67±0.01</td>
<td>240.08±0.008</td>
<td>146.04±0.001</td>
</tr>
<tr>
<td>Total phenolic content</td>
<td>196.70±0.01</td>
<td>158.54±0.01</td>
<td>312.64±0.005</td>
<td>219.02±0.004</td>
</tr>
<tr>
<td>Total flavonoid content</td>
<td>230.41±0.01</td>
<td>551.22±0.002</td>
<td>643.22±0.001</td>
<td>454.00±0.003</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± standard deviation (n = 3).

4. CONCLUSIONS

*P. indica* Less. tea demonstrates the great antioxidant activity before digestion. Although some antioxidant effect will reduce after digestion. However, *P. indica* Less. tea was still effective activity. The digestive process in GI tract possibly influenced to the antioxidant activity and bioactive compounds of *P. indica* Less. tea. This tea may be considered as an alternative healthcare to consumer. But the study of the human digestive system is difficult because there are many factors involved, such as pH, digestive enzymes and the stability of chemical structure of bioactive compounds in this herbal tea.

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REFERENCES


