A case study of Thai Herbal Formula “Tri-Ka-La-Phis”
and Antioxidant activities

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Abstract

Tri-Ka-La-Phis Herbal Formula is a Thai traditional herbal formula that has long been used in Thai traditional medicine as body tonic, natural aphrodisiac, the treatment of febrile seizure and blood disease. This formula consists of three crude drugs as followed; holy basil’s root, galangal’s rhizome and finger root. However, there is still border of knowledge between traditional indication and scientific evidence. The lack of scientific supports lead to the boundary of using this formula between traditional and modern medicine. Therefore, this research aims to provide the scientific data by measuring antioxidant and total phenolic content. This formula was prepared by mixing all three herbs in the proportion 1:1:1, decoction with water and dried using freeze dry method. The obtained extract was analyzed by antioxidant activity through DPPH assay and FRAP assay. Another part of extract was used to analyze the total phenolic content using folin ciocalteu method. The results showed that Tri-Ka-La-Phis Herbal Formula showed high antioxidant activity and total phenolic content. The results from this study can be used as promising data for corresponding the traditional indication and scientific confirmation.

Keywords: Antioxidant Activities; Tri-ka-la-phis; Herbal Formula
1. Introduction

Traditional medicine is defined as “the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness”. As at the state of art, herbal medicines have been using since very long time in the oriental countries including China and India as well as western countries. Medicinal plants have been using for many purposes including medicine, healthy food and cosmetics. The study of traditional Thai medicine is increasing in Thailand with the focus to use traditional wisdom to treat or to prevent the diseases as well as to promote health. However, the border of knowledge between traditional and modern medicine are still needed to be authenticate. The lacking of scientific evidence causes the incomprehensibility to connect the knowledge between two kinds of medicine. In Thai Traditional Medicine encompass of single herbs and formula herbs using for treatments. Tri-Ka-La-Phis herbal formula is counted as imperative formula for using as therapeutic medicine. This formula consists of three crude drugs as followed; holy basil’s root (Ocimum tenuiflorum L.) (Figure 1), galangal ‘s rhizome (Alpinia galanga (L.) Willd.) (Figure 2) and finger root (Boesenbergia rotunda (L.) Mansf.) (Figure 3) The medical properties of this formula are tonic, anti-influenza, blood detoxification and aphrodisiac effect decoction with water for drinking. Nowadays, the perception of using in modern medicine is limited according lacking of scientific data support. This leads to the border of traditional and western medicine. This study aims to cross border of this feature by studying to provide the scientific evidence of Tri-Ka-La-Phis herbal formula. We anticipate that this study will construct the synchronizing data of traditional wisdom and scientific substantiation.
2. Methodology

2.1 Preparation of sample extract

In our study, Herbal ingredients of Tri-Ka-La-Pis formula are locally available medicines. This formula can be prepared by mixing all three herbs in the proportion 1:1:1, decoction with water until the water volume is reduced into one third or so called boil 3 take 1 and dried using freeze dry method. Then, filtered to collect the supernatant and freeze dry to yield the extract and kept at -20°C.

2.2 DPPH assay (modified from Yamazaki et al., 1994)

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay modifications from Yamazaki et al., 1994. Briefly, 0.2 mM DPPH solution was mixed with Tri-Kha-La-Phis extract prepared with various concentration ranged from 1 μg/ml-1000 μg/ml in 96well plate and incubated in the dark at room temperature for 30 minutes. The absorbance of the mixture was then measured at 517 nm. Trolox® was used as a positive control. The ability of the sample to scavenge DPPH radical was determined from inhibition concentration at 50 % (IC50) as follows: IC50 = [(A0-A1)/A0] ×100

2.3 FRAP assay

Ferric reducing or antioxidant power (FRAP) was modified from Benzie et al.,1996. FRAP reagent was prepared from acetate buffer pH 3.6, TPTZ (2,4,6Tripyridyl-s-triazine) 10 mM in HCl 40 mM and Ferric chloride 20 mM. All reagents were mixed in with the ratio of 10/1/1 (v/v). The reagent was mixed with the extracts of Tri-Pha-La-Phis in various concentrations. The absorbance of the mixture was then measured at 593 nm. Trolox® was used as a positive control. The absorbance was compared to the standard curveof Fe (II) Sulphate7-hydrate and the antioxidant activity was reported as Trolox Equivalence Antioxidant Capacity (TEAC).
2.4 The total phenolic

Total phenolic content of the extract was determined by the Folin–Ciocalteu method modified from Yamazaki et al. (1994). Briefly, of Tri-Ka-La-Phis extract (1 mg/mL) were made with distilled water, mixed thoroughly with Folin–Ciocalteu reagent followed by the addition of sodium carbonate. The mixture was allowed to stand for a further 30 minutes and absorbance was measured at 750 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as µg/ml of gallic acid equivalent.

3. Discussion and Conclusion

The results showed that total antioxidant activity and total phenolic content showed high antioxidant activity and total phenolic content as shown in Table 1.

As the results showed in table 1, Tri-Ka-La-Phis herbal formula exhibited the high antioxidant properties when compared to other studies (Alafiatayo, A.A., Syahida, A. and Mahmood, Ma, 2014; Barik, B.R., Kundu, A.B. and Dey, A.K., 1987; Benzie, I. and Strain, J., 1996; Brighente, I. et al., 2007; Chew, Y.L. et al., 2008; Deepak, M.K. et al., 2015 and Wangcharoen, W. and Morasuk, W., 2007). The antioxidant activity and total phenolic content are related to tonic, anti-influenza, blood detoxification and aphrodisiac effect (Wattanathorn, J., 2012). We suggested that the results from this study can be used as promising data for declaring the traditional wisdom. Tri-Ka-La-Phis herbal formula may be considered to use as an alternative medicine both in traditional and modern treatment manner.

Table 1: Antioxidative properties (DPPH and FRAP assays) and the total phenolic content of Tri-Ka-La-Phis herbal formula

<table>
<thead>
<tr>
<th>Experimental tests</th>
<th>Results</th>
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<tbody>
<tr>
<td>DPPH assay</td>
<td>IC50= 137.60 µg/ml</td>
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<tr>
<td>FRAP assay</td>
<td>60.04 mg Trolox/g crude extract</td>
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<tr>
<td>The total phenolic</td>
<td>332.63 µgGAE/mg</td>
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References


