

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF DIFFERENT FORMULA OF HEART TONIC HERBAL TEAS

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Abstract: Heart tonic herbal teas were developed to select herbal teas to enhance cardiovascular health. The herbal teas were separated into 2 formulas: first, *Pleurotus ostreatus*: *Pandanus amaryllifolius*: *Oryza sativa* cv. KDML 105 (brown rice): *Oryza sativa* cv. KDML 105 (leaves): *Clitoria ternatea*; 1: 1:1:1:1 (w:w:w:w;w) and second, *Ganoderma lucidum*: *Caesalpinia sappan*: *Aegle marmelos*: *Chrysanthemum indicum*: *Carthamus tinctorius*; 1:1:1:1:1 (w:w:w:w;w), which were extracted with aqueous infusion. Phytochemical screening was used to examine flavonoid and phenolic acid compounds, using High-Performance Liquid Chromatography (HPLC), and the total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined. The antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Ferric reducing antioxidant Power (FRAP) assay. The results showed that the second formula contained significantly higher TPC, TFC, flavonoid compounds and phenolic compounds than the first formula. Antioxidant activities with DPPH and FRAP assay of the second formula (16.88 ± 0.25 mgTE/g and 62.97 ± 2.45 mM Fe²⁺ E/g, respectively) was significantly higher than the first formula (5.41 ± 0.03 mgTE/g and 13.57 ± 1.10 mM Fe²⁺ E/g, respectively). These findings indicated that the second formula of the heart tonic herbal tea has an inhibitory free radical effect and has the potential to improve cardiovascular health following its consumption.

Keywords: Heart Tonic Herbal Teas, CVDs, Phytochemical screening, Antioxidant.

Introduction

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels and they include coronary heart diseases, cerebrovascular diseases, rheumatic heart diseases, peripheral arterial diseases, congenital heart diseases, deep vein thrombosis and pulmonary embolism and other conditions. CVDs are the No. 1 cause of death globally, taking an estimated 17.9 million lives each year (WHO, 2017). Previously, it was predicted that by 2020, CVDs would be the most common cause of death (Trivedi & Nehra, 2004). The major risk factors for CVDs include family history, sex, raised lipid levels, hypertension, obesity, cigarette smoking, hypercholesterolemia, diabetes, and other lipid abnormalities. According to the National Health Survey of Thailand, about 20,000 deaths occur per year in the country due to CVDs. Many

synthetic drugs have been commonly used for the treatment of these diseases, but herbal medicines remain the popular choice in Third-World countries (Akhtar *et al.*, 2013). According to a World Health Organisation (WHO) survey, approximately 60% of the world's population rely on traditional herbal medicines (Zhang, 2000).

Free radicals cause many illnesses in humans, especially chronic diseases, such as cancer, heart disease, cerebrovascular disease, hypertension, kidney disease, cataract, and gout, through multiple mechanisms (Chan *et al.*, 2006). Oxidative stress has been shown to be actively involved in the development of CVDs, as well as cardiovascular-related complications by dysfunction of vascular smooth muscle cells, endothelial cells, and mononuclear cells, which can ultimately lead to CVDs (Higashi *et al.*, 2006;

Zhang *et al.*, 2014). In recent years, numerous studies have reported that phytochemicals from plants have antioxidant potential, which are risk factors for many diseases (Duthie & Crozier, 2000).

Herbal teas, or more accurately known as tisanes, are mixtures of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other plant parts that give them their flavor and provide therapeutic benefits (Ravikumar, 2014). In Thailand, they have gained popularity in recent years and are available in a variety of health food stores. Many herbal companies are interested in the production and distribution of herbal teas made from a variety of medicinal plants. Herbal teas are sold as ground herbs packed in tea bags, which can be consumed by steeping them in hot water. In addition, they are popular due to their pleasant fragrance, antioxidant properties and therapeutic applications (Chan *et al.*, 2012).

Heart tonic herbal teas have been developed to be an alternative in the enhancement of cardiovascular health and prevention of cardiovascular diseases. In this study, we selected 2 herbal teas with equal amounts of different herbal components in order to compare their phytochemical composition and antioxidant capacity. The first formula is composed of *Pleurotus ostreatus*, *Pandanus amaryllifolius* (leaf), *Oryza sativa* cv. KDML 105 (brown rice), *Oryza sativa* cv. KDML 105 (leaf) and *Clitoria ternatea* (flower). The second formula is composed of *Ganoderma lucidum*, *Caesalpinia sappan* (heartwood), *Aegle marmelos* (fruit), *Chrysanthemum indicum* (flower) and *Carthamus tinctorius* (flower).

A literature review related to the treatment of cardiovascular disease found that the phytochemical of *P. ostreatus* (Oyster mushroom) extract contained terpenoids, tanins, steroidal glycosides, carbohydrates, amino acid, vitamin B, and phenolic compounds (Iwalokun *et al.*, 2007). It has been shown to inhibit the activity of Type-2 diabetes in rats (Zhang *et al.*, 2016) and anti-hyperlipidaemic in hypercholesterolemic rats (Bobek *et al.*, 1997; Alam *et al.*, 2011), as well decrease the levels of blood pressure and

blood glucose in people (Choudhury *et al.*, 2015). The extract of *P. amaryllifolius* Roxb. leaves contains several compounds that act as a natural antioxidant and antidiabetic agents, such as essential oils, tocopherols, tocotrienols, alkaloid fatty acids, esters non-specific lipid transfer protein, carotinoids and flavonoids (Chiabchalard *et al.*, 2015). *O. sativa* cv. KDML 105, or Thai jasmine rice, is known as one of the highest quality rice varieties in Thailand (Semsang *et al.*, 2012). In addition, various parts of the rice, such as seeds, paddy, leaves, roots, rice stalks and boiling milk-like water, are used as Thai herbs to treat diseases. The seeds of Thai jasmine rice contain phytochemicals, such as carbohydrates, γ -aminobutyric acid (GABA), vitamin B, phytic acid, tocotrienols and phenolic compounds (Sripum *et al.*, 2017). Preliminary phytochemical screenings of the extract of *C. ternatea* flowers showed that they contained tannins, phlobatannin, carbohydrates, saponins, triterpenoids, phenols, flavanoids, flavonol glycosides, proteins, alkaloids, anthraquinone, anthocyanins, cardiac glycosides, stigmast-4-ene-3,6-dione, volatile oils and steroids (Al-Snafi, 2016). It has been shown to have antihyperglycemic and antihyperlipidemic effects in alloxan-induced diabetic rats (Daisy *et al.*, 2009).

The phytochemical of *G. lucidum* extract contains carbohydrates, glycosides, triterpenoids, steroids, phenolic compounds, and tannins, which decreased blood pressure and lipid levels in spontaneously hypertensive rats (Kabir *et al.*, 1998). The extract of *C. sappan* heartwood contains tannin, phenolic compounds, and several aromatic compounds, such as brazilin, brasilein, phellandrene, ocimene, sappanin and caesalpin (Pawar *et al.*, 2008). Brazilin isolated from *C. sappan* heartwood induces vasodilation of rat aorta (Yan *et al.*, 2015). The bael fruit (*A. marmelos*) contains many functional and bioactive compounds, such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, and terpenoids (Charoensiddhi & Anprung, 2008), which have been shown to act as an anti-diabetic (Kamalakkannan & Prince, 2003) and antihyperlipidaemic in streptozotocin diabetic rats (Kamalakkannan & Prince, 2004). The

phytochemicals of *C. indicum* flower extract contains flavonoids, terpenoids, phenolic compounds, vitamin and amino acid, which has anti-hypertensive and anti-stroke effects in stroke-prone spontaneously hypertensive rats (Zhao *et al.*, 2008). Furthermore, caryophyllene, p-allyltoluene, 1-acetoxytetralin and heneicosane have been identified as the major components of essential oils of *C. tinctorius* flowers, which have hypolipidemic effects (Rahimi *et al.*, 2014) and they reduce the blood pressure (Prasattong *et al.*, 2014) in diabetic rats.

Nevertheless, these herbal tea formulas were first developed as an alternative to enhancing cardiovascular health. Therefore, this study aims to determine the phytochemical (TPC, TFC and HPLC assay) and antioxidant activities (DPPH and FRAP assay) for confirmed pharmaceutical preliminaries, as well as to compare the phytochemical composition and antioxidant capacity of the two formulas of the best qualified heart tonic herbal teas.

Materials and methods

Materials

The heart tonic herbal teas were developed based on equal amounts of different herbal ingredients to compare their phytochemical composition and antioxidant capacity. The first formula was composed of *Pleurotus ostreatus*: *Pandanus amaryllifolius* (leaves): *Oryza sativa* cv. KDML 105 (brown rice): *Oryza sativa* cv. KDML 105 (leaves): *Clitoria ternatea* (flower); 1:1:1:1:1 (w:w:w:w;w). The second formula was composed of *Ganoderma lucidum*: *Caesalpinia sappan* (heartwood): *Aegle marmelos* (fruits): *Chrysanthemum indicum* (flowers): *Carthamus tinctorius* (flowers); 1:1:1:1:1 (w:w:w:w;w). All the fresh materials were cleaned and dried at 45-60°C for 48 h in a hot air oven and then powdered as shown in Table 1.

Preparation of Extracts

For extraction from teas, 1 g of tea in powder form was extracted by immersing in 75 mL of water at 80 °C for 15 min (1:75 w/v). Extracts

were filtered through a 0.45 µm membrane filter. The extracts were separated into two parts. Part 1 was stored at 4°C for analysis of TPC, TFC, DPPH and FRAP assay. Part 2 was stored at -18°C for analysis of HPLC. Analyses of aqueous tea extracts were done in triplicate.

Determination of Total Phenolic Content (TPC)

The total phenolic content was determined according to a modified procedure from Singleton *et al.* (1999). The extracts of the herbal tea formulas (100 µL) were oxidized with 500 µL of 0.2 N Folin-Ciocalteu's reagent and neutralized by adding 400 µL of 7.5% Na₂CO₃. The absorbance was measured at 765 nm by a UV-Vis spectrophotometer after being mixed and incubated in room temperature for 30 min. The results were expressed as gallic acid equivalent (mgGE/gExt).

Determination of Total Flavonoid Content (TFC)

The flavonoid content was estimated using the aluminum chloride colorimetric method (Sharma & Agarwal, 2015). The extracts from the herbal tea formulas (500 µL), 2,000 µL distilled water and 150 µL 5% NaNO₂ solution were added. After 6 min, a 150 µL 10% AlCl₃ solution was added and kept for another 6 min. To this reaction mixture, 2,000 µL 4% NaOH solution and 200 µL water was added to make up the final volume of 5,000 µL. The reaction mixture was mixed well and allowed to stand for 15 min, after which absorbance was recorded at 510 nm. The total flavonoid content (TFC) was calculated from a standard rutin equivalent (mgRE/gExt).

High-Performance Liquid Chromatography (HPLC) Analysis

Analyses were conducted to compare the amount of some flavonoid and phenolic acid compounds that were potentially beneficial in the herbal tea formulas using the High-Performance Liquid Chromatography (HPLC) method according to Jorjong *et al.* (2015). The HPLC-DAD system (Shimadzu, Kyoto, Japan), comprising Shimadzu LC-20AC pumps, a SPD-M20A diode array

Table 1: Details of the composition, parts used, ratio, harvesting time, drying and the source of raw materials

Materials	Parts Used	Ratio	Harvest time	Drying	Raw material source (in Thailand)
1st Formula					
<i>Pleurotus ostreatus</i>	Fruiting body	1	3 – 4 month	60°C /48 h	Lom and Fon Organic Farm Co., Ltd., Udon Thani province
<i>Pandanus amaryllifolius</i>	Leaves	1	-	45°C /48 h	Thong In pharmacy Co.,Ltd., Maha Sarakham province
<i>Oryza sativa</i> cv. KDML 105	Brown rice	1	6 - 7 month	45°C /48 h	farmer in Kham Rieng sub district, Kantharawichai district, Maha Sarakham province
<i>Oryza sativa</i> cv. KDML 105	Leaves	1	3 – 4 month	45°C /48 h	farmer in Kham Rieng sub district, Kantharawichai district, Maha Sarakham province
<i>Clitoria ternatea</i>	Flowers	1	-	45°C /48 h	Thong In pharmacy Co.,Ltd., Maha Sarakham province
2nd Formula					
<i>Ganoderma lucidum</i>	Fruiting body	1	3 – 4 month	60°C / 48 h	Chok Taweasap Mushroom Farm, Maha Sarakham province
<i>Caesalpinia sappan</i>	Heart wood	1	-	45°C / 48 h	Thong In pharmacy Co.,Ltd., Maha Sarakham province
<i>Aegle marmelos</i>	Fruits	1	-	45°C / 48 h	Thong In pharmacy Co.,Ltd., Maha Sarakham province
<i>Chrysanthemum indicum</i>	Flowers	1	-	45°C / 48 h	Thong In pharmacy Co.,Ltd., Maha Sarakham province
<i>Carthamus tinctorius</i>	Flowers	1	-	45°C / 48 h	Thong In pharmacy Co.,Ltd., Maha Sarakham province

detector and an Apollo C-18 column (Alltech Associates, Deerfield, IL, USA) (4.6 mm x 250 mm, 5 µm) protected with guard column Inertsil ODS-3 (4.0 mm x 10 mm, 5 µm) (GL Science Inc., Tokyo, Japan) were used for the analysis of flavonoids and phenolic acids. The mobile phase for flavonoids used acetonitrile/deionized water (2/97.8, v/v) containing 0.2% phosphoric acid (solvent A) and acetonitrile/deionized water (97.8/2, v/v) containing 0.2% phosphoric acid (solvent B) at a flow rate of 0.6 ml/min and 40°C column temperature. The UV-Vis spectra were detected at 254 nm. (Butkhup & Samappito, 2008). The mobile phase for phenolic acids consisted of acetonitrile (solvent A) and phosphoric acid in deionized water pH 2.58 (solvent B) at a flow rate of 0.8 ml/min and 40°C column temperature. The content of flavonoid

compounds were catechin, myricetin, quercetin and kaempferol, and the phenolic compounds, including gallic acid, syringic acid and cumaric acid, were analyzed by comparing the retention time and spectrum, as well as standard addition.

Antioxidant Activity by DPPH Assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacities of extracts were estimated by the reduction of the reaction color between the DPPH solutions and sample extracts, as previously described by a prior method by Ursini *et al.*(1994). The DPPH was dissolved in ethanol to a concentration of 0.039 mg/mL. The formula of extract at various concentrations was diluted with distilled water to provide sample solutions, 100 µL of the sample solution,

following which 900 μL DPPH (0.1 mM) working solution. After a 30 min reaction, which was kept in the dark at ambient temperature, the absorbance of the solution was measured at 515 nm. In this study, Trolox® was used as a standard substance. Blanks were run in each assay. The DPPH radical ability was expressed as IC_{50} (mg/mL) and the inhibition percentage was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_s) / A_0] \times 100$$

Where A_0 of control is the absorbance of the solvent mixed with DPPH solution and A_s is the absorbance of the extract solution.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted according to previous method (Benzie & Strain, 1996). The working solution was prepared by mixing 25 mL of acetate buffer pH 3.6 (3.1 g of $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ and 16 mL of CH_3COOH) to a concentration of 300 mM, 2.5 mL TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.5 mL of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution and incubating at 37 °C before use. Samples have a concentration of 1500 $\mu\text{g/mL}$ (200 μL) and could react with 2.5 mL of the working solution for 30 min in dark at 37°C. Absorbance was measured at 593 nm (n=3) using a UV-Vis spectrophotometer. Ferrous sulfate (FeSO_4) was used as standard to establish a standard curve. The FRAP antioxidant activity was expressed as mM of Fe^{2+} equivalents per g of samples (mM Fe^{2+}/g).

Statistical Analysis

All assays were expressed as mean \pm standard deviation (SD) from five separate experiments (n =5). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered to be significant.

Results and Discussion

Total phenolic compounds and total flavonoid contents

The total phenolic contents (TPC) were estimated using gallic acid, and total flavonoid contents (TFC) were estimated using rutin as a standard. Following TPC analyses, it was found that the second formula ($26.21 \pm 1.18 \text{ mgGE/gExt}$) was significantly higher than the first formula ($8.00 \pm 0.14 \text{ mgGE/gExt}$). Moreover, TFC from the second formula ($24.32 \pm 1.12 \text{ mgQE/gExt}$) still had significantly more content than the first formula ($3.87 \pm 0.55 \text{ mgGE/gExt}$) (Table 2). Phenolic compounds from plants, such as flavonoid, have antioxidant potential, which are risk factors for disease (Duthie & Crozier, 2000). Furthermore, phenolic compounds are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants with variable properties, and are thought to have positive effects on human health (Saeed et al., 2012).

A partial review of the literature concerning the phenolic and flavonoids of the herbs in the second formula found that the ethanol extracts of *Aegle marmelos* fruits have high contents of TPC ($87.34 \pm 4.44 \text{ mgGE/g DW}$) and TFC ($15.20 \pm 0.51 \text{ mgCE/g DW}$) (Charoensiddhi & Anprung, 2008), and *Ganoderma lucidum* extract had TPC (40.29 mgGE/gExt) and TFC ($469.9 - 627.7 \mu\text{g/gExt}$) (Gasecka et al., 2016b). It has been reported that the aqueous extract of *Pleurotus ostreatus*, which is in the first formula, showed TPC ($9.64 \pm 0.33 \text{ mg/gExt}$) and TFC ($2.11 \pm 0.19 \text{ mg/gExt}$) (Gasecka et al., 2016a). According to Ghasemzadeh & Jaafar (2014), studies on *Pandanus amaryllifolius* leaves state that methanol extract had TPC at 1.78 mg/g DW and TFC at 6.601 mg/g DW.

Flavonoids and Phenolic Acids

Analysis of some flavonoid and phenolic acid composition that were beneficial to the health of herbal tea formulas were performed using reversed-phase HPLC. Generally, the same

Table 2: The total phenolic contents (TPC) and total flavonoid contents (TFC) of different formulas of heart tonic herbal teas prepared through aqueous infusion

Samples	TPC (mgGE/gExt)	TFC (mgRE/gExt)
1 st Formula	8.00 ± 0.14 ^a	3.87 ± 0.55 ^a
2 nd Formula	26.21 ± 1.18 ^b	24.32 ± 1.12 ^b

TPC was measured with gallic acid equivalents (mgGE/gExt). TFC was measured with rutin equivalent (mgRE/gExt). Different superscript letters (a, b) in column represented significant differences ($p < 0.05$).

flavonoid and phenolic acid compounds were present in all plant species, but there were differences in relative levels (Jorjong *et al.*, 2015). In this study, a comparison was made between some of the flavonoids contents of different formulas of heart tonic herbal teas prepared through aqueous infusion consisting of catechin, myricetin, quercetin and kaempferol. The results showed that the first formula had significantly less flavonoid constituents than the second formula. The contents of catechin and myricetin in the first formula were 3946.26 ± 98.89 and 23.06 ± 0.03 $\mu\text{g/g}$, respectively. The contents of catechin, myricetin and quercetin in the second formula were 36985.68 ± 98.80 , 29.10 ± 0.01 and 53.24 ± 0.01 $\mu\text{g/g}$, respectively. However, kaempferol could not be detected in both formulas. In addition, quercetin is not found in the first formula (Table 3). Reviews of some herbs in the second formula, such as ethanol extracts from *Ganoderma lucidum*, detected flavonoids constituents consisting of rutin, quercetin and kaempferol (Gąsecka *et al.*, 2016b). The extracts of *Caesalpinia sappan* heartwood contain many flavonoids, such as ombuin, rhamnetin, sappan chalcone and sappanol (Badami *et al.*, 2004). The ethanol extracts of flowers of *Carthamus tinctorius* have kaempferol, 6-hydroxyl kaempferol and quercetin (Kazuma *et al.*, 2000). Reviews of some herbs in the first formula reported that the extract of *Pleurotus ostreatus* contains flavonoids, including myricetin and naringin (Kim *et al.*, 2008). Kaempferol, quercetin and myricetin were isolated from the petals in the flowers of *Clitoria ternatea* (Al-Snafi, 2016).

We also compared the phenolic acid content of different formulas from the heart tonic herbal teas prepared through aqueous

infusion consisting of gallic acid, syringic acid and cumaric acid. The results showed that the first formula had significantly less phenolic acid constituents than the second formula. The first formula consisted of gallic acid, syringic acid and cumaric acid at 1667.99 ± 91.20 , 52.15 ± 0.01 and 66.97 ± 0.01 $\mu\text{g/g}$, respectively. The second formula consisted of gallic acid, syringic acid and cumaric acid at 3793.55 ± 89.71 , 65.80 ± 0.01 and 84.51 ± 0.01 $\mu\text{g/g}$, respectively (Table 4). Past studies have reported that the extracts of *Ganoderma lucidum* in the second formula had phenolic acids constituents, consisting of chlorogenic acid, syringic acid, ferulic acid, *p*-coumaric acid, caffeic acid, 4-hydroxybenzoic acid and gallic acid, protocatechuic acid, vanillic acid, sinapic acid and *t*-cinnamic acid (Gąsecka *et al.*, 2016b), while the extracts of *Pleurotus ostreatus* in the first formula had shown phenolic acid constituents, which consisted of chlorogenic acid, syringic acid, ferulic acid, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic acid and gallic acid (Woldegiorgis *et al.*, 2014).

Antioxidant activity

The radical scavenging activities of different formulas of the heart tonic herbal teas by prepared through aqueous infusion were measured using two different assays, namely DPPH and FRAP, as shown in Table 5. The results found that the antioxidant activity the second formula (16.88 ± 0.25 mgTE/g and 62.97 ± 2.45 mM Fe²⁺ E/g, respectively) detected through DPPH and FRAP was significantly higher than the first formula (5.41 ± 0.03 mgTE/g and 13.57 ± 1.10 mM Fe²⁺ E/g, respectively). This is consistent with investigations of phytochemical substances, such as flavonoid and phenolic, which was found to be higher in the second formula than

Table 3: The flavonoid contents ($\mu\text{g/g DW}$) of different formulas of heart tonic herbal teas prepared through aqueous infusion

Samples	Catechin ($\mu\text{g/g DW}$)	Myricetin ($\mu\text{g/g DW}$)	Quercetin ($\mu\text{g/g DW}$)	Kaempferol ($\mu\text{g/g DW}$)
1 st Formula	3946.26 \pm 98.89 ^a	23.06 \pm 0.03 ^a	ND	ND
2 nd Formula	36985.68 \pm 98.80 ^b	29.10 \pm 0.01 ^b	53.24 \pm 0.01	ND

Total catechin, myricetin, quercetin and kaempferol, ND = not determined.

Different superscript letters (a, b) in column represented significant differences ($p < 0.05$).

Table 4: The phenolic acid content ($\mu\text{g/g DW}$) of different formulas of heart tonic herbal teas prepared through aqueous infusion

Samples	Gallic acid ($\mu\text{g/g DW}$)	Syringic acid ($\mu\text{g/g DW}$)	Cumaric acid ($\mu\text{g/g DW}$)
1 st Formula	1667.99 \pm 91.20 ^a	52.15 \pm 0.01 ^a	66.97 \pm 0.01 ^a
2 nd Formula	3793.55 \pm 89.71 ^b	65.80 \pm 0.01 ^b	84.51 \pm 0.01 ^b

Total gallic acid, syringic acid and cumaric acid, ND = not determined.

Different superscript letters (a, b) in column represented significant differences ($p < 0.05$).

the 1st formula. The phenolic compounds from plants, such as flavonoid and phenolic acids, have antioxidant potential, which is a risk factor for disease (Duthie & Crozier, 2000).

Previous studies have found that all herbs have powerful antioxidants. This result related well to the study by Yim *et al.* (2010), in which the aqueous extract of *Pleurotus ostreatus* showed an increase in antioxidant activity with the increase of concentration of extract of both DPPH (63.20%), and ABTS⁺ (87.29%), β -carotene-linoleate bleaching (83.51%) and FRAP methods (1.45 mM FE/100 g). The propylene glycol extract of *Pandanus amaryllifolius* leaves showed high antioxidant

activity, with an IC₅₀ value of DPPH of 0.810 mg/mL and the inhibition of peroxidation of linoleic acid of up to 90.1% (Jimtaisong & Krisdaphong, 2013). The aqueous flowers extract of *Clitoria ternatea* had antioxidant activity with an IC₅₀ value by DPPH (1.00 mg/mL), which is higher than the ethanol extract (4.00 mg/mL) (Kamkaen & Wilkinson, 2009). According to Kozarski *et al.* (2011), polysaccharide extracts of *Ganoderma lucidum* had antioxidant activity with an EC₅₀ value by DPPH of 7.07 mg/mL. Brazilin isolated from the heartwood of *Caesalpinia sappan* had antioxidant activity with an IC₅₀ value by ABTS⁺ of 28.796 $\mu\text{g/mL}$ (Wetwitayaklung *et al.*, 2005).

Table 5: The DPPH and FRAP radical scavenging activities of different formulas of heart tonic herbal teas prepared through aqueous infusion

Samples	DPPH (mgTE/g)	FRAP (mM Fe ²⁺ E/g)
1 st Formula	5.41 \pm 0.03 ^a	13.57 \pm 1.10 ^a
2 nd Formula	16.88 \pm 0.25 ^b	62.97 \pm 2.45 ^b

DPPH radical scavenging activity was used with Trolox as positive control for comparison. FRAP radical scavenging activity was reducing ferrous ion (Fe²⁺) radical from the heart tonic herbal teas.

Different superscript letters (a, b) in column represented significant differences ($p < 0.05$).

Conclusions

Each formula of the heart tonic herbal teas was shown to have antioxidant activity based on TPC, TFC, DPPH, and FRAP. Flavonoid compounds, identified by HPLC, consisted of catechin, myricetin and quercetin while phenolics were gallic acid, syringic acid and cumaric acid. We found that the second formula contained significantly higher TPC, TFC, flavonoid compounds and phenolic compounds than the first formula. Kaempferol could not be detected in both formulas. In addition, quercetin was not found in the first formula. It can be concluded that the first heart tonic herbal tea formula does have inhibitory-free radical effects and has the potential to assist in cardiovascular health via herbal tea consumption. However, further investigations are needed to identify and characterize the major active substance *in vivo* through clinical research, as well as determining related biological activities and the effectiveness of other extraction methods.

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