

Antioxidative Properties of White and Red Flowered Agathi (*Sesbania grandiflora*) Tea and Tea Extracts

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Abstract

The study aimed to examine the effect of agathi (*Sesbania grandiflora*) variety (i.e. red and white flower varieties), flower with and without pollen, and infusion times on the total phenol and tannin contents, and antioxidative activity (DPPH free radical scavenging and ferric reducing antioxidant power; FRAP) of agathi teas. The total phenol content, DPPH, and FRAP of the red flower agathi in form of dried tea were higher than those of the white variety ($p < 0.05$). The total phenol content of red and white flowered agathi teas were remained about 77 and 55 %, respectively (as dry basis weight) compared to the fresh flower agathi. In addition, the antioxidative activity of the agathi tea extracts significantly increased with increasing infusion time. The DPPH and FRAP of the tea extracts of flower agathi with pollen were slightly higher than those of the flower agathi without pollen tea extracts. After infusion the agathi tea with hot water (95 °C) for 10 min (time interval of 2 min), total phenol content, DPPH, FRAP, and tannin content of the red flowered agathi with pollen were the highest among all flower agathi teas ($p < 0.05$). Therefore, the agathi tea should be infused at 95 °C for 10 min to gain more bioactive compounds. The results indicated that the red flowered agathi tea had efficient antioxidative activity that they could be used as a potential source of natural antioxidants.

Keywords: Antioxidative properties, tannin, agathi, tea, tea extract

Introduction

Agathi (*Sesbania grandiflora*) is a common plant found in Asian countries, especially in India, Malaysia, and Thailand, and its flower and leave are normally consumed. It is adapted to both wet and dry regions of the tropics and is frost sensitive. It has large pea flowers which range in color from white and pink through to red. The flower agathi is rich in antioxidative properties as a result of polyphenols and flavonoids [1]. It has been reported that the red flowered agathi had high in anthocyanin content resulting in anti-inflammatory and antinociceptive activities [2]. Apart from its flower, the antioxidant compounds, such as gallic acid, caffeic acid, kaempferol, quercetin, and rutin, in agathi leaves were also found [3]. Hence, the antioxidant activity of the red flowered agathi were higher than that of the white flowered agathi because of the flower pigments, known as bioactive compounds such major phenolic compounds, to act as anti-cancer and anti-Alzheimer's [4].

Tea is one of the most widely consumed non-alcoholic beverages that rich in tannins, commonly referred to tannic acid, which are water-soluble polyphenol compounds. It is ordinary type from *Camelia sinensis*, however it could be made from edible stems, flowers and leaves of plants in forms of fresh and dried as herbal teas. They are consumed by infusion in boiling water to gain their fragrance, color and antioxidant properties [5-7]. Herbal teas could be considered as an alternative source of antioxidants to provide a health benefit due to polyphenol compounds present in plants like fruits and vegetables that

could be applied as dietary supplements [8], such as stevia (*Stevia rebaudiana*), mulberry (*Morus alba*), safflower (*Carthamus tinctorius*), senna (*Cassia angustifolia*), roselle (*Hibiscus sabdariffa*), ginger (*Zingiber officinale*), and finger root (*Boesenbergia rotunda*) teas [9,10]. The polyphenol compounds present in herbal tea may act as antioxidant, antimicrobial, anticarcinogenic and antimutagenic. On the other hand, a high concentration of tannin is often considered as antinutritional by inhibition of digestive enzymes, and preventing in vitamin and mineral utilization [11,12]. In general, phenolic compounds, which consist of a hydroxyl group directly attached to an aromatic ring, are the most abundant antioxidative components in most plant-based foods. They have a wide variety of structures, such as simple phenolic, phenolic acid and polyphenolic, therefore they can exhibit different functions, e.g. coloring, sensory, and biological functions as bioactive components having health benefits [13,14].

In the recent years, the study of antioxidant activity of many herbal tea plants is still limited. Therefore, this research aimed to determine bioactive compounds (phenolic and tannin) and antioxidant activity (DPPH free radical scavenging and ferric reducing antioxidant power; FRAP) of 2 agathi (*S. grandiflora*) varieties, including white and red flower varieties, in forms of tea (dried flower agathi) and tea extracts (water-soluble extract).

Materials and methods

Agathi tea processing

Two varieties of mature flower agathi (*S. grandiflora*), i.e. white and red flowered agathi, were collected from Phra Nakhon Si Ayutthaya, Thailand. The freshly collected plant materials were immediately clean, taken of the pollen (for the sample without pollen), washed thoroughly in running water and allowed to drain. After that, they were sliced into small pieces, placed on a tray and dehydrated in a cabinet tray drier at 60 °C until the weight remains constant, which were dried for 4 h 30 min to obtain dried flower agathi with roughly 13 % moisture content. The agathi tea samples were then stored at -18 °C in a dark container for determinations of total phenol content, tannin content, and antioxidant activity (namely DPPH and FRAP).

Acceptance test of agathi teas

The sensory evaluation of 4 developed agathi tea products (i.e., with and without pollen white and red flowered agathi) was carried out. One gram of each product was infused in hot water (95±2 °C). Sensory evaluation was done at room temperature with ambient light by 30 panelists. Five attributes were evaluated (i.e., color, flavor, taste, after-taste, and overall acceptability) using a 9-point hedonic scale where 1 meaning dislike extremely, 2 meaning dislike much, 3 meaning dislike moderately, 4 meaning dislike slightly, 5 meaning neither dislike nor like, 6 meaning like slightly, 7 meaning like moderately, 8 meaning like much, and 9 meaning like extremely.

Preparation of agathi tea infusions

The effect of infusion time on antioxidative properties of agathi teas was examine as following; 4 types of agathi teas (i.e., with and without pollen white and red flowered agathi) were prepared by taking 1 g of tea sample in a sealed tea bag into 200 ml hot water (95±2 °C) to provide water-soluble tea extracts. After that, the tea extracts were collected for 0, 2, 4, 6, 8 and 10 min in triplicate, and infusions were allowed for 15 min with constant stirring. Tea extracts were filtered using Whatman No. 1 filter paper, and stored at -18 °C in a dark place for antioxidative property determinations, namely total phenolic content, total tannin content and antioxidant activity (DPPH and FRAP) of tea extract samples.

Extraction for antioxidant activity determinations

The extracts for antioxidative property determinations were prepared as following; the agathi tea and water-soluble tea extracts were extracted with chilled 80 % acetone solution in a blender for 10 min to mainly contain the extraction of polar compounds. The slurry was vacuum-filtered over a Whatman no. 1 filter paper [modified from 15,16]. The extracts were stored in dark at -18 °C for further analysis. The

analyses of aqueous acetone extracts were done in triplicate, and the chemical for determination might be freshly prepared.

Total phenol content analysis

The Folin-Ciocalteu's reagent was used for total phenol content determination [17]. A 0.3 ml of aqueous acetone extract was added to 1.5 ml of Folin-Ciocalteu's reagent and 1.2 ml of 7.5 % sodium carbonate. After vortexing, the samples stabilised for 30 min at room temperature, and the total phenol content was determined by using a spectrophotometer at a wavelength of 765 nm. The standard curve was prepared using solutions of gallic acid (GA) and then total phenol content is expressed as mg GA equivalents in the sample.

DPPH free radical scavenging activity analysis

The DPPH assay can be used to determine the free radical scavenging capacity of the sample. A 4 ml of 80 % ethanolic 0.6 mM of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was mixed with 1 ml of the extract. The absorbance was measured at 515 nm after standing for 3 h (modified from [14]). The series of GA solutions were prepared for the standard curve, and DPPH value is expressed in mg GA equivalents in the sample.

Ferric reducing antioxidant power (FRAP) analysis

The FRAP determination of the extracts was determined according to the method of Lim *et al.* [17]. A 1 ml of the extract was mixed with 2.5 ml 0.2 M phosphate buffer at pH 6.6 and 2.5 ml 1 % potassium ferricyanide. The mixtures were incubated in a water bath at 50 °C for 20 min, and then 2.5 ml 10 % trichloroacetic acid was added. After that, 2.5 ml of the mixture was taken, and 2.5 ml distilled water and 0.5 ml 1 % FeCl₃ was added. The absorbance at 700 nm was measured after standing for 30 min at room temperature.

Total tannin content analysis

Total tannin content was examined by Folin-Ciocalteu's method with tannic acid as reference (adapted from [18]). A 0.2 ml of extract was added to a mixture of 2.5 ml of distilled water, 0.2 ml of Folin-Ciocalteu's reagent and 2 ml of 7 % Na₂CO₃. After vortexing, the samples stand for 30 min, and the total tannin content was determined by using an UV/Visible spectrophotometer at a wavelength of 700 nm. The standard curve was prepared using solutions of tannic acid and the results were expressed in mg tannic acid equivalents.

Statistical analysis

In the study, the data obtained from 3 replications were analyzed and shown as mean±standard deviation (SD). The significant differences among means of all treatments were subjected to analysis of variance (ANOVA), and the confidence limits used in this study were based on 95 % ($p < 0.05$).

Results and discussion

The antioxidative properties of the white and red flowered agathi tea

The untreated white and red flowered agathi samples were subjected to determine the antioxidative properties, and the results illustrate in **Figure 1**. The moisture content of all flower agathi samples was comparable values (about 90 %). Total phenol and tannin contents, and antioxidative properties (DPPH and FRAP) of the fresh red flowered agathi were significantly higher than those of the white variety ($p < 0.05$). The DPPH of red flowered agathi was 3.4 times higher than that of the white flowered agathi, and the FRAP values of untreated white and red flowered agathi samples were 0.51 and 1.68, respectively. According to the results, a high antioxidant activity in the red flowered agathi might be due to it contain phytochemical compounds, such as phenolic compounds, flavonoids and anthocyanins, etc., which have the ability to neutralize free radicals, resulting in many disease prevention and health promotion [1,2]. The results indicated that flower agathi could be considered as a potential antioxidant activity source like

found in some edible flowers, such as *Curcuma sessilis* Gage, *Dolichandrone serrulata* (DC.) Seem, and *Telosma minor* Craib. [19]. It was also found that flower of *Tupistra albiflora* K. Larsen showed remarkable antioxidant properties due to high content of essential oils, and rich in nutritional values such as protein, thiamin, riboflavin, and pyridoxine [20].

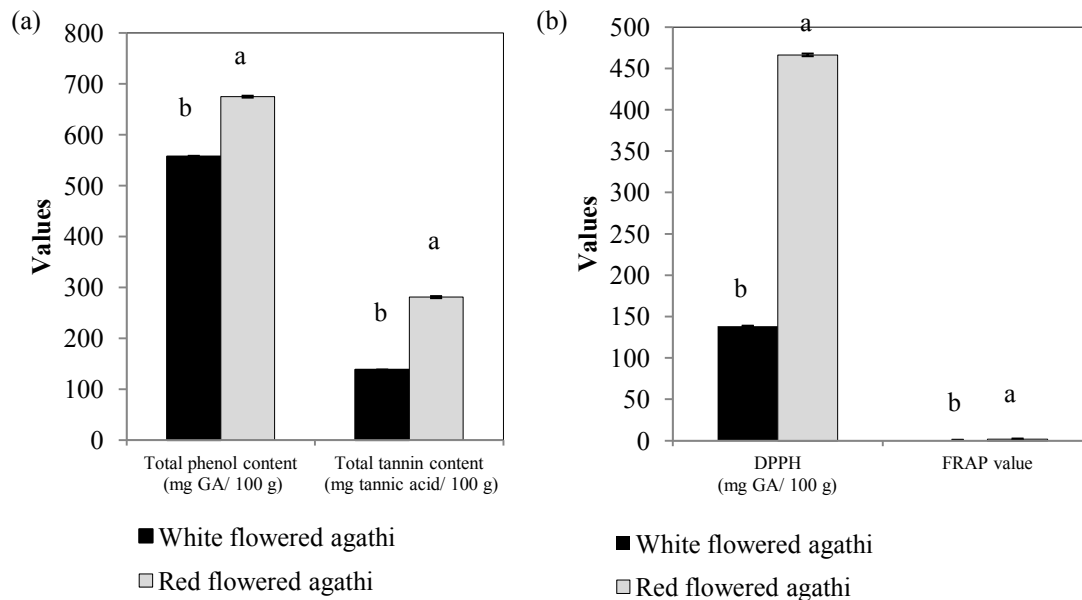


Figure 1 The antioxidative properties of the untreated white and red flowered agathi (dry basis weight); (a) total phenol and tannin contents, and (b) DPPH free radical scavenging method and ferric reducing antioxidant power (FRAP) values.

In the study, 4 samples of flower agathi, i.e. the white flowered agathi with pollen, the white flowered agathi without pollen, the red flowered agathi with pollen, and the red flowered agathi without pollen, were dried at 60 °C for 4 h 30 min to obtain the dried flowered agathi, which referred as agathi teas. The bioactive compounds of the dried agathi were examined, and the results show in **Table 1**. The moisture content of all flower agathi samples decreased from about 90 to 13 % after drying. Total phenol and tannin contents, and antioxidative properties (DPPH and FRAP) of the red flowered agathi tea were significantly higher than those of the white variety ($p < 0.05$). The higher FRAP value indicates an increasingly of the reducing power. The agathi teas with higher phenol content had stronger antioxidant activities. In addition, the antioxidative properties of the flower agathi with pollen tea were also slightly higher than those of the flower agathi without pollen tea. After drying, the total phenol content of the white and red flowered agathi tea remained up to 54.58 and 76.56 %, respectively, compared to the fresh agathi (dry basis). However, the total phenolic content of the red flowered agathi tea (516.56 - 562.53 mg GA/ 100 g, dry basis) had much higher than finger root (*B. rotundas*) tea (190 mg GA/ 100 g), whereas it had slightly lower than roselle (*H. sabdariffa*) tea (660 mg GA/ 100 g) [10]. These teas could be considered as herbal teas having antioxidant activities. This finding is in agreement with Siddhuraju *et al.* [21]. The drying temperature is also important to control the product quality to maintain the antioxidant activity. However, it has been reported that the antioxidant activities of the *C. sinensis* teas had much higher than some fresh vegetables, such as broccoli flowers, red bell pepper, onion, corn, sweet potato, and carrot [22]. In addition, Jung *et al.* [23] found that the antioxidant properties of dried persimmon decreased compared to the fresh persimmons through a drying [24]. In conclusion, the major results in

this study indicated that flower agathi showed a high content of polyphenols, having antioxidant potential. Apart from agathi tea, the flower agathi might be useful in dietary supplement for food applications, such as bakery products (i.e. cookie and bread).

Table 1 The antioxidative properties of the white and red flowered agathi tea (dry basis weight).

Agathi tea	Antioxidative properties			
	Total phenol content (mg GA/ 100 g)	DPPH (mg GA/ 100 g)	FRAP value	Total tannin content (mg tannic acid/ 100 g)
White flowered agathi tea				
With pollen	318.80±3.88 ^c	22.30±0.15 ^b	1.12±0.05 ^c	19.41±0.28 ^b
Without pollen	304.62±4.85 ^d	12.71±0.49 ^c	0.88±0.01 ^d	15.40±0.16 ^c
Red flowered agathi tea				
With pollen	562.53±4.28 ^a	28.78±0.07 ^a	1.52±0.05 ^a	20.79±0.31 ^{ab}
Without pollen	516.56±5.44 ^b	28.72±0.04 ^a	1.37±0.02 ^b	19.85±0.21 ^b

Values are means of triplicate measurements ± SD (n=3) and the superscripts with the different letters within the same column are significantly different ($p < 0.05$).

The acceptance test of the white and red flowered agathi tea

The acceptance of tea extracts of white flowered agathi with pollen, the white flowered agathi without pollen, the red flowered agathi with pollen, and the red flowered agathi without pollen after infusion in hot water were examined by sensory evaluation. The sensory scores (9-hedonic scale) of these water-soluble agathi tea extracts are shown in **Table 2**. It was found that tea from both varieties of flower agathi was preferred in terms of color, flavor, taste, after-taste and overall acceptability of the tea, which the panelists like the products moderately (scores were between 6.38 and 7.26). The sensory score of all attributes of 4 products were not significantly different ($p > 0.05$). This indicates that agathi tea with pollen was also preferred, even the pollen of the agathi flower is bitter. For color attribute, the red flowered agathi showed the reddish color due to the presence of anthocyanins, which is a water-soluble compound, and brilliantly red-purple pigments.

Table 2 The sensory score of the water-soluble white and red flowered agathi tea extracts.

Agathi tea extract	Sensory score				
	Color ^{ns}	Flavor ^{ns}	Taste ^{ns}	After-taste ^{ns}	Overall acceptability ^{ns}
White flowered agathi tea					
With pollen	6.76±1.33	6.74±1.12	6.46±1.36	6.40±1.50	6.68±1.19
Without pollen	6.88±1.29	6.66±1.35	6.60±1.31	6.52±1.45	6.70±1.22
Red flowered agathi tea					
With pollen	7.10±1.05	6.94±1.11	6.38±1.29	6.70±1.43	6.82±1.02
Without pollen	7.26±1.21	6.86±1.14	6.78±1.25	6.68±1.36	7.02±1.13

Values are means ± SD (n=30) and ^{ns} means were not significantly different ($p > 0.05$).

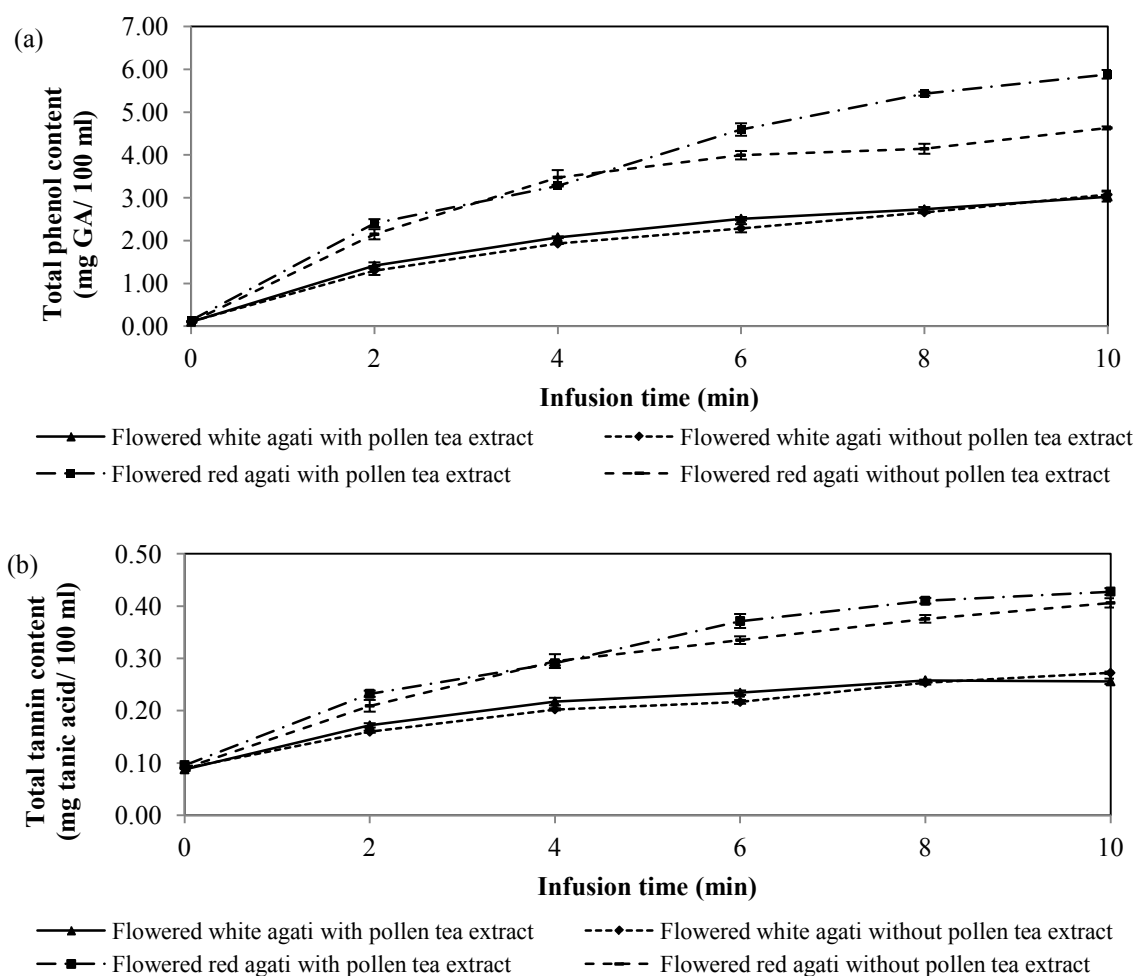


Figure 2 The influence of different infusion times on (a) total phenol content and (b) total tannin content of the white and red flowered agathi tea extract.

Effect of infusion times on the changes of antioxidative properties of the white and red flowered agathi tea extract

Four tea samples (i.e., the white flowered agathi with pollen, the white flowered agathi without pollen, the red flowered agathi with pollen, and the red flowered agathi without pollen teas) were infused in hot water at 95 °C for 10 min, collected the extract samples every 2 min, and determined the antioxidative properties of the flower agathi tea extracts. Total phenol and tannin contents of these 4 samples are shown in **Figure 2**, and the antioxidant activity (DPPH and FRAP) of studied agathi tea extracts are illustrated in **Figure 3**. Total phenol, tannin content, DPPH and FRAP of the tea extracts of both agathi varieties significantly increased when infusion time longer. After infusion for 10 min, these antioxidative properties of all samples were the highest ($p < 0.05$). This indicates that the infusion time affected bioactive compounds in tea extract. The high antioxidative properties in the red agathi tea extract were also observed compared to that of the white agathi tea extract. Hence, the antioxidative properties in the flower agathi with pollen tea extract were slightly higher than in the tea extract without pollen.

In addition, total tannin content of the red flower agathi for 10 min of infusion contained about 4 times higher compared to the initial infusion time (**Figure 2(b)**). The finding results are in good

agreement with the results of Rehman *et al.* [25] that the content of tannin, which could be found in tea extract because they are water-soluble polyphenols, of tea extract increased with an increase in boiling time. However, the excess content of tannin might course in decrease in sensory properties and also act as antinutritional.

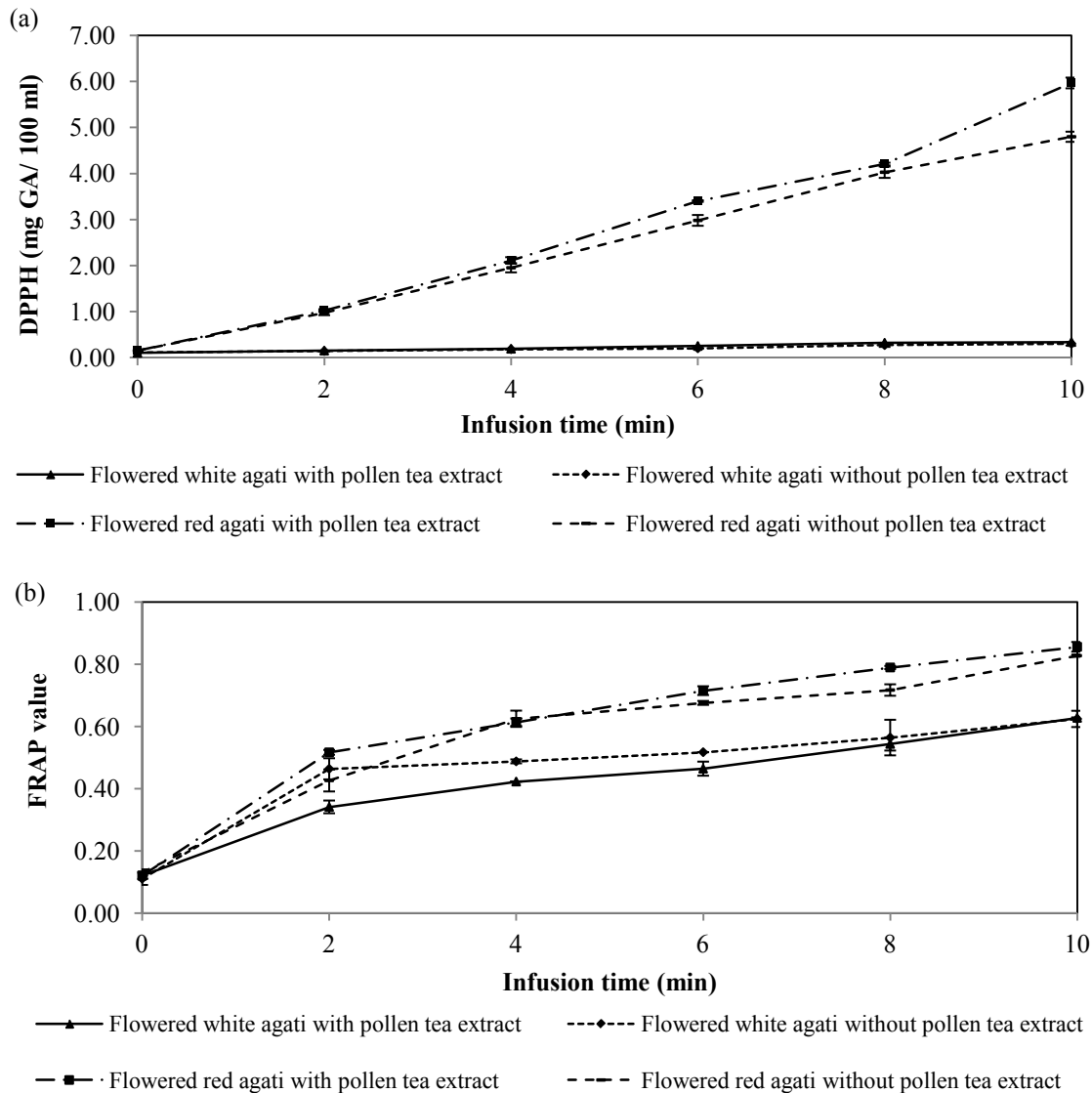


Figure 3 The influence of different infusion times on antioxidant activity by using (a) DPPH free radical scavenging method and (b) ferric reducing antioxidant power (FRAP) assay of the white and red flowered agathi tea extract.

Conclusions

The bioactive compounds of the tea made from 2 flower agathi (*S. grandiflora*) varieties, i.e. white and red flowered agathi were significantly observed. An excellent antioxidant activity and a high content of bioactive compounds were found in the untreated and dried (referred to the flower agathi tea) of the red flowered agathi, particularly in the flower agathi with pollen. Hence, the infusion time affected bioactive compounds and antioxidant activity in the water-soluble flowered agathi tea extract. Total phenol content, tannin content, DPPH and FRAP of all samples of water-soluble flowered agathi tea extracts gradually increased when infusion time longer. Therefore, the flower agathi tea, especially the red flowered agathi with pollen tea, could be recommended as a healthy beverage.

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