



Effect of infusion conditions on total phenolic content and antioxidant activity in *Centella asiatica* tea

[Kesan keadaan seduhan terhadap jumlah kandungan fenol dan aktiviti antioksidasi air teh pegaga (*Centella asiatica*)]

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Keywords: *Centella asiatica* tea, temperature, steeping time, antioxidant activity, total phenolic content

Abstract

The effect of different infusion conditions (water temperature, steeping time and multiple infusions) on the total phenolic content (TPC) and antioxidant activity (AOA) of *Centella asiatica* tea were studied. Total phenolic content and antioxidant activity were determined spectrophotometrically using Folin-Ciocalteu method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay respectively. The extraction efficiency of these compounds strongly depends on the infusion conditions. The results indicated that higher water temperature and shorter steeping time are the best combination for the extraction of TPC and AOA of *C. asiatica*. The highest TPC and AOA were detected in *C. asiatica* tea infused at 100 °C for 5 min and 80 °C for 10 min, while the lowest TPC and AOA were detected in infusions at 60 °C for 3 min. The study also showed that TPC and AOA were higher in the first infusion rather than second and third infusions. However, *C. asiatica* tea can be re-infused with the same leaves although the TPC and AOA decreased in the later infusions.

Introduction

Freshly prepared infusion of dried leaves, buds, flowers, seeds and roots of plants have been gaining popularity in recent years. Hundreds of different herbal teas are sold in health food stores either as pure or blended products such as pegaga (*Centella asiatica*), misai kucing (*Orthosiphon stamineus*), lemon grass (*Cymbopogon citratus*), bitter gourd (*Momordica charantia*) and mas cotek (*Ficus deltoidea*). These tropical herbal teas are appreciated because of its attractive aroma and taste characteristics as well as beneficial health effects.

Herbal teas beneficial health effects are thought to stem from polyphenols with antioxidant properties. Many studies have shown that polyphenol compounds extracted from herbal plants are good antioxidants against lipid peroxidation in phospholipid bilayers (Terao et al. 1994; Naithani et al. 2006; Aoshima et al. 2007), in biological systems (Guo et al. 1996) and against tumourigenesis and DNA damage (Anderson et al. 2001). Besides, herbal tea is also reported to reduce serum cholesterol levels and inhibit hypertension and mutagenesis (Yokozawa et al. 2002).

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Centella asiatica is a tropical medicinal plant with a long history of therapeutic uses for conditions such as dermal disorders, venous insufficiency and microangiopathy (Incandela et al. 2001). The plant contains chemicals called triterpenes such as asiaticoside, madecassoside, asiatic acid and madecassic acid that possess various physiological effects (Huang et al. 2004). Many studies have shown that it can be used as a tonic and for anti-inflammatory, diuretic, laxative, sedative and wound healing purposes (Guo et al. 2004; Li et al. 2007). It can be used to treat skin infections including leprosy and skin ulcers and appears to activate blood cleaning and immunity while stimulating deep replacement. It can also be used as a nerve tonic to improve memory and reduce mental fatigue (Inamdar et al. 1996).

In Malaysia, *C. asiatica* or more familiar known as 'pegaga' (local name) is commonly eaten raw as 'ulam' in the Malay cuisine. With the advent in food processing and packaging technology, this herbal plant has been processed into other convenience products, the most common of which is *C. asiatica* tea.

Recently, the tea prepared by infusing tea leaves in lower water temperature has become a new choice in Taiwan, in addition to traditionally prepared by infusing them in hot water (Lin et al. 2008). Researchers have found that using boiling water and longer steeping times increases the amount of polyphenols in green tea, however, there is no research carried out on herbal teas. Thus, the main objective of this study was to determine the effect of infusion conditions (temperature of water, steeping time and multiple infusions) on the extractability of total phenolic compounds and antioxidant activity in *C. asiatica* tea.

Materials and methods

Preparation of *Centella asiatica* tea

Centella asiatica tea was prepared based on the process reported by Siah et al. (2010). Fresh *C. asiatica* plants were obtained from

a grower at Paya Rumput, Malacca. The plants were trimmed to discard roots and dried leaves and subsequently washed twice in a rotary washer for 10 min each. They were then fed to a fabricated centrifuge to get rid of excessive water to shorten the time required to dry the leaves. Subsequently, these leaves were placed in trays and dried in the oven at 60 °C. The leaves were turned every 2 h to dry them evenly. The drying process took about 16–20 h to reach a moisture content of 8%. The dried leaves were then grounded and packed in cellulose sachets with 2.5 g each using the Vertical-Form-Fill-Seal machine. The sachets were sealed in low density polyethylene/aluminum/low density polyethylene (LDPE/AL/LDPE) bags until further use.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma Chemical Co. (USA). Folin-Ciocalteu reagent and methanol were purchased from Merck (Germany). All other chemicals used were of standard analytical grade.

Preparation of *Centella asiatica* infusions

In order to study the effect of different infusion conditions on the extractability of total phenolic content and antioxidant activity from *C. asiatica* tea, the following infusion procedures were applied:

- Different water temperature and steeping time: tea sachets (2.5 g) were infused in 200 ml of distilled water at 60, 80 and 100 °C. The infusions were left for 3, 5, 10, 30 and 60 min and stirred with a glass rod occasionally.
- Multiple infusions: tea sachets (2.5 g) were infused in 200 ml of distilled water at 100 °C for 5 min to produce first infusion. The same tea sachet was taken out and infused in another 200 ml of 100 °C distilled water for 5 min repeatedly to produce the second and third tea infusions.

Preparation of infusion extracts for analysis

Two ml of *C. asiatica* infusions were extracted with 20 ml of 60% methanol. The mixture was placed in a conical flask (wrapped with an aluminum foil) and kept overnight in a refrigerator at 4 °C. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract which was used for all analyses.

Determination of total phenolic content

Total phenolic content (TPC) of *C. asiatica* infusion extracts was determined according to the Folin-Ciocalteu method described by Singh et al. (2002), with some modifications. Methanol solution of the tea extract (0.5 ml) was mixed with a 10-fold dilution of Folin-Ciocalteu reagent (0.5 ml). The mixture was homogenized with a vortex and incubated for 5 min at room temperature before the addition of a sodium carbonate solution (1 ml, 20% w/v). The absorbance of the mixture was measured at 725 nm using UV/VIS spectrophotometer (Perkin-Elmer Lambda 25, USA) after standing for 40 min at room temperature. Gallic acid solutions (0.5 ml) in concentrations of 0.0–0.20 mg/ml were used to prepare a calibration curve. The estimation of phenolic content in the extract was carried out in triplicate. Results were expressed as gallic acid equivalents (GAE), which reflect the phenolic content as the amount of gallic acid in mg per litre of infusions.

Determination of antioxidant activity

The antioxidant activity of the *C. asiatica* infusions was evaluated by the DPPH free radical-scavenging method. The DPPH free radical-scavenging activity measurements were carried out according to the procedure of Lee et al. (1996), with some modifications. An aliquot of 200 µl sample extract or control sample (60% methanol) was added to 1 ml of 0.2 mM DPPH in anhydrous methanol. The mixture was homogenized by vortexing for 5 min and left to stand in the dark at room

temperature for 30 min. The absorbance of the samples and control was measured at 517 nm with 60% methanol as a blank using UV/VIS spectrophotometer (Perkin-Elmer Lambda 25, USA). For each sample, three separate determinations were carried out. The antioxidant activity was expressed as the scavenging percentage, calculated using the following formula,

$$\text{Scavenging (\%)} = \left\{ \frac{A_o - A_1}{A_o} \right\} \times 100$$

where A_o is the absorbance of the control sample, and A_1 the absorbance of the sample.

Statistical analysis

Data are presented as mean \pm SD for three separate determinations for each treatment. Experimental data were analysed using Analysis of Variance (ANOVA) and the significant differences among means were determined by Duncan Multiple Range Test (DMRT) at 5% levels using the Statistical Analysis System (SAS 9.1) computing program.

Results and discussion

Effect of water temperature and steeping time on total phenolic content

Phenolic compounds are ubiquitously present in vegetables, fruits, herbs, tea and juices; thus, they are an integral part of the human diet. Recently, they have received much attention since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain forms of cancer (Prior and Cao 2000; Kaur and Kapoor 2001). The use of herbal remedies, including the centella tea is popular as an alternative to standard Western allopathic medicine. Herbal teas are easy to make and constitute one of the most common methods of taking plant medicine.



Effect of infusion conditions on TPC and AOA

Table 1. Total phenolic content (mg/litre GAE) of *Centella asiatica* tea infused under different temperatures and steeping time

Steeping time	60 °C	80 °C	100 °C
3 min	30.20 ± 1.14bcC	40.91 ± 0.62cB	43.13 ± 0.14bA
5 min	30.06 ± 0.20cC	42.43 ± 0.63bB	45.99 ± 0.83aA
10 min	31.43 ± 0.99abC	45.53 ± 0.60aA	42.73 ± 0.97bB
30 min	32.06 ± 0.19aC	45.28 ± 0.60aA	42.31 ± 0.64bB
60 min	32.26 ± 0.41aC	45.84 ± 1.17aA	42.14 ± 0.26bB

a – c Means within a column with the same letters are not significantly different at 5% level

A – C Means within a row with the same letters are not significantly different at 5% level

The polyphenols have been reported to be the important antioxidant compounds in tea extracts by a number of researchers (Lin et al. 1996; Rice et al. 1996; Zhu et al. 2002). Our results confirmed the previous published reports (Lachman et al. 2003; Cheong et al. 2005; Rusak et al. 2008; Komes et al. 2010) that TPC in tea correlated with infusion temperature and time. As shown in *Table 1*, the TPC in *C. asiatica* tea increased with higher temperature and longer steeping times, and if lower temperatures were to be used, longer steeping times were required. The highest TPC (45.99 ± 0.83 mg/litre GAE) occurred with 5 min steeping at 100 °C and the lowest content (30.20 ± 1.14 mg/litre GAE) was obtained with 3 min steeping at 60 °C.

Sharma et al. (2005) brewed 3 g of green tea in 125 ml of 80 and 100 °C water for 3 min and found that tea infused at a higher temperature (100 °C) had higher level of gallic acid than those at lower temperatures, which was in agreement with our results. Other researchers also found that the extraction of the phytochemical compounds, namely flavonoid, was completed at about 4 min (Arts et al. 2000; Lakenbrink et al. 2000) which conformed with our results in this finding which showed that TPC was the highest after the leaves were infused for 5 min.

The highest level of TPC at 100 °C infusion was established after 5 min of steeping, but at 60 °C and 80 °C infusions, the highest readings were reached after

infusion for 10 min. Although longer steeping time at these two temperatures showed increased in TPC, there were no significant differences ($p > 0.05$) from that obtained at 10 min steeping time. Adverse results were observed in tea infused at 100 °C where steeping time beyond 5 min caused a decrease in TPC. The results obtained are in accordance with previous studies by Cheong et al. (2005) and Perva et al. (2006) who showed that prolonged infusion time at high temperature can lead to degradation of phenolic compounds.

Effect of water temperature and steeping time on antioxidant activity

Antioxidant activity (AOA) of *C. asiatica* tea prepared by different infusion conditions is shown in *Table 2*. Tea infused at 100 °C for 5 min and 80 °C for 10 min exhibited the highest AOA (52.8%), while tea infused at 60 °C for 3 min exhibited the lowest (32%). It was conjectured that the ground *C. asiatica* tea leaves could not swell completely at 60 °C water temperature for 3 min and had lower amount of AOA extracted. They would probably have swelled entirely at 100 °C for 5 min and 80 °C for 10 min, and therefore, the AOA at these two temperatures showed the highest readings. The order of AOA of *C. asiatica* tea influenced by different water temperatures was in accordance with the TPC determined by the Folin-Ciocalteu method. The increase in AOA was consistent with the increase of water temperature used for the extraction. These results implied that



Table 2. Total antioxidant activity (%) of *Centella asiatica* tea infused under different temperature and steeping time

Steeping time	60 °C	80 °C	100 °C
3 min	32.20 ± 0.40bC	44.31 ± 0.45cB	51.71 ± 1.15aA
5 min	32.66 ± 0.89abC	46.53 ± 0.78bB	52.82 ± 0.26aA
10 min	32.77 ± 0.95abC	52.79 ± 0.22aA	50.14 ± 0.27bB
30 min	33.34 ± 1.40abC	52.52 ± 0.71aA	49.75 ± 0.68bB
60 min	34.11 ± 0.89aC	52.32 ± 1.23aA	50.18 ± 0.35bB

a – c Means within a column with the same letters are not significantly different at 5% level

A – C Means within a row with the same letters are not significantly different at 5% level

Table 3. Total phenolic content and total antioxidant activity of *Centella asiatica* tea after multiple infusions

Number of extractions	TPC (mg/litre GAE)		Total antioxidant activity (%)	
		% extracted		% extracted
1 st infusion	45.39 ± 0.46a	62.06	52.99 ± 1.23a	62.67
2 nd infusion	16.01 ± 0.39b	21.89	18.03 ± 0.36b	21.33
3 rd infusion	11.74 ± 0.54c	16.05	13.53 ± 0.76c	16.00

a – c Means within a column with the same letters are not significantly different at 5% level

the antioxidant potential of *C. asiatica* tea is directly related to their phenolic content. Yen et al. (1993) also found that the AOA of peanut hull correlated with its TPC.

Therefore, the higher content of TPC at 100 °C infusions might explain their high antioxidant properties.

Prolong steeping time from 3 min to 10 min significantly ($p < 0.05$) increased the AOA of the *C. asiatica* tea infused at 80 °C. However, after 30 min and 60 min of steeping time, the AOA did not show any significant increase from that obtained at 10 min. Whereas, *C. asiatica* tea infused at 100 °C with steeping time for 3 min and 5 min did not have significant effect ($p > 0.05$) on its AOA but further steeping for 10, 30 and 60 min caused the AOA to decrease significantly ($p < 0.05$). According to Pinelo et al. (2004), the increase in the AOA could be explained by the strong tendency of polyphenols to undergo polymerization reactions. When the degree of polymerization exceeds a critical value, the increased molecular complexity and steric hindrance reduce the availability of hydroxyl groups in reactions with radicals, which causes a decrease in the antioxidant activity.

This may explain the observed decrease in AOA of the extracts, which occurred after the initial increase in this study.

Effect of multiple infusions on total phenolic content and antioxidant activity

In Japan, China and some other Southeast Asian countries, people are accustomed to making tea infusions three or four times with the same tea leaves. The results obtained for multiple infusions from this study are shown in Table 3. TPC and AOA from the first infusion were significantly higher ($p < 0.05$) than in second and third infusions. The first infusion has TPC and AOA of 45.39 ± 0.46 mg/litre GAE and 52.99 ± 1.23%, respectively. In the second infusion, TPC and AOA decreased significantly to 16.01 ± 0.39 mg/litre GAE and 18.03 ± 0.36% and these readings further decreased to 11.74 ± 0.54 mg/litre GAE and 13.53 ± 0.76% at third infusion.

The percentage of TPC and AOA extracted in each infusion was calculated based on a total of three infusions and is shown in Table 3. In the first infusion, 62% of TPC and AOA were extracted compared to 21% and 16% in second and third



Effect of infusion conditions on TPC and AOA

infusions respectively. As mentioned earlier, *C. asiatica* tea leaves swelled completely after infusion at 100 °C for 5 min and thus TPC and AOA were released most at the first infusion. The results of this study indicated that although TPC and AOA were mainly extracted after the first infusion, substantial amounts of TPC and AOA were still available in the second and third infusions.

Conclusion

Although herbal tea is a rich source of bioactive compounds, the extraction efficiency of these compounds strongly depends on the infusion conditions. The results showed that higher water temperature and short steeping time are the best combinations for the extraction of bioactive compounds from *Centella asiatica* tea. The maximum extraction efficiency from *C. asiatica* tea was achieved at 100 °C for 5 min or 80 °C for 10 min. The study also showed that *C. asiatica* tea can be re-infused although the first infusion showed higher levels of TPC and AOA, and gradually decreased in later infusions.

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Effect of infusion conditions on TPC and AOA

Abstrak

Kesan keadaan seduhan yang berbeza (suhu air, masa rendaman dan rendaman berulang kali) terhadap jumlah kandungan fenol (TPC) dan aktiviti antioksidan (AOA) air teh pegaga telah dijalankan. Jumlah kandungan fenol dan aktiviti antioksidan ditentukan secara spektrofotometrikal melalui kaedah Folin-Ciocalteu dan 2,2-difenil-1-pikrilhidrazil (DPPH). Keberkesanan pengekstrakan kedua-dua komponen ini sangat bergantung kepada keadaan seduhan. Keputusan yang diperoleh menunjukkan bahawa gabungan suhu air yang tinggi dengan masa rendaman yang pendek ialah yang terbaik untuk pengekstrakan komponen bioaktif daripada teh pegaga. Tahap TPC dan AOA yang paling tinggi dikesan pada air teh pegaga yang diseduh pada suhu 100 °C dan direndam selama 5 minit atau pada suhu 80 °C dengan rendaman selama 10 minit. Bacaan TPC dan AOA yang paling rendah pula dikesan pada air teh pegaga yang diseduh pada suhu 60 °C dengan rendaman selama 3 minit. Keputusan kajian juga menunjukkan bahawa bacaan TPC dan AOA lebih tinggi pada air teh pegaga dalam seduhan pertama berbanding dengan seduhan kedua dan ketiga. Walau bagaimanapun, teh pegaga boleh diseduh berulang kali dengan menggunakan daun teh yang sama sungguhpun kandungan TPC dan AOA telah berkurangan dalam seduhan seterusnya.



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