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From Herbal Teabag to Infusion—Impact of Brewing on Polyphenols and Antioxidant Capacity

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Abstract: Herbal teas, which are a rich and diverse source of polyphenols, have been widely consumed due to their association with various health benefits. Preparation techniques can significantly affect the level of polyphenols in a cup of tea. Thus, this study investigated the impact of different preparation techniques, including brewing time in hot water, microwave-assisted extraction with cold and hot water (cold and hot MAE) for both radiation time and power, and laboratory testing condition on extractability of polyphenols in infusion from a teabag. The results showed that brewing time using hot water significantly affected the extractability of polyphenols and antioxidant activity. Cold and hot MAE conditions also significantly affected the extractability of polyphenols and antioxidant activity from a teabag infusion. Hot brewing at 7 min and cold MAE at full power with second boiled (1.93 min on and 1 min off radiation) are recommended for the preparation of herbal tea from a teabag, as these conditions had comparable extractability of polyphenols and antioxidant activity in comparison with other preparation techniques. There are over 20 major chromatogram peaks, of which 7 were identified as gallic acid, catechin, caffeic acid, ferulic acid, epicatechin gallate, quercetin, and kaempferol, revealing potential health benefits of this herbal tea.

Keywords: herbal tea; polyphenols; extraction; phenolic compounds; antioxidant

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1. Introduction

Herbal teas have been consumed in many countries for centuries, and their consumption is continuing to rise due to their association with health benefits [1]. Herbal teas are different from traditional teas, which is popular beverage with many people, such as green tea, black tea, oolong tea, yellow tea, Pu-erh tea, white tea, and scented tea [2]. These standard teas are produced from the leaves of *Camellia sinensis*, whereas herbal teas are made from the leaves, flowers, seeds, fruits, bark, stems and roots of other plants [3]. Herbal teas are made from plant materials but are not entirely herbal medicines. Similar to standard teas, herbal teas are mainly consumed in the form of infusion of dried herbs brewed in hot water [4]. Herbal teas can be classified based on their types, such as cinnamon, dandelion, chamomile, ginger, turmeric, blends, and others. Alternatively, they can be categorized based in their health functions, such as cognitive health, gut and digestive health, multifunctional, and others. In addition, they are also classified based on their forms of use including teabags, loose leaves, and ready-to-drink herbal teas.

Herbal teas contain a rich and diversified source of polyphenols as they are prepared from different plant materials. For example, cinnamon tea is a rich source of procyanidins and catechins [5]. Dandelion tea contains high level of various phytochemicals including flavonoids and phenolic acids [6]. Chamomile tea is rich in flavonoids, such as apigenin, quercetin, patuletin, luteolin and their glucosides [7]. Peppermint tea has high levels of rosmarinic acid and flavonoids. Ginger tea has high levels of gingerol and shogaol [8].

Due to their high and diversified polyphenols, herbal teas have been known to possess antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, anti-atherogenic, anti-aging, cardioprotective, chemopreventive, hepatoprotective and neuroprotective activities [3]. Drinking herbal teas have been linked with various health benefits, such as prevention and treatment of various diseases, such as diarrhea, dysentery, cough, respiratory disorders, colds, influenza, cough, and fever [9].

It is well known that availability of polyphenols or phenolic compounds, which are linked with health benefits, in herbal tea is primarily dependent on preparation techniques. As such, it is important to understand the impact of different brewing methods and conditions on extractability of phytochemicals and antioxidant activity from a teabag to infusion, to identify the most suitable conditions for preparation of a high-quality cup of tea.

2. Materials and Methods

2.1. Materials

Herbal teabags were donated by Anteaox, a local company in Hunter Valley, NSW, Australia. Weight of herbal teabags were 2.15 ± 0.05 g. The herbal teabags were prepared from a mixture of herbs, including *Trifolium pratense* L., *Syzygium Aromaticum* L., *Cinnamomum cassia* L., *Camellia senensis* L., *Illicium verum Hook* F., *Curcuma longa* L., *Mentha spicata* L., *Polygonum cuspidatum*, and *Carum carvi* L. This herbal tea has a moisture content of 9.7% and water activity of 0.675. The teabags were stored in a sealed container and stored at −18 °C for further analysis.

All chemicals used in this study were of analytical or HPLC grade. Methanol and acetonitrile (HPLC grade) were purchased from Merck (Bayswater, VIC, Australia). Folin–Ciocalteu's phenol reagent, anhydrous sodium carbonate, sodium nitrite, ferric chloride, gallic acid, catechin, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), (6)26-hydroxy-2,5,7,8 tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ascorbic acid were purchased from Sigma-Aldrich Pty Ltd. (Castle Hill, NSW, Australia). Sodium acetate trihydrate and hydrochloric acid were purchased from Bacto Laboratories Pty Ltd. (Mt Pritchard, NSW, Australia). Aluminium chloride and sodium hydroxide was obtained from Thermo Fisher Scientific Pty Ltd. (Scoresby, VIC, Australia).

2.2. Methods

2.2.1. Experimental Design

Each teabag was extracted in 250 mL of water, which is an equivalent volume to a cup or a mug [10]. Four extraction techniques were applied for comparison of extraction efficiency of phenolic compounds, flavonoids and antioxidant activity as shown in Figure 1. To achieve total extraction of polyphenols, each teabag was moved up and down 10 times for each tested condition and was removed from the infusion and gently squeezed to remove captured water. The tea infusion was cooled on ice to room temperature (RT, 22 ± 1 °C) to minimize degradation, and then filtered using a Whatman No. 1 filter paper (Lomb Scientific, Taren Point, Australia) to remove the solids. The infusion was then stored at 4 °C for analysis of phenolics and antioxidant activity. All analyses were carried out in triplicate.

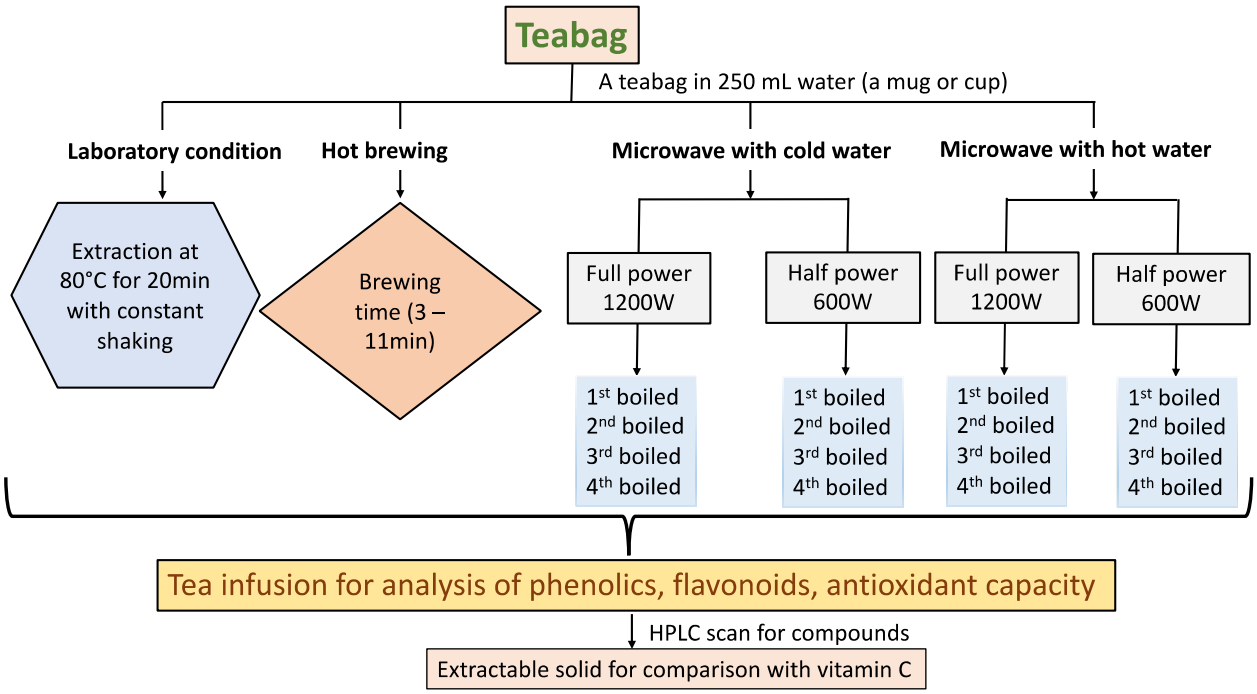

Figure 1. Experimental design of the current study.

A hot brewing technique was employed to indicate the impact of traditional brewing tea on extractability of phenolics and antioxidant activity. As brewing time is the most critical factor for this technique, as such a range of brewing time from 3 to 11 min was investigated. An herbal teabag was put in a mug, it was followed by adding 250 mL of boiled water. Extraction for each tested condition was done in triplicate. After completion of the brewing, the tea infusion was cooled and filtered for further analysis, HPLC scanning for individual compounds, and preparation of the solid extract for comparison of antioxidant with vitamin C.

A household microwave (1200 W, Frequency 2450 MHz, Sharp Carousel, Tokyo, Japan) was employed to assist extraction using hot water or cold water. For either hot or cold water, two microwave power levels, including full power at 1200 W and half power 600 W, were applied for comparison as these are more feasible and applicable for a household preparation. A teabag was put in a mug, it was followed by addition of 250 mL of either cold or boiled water. The mug was put in the microwave and heated to the first boiling observed, rested 1 min to prevent overflowing, then further heated for the second boiling, then subsequent resting 1 min and further heating to the third and fourth boiling. Each tested condition was conducted separately and was performed in triplicate. After completion of extraction, the tea infusion was cooled and filtered for further analysis. At RT of 20 °C, the radiation and resting time for each MAE condition are shown in Table 1.

A laboratory condition was also tested to compare the extraction efficiency between different extraction techniques. An optimal condition for extraction of tea at 80 °C for 20 min [11] was employed for extraction of the herbal teabag. A herbal teabag was put in a mug, followed by addition of 250 mL of water preheated at 80 °C and incubation at 80 °C for 20 min in a temperature-controlled shaking water bath (Ratek Instruments, Boronia, VIC, Australia) set at 60 rpm. After completion of the extraction, tea infusion was cooled and filtered for further analysis.

Table 1. Radiation and resting time of the MAE with hot and cold water at full and half microwave power under ambient temperature of 20 °C.

MAE Extraction	Boiling	Radiation Time	Resting Time [1]
MAE cold at 1200 W	First boiled	1.83 min	1 min
MAE cold at 1200 W	Second boiled	10 s	1 min
MAE cold at 1200 W	Third boiled	10 s	1 min
MAE cold at 1200 W	Fourth boiled	10 s	
MAE cold at 600 W	First boiled	3.83 min	1 min
MAE cold at 600 W	Second boiled	20 s	1 min
MAE cold at 600 W	Third boiled	20 s	1 min
MAE cold at 600 W	Fourth boiled	20 s	
MAE hot at 1200 W	First boiled	20 s	1 min
MAE hot at 1200 W	Second boiled	10 s	1 min
MAE hot at 1200 W	Third boiled	10 s	1 min
MAE hot at 1200 W	Fourth boiled	10 s	
MAE hot at 600 W	First boiled	50 s	1 min
MAE hot at 600 W	Second boiled	20 s	1 min
MAE hot at 600 W	Third boiled	20 s	1 min
MAE hot at 600 W	Fourth boiled	20 s	

[1] Resting time is the interval between each boiling when microwave was off to prevent water overflowing to microwave.

2.2.2. Analysis of Total Phenolic Content and Total Flavonoid Content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method, which was modified from a previous study [12]. An amount of 2.5 mL of 10% (*v*/*v*) Folin–Ciocalteu reagent was added to 0.5 mL of diluted tea infusion. The mixture was added with 2 mL of 7.5% (*w*/*v*) Na_2CO_3, then mixed and left at RT for 1 h before measurement of the absorbance at 765 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Mulgrave, VIC, Australia). Gallic acid Purity of over 97.5%, Sigma-Aldrich Pty Ltd., Castle Hill, NSW, Australia) was used as the standard and the results were expressed as mg of gallic acid equivalents per g of herbal tea (mg GAE/g).

Total flavonoid content (TFC) was measured as described previously [13] with modification. An amount of 0.5 mL of diluted tea infusion was added with 2 mL of H_2O and 0.15 mL of 5% (*w*/*v*) $NaNO_2$ and left at RT for approximately 6 min. The mixture was added with 0.15 mL of 10% (*w*/*v*) $AlCl_3$ and left at RT for further 6 min. The solution was mixed with 2 mL of 4% (*w*/*v*) NaOH and 0.7 mL of H_2O and left at RT for 15 min before measurement of the absorbance at 510 nm. Catechin (Purity of over 98%, Sigma-Aldrich Pty Ltd., Castle Hill, NSW, Australia) was used as the standard and the results were expressed as mg of catechin equivalents per gram of sample (mg CE/g).

2.2.3. Measurement of Antioxidant Activity

Three in vitro antioxidant assays were used to measure antioxidant activity of the tea infusion, tea extract, and vitamin C as each assay has advantages and limitations, thus it is appropriate to employ more than one assay to increase rigor and confidence of data. This study applied the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity and ferric reducing antioxidant power (FRAP) as described in a previous study [14] for measurement of antioxidant activity. Trolox (Purity of over 97%, Sigma-Aldrich Pty Ltd., Castle Hill, NSW, Australia) was used as the standard for a calibration curve and the results were expressed as mg of Trolox equivalents per g of sample (mg TE/g).

2.2.4. Scanning of Phytochemicals in Tea Infusion

A HPLC system coupled with a photodiode array detector (SPD-M40) was applied to scan major phytochemicals in the tea infusion. It was revealed that most of the major

phytochemical compounds were well-absorbed at 280 nm; thus, this wavelength was chosen for scanning major phytochemical compounds in the tea infusion. The tea infusion as filtered using a 0.45 µm Phenex syringe filter (Phenomenex), and then 25 µL was injected into the Shimadzu HPLC system coupled with a Luna 5u Phenyl-Hexyl 3 × 250 mm 5-micron column (Phenomenex Australia Pty. Ltd., Lane Cove, NSW, Australia), which was maintained at 35 °C. The mobile phases consisted of solvent systems A: deionized water-formic acid, 99.9:0.1 (*v*/*v*), and solvent B: acetonitrile. A flow rate of 0.7 mL/min and gradient was scheduled as follows: 0% solvent B for first 10 min; solvent B increased to 40% in following 35 min, then increased to 60% in further 15 min, and remained at 60% for the next 10 min. Finally, solvent B decreased to 0% in final 10 min and the post run of 5 min before the next injection. Gallic acid (≥97.5%), catechin (≥98%), caffeic acid (≥98%), ferulic acid (≥98%), epicatechin gallate (≥98%), quercetin (≥9 5%), and kaempferol (≥97%) purchased from Sigma-Aldrich Pty Ltd. (Castle Hill, NSW, Australia) were used as standards. Individual compounds were identified by comparison with the retention time of the pure standards.

2.3. Statistical Analysis

All experiments were conducted with triplicate replicates as minimum. The statistical analyses were conducted using statistical software IBM SPSS Statistics 24 (version 24.0.0.1) (Lane Cove, NSW, Australia). Differences were evaluated using one-way analysis of variance (ANOVA) and Tukey post hoc tests. Differences were considered to be significantly different at $p < 0.05$.

3. Results and Discussion

3.1. Impact of Hot Water Brewing Condition

Brewing time is very important in producing a high-quality cup of tea. Infusion can be as short as 30 s for a light cup of tea, or longer than 10 min, which would result in a strong cup of tea. Therefore, it is important to understand the impact of brewing time on extractability of phytochemicals from the herbal tea to identify the suitable brewing time. The effects of various brewing time on extractability of TPC and TFC were tested, and the results are shown in Figure 2. The results showed that levels of TPC and TFC increased when brewing time increased from 3 min to 7 min, then did not change when brewing time exceeded 7 min. These findings revealed that extractability of TPC and TFC was influenced by brewing time; however, their stability was not affected by brewing time for the first 11 min. Therefore, the most suitable time for brewing the herbal teabag is 7 min. It could be suggested that consumers brew herbal tea for 7 min or longer to obtain high levels of phenolic compounds and flavonoids in the tea infusion. Our results agreed with previous findings that phenolic content increased when brewing time increased from 0.5 to 5 min and the best brewing time was 6–8 min [10,15,16].

McAlpine and Ward [17] tested the impact of brewing time on 8 different types of tea and also found that more TPC can be extracted with longer brewing time, but the majority of TPC was extracted in the first 5 min regardless of the tea types. This can be explained that brewing temperature steadily decreased during regular brewing process (Figure 2), thus it is not a critical factor affecting extractability of phenolics. However, brewing time is a critical factor as it requires a sufficient time for the dried plant materials to absorb water into their matrix, then the phytochemicals can be diffused into the water. Therefore, more phenolics are diffused to the infusion in the first 5 min and not much after 8 or 10 min. Thus, it does not require a long brewing time, which might affect the quality of tea in terms of cold temperature and unfavorable sensory quality, while no more phenolics can be extracted to the infusion.

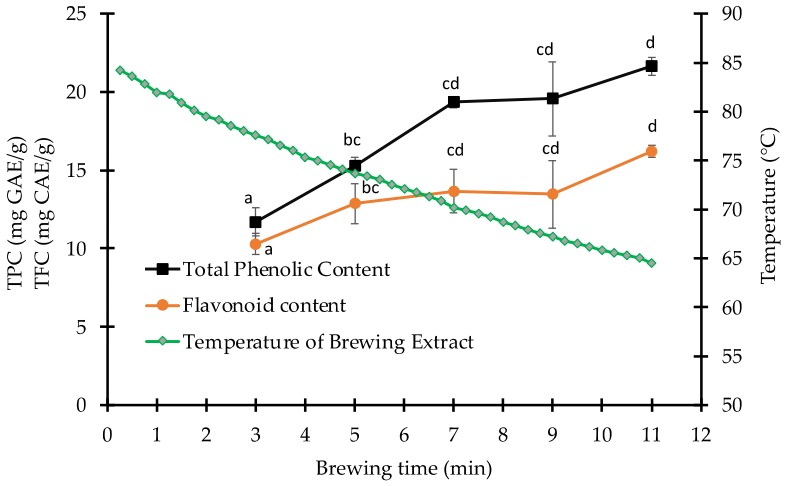

Figure 2. Extractability of total phenolic content (TPC) and total flavonoids (TFC) and the change of infusion temperature at different brewing time. Data are means ± standards deviations. Data for each phytochemical group (TPC or TFC) not sharing similar letters are significantly different at $p < 0.05$.

Results from three antioxidant assays revealed that brewing time also affect antioxidant activity of the herbal tea infusion (Figure 3). Antioxidant activity of the tea infusion brewed for 7 min was significantly higher than that of the infusion brewed for 3 min; however, antioxidant activity at 7 min was not significantly different when the herbal tea is brewed longer for 9 min or 11 min. Previous studies also found that increasing brewing time can increase antioxidant activity [15,17]. The change of antioxidant activity was similar to that of the TPC, and this can be explained by the main antioxidant contributors in herbal tea infusion are phenolic compounds. The results from antioxidant assays further confirm that brewing time for a minimum 7 min is sufficient for extraction of phenolics to obtain a tea infusion with potent antioxidant activity.

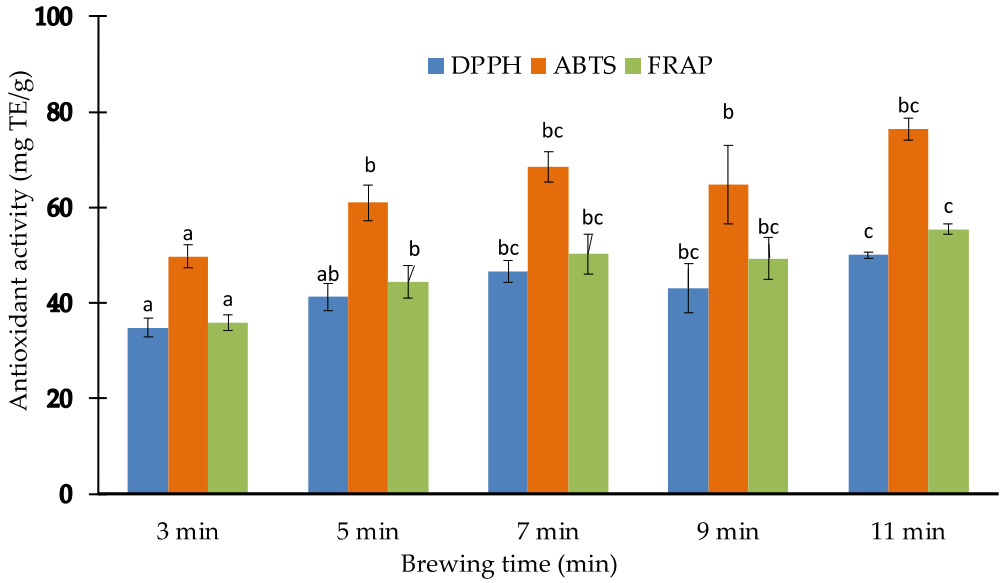

Figure 3. Antioxidant properties of tea infusion brewed at different time. Data are means ± standards deviations. Data for each antioxidant assay (DPPH, ABTS or FRAP) not sharing similar letters are significantly different at $p < 0.05$.

3.2. Impact of Microwave Assisted Extraction with Hot Water

The household microwave oven is a very popular kitchen device that is used for defrosting, heating, and cooking food. Microwave assisted extraction (MAE) was found to effectively extract phenolics from various plant materials, where radiation time and power were reported to be the major influencing factors [16]. The results (Figure 4) indicated that extractability of TPC increased when radiation for first or second boiled for both full and half power (1200 W and 600 W, respectively). However, extractability did not increase after radiation for third boiled or more. Similarly, extractability of TFC increased and reached plateau after the third boiled using MAE with hot water. Our findings were in agreement with results in a previous study on application of MAE with hot water for extraction of teabags, and this study also indicated longer radiation time, resulted in more catechins being extracted from the tea [16]. This can be explained by more cells being ruptured when longer radiation time was applied [18], thus more TPC and TFC can be released into the water.

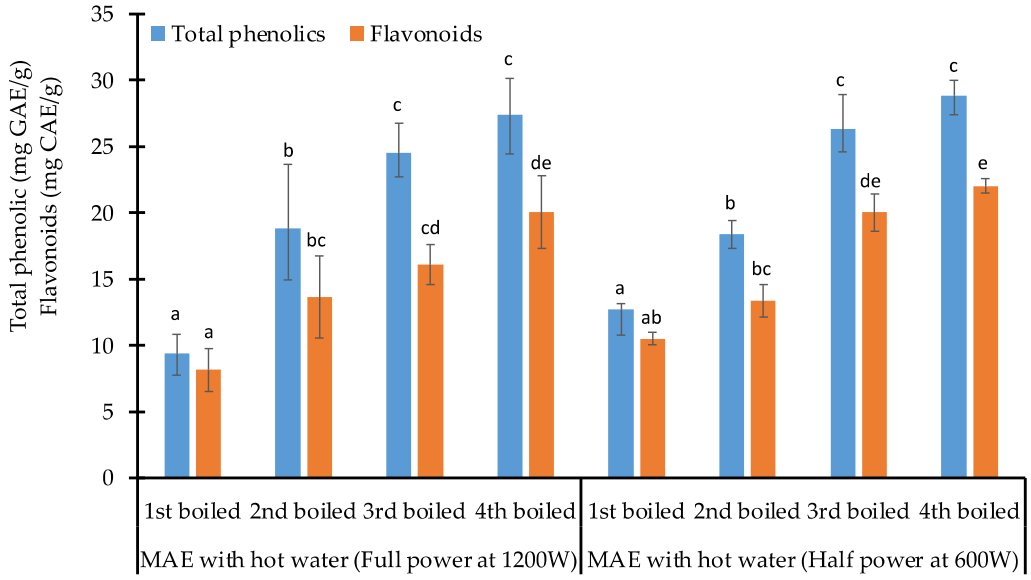

Figure 4. Extractability of total phenolic content (TPC) and total flavonoids (TFC) of herbal tea infusion prepared at different microwave assisted conditions with hot water. Data are means ± standards deviations. Data for each phytochemical group (TPC or TFC) not sharing similar letters are significantly different at $p < 0.05$.

Results from ABTS and FRAP assays (Figure 5) revealed that antioxidant activity of the herbal tea extract increased and was stable after the third boiled for both full and half power. However, results from the DPPH assay showed that antioxidant continue to increase when longer radiation was applied. The changes in antioxidant activity are similar to the change of TPC and TFC, and these further confirm that TPC and its second metabolite TFC are major antioxidant contributors in this herbal tea. Based on the results from extractability of TPC and TFC and antioxidant activity, it can be concluded that the most suitable condition for extraction of herbal teabag using MAE with hot water is radiation for the third boiled after adding hot water to a teabag. It should be noted that the "off-time" is the same 3 min for different powers, but radiation time is different for different power. With full power (1200 W), total radiation time is 40 s, whereas it is 90 s for half power (600 W), as higher the power boils water quicker than lower power.

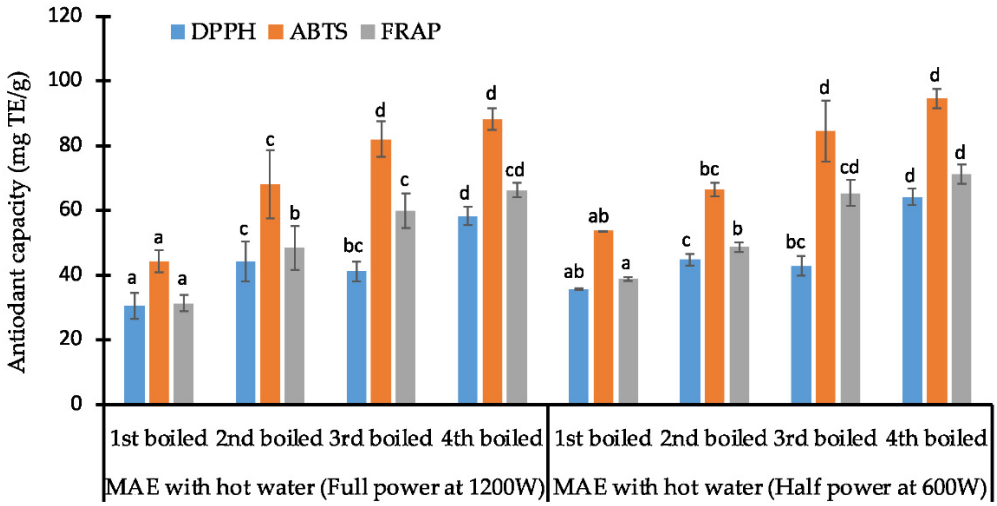

Figure 5. Antioxidant activity of herbal tea infusion prepared at different microwave assisted conditions with hot water. Data are means ± standards deviations. Data for each antioxidant assay (DPPH, ABTS, or FRAP) not sharing similar letters are significantly different at $p < 0.05$.

3.3. Impact of Microwave Assited Extraction with Cold Water

In a busy modern society, it may be more convenient to make a teacup using microwave from cold water as it saves time waiting for the water to boil as infusion is achieved during heating. As such, this hypothesis was tested to determine validity. This study found that when herbal tea bags were extracted using MAE from cold water start that longer radiation time increased extractability of TPC and TFC for both full and half power. However, extractability of TPC and TFC did not significantly increased after the second boiled. It could be hypothesized that the long heating time to boil water from cold (3.83 min for half power and 1.83 min for full power), following by 1 min rest and 10 or 20 s of radiation, was sufficient enough for extraction of most TPC and TFC into the solvent. Therefore, most of TPC and TFC were extracted after the second boiled (Figure 6).

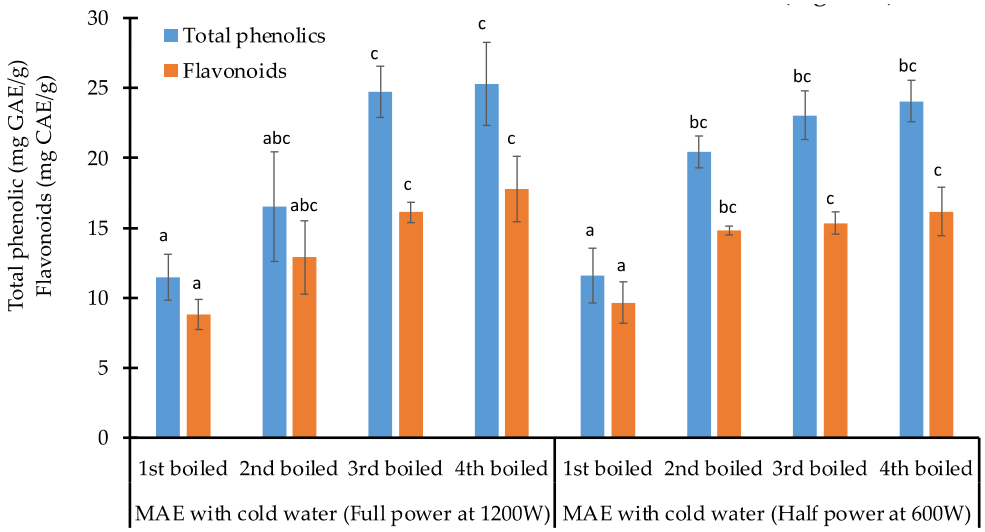

Figure 6. Extractability of total phenolic content (TPC) and total flavonoids (TFC) of herbal tea infusion prepared at different microwave assisted conditions with cold water. Data are means ± standards deviations. Data for each phytochemical group (TPC or TFC) not sharing similar letters are significantly different at $p < 0.05$.

The results from three antioxidant assays (Figure 7) revealed that antioxidant activity significantly increased from the first boiled to second boiled and remained when the teabag

was extracted at half power (600 W). The trend was similar to the extractability of TPC and TFC. However, the trend of antioxidant activity was different when the teabag was extracted at a full power (1200 W). Results from ABTS and FRAP assays showed that antioxidant activity of the tea extract increased and remained after the third boiled, whereas results from DPPH assay indicated that the highest antioxidant activity was achieved after the fourth boiled. DPPH scavenging capacity of the tea extract at second boiled was 26% less in comparison with that of the fourth boiled; where as ABTS and FRAP capacity at second boiled was 27% less as compared to that of the third boiled. Difference in the trend of antioxidant activity with extractability of TPC and TFC can be explained by the different composition of individual phenolic compounds, which were released after the third and fourth boiled and exhibited stronger antioxidant activity. Based on the extractability (Figure 6) and antioxidant activity (Figure 7), it is suggested that the most suitable condition for extraction of herbal teabag using MAE with cold water is radiation for the second boiled after adding cold water to a teabag. It would be convenient to do as it is very easy and quick with a total of heating and resting time of 2.93 min for full power (1200 W) and 5.03 min for half power (600 W).

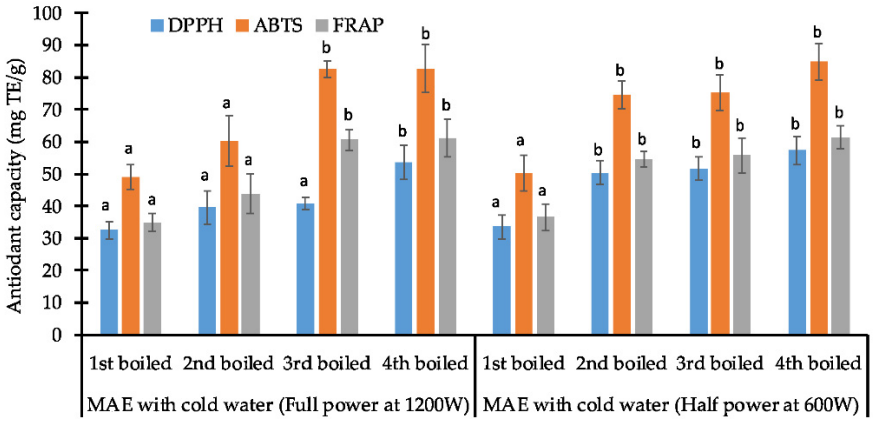

Figure 7. Antioxidant activity of herbal tea infusion prepared at different microwave assisted conditions with cold water. Data are means ± standards deviations. Data for each antioxidant assay (DPPH, ABTS, or FRAP) not sharing similar letters are significantly different at $p < 0.05$.

3.4. Comparison on Extractability of Polyphenols from Herbal Teabag Using Different Extraction Methods

The extractability of bioactive compounds can be significantly affected by normal brewing, traditional and advanced extraction techniques [16,18]. This study compared the extractability of TPC and TFC from a teabag to water using different extraction methods and the results are shown in Figure 8. Brewing at 7 min had similar extractability of TPC in comparison with that of the laboratory condition, MAE with cold water at full or half power. However, brewing at 7 min had lower extractability than MAE with hot water at full or half power. Extractability at 7 min had approximately 20–25% less extractability in comparison with MAE with hot water. Extractability of TPC of MAE with cold water at full power was comparable with that of all other extraction methods, whereas MAE with cold water at half power has similar extractability of TPC with laboratory condition, brewing at 7 min and MAE with cold water at full power. However, extractability of TPC was significantly less than that of MAE with hot water at full and half power. It is interesting to note that the extractability of TFC was not significantly different between the different extraction methods. Based on the results (Figure 8), it is suggested that the herbal teabag can be simply extracted using hot water at 7 min or it can be alternatively extracted using a household microwave with cold water at full power for second boiled. Using MAE was effective with a short time of 2.93 min (1.93 min of radiation time and 1 min of resting time).

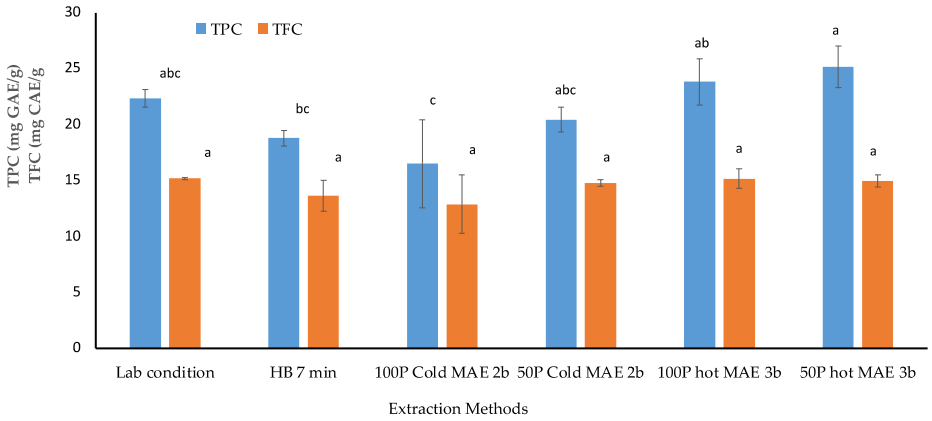

Figure 8. Levels of total phenolic content (TPC) and total flavonoids (TFC) of herbal tea infusion prepared from different extraction methods. Data are means ± standards deviations. Data for each phytochemical group (TPC or TFC) not sharing similar letters are significantly different at $p < 0.05$.

Results from three antioxidant assays were slightly different to the trend of TPC and TFC extractability (Figure 9). DPPH assay revealed that brewing at 7 min has lower antioxidant activity than that of laboratory extraction condition and MAE with cold water at half power. However, this assay showed that brewing at 7 min had similar antioxidant activity as compared to MAE with cold water at full power of MAE with hot water at full or half power. Results from ABTS and FRAP assay revealed that hot brewing at 7 min had comparable antioxidant activity to laboratory extraction condition, MAE with cold water at full or half power, and MAE with hot water at full power. Results from DPPH assay also showed that MAE with cold water at full power has similar antioxidant activity to brewing at 7 min, MAE with cold water at half power, and MAE with hot water at full or half power. Results from ABTS and FRAP assay indicated that MAE with cold water at full power has comparable antioxidant activity as compared to that of laboratory extraction condition, brewing at 7 min, MAE with cold water at half power, and MAE with hot water at full power. The results from three antioxidant assays further confirmed that brewing at 7 min or extraction using a household microwave with cold water at full power for second boiled are suitable for preparation of an herbal teacup, which has high polyphenols and potent antioxidant activity.

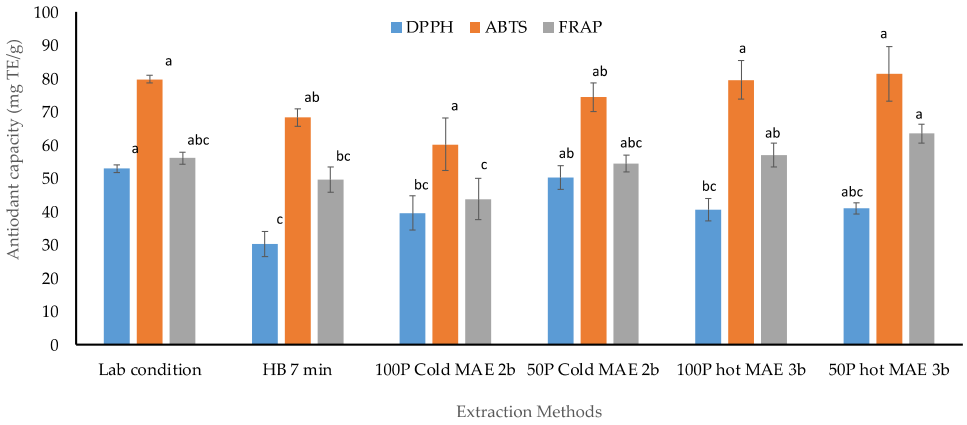

Figure 9. Antioxidant properties of herbal tea infusion prepared from different extraction methods. Data are means ± standards deviations. Data for each antioxidant assay (DPPH, ABTS, or FRAP) not sharing similar letters are significantly different at $p < 0.05$.

3.5. Comparison on Antioxidant Properties of Herbal Tea Extract with Vitamin C and Scaning Its Phytochemicals Compounds

Antioxidant activity of the dried extracts prepared from normal brewing at 7 min and MAE with cold water at full power for second boiled was compared with that of vitamin C. The results (Figure 10) showed that antioxidant activity of these extracts is approximately 4 times less powerful in comparison with that of pure vitamin C. As the extracts are crude and they contain a large portion of non-phenolic antioxidants and only 13–15% of these crude extracts are phenolic antioxidants, antioxidant activity of the herbal tea extracts is potent. Antioxidant activity can be significantly improved if composition of phenolic antioxidants is improved.

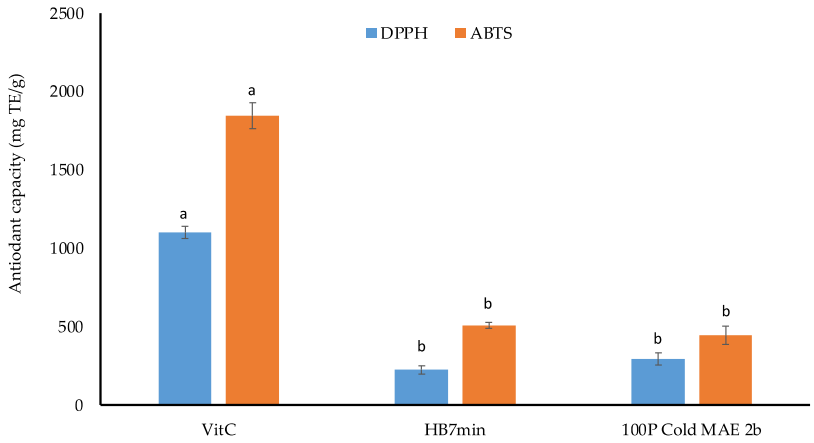

Figure 10. Antioxidant activity of vitamin C in comparison with herbal tea extracts prepared from hot brewing for 7 min, microwave assisted extraction with cold water at full power. Data for each antioxidant assay (DPPH or ABTS) not sharing similar letters are significantly different at $p < 0.05$.

Results from HPLC analysis revealed that there are over 20 major chromatogram peaks, of which 7 were identified using reference standards (Figure 11). Gallic acid was identified at 6.06 min, catechin at 27.14 min, caffeic acid at 27.63 min, ferulic acid at 34.10 min, epicatechin gallate at 35.47 min, quercetin at 42.65 min, and kaempferol at 46.87 min. These phytochemicals have been linked with various health benefits, such as prevention of cancer and cardiovascular diseases [19]. Therefore, drinking this herbal tea can potentially link with health benefits. Future studies are recommended to isolate and identify other unknown phytochemicals and test the link of this herbal tea with various health benefits.

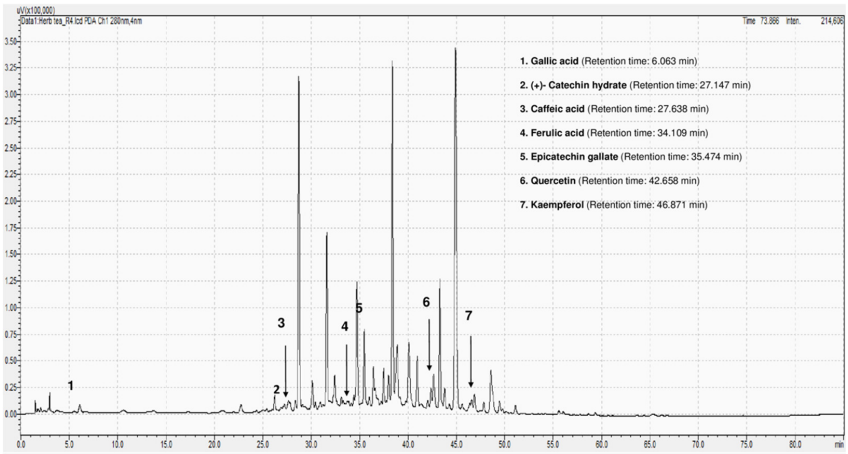

Figure 11. Chromatogram of herbal tea infusion prepared from hot brewing for 7 min. Detection was conducted using a PDA detector at 280 nm.

4. Conclusions

Preparation techniques have strong impact on extractability of polyphenols from a herbal teabag to infusion, thus they significantly affect availability of polyphenols and antioxidant activity of a cup of tea. Normal brewing using hot water for 7 min or cold MAE at full power to second boil are suggested to prepare a cup of tea form a herbal teabag, as infusion prepared under these conditions has comparable levels of polyphenols and antioxidant activity. In addition, it is convenient and easy to brew a cup of tea under these conditions. There are 20 major chromatogram peaks, of which 7 were identified using reference standards, revealing that this herbal tea can link with health benefits. It should be noted that this study has not conducted sensory evaluation on the tea infusion. Future studies are recommended to further conduct sensory evaluation of the tea infusion and test potential link of the tea infusion with health benefits to confirm its quality and health association.

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