





Effect of Extraction Time on the Total Phenolic Content, *In Vitro* Antioxidant and Antidiabetic Properties of Commercial Herbal Teas

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Abstract	Article History
<p>The health benefits of herbal teas are associated with their polyphenol compounds. Total phenolic content (TPC), total antioxidant capacity (TAC) and potential antidiabetic properties were measured in seven herbal teas at 3, 5 and 10 min at 100 °C by Folin–Ciocalteu's method, ABTS radical scavenging and alpha-amylase activity inhibition, respectively. This study also evaluated the influence of brewing time and tea form (loose-leaf vs. bagged) on these attributes. The findings indicated distinct variations of TPC among the herbal teas, arranged in the order of Tulsi > Liquorice > loose-leaf Camomile > bagged Camomile > Nettle > Fennel > and Ginger tea. In particular, the phenolic compounds had a positive linear correlation with TAC. Both extraction time and the form of tea had significant effects on TPC, TAC and antidiabetic property.</p> <p>Keywords: Herbal teas, extraction time, phenolic content, antioxidant activity and alpha amylase inhibition</p>	<p>Received: 10 Jun 2025 Accepted: 18 Jun 2025 Published: 20 Jun 2025</p>  <p>Scan QR code to view*</p> <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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1. Introduction

For centuries, herbal teas have served as alternative medicine in eastern countries due to their therapeutic and healing properties (Tandon and Yadav, 2017). In recent years, the desire for a healthier lifestyle has led to the widespread consumption of these herbal teas in western countries as well. The popularity can be attributed to their affordability, minimal side effects, and gentler biological impact compared to traditional medicines. Herbal teas play a pivotal role in offering various health benefits including antioxidative, anti-inflammatory, anticancer, anti-anaemic, hypoglycaemic, and metabolic regulatory effects (Pohl et al., 2016). These positive impacts are largely attributed to the presence of phenolic compounds within the teas. Notably, polyphenols also have the ability to regulate postprandial glycemia by inhibiting α -amylase enzyme activity (Fu et al., 2017), which can reduce the risk of type 2 diabetes mellitus (T2DM).

This study aims to determine the total phenolic content and the *in vitro* antidiabetic effects and antioxidant activities in the infusions of seven commercial herbal teas. The herbal tea

exhibiting the highest values in terms of total phenolic content (TPC), total antioxidant capacity (TAC), and antidiabetic properties was subjected to High-Performance Liquid Chromatography (HPLC) analysis.

2. Materials and Methods

2.1. Herbal teas

Seven commercial herbal tea products were selected, including loose-leaf and bagged Camomile (*Matricaria recutita*), Fennel (*Foeniculum vulgare*), Nettle (*Urtica dioica*), Tulsi (*Ocimum tenuiflorum*), Ginger (*Zingiber officinale*) and Liquorice (*Glycyrrhiza glabra*). All tea samples were purchased from Holland and Barrett, UK, during June 2023.

2.2. Chemicals

Commercial standards of gallic acid, catechin, p-coumaric acid, ferulic acid, sinapic acid, Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), phosphate buffered saline (PBS), ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate

(K₂S₂O₈), Acarbose, alpha-amylase and 2-chloro-4-nitrophenyl α -D-maltotriose (CNP-G3) were obtained from Sigma-Aldrich. Co., Ltd., Dorset, U.K. All chemicals used in the study were analytical or HPLC grade.

2.3. Methods

2.3.1. Preparation of herbal tea infusions

200 ml of deionised water was added to a labelled beaker and pre-heated in a water bath to 100°C. The infusions of herbal

teas were prepared by infusing one tea bag or two tea spoons of loose-leaf tea (according to recommendations on its label) in 200 ml of boiling deionised water for 3, 5 or 10 minutes without mechanical agitation (Figure 1). The tea bag or loose-leaf tea was then removed when it reached the extraction time and the solution was left to cool at room temperature. The prepared tea samples were centrifuged at 4000 rpm for 5 minutes and then stored in the freezer overnight for further assessment.

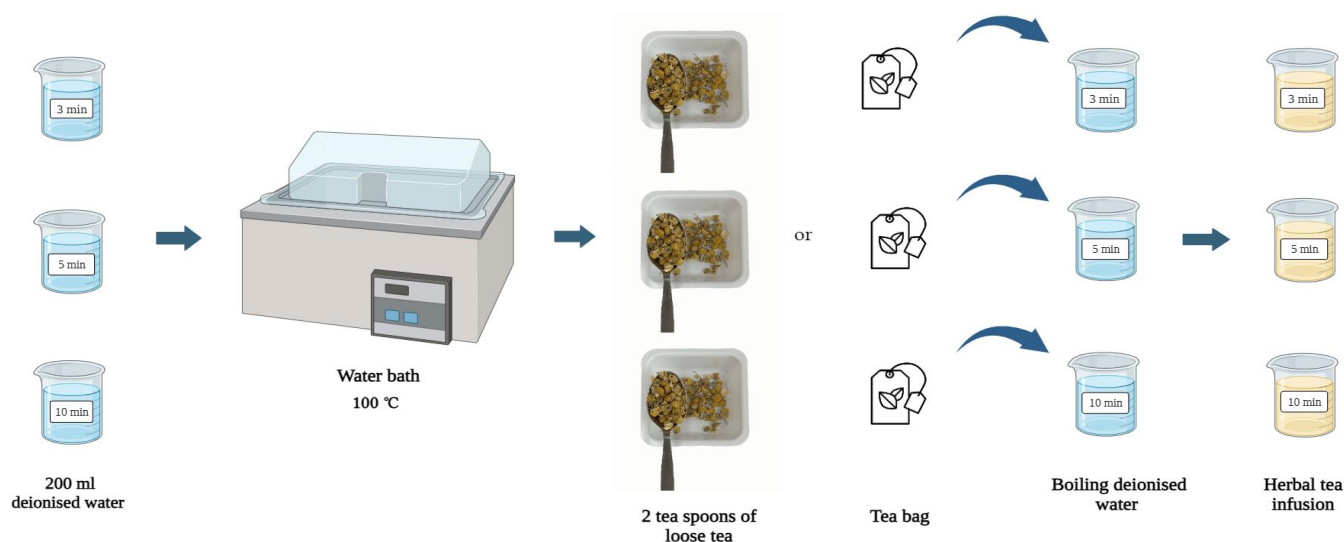


Figure 1: Preparation of Herbal Tea Infusions

2.3.2 Determination of total phenolic content (TPC)

The amount of TPC was determined according to the Folin-Ciocalteu colorimetric method with some modifications (Ifie et al., 2016), using gallic acid as the standard and expressing the results as mg of gallic acid equivalents per ml of herbal tea (mg GAE/ml). Standard solutions were prepared from a 0.1 mg/ml gallic acid stock solution to make a concentration range of 0, 20, 40, 60, 80 and 100 μ g/ml with distilled water. Tea samples were prepared by 1:10 dilution with distilled water. To 1 ml of diluted tea or standard, 5 ml of 10% (v/v) Folin-Ciocalteu reagent was added and mixed on a whirlimix for 1 minute. The mixture was left to stand at room temperature for 5 minutes, then added with 4 ml of 7.5% (w/v) sodium carbonate and mixed on a whirlimix for 1 minute before being left to stand in a 50°C water bath for 30 minutes. After 30 minutes, the absorbance of the mixed solution was measured at 765 nm using a UV-vis spectrophotometer. All samples were assessed in two sets of triplicates and presented as mean \pm SD (n = 6).

2.3.3. Measurement of total antioxidant capacity (TAC)

The Trolox Equivalent Antioxidant Capacity (TEAC) assay based on Arts et al., (2004) was used to measure the TAC of herbal teas and the results were expressed as mg of Trolox equivalents per herbal tea (mg TE/ml). In this assay, TAC was evaluated by the ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) free radical-scavenging activity of herbal teas in 5 minutes. The ABTS reagent was prepared by mixing 96.2 mg ABTS and 16.5 mg potassium persulfate in distilled water to make up to the volume of 100 ml, then stored in the dark for 12–16 hours. Before use, the ABTS solution was diluted with

10% (v/v) PBS to obtain an absorbance of 0.7 ± 0.02 at 734 nm for analysis. The results were expressed as mg of Trolox equivalents per ml of herbal tea (mg TE/ml). Standard solutions of 0, 20, 40, 60, 80 and 100 μ g/ml were prepared from 0.1 mg/ml Trolox solution with 10% (v/v) PBS solution. Herbal tea infusions were diluted 2-fold before assessment. The standard TAC was evaluated using the same procedure as the samples. 10% (v/v) PBS solution was used as a blank. For analysis, 40 μ l of standard or sample solution was added into 2 ml of ABTS in the cuvette, then the cuvette was covered with a small piece of parafilm, followed by manual mixing. The mixture was then allowed to sit at room temperature for 5 min. The absorbance of the standard and samples was read at 734 nm with 2 ml of ABTS and 40 μ l 10% (v/v) PBS as the reagent blank. All tests were done in duplicate and exported as mean \pm SD (n = 2).

2.3.4. Evaluation of antidiabetic property

According to the method conducted by Deen et al. (1988) with some modifications, the antidiabetic property of herbal tea samples was estimated by inhibitory effects on the α -amylase enzyme activity. The α -amylase inhibition assay was conducted with 2-chloro-4-nitrophenyl α -D-maltotriose (CNP-G3) as substrate and Acarbose as positive control. CNP-G3 solution (2mM) was prepared by dissolving CNP-G3 in phosphate buffer (pH 6.9, 50mM) and kept in freezer at -20°C until required. 2-fold dilutions of Acarbose standard solution including 0, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 mM were prepared from 100mM Acarbose stock solution. A series of tubes was added with 500 μ l of Acarbose standard solution, respectively, and 1 ml of α -amylase (100U/ml). The tubes were incubated in a water bath at 37 °C for 10 minutes, then

added with 500 μ l of 2mM CNP-G3 solution. The reaction mixture was incubated at 37 °C for 10 minutes and the reaction was stopped by placing the test tubes in a boiling water bath (100 °C) for 10 minutes before cooling to room temperature. Thereafter, the reaction mixture was diluted by adding 6 mL of phosphate buffer (pH 6.9, 50mM) and the absorbance was measured at 405 nm. For sample measurement, 500 μ l of tea infusion was directly added with 1 ml of α -amylase (100U/ml), followed by the same procedure as Acarbose control. The inhibitory effect was calculated according to the following equation: Inhibition (%) = (Absorbance at 100% α -amylase activity of control - Absorbance α -amylase activity of sample)/Absorbance at 100% α -amylase activity of control \times 100. All tests were done in duplicate and presented as mean \pm SD (n = 2).

2.3.6. Statistical analysis

Calibration curves for gallic acid standard, Trolox standard and correlation among TPC, TAC and antidiabetic property were performed by Pearson's Correlation Coefficient. The effects of tea types, extraction times and the forms of teas for TPC, TAC and antidiabetic property were evaluated by two-way analysis of variance (ANOVA) test using IBM SPSS

software (v29.0; SPSS Inc., Chicago, Illinois, USA). Statistically significant difference was considered at $p < 0.05$.

3. Results

3.1. Total phenolic content

Seven selected herbal teas at extraction times of 3, 5 and 10 minutes were assessed for TPC using the Folin-Ciocalteu method. The calibration curve generated from the analysis of the gallic acid standard was linear with $y = 0.0109x + 0.0193$, $R^2 = 0.9951$. Among all samples, the amount of TPC was ranged from 0.009 to 0.383 mg GAE/mL. The highest average TPC was found in the Tulsi tea infusion (0.317 ± 0.048 mg GAE/mL), followed by Liquorice (0.158 ± 0.094 mg GAE/mL) > loose-leaf Camomile (0.102 ± 0.034 mg GAE/mL) > bagged Camomile (0.093 ± 0.016 mg GAE/mL) > Nettle (0.070 ± 0.008 mg GAE/mL) > Fennel (0.044 ± 0.009 mg GAE/mL) > and Ginger tea (0.015 ± 0.007 mg GAE/mL). As demonstrated in Figure 2, progressive increases in TPC with extraction time were observed for each type of tea. The majority of polyphenols were released within 5 min of extraction time for most of herbal tea samples. In contrast, the TPC of Liquorice tea at 10 minutes of extraction time was about 3 times higher than at 5 minutes.

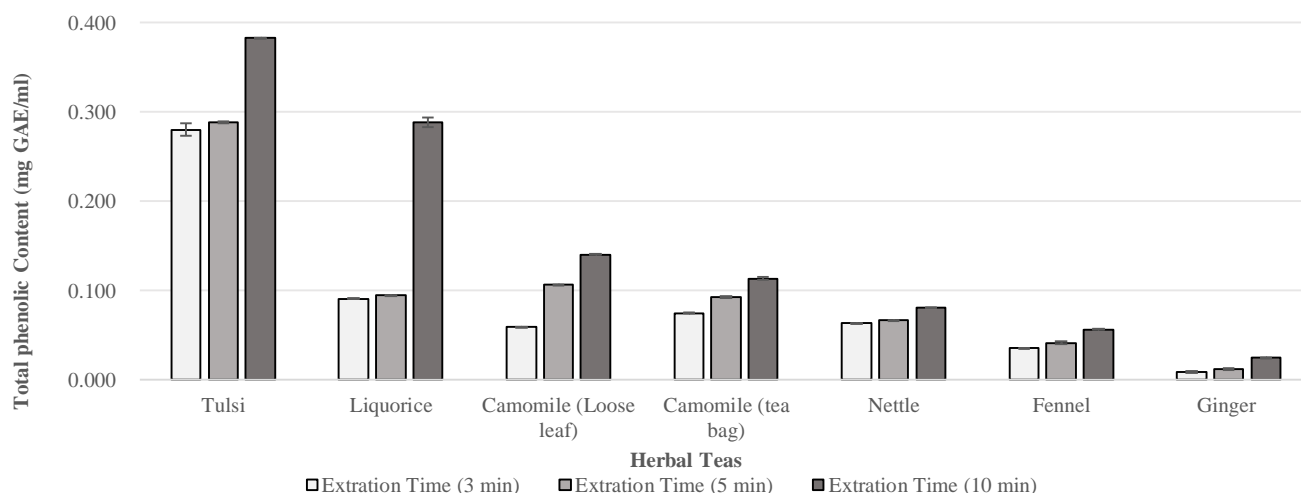


Figure 2: Total phenolic content of herbal teas

The two-way ANOVA test data indicated that both factor 1 (Tea type) ($F = 40827.48$, $p < 0.001$) and factor 2 (Extraction time) ($F = 12266.36$, $p < 0.001$) had statistically significant effects on TPC. The results also revealed that there was a significant interaction effect, which further suggested that the relationship between extraction time and TPC strongly depends on the type of herbal tea. Moreover, the F-value for tea type was larger than the F-value for extraction time, which revealed that the effect of tea type was stronger than that of extraction time on TPC. Additionally, the form of tea (loose leaf or bagged) had statistically significant effects on TPC ($F = 727.94$, $p < 0.001$) and a significant interaction effect between tea form and extraction time was observed ($p < 0.001$).

3.2. Total antioxidant capacity

The TAC of seven herbal tea samples was measured as ABTS free radical-scavenging activity using the TEAC method. The calibration curve generated from the analysis of the Trolox standard was linear with $y = 0.0019x$, $R^2 = 0.9996$. In selected herbal teas, antioxidant activity varied from 0.064 ± 0.001 mg TE/mL to 0.704 ± 0.004 mg TE/mL. The highest average TAC was found in the Tulsi tea infusion (0.655 ± 0.039 mg TE/mL), followed by loose-leaf Camomile (0.382 ± 0.119 mg TE/mL) > Liquorice (0.332 ± 0.040 mg TE/mL) > bagged Camomile (0.239 ± 0.033 mg TE/mL) > Nettle (0.198 ± 0.012 mg TE/mL) > Fennel (0.166 ± 0.022 mg TE/mL) > and Ginger tea (0.081 ± 0.020 mg TE/mL). The outcome was consistent for all samples in that the TAC increased with extraction time as shown in Figure 3.

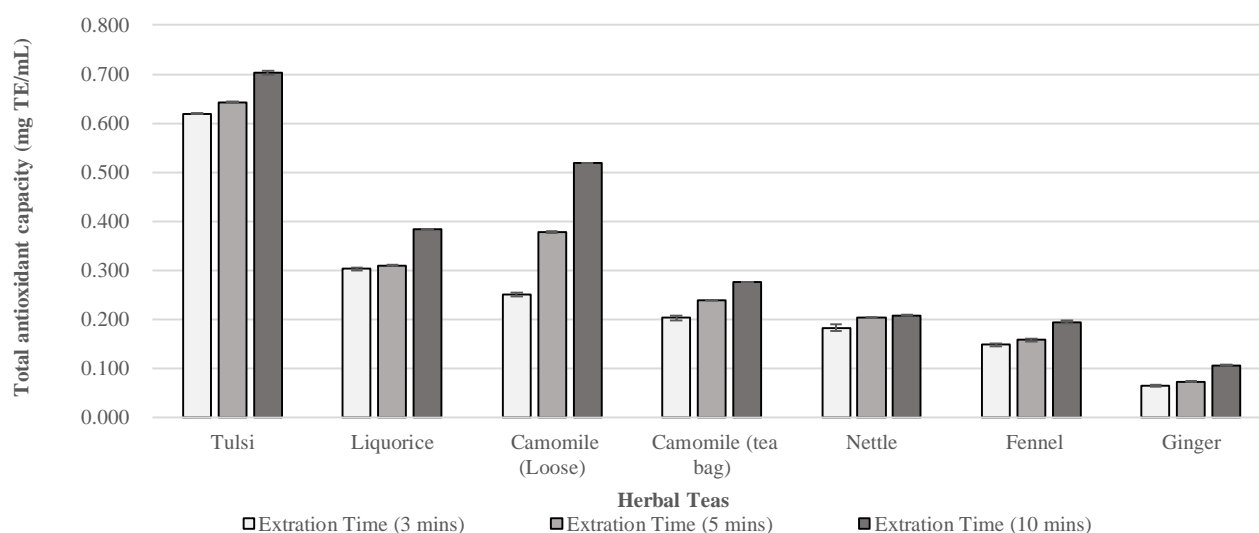


Figure 3: Total antioxidant capacity of herbal teas

Based on the two-way ANOVA test results, notable effects of Type of Herbal Tea ($F=31765.20$, $p<0.001$) and Extraction Time ($F=4124.69$, $p<0.001$) on TAC were observed. Furthermore, the effect of Tea Form ($F=8536.62$, $p<0.001$) was stronger than Extraction Time ($F=4014.82$, $p<0.001$) as Tea Form had larger F value. Thus, indicating that the type and form of herbal teas should be considered as significant factors when evaluating TEAC.

3.3. The antidiabetic property

The antidiabetic property was measured as the inhibition (%) of herbal teas to α -amylase enzyme activity. In the assay,

inhibitory effect of herbal teas was ranged from 68.9% to 87.9%. The highest average inhibition (%) was found in the Tulsi infusion (87.62 ± 0.38), followed by Liquorice (78.15 ± 0.12) > bagged Camomile (74.53 ± 3.15) > loose-leaf Camomile (74.71 ± 2.02) > Nettle (72.17 ± 1.97) > Fennel (69.06 ± 1.39) > and Ginger tea (67.23 ± 2.52). As shown in Figure 4, the inhibitory effects were increased with extraction time for all herbal tea samples. The standard deviations of all samples at all extraction times were substantially low, suggesting that the variability between test sets was low.

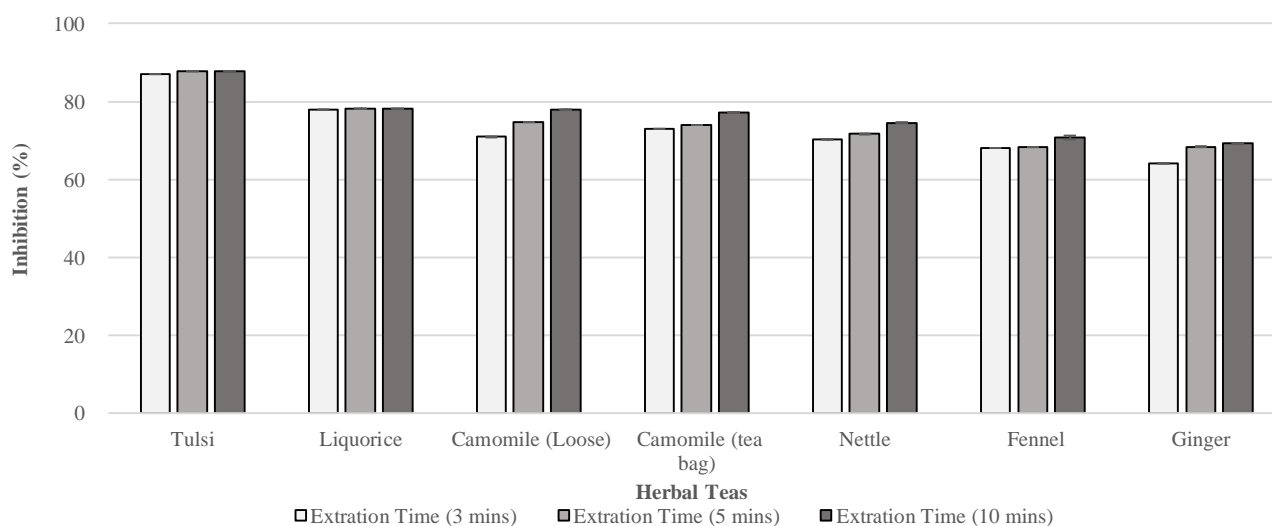


Figure 4: The antidiabetic property of herbal teas

According to the two-way ANOVA test results, statistically significant effects were observed for factor 1 (Tea type) ($F=18005.84$, $p<0.001$) and factor 2 (Extraction time) ($F=4311.05$, $p<0.001$) on inhibition of α -amylase enzyme activity. In addition, Tea form ($F=73.92$, $p=0.0001$) also had significant effect on the antidiabetic activity. However, unlike Tea type, the effect of Tea form was relatively weaker than

Extraction time ($F=29713.92$, $p<0.001$) as the F value was smaller.

Discussion

4.1. TPC, TAC and association between TPC and TAC

In the present study, herbal tea samples exhibited a wide range of TPC amount and notable differences in TPC were observed

for all tea types at all extraction times with 42-fold of variation. The highest amount of average TPC was found in Tulsi tea and the lowest amount was found in ginger tea, with the amount of Tulsi tea was about 21 folds higher than that of Ginger tea. However, previous studies conducted by Alam et al. (2018) and Rabiul Islam et al. (2013) revealed that the polyphenol content in the Ginger tea infusion was slightly higher than that of in Tulsi tea infusion.

Nettle and Fennel teas has relatively low TPC amount and the amount of TPC in Nettle infusion was larger than that of Fennel. Unlike the present study, it was previously detected by Folin-Ciocalteu method that the TPC values in water extracts of Nettle and Fennel were similar with 0.803 mg GAE/mL and 0.807 mg GAE/mL, respectively (Albayrak et al., 2012). The observed variations of TPC may be due to difference in geographical origin, species and growing conditions (e.g., climate and maturity) of tea samples (Zhao et al., 2017). Additionally, the phenolic composition of commercial teas could be varied from wild teas (Dias et al., 2013), which can contribute to the manufacturing process, such as rolling and drying.

The findings of present study suggested that tea type had a substantial influence on the TAC, which is mainly due to different polyphenols have a diverse range of antioxidant activity. A tea with a relatively lower TPC could potentially have a higher antioxidant capacity, as can be seen in the results of the study with Liquorice showing higher TAC than that of loose-leaf Camomile at a 10-minute extraction time. Notably, polyphenols are heat sensitive and an increase in hot extraction time can lead to a reduction in polyphenol content, which further affects the antioxidant capacity. For example, catechins exhibit low thermal stability when extracted at 100 °C and tend to degrade with prolonged extraction time (Volf et al., 2014). Catechin was identified in water extracts of Liquorice root (32.40 mg/100g) (Alfauomy et al., 2020), while no catechin was detected in Camomile tea origin from Brazil (Matsubara and Rodriguez-Amaya, 2006). Therefore, at a short extraction time (3 min), the TAC for Liquorice was higher than that of loose-leaf Camomile.

The correlation analysis results in present study revealed strong positive linear associations between TPC and TAC of herbal teas at 3 min ($R^2 = 0.9618$) and 5 min ($R^2 = 0.9482$), which suggested that phenolic compounds may be one of the primary components contributing to antioxidant activities in herbal tea infusions. However, the association weakened at 10 min ($R^2 = 0.7521$), likely because the antioxidant properties greatly influenced by the degradation of polyphenols as mentioned above. The relationship between the total polyphenol content and the antioxidant capacity was also confirmed using the ferric thiocyanate (FTC) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) tests, with high correlation coefficients $R^2 = 0.9935$ and $R^2 = 0.9141$, respectively (Anesini et al., 2008). A study conducted for 51 herbal tea origin from China also indicated that the positive linear correlation was exhibited between TPC and TAC assessed by TEAC and ferric reducing antioxidant power (FRAP) assays

with $R^2 = 0.7929$ and $R^2 = 0.8043$, respectively (Fu et al., 2011).

4.2. The antidiabetic property of herbal teas

All herbal teas significantly decreased α -amylase activity in different extraction time and the inhibitory effect was dependent on the type of tea. Tulsi tea infusion exhibits the highest antidiabetic property, followed by Liquorice tea. The effect of Tulsi tea on α -amylase enzyme activity have not been evaluated in previous studies. However, the aqueous extract of Tulsi was detected that significantly reduced the fasting blood glucose level in diabetic rats (Suanarunsawat et al., 2014; Hussain et al., 2001) and a fall of HBA1c was also contributed by the antidiabetic property of Tulsi (Devra et al., 2012). The α -amylase inhibitory activity of glycyrrhizin, the main constituent of Liquorice was determined with 34.9% compared to Acarbose standard control (Xiao-Ping et al., 2010). The difference of inhibition rate maybe due to the variation of extraction method and other compounds in Liquorice tea can increase the inhibitory effect. Due to the fact that the interactions between different components of herbal teas can lead to synergistic or antagonistic effects on biological activities (e.g., inhibition of α -amylase activity), the mechanism of antidiabetic components in tea needs to be further elucidated.

4.3. The effects of brewing time and the form of tea

In terms of extraction time, the results of the present study suggested that for all herbal tea samples, the TPC, TAC and α -amylase activity inhibition were increased with a longer extraction time. The extraction time was studied by Cleverdon et al. (2018) from 1 to 10 min and progressive increases in TPC were observed for all tea samples, which is consistent with the present study. In addition, they also revealed that about 80%–90% of the phenolic content was released from tea bags in the first 5 min of extraction for the majority of tea. In the present study, only Liquorice tea released about twice as much phenolic content at 5–10 min as in the first 5 min. Therefore, to ensure adequate release of polyphenols, the brewing time for herbal teas should be at least 5 min. Furthermore, the relationship between extraction time and antioxidant activity is influenced by several factors, including the temperature and pH of the extraction solution. According to Hajiaghaalipour et al. (2016), it was detected that the highest antioxidant activity in some teas would be observed during prolonged cold brewing. More work should be conducted to understand the effect of brewing time on the extraction rate of phytochemicals from herbal teas and determine the appropriate brewing time. In regard to the form of tea, loose-leaf Camomile tea exhibited higher TPC, TAC and α -amylase activity inhibition than those in bagged Camomile tea. The primary factor is the existence of resistance resulting from the tea bag material, which has the potential to impede the transportation of the soluble components into the infusion (Astill et al., 2001). Additionally, commercial bagged tea often uses small particles of leaf, which has a higher processing degree than loose-leaf tea. Wen et al. (2023) suggested that phenolic content and the inhibitory effect of α -amylase activity can be reduced during the processing of tea, which may contribute to the significant loss of some polyphenols (e.g., catechin).

4. Conclusion

The phenolic content, antioxidant and antidiabetic activities of herbal tea infusions were sensitive to the prolonged extraction time. At least 5 min of hot brewing are recommended for most herbal teas. Seven commercial herbal teas all exhibited health benefits, with Tulsi having the most potent levels.

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