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CORRELATION of MAJOR COMPONENTS and RADICAL SCAVENGING ACTIVITY of COMMERCIAL TEA DRINKS

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Key words: tea drinks, catechins, free radical scavenging, sugar, caffeine

ABSTRACT

Correlation of major components with free radical scavenging activities of 27 commercial tea drinks prepared from green, Oolong, and black teas were investigated. Green tea drinks contained the highest level of total catechins including epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG), and gallocatechin gallate (GCG), while these components were low in black tea drinks and moderate in Oolong tea drinks. EGC and EGCG were the major catechins in the three types of tea drinks, and gallic acid showed the highest abundance in black tea drinks. Caffeine concentrations were comparable in the three types of tea drinks. The scavenging abilities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals were used to determine the antioxidant potential of tea drinks, and the ranked order of potency was green tea > Oolong tea > black tea. Total phenolics, total catechins, and EGC of tea drinks were positively and significantly (r > 0.8) correlated to the scavenging abilities against DPPH and ABTS radicals. Our results show that green tea drinks have higher free radical scavenging activity than black and Oolong tea drinks, which may be related to their high levels of total phenolics and catechins.

Key words: tea drinks, catechins, free radical scavenging, total phenolics, caffeine

(因去除關鍵字”糖”，所以改用”總酚”取代)

市售茶飲料主要成份與清除自由基活性之相關性

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摘要

本研究調查27種包含綠茶、烏龍茶及紅茶所製備之市售茶飲料的主要成份與清除自由基活性之相關性。綠茶飲料含最高總兒茶素含量，包含epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG)及gallocatechin gallate (GCG)，而這些成份則在紅茶飲料中含量低，在烏龍茶飲料中則為中等含量。EGC與EGCG為三種茶飲料中主要之兒茶素成份，而紅茶中含有最大量之沒食子酸(gallic acid)。三種茶飲料含有大約等量之咖啡因濃度。利用捕捉1,1-diphenyl-2-picrylhydrazyl (DPPH)及2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)自由基能力來測定茶飲料之抗氧化能力，並且發現其能力依序分別為綠茶>烏龍茶>紅茶。茶飲料中總酚、總兒茶素及EGC含量與捕捉DPPH及ABTS自由基能力具有正向且高度($r > 0.8$)相關。我們的結果顯示綠茶飲料比紅茶及烏龍茶飲料有較高清除自由基活性可能與其有較高之總酚及兒茶素含量有關。

關鍵字： 茶飲料，兒茶素，清除自由基，總酚，咖啡因

INTRODUCTION

Tea is one of the most widely consumed beverages in the world. Three types of tea are commercially available in Taiwan. They are: green tea (unfermented), Oolong tea (partially fermented) and black tea (fully fermented). The manufacturing conditions differ, which influence the chemical composition and taste. For green tea, the tea leaves are steamed to inactivate polyphenol oxidase to minimize oxidation before further processes. For black tea, the tea leaves undergo full oxidation in the fermentation process. Oolong tea is manufactured by partial oxidation of the tea leaves, intermediate between the processes used for the preparation of green and black teas[1,2]. Epidemiological studies suggest that the polyphenolic compounds present in tea leaf have the benefits of reducing the risk of various diseases such as cancer and cardiovascular diseases[3,4]. The principal hypothesis is linked to the strong free radical-scavenging and antioxidant properties of their polyphenols[5]. Catechins and gallic acid (GA) are considered as the active components of tea polyphenols responsible for the beneficial effects on human health[6]. However, the oral bioavailability of these polyphenols was found to be relatively poor[6,7]. Among various kinds of teas, green tea contains the highest amounts of catechins due to lacking fermentation in the manufacturing process. Four major catechins found in tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). EGCG is the most abundant catechin in tea and is believed to be the most bioactive component[8]. In addition to catechins, tea also contains moderate amount of caffeine (CA), a central nervous system (CNS) stimulant [9].

Canned and bottled tea drinks are becoming more popular worldwide due to their convenience. The catechins in tea drinks, however, may change during manufacture

process and storage. For instance, degradation and/or epimerization of epicatechins by heat treatment may occur in tea drinks [10,11], and the concentration of GCG present in green tea drinks is likely to increase due to the epimerization of EGCG during autoclaving[11]. The addition of ascorbic acid to green tea drinks was shown to stabilize catechins[12]. In addition, catechins are labile in alkaline solution. Catechins in green tea at pH values of > 6 degrad with storage time[11,13]. These results indicate catechins are not always stable during the manufacturing process and storage.

In this study, we evaluated the major components of 27 tea drinks, including green, Oolong, and black tea, commercially available in Taiwan and their correlations to free radical scavenging activities. Free radical scavenging activities of the tea drinks were evaluated by examining the scavenging abilities against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals. Correlations between the major components including catechins, GA, CA, ascorbic acid, and sugars (sucrose, fructose, and glucose) and free radical scavenging activities of the tea drinks were determined to verify the contribution of each component to the antioxidant activity. This is the first study concerning the major components of three types of commercial tea drinks and their correlations to free radical scavenging activity.

MATERIALS AND METHODS

I. *Chemicals and Reagents*

EC, EGC, EGCG, ECG, GA, gallocatechin gallate (GCG), and CA, Folin-Ciocalteu reagent, DPPH, ABTS, aluminum chloride hexahydrate, sodium ascorbate, formic acid, and ammonium acetate were all obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

II. *Tea Drinks*

Twenty-seven commercial bottled tea drinks, including green, Oolong, and black tea, were purchased in Taichung. The ingredient labeled of the tea drinks are described in Table 1. The tea drinks were filtered with a 0.45-μm filter, and the filtrate was used as test sample.

III. *Determination of the Concentration of Total Phenolics*

The concentrations of total phenolics in the tea drinks were determined using the Folin-Ciocalteu reagent[16]. A 20 μL sample was mixed with 90 μL of Folin-Ciocalteu reagent. The mixture was shaken vigorously for 3 min, and then 175 μL of 7.5% Na_2CO_3 (w/v) was added. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 750 nm using an ELISA reader (Bio-Tek instruments, Inc., Winooski, VT, USA). Gallic acid (GA) was chosen as a standard to construct a standard curve. The total phenolics in tea drinks are expressed in μg of gallic acid equivalents/mL.

IV. *Determination of the pH Value*

The pH of the tea drinks was measured using a SUNTEX microprocessor pH meter sp-2200 (Taipei, Taiwan).

V. *Determination of EGC, EGCG, GCG, EC, ECG, GA, and CA*

EGC, EGCG, GCG, EC, ECG, GA, and CA concentrations in the tea drinks were determined according to the method of Cabrera *et al.*[6] with modifications. The filtrate of a tea drink was injected into a Hitachi-L7400 Series LC System (Tokyo, Japan) equipped with an ultraviolet (UV) detector set at 280 nm. An Agilent Extend-C18 reversed-phase column (5 μm, 250 × 3.0 mm) was used. The mobile phase consisted of 10 mM ammonium acetate containing 0.5% formic acid (Solvent A) and methanol containing 0.5% formic acid (Solvent B). The flow rate was 0.8 mL/min, and the total running time was 50 min. The gradient system was 2% B (0-1 min), 2%

B to 43% B (1-27 min), 43% B to 90% B (27-33 min), 90% B to 2% B (33-40 min), and 2% B (40-50 min). The column temperature was at ambient temperature (25 °C). The retention times of GA, EGC, CA, EGCG, EC, GCG, and ECG were 5.0, 15.8, 17.9, 19.4, 20.6, 21.6, and 24.3 min, respectively. The concentrations of these compounds in the tea drinks were determined with the calibration curves of authentic standards. The calibration curves were linear over a concentration range of 0.2-300 μg/mL with correlation coefficients ≥ 0.999 for all compounds. The accuracy of the method was evaluated from the recovery assays which were calculated from the measured concentration vs. nominal concentration by the following equation:

Accuracy (%) = (Measured concentration ÷ Nominal concentration) × 100.

The acceptance criterion of accuracy was 95 - 105%. The precision of the method was expressed as the percent (%) relative standard deviation (RSD). The RSD (%) = Standard deviation of the QC group (accuracy) ÷ Average accuracy of the QC group × 100. The acceptance criterion was ≤ 10%. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as the concentration giving a signal equal to 3 times and 10 times the signal/noise (S/N) ratio, respectively.

VI. *Determination of Ascorbic Acid*

Ascorbic acid concentration in the tea drinks was determined following the method by de Quirós *et al.*[17]. Sample preparations and the mobile phase solvents used were the same as used for the determination of catechins. A Supelco C18 column (5 μm, 250 × 4.6 mm) was used. The gradient system was: 10% B (0-3 min), 10% B to 90% B (3-4 min), 90% B (4-6 min), 90% B to 10% B (6-7 min), and 10% B (7-16 min). The effluent was monitored using a UV detector set at 245 nm. The flow rate was 0.8 mL/min, and the total running time was 16 min. The retention time of ascorbic acid was 3.1 min. The concentration of the calibration curve ranged 12.5-500 μg/mL with correlation coefficients ≥ 0.999. The concentration of ascorbic acid in the

tea drinks was calculated by comparing the peak area of ascorbic acid in the drinks with that of the standard curve.

VII. *Determination of Sucrose, Fructose, and Glucose Contents*

Sucrose, fructose, and glucose concentrations in the tea drinks were determined by an enzymatic assay kit method according to the instruction of the manufacturer (Megazyme, Bray, Ireland).

VIII. *Determination of the Scavenging Abilities of the Tea Drinks against the DPPH and ABTS Radicals*

Tea drink samples were diluted 200 and 400 folds, respectively, with deionized water prior to analysis.

The scavenging activity of tea drinks against the DPPH free radical was determined by the method of Zhao *et al.*[18] with some modifications. An aliquot (224 μL) of diluted tea drink sample was added to 56 μL of an 80 μM DPPH solution in methanol. The mixture was then shaken vigorously and allowed to stand at room temperature for 30 min. Deionized water (224 μL) only was used as control. The absorbance of the reaction mixture was measured at 517 nm (Bio-Tek instruments, Inc., Winooski, VT, USA).

The scavenging activity of tea drinks against the ABTS free radical was determined by the method of Ozgen *et al.*[19] with some modifications. The ABTS free radical solution was prepared by dissolving 7 mM of ABTS and 2.45 mM of potassium persulfate in a 20 mM acetic acid solution. This mixture was allowed to stand for 16 h at room temperature in the dark to reach a stable oxidative state. The ABTS solution was then diluted with 20 mM acetic acid solution (pH 4.5) to obtain a solution with an absorbance of 0.700 ± 0.01 at 734 nm (Bio-Tek instruments, Inc., Winooski, VT,

USA). The ABTS solution (20 μL) was added to 230 μL of the tea drink sample solution. Deionized water (230 μL) only was used as the experimental control. The mixture was then shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance of the reaction mixture was measured at 734 nm.

The percent inhibition of the DPPH and ABTS radicals was calculated according to the following formula:

Inhibition (%) = (Absorbance of control- Absorbance of test sample/Absorbance of control) ×100.

Effects of the components of tea drinks including ascorbic acid, GA, EGCG, and GCG on the inhibition of DPPH and ATBS radicals were evaluated, and the concentration (μg/mL) of these components required to deplete the amount of both radicals by 50% (IC_{50} value) were determined. The maximum inhibitory actions of the scavenging abilities against the DPPH and ABTS radicals by tea drinks were 82% and 95%, respectively.

IX. *Data Analysis*

All experiments were performed, at least, in triplicate. Data are presented as the mean ± SD. A linear regression analysis was performed, and the correlation coefficient, *r*, between the scavenging abilities of the free radicals (DPPH and ABTS) and tea components was determined.

RESULTS AND DISCUSSION

I. *Total Phenolics and the Scavenging Abilities against the DPPH and ABTS Radicals*

The contents of total phenolics in the 27 commercial tea drinks (Table 2) ranged 130-639 μg/mL. The highest level was a green tea drink (639 μg/mL; Sample 3). The mean total phenolics in the three types of tea drinks were green tea (485 ± 94 μg/mL) > Oolong tea (348 ± 14 μg/mL) > black tea (236 ± 70 μg/mL). The scavenging abilities of the tea drinks on DPPH and ABTS radicals were used to determine their antioxidant activities[18,19]. The percent inhibition against DPPH and ABTS free radicals generation is shown in Table 2. Due to the high scavenging activity of the tea drinks against the DPPH and ABTS free radicals, all tea drinks were diluted 200 and 400 folds for the DPPH and ABTS assays, respectively. The scavenging activity of the 27 tea drinks against DPPH and ABTS free radicals ranged 24.2-70.6% and 23.0-91.7%, respectively. As expected, the green tea drinks showed the highest scavenging activity against DPPH (57.4 ± 7.9 %) and ABTS (75.3 ± 10.0 %) free radicals followed by Oolong tea drinks (DPPH: 45.6 ± 9.7 %; ABTS: 62.9 ± 13.5 %). Black tea drinks were found to have the lowest scavenging activity (DPPH: 28.9 ± 4.6 %; ABTS: 38.7 ± 11.2 %). The higher antioxidant activity observed in green tea drinks is probably due to higher content of total phenolics. Catechins, the major components of polyphenols, are probably the major contributor to the antioxidant activity of green tea drinks[1,2].

II. *Concentrations of Catechins (EGC, EGCG, GCG, EC, ECG), GA, and CA in Tea Drinks*

Figure 1 shows the HPLC-UV chromatograms of reference standards and representative samples of green (Sample No. 10), Oolong (Sample No. 17) and black (Sample No. 27) tea drinks. Table 3 summarizes the analytical data of the standard curves. Table 4 shows the pH and the concentrations of EGC, EGCG, GCG, EC, ECG, GA, CA and ascorbic acid in each commercial tea drink**.** Green tea drinks have the highest concentrations of catechins, followed by Oolong tea drinks. Black tea drinks had the lowest concentrations of catechins. This observation is in agreement with the

degree of fermentation of tea during the manufacturing process [1,2]. EGC was identified as the most abundant catechin in all tea drinks except for Samples 10, 17, and 27. The mean values of each catechin in the three types of tea drinks was EGC > EGCG > GCG > ECG = EC. This result is inconsistent with a previous study that EGCG was found to be the most abundant catechin in green tea[2] as well as in green tea drinks[20]. Tsai *et al.*[21] reported relatively close values on a dry-weight basis (%, w/w) for EGCG (5.1 ± 2.3 %) and EGC (4.5 ± 1.5 %) in different varieties of commercial green teas in Taiwan. Therefore, the cultivated variety of teas rich in EGC may be a contributing factor for the high concentration of EGC observed in the commercial tea drinks in Taiwan. It has been reported that the GCG concentration of total catechins could increase from approximately <1.5% of normal level to as much as 50% in some tea drinks[11]. However, in this study, a moderate amount of GCG (54 ± 21 μg/mL; accounting approximately 14.5% of total catechins) was observed in the green tea drinks. The high GCG concentration found in the green tea drinks is probably derived from the thermal conversion of EGCG during autoclaving[11,22].

The stability of catechins in tea drinks varies with the ingredients and pH of the tea drinks[11,12]. Sucrose appears to have no effect but ascorbic acid exerts a protective effect from degradation of catechins[11]. The catechins stability in tea drinks is sensitive to pH[12]. At pH 4, catechins were found stable for 28 days at 25 °C[13] while stable only 20 min at 120 °C [11]. Catechins were also found to degrade gradually or underwent epimerization at pH 6. In this study, green tea drinks except Sample 1 had pH > 6 (mean value: 6.3 ± 0.3). The concentrations of ascorbic acid in green tea drinks vary widely, and it was higher than those in the Oolong and black tea drinks (Table 4). It was shown that supplementation of ascorbic acid to a concentration of 0.2 mg/mL cause little or no change in the pH of the tea drink, but the stability of catechins can be significantly improved[12]. Thus, the higher ascorbic acid

supplemented in green tea drinks than that in Oolong or black tea drinks may reduce the degradation of catechins during storage.

In this study, GA was detected in all tea drinks tested (2.4-64.4 μg/mL). Black tea drinks were found to have the highest concentration of GA (24.9 ± 18.4 μg/mL). This result was accompanied by notably lower pH values of black tea drinks (5.5 ± 0.9) than those of green (6.3 ± 0.3) and Oolong (6.3 ± 0.2) tea drinks. Fernández *et al.*[23] analyzed the contents of GA in 45 tea samples and found higher GA and lower catechin levels in fermented teas. Zuo *et al.*[2] reported that the fermentation process enhanced the liberation of GA from galloylcatechins in green tea and that both Pu-erh and black teas contain remarkably high level of GA. Since the pKa of GA is at 4.4, which may result in proton dissociation of the carboxyl group of GA from the galloyl moiety of some catechins in tea drinks, it is thus suggested that the lower pH observed in black tea drinks might be related to the higher GA level.

Concentrations of CA in all tea drinks varied (49.8-205 μg/mL) but the mean concentrations among the three types of tea drinks are comparable (green tea: 137 ± 29 μg/mL; black tea: 136 ± 50 μg/mL; and oolong tea: 144 ± 41 μg/mL). Fernández *et al.*[23] and Cabrera *et al.*[6] reported that CA was higher in black tea than green and Oolong teas. The reasons why CA was higher in black teas and comparable among the three types of tea drinks were unknown. Since the manufacture processes of the tea drinks, such as amounts of tea used, brewing methods and sterilization, etc., may vary, resulting profile of CA and catechins in tea drinks may not be the same as that reported in tea leaves. Nakagawa *et al.*[20] has reported the similar observation. The same study also showed that co-administration of CA and EGCG (0.7-3 mg CA and 1.6 mg EGCG per kg of body weight) increased the bioavailability of EGCG in humans by 36-60% [20]. These results suggest that CA in green tea drink may enhance the bioavailability of catechins especially that of the EGCG.

III. *Concentrations of Sugars in Tea Drinks*

Because tea catechins and alkaloids (caffeine and theobromine) contribute to the bitterness of tea drinks, some commercial tea drinks are supplemented with sugars to mask the bitter taste. Table 5 shows the concentrations of glucose, sucrose, and fructose in the tea drinks. Sucrose was the main sweetener in these tea drinks. All black tea and some green and Oolong tea drinks were supplemented with sucrose. In addition to sucrose, Sample 12 was also found supplemented with glucose (18.3 g/L) and fructose (22.4 g/L). It was demonstrated that sugar-sweetened beverages are a significant contributor to weight gain and can lead to increased risks of type 2 diabetes mellitus and cardiovascular diseases[14,15]. This result implies that sugar-sweetened tea drinks may counteract the beneficial effects of unsweetened tea drinks in reducing the risk of these diseases..

IV. *Correlation Coefficient Analyses between Tea Components and Antioxidant Activities*

The principal hypothesis is that the putative benefits of tea are associated with the strong free radical-scavenging and/or antioxidant properties of their polyphenols, especially catechins[5]. For all tea drinks, there was a strong correlation between the antioxidant activities and total phenolics (DPPH: $r = 0.80$; ABTS: $r = 0.88$) (Table 6). Regression correlation coefficients also showed important contributions of the contents of EGC (DPPH: $r = 0.82$; ABTS: $r = 0.87$), GCG (DPPH: $r = 0.69$; ABTS: $r = 0.80$), ECG (DPPH: $r = 0.59$; ABTS: $r = 0.65$), and EC (DPPH: $r = 0.80$; ABTS: $r = 0.76$). The free radical scavenging activities also had high correlations with total catechins (DPPH: $r = 0.79$; ABTS: $r = 0.87$). The ascorbic acid contents only weakly influenced the antioxidant potencies of the tea drinks (DPPH: $r = 0.28$; ABTS: $r = 0.3$). GA showed the greatest contribution to the free radical scavenging activities only in black tea drinks (DPPH: $r = 0.95$; ABTS: $r = 0.77$). In sugar-sweetened tea

drinks, the total sugar content showed weak or negative correlations with the free radical scavenging activities (DPPH: r = -0.22; ABTS: r = -0.16) (data not shown). These results indicated that the greatest contribution of phenolic compounds to the free radical scavenging activities of the tea drinks, especially green and Oolong tea drinks, were from EGC, EC, ECG, and GCG. It was interesting to note that CA also had a high correlation with free radical scavenging activities in green tea drinks (DPPH: r = 0.74; ABTS: r = 0.78). However, in this study, CA at concentrations of up to 200 μg/mL had no significant effect on the inhibition of DPPH and ABTS radicals (< 3%) (data not shown). This result can be explained by the fact that tea catechins and CA are extracted out simultaneously by hot water during the brewing process.

Catechins were found to effectively scavenge various radical species (i.e. hydroxyl and DPPH radicals) although their respective scavenging abilities tended to differ somewhat according to the type of the radical species tested[24]. The important structure features of catechins for scavenging the DPPH radical are *ortho*-trihydroxy group in the B ring and a galloyl moiety at the 3 position and, therefore, the DPPH scavenging abilities of EGC ECG and EGCG are stronger than other catechins[25]. EGCG appeared to be the most effective regardless of the radical species[24, 26]. In contrast to a previous study which indicated that EGCG in green tea has a high correlation (r > 0.8) with the DPPH-scavenging activity[27], in this study, EGCG in green tea drinks showed a relatively weak influence on the free radical scavenging activities (DPPH: r = 0.39; ABTS: r = 0.44). One of the possible explanations for this observation is the partial conversion of EGCG to GCG in tea drinks. This speculation might be supported by the observation that EGCG+GCG also had high correlations with the antioxidant activities in green (DPPH: r = 0.69; ABTS: r = 0.79) and Oolong tea drinks (DPPH: r = 0.78; ABTS: r = 0.79). Moreover, similar inhibitory potencies (IC_{50} values) of EGCG and GCG against DPPH (EGCG: 3 μg/mL; GCG: 3 μg/mL)

and ABTS (EGCG: 2.5 μg/mL; GCG: 2.3 μg/mL) radicals were noted (Table 2). Similar antioxidant potencies between EGCG and GCG for both human low-density lipoprotein oxidation and DPPH free radical assays were reported by Xu *et al.*[22]. These results indicated that in green and Oolong tea drinks, the epimerization from EGCG to GCG might not cause any loss of their antioxidant capacities, but could reduce the weight of EGCG as a major contributor to antioxidant activity. On the other hand, the green tea drinks have higher concentration of EGC than the Oolong and black tea drinks (Table 4); however, the lower correlation between the free radical scavenging activities and the EGC level was found in green tea drinks than in the Oolong and black tea drinks (Table 6). These results suggested that components other than catechins, such as ascorbic acid and unknown phenolic compounds, in green tea drinks may also contribute to the antioxidant activity and reduce the correlation between the free radical scavenging activities and the EGC level. Nevertheless, EGCG and EGC, due to their high radical scavenging abilities and high levels, were suggested as the major antioxidants in green tea drinks.

It is noteworthy that the total phenolic contents and the inhibiting activities against DPPH and ABTS free radicals of green tea drinks were approximately 2-fold higher than that of black tea drinks (Table 2). However, the difference on total catechins between green and black tea drinks was much greater (8.8-fold) than the difference on the inhibiting activities against DPPH and ABTS radicals (Table 4). This result clearly indicates that components other than catechins in black tea drinks may also contribute to the free radical scavenging activities. It was demonstrated that theaflavins, the fermentation product of catechins in black tea, and catechins in green tea are equally effective antioxidants, suggesting that the fermentation process may not greatly reduce the antioxidant properties[28]. In this study, our data also showed similar free radical scavenging activities between GA and catechins (Table 2).

Therefore, even the total phenolics and free radical scavenging activities of black tea drinks were lower than those of green tea drinks, their antioxidant potentials cannot be ignored.

Tea polyphenols, especially catechins, were demonstrated to prevent oxidative modifications of cellular lipids, proteins, and nucleic acids by multidirectional antioxidant actions[29]. Thus regular consumption of tea drinks may provide beneficial effects in reducing the risk of chronic diseases such as cardiovascular disease and cancers. Assuming an average tea consumption to be one to two bottles (approximately 600 mL per bottle) per day, the total phenolics intake from green tea drinks would be approximately 0.29-0.58 g/day, which is close to one half of that of a typical American diet (1 g/day)[30]. It is suggested that commercial tea drinks represent an excellent source of phenolic compounds that exhibit important antioxidant activities.

In summary, total phenolics, total catechins, and EGC were major contributors of tea drinks to the scavenging abilities against DPPH and ABTS radicals. Green tea drinks have higher free radical scavenging activity than black and Oolong tea drinks due to higher levels of total phenolics and catechins. Our data also show that commercial tea drinks, especially the green tea drink, can be an important dietary source of polyphenols with antioxidant activity.

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Table 1. Product name and ingredients on the labels of the commercial tea drinks.

Sample number	Product name	Ingredient label
Green tea		
1	Kutao Super Green Tea	Fiber
2	Cha Li Won Japanese Style Green Tea Sugar Free	Flavor, vitamin C
3	AGV Health and Oil Trim	Fiber
4	Royal Tea Garden Tea Japanese Sugar-free Green Tea	-
5	Every Morning Green Tea	Fiber, flavor, vitamin C
6	Uni-President Green Tea	Flavor, vitamin C
7	Life Foam Green tea	Sugar, flavor
8	Kuang Chuan Cold Brew Green Tea	Sugar, flavor, vitamin C
9	Y.E.S Health and Oil Trim	Vitamin C
10	Pure Tea Sugar-free Green Tea	Flavor
Black tea		
11	Kuang Chuan Cold Brew Black Tea	Sugar, flavor, vitamin C
12	Taisun Iced Black Tea	Sugar, flavor, malic acid
13	Cha Li Won English Tea	Sugar, flavor, vitamin C
14	Life Foam Black Tea	Sugar, flavor
15	Mine Chine Black Tea	Sugar, flavor, vitamin C
16	Uni-President Black Tea	Sugar, flavor, vitamin C
17	Pure Tea Black Tea	Sugar, flavor
Oolong tea		
18	Fantastic Tea	Vitamin C
19	Every Morning Black Oolong Tea	Fiber, vitamin C
20	Kuang Chuan Cold Brew Oolong Tea	Sugar, flavor
21	Dong Ding Oolong Tea	Sugar, vitamin C
22	Uni-President Oolong Tea	Flavor, vitamin C
23	Cha Li Won Alishan Oolong Tea	Flavor, vitamin C
24	Royal Tea Garden Tea Cold Mountain Oolong Tea	-
25	Cha Li Won Ching Sing Oolong Tea	Flavor, vitamin C
26	Cha Li Won Pekoe Oolong Tea	Sugar, vitamin C
27	Pure Tea Oolong Tea	Sugar, flavor, vitamin C

Table 2. Total phenolics and the antioxidant activities of 27 commercial tea drinks[a]

	Total phenolics (μg/mL)	Inhibition of free radicals	
		DPPH (%)	ABTS (%)
Green tea			
1	383 ± 35	58.6 ± 0.9	68.9 ± 0.5
2	491 ± 57	53.3 ± 2.4	71.9 ± 3.2
3	639 ± 69	67.2 ± 4.8	91.7 ± 0.8
4	345 ± 60	45.8 ± 3.1	64.9 ± 0.1
5	596 ± 85	70.6 ± 1.9	90.2 ± 1.6
6	452 ± 79	53.0 ± 1.8	70.8 ± 1.7
7	437 ± 40	51.8 ± 2.6	72.0 ± 1.0
8	467 ± 10	50.9 ± 1.3	62.0 ± 4.7
9	466 ± 8	60.4 ± 1.7	79.0 ± 0.3
10	577 ± 68	62.9 ± 1.2	81.3 ± 0.3
Mean ± SD	485 ± 94	57.4 ± 7.9	75.3 ± 10.0
Black tea			
11	180 ± 13	28.1 ±.4.4	28.6 ± 0.4
12	130 ± 0	24.7 ± 1.3	23.0 ± 0.1
13	315 ± 13	57.2 ± 5.7	56.3 ± 0.5
14	234 ± 9	26.8 ± 3.8	40.6 ± 0.5
15	262 ± 16	34.0 ± 1.3	42.2 ± 0.9
16	211 ± 13	24.2 ± 1.6	34.5 ± 1.1
17	323 ± 13	33.2 ± 2.9	45.9 ± 0.5
Mean ± SD	236 ± 70	28.9 ± 4.6	38.7 ± 11.2
Oolong tea			
18	373 ± 14	50.9 ± 3.4	67.7 ± 0.5
19	467 ± 16	52.8 ± 2.7	74.3 ± 1.2
20	324 ± 29	42.6 ± 1.5	59.0 ± 1.1
21	242 ± 16	39.4 ± 1.8	49.1 ± 0.2
22	233 ± 6	30.9 ± 2.5	42.6 ± 0.3
23	480 ± 17	59.1 ± 2.4	80.9 ± 0.1
24	342 ± 26	51.6 ± 1.4	62.7 ± 0.5
25	372 ± 7	50.4 ± 0.5	69.6 ± 0.2
26	273 ± 3	32.5 ± 2.9	45.9 ± 0.5
27	369 ± 53	58.1 ± 3.5	77.2 ± 0.4
Mean ± SD	348 ± 14	45.6 ± 9.7	62.9 ± 13.5
Reference standards [b]			
Ascorbic acid (5 μg/mL)		37.4 ± 1.6	42.2 ± 0.7
Gallic acid (5 μg/mL)		70.7 ± 1.8	80.1 ± 0.7
EGCG (5 μg/mL)		73.7 ± 1.2	79.7 ± 0.3

1426-100.1.10 GCG (5 μg/mL)	74.3 ± 2.4	87.7 ± 0.2

[a] Tea drinks were diluted 200 and 400-folds with deionized water for the DPPH and ABTS assays, respectively. Data are expressed as the mean ± standard deviation (n = 3).

[b] IC_{50} values of ascorbic acid, gallic acid (GA), epigallocatechchin gallate (EGCG), and gallocatechin gallate (GCG) for the DPPH assay were 4.9, 2.5, 2.3, and 2.2 μg/mL, respectively. The corresponding values of ascorbic acid, GA, EGCG, and GCG for the ABTS assay were 5.9, 2.1, 2.4, and 2.2 μg/mL, respectively.

Table 3. Calibration curve equations, correlation coefficients, limit of detection (LOD), limit of quantification (LOQ), reproducibility (RSD in %), and recovery of seven tea drink components examined in this study

Component	Calibration curve equation (n=4)	Correlation Coefficient (n = 4)	Linear range (μg/mL) (n = 4)	LOD (μg/mL) (n = 4)	LOQ (μg/mL) (n = 4)	RSD (%) (n = 10)	Recovery (%) (n = 4)
GA	y = (0.0223 ± 0.0002)x - (4.9503 ± 0.4678)	0.9998	0.2-100	0.0125	0.05	0.6	99
EGC	y = (0.0015 ± 0.0000)x - (0.3932 ± 0.3162)	0.9999	0.4-250	0.2	0.4	1.0	108
CA	y = (0.0234 ± 0.0004)x - (10.2826 ± 7.5065)	0.9999	0.6-200	0.0375	0.15	0.4	101
EGCG	y = (0.0092 ± 0.0002)x - (12.3443 ± 1.1374)	0.9992	0.6-300	0.075	0.15	0.5	105
EC	y = (0.007 ± 0.0001)x - (1.4979 ± 0.9939)	0.9999	0.4-100	0.1	0.4	0.6	99
ECG	y = (0.0209 ± 0.0003)x - (9.6684 ± 1.2561)	0.9990	0.2-50	0.025	0.05	0.5	101
GCG	y = (0.0209 ± 0.002)x - (13.5156 ± 7.1249)	0.9999	0.2-200	0.1	0.2	0.7	110

GA, gallic acid; EGC, epigallocatechin; CA, caffeine; EGCG, epigallocatechin gallate; EC, epicatechin; ECG, epicatechin gallate; GCG, gallocatechin gallate.

1426-100.1.10

Table 4. Concentrations (μg/mL) of catechins, gallic acid (GA), caffeine (CA), ascorbic acid and pH of tea drinks

	pH	GA	EGC	CA	EGCG	GCG	EC	ECG	Total catechins	Ascorbic acid
Green tea										
1	5.8	5.6 ± 0.6	122 ± 9	108 ± 8	87 ± 6	44 ± 3	10.9 ± 1.0	9.1 ± 0.5	273 ± 20	204 ± 10
2	6.7	8.8 ± 0.3	177 ± 3	118 ± 4	95 ± 3	48 ± 1	15.1 ± 0.3	12.6 ± 0.3	347 ± 7	46.8 ± 40.2
3	6.1	7.9 ± 0.1	186 ± 3	181 ± 2	142 ± 1	103 ± 1	16.7 ± 0.1	23.2 ± 1.1	471 ± 4	484 ± 33.9
4	6.3	4.7 ± 0.1	113 ± 1	96 ± 1	90 ± 1	43 ± 0	11.9 ± 0.2	9.9 ± 0.0	267 ± 1	223 ± 9
5	6.6	6.5 ± 0.1	214 ± 8	173 ± 6	165 ± 6	78 ± 3	22.0 ± 0.6	17.5 ± 0.5	497 ± 18	251 ± 24
6	6.3	9.8 ± 0.2	165 ± 2	125 ± 3	93 ± 1	45 ± 1	13.8 ± 0.4	13.1 ± 0.2	331 ± 4	98.1 ± 27.2
7	6.1	8.0 ± 0.7	167 ± 1	128 ± 1	98 ± 0	53 ± 1	15.3 ± 0.1	18.2 ± 0.1	352 ± 1	156 ± 9
8	6.3	10.6 ± 0.7	152 ± 1	131 ± 2	41 ± 6	30 ± 2	19.5 ± 0.2	21.6 ± 0.3	263 ± 7	4.5 ± 3.7
9	6.0	21.1 ± 0.4	162 ± 0	140 ± 4	131 ± 1	43 ± 1	22.8 ± 0.4	14.1 ± 0.3	372 ± 3	99.7 ± 6.4
10	6.6	2.4 ± 0.1	173 ± 17	173 ± 9	278 ± 38	49 ± 1	26.1 ± 1.0	35.5 ± 1.2	512 ± 56	21.1 ± 17.1
Mean ± SD	6.3 ± 0.3	8.6 ± 5.0	163 ± 29	137 ± 29	122 ± 65	54 ± 21	17.4 ± 5.0	17.5 ± 7.9	369 ± 95	159 ± 143
Black tea										
11	5.9	16.5 ± 0.5	23.7 ± 0.3	152 ± 7	2.1 ± 0.0	1.5 ± 0.2	4.0 ± 0.1	1.2 ± 0.1	32 ± 1	6.2 ± 2.0
12	3.4	7.2 ± 0.3	6.7 ± 0.8	50 ± 2	3.0 ± 0.1	0.1 ± 0.0	1.4 ± 0.0	1.8 ± 0.1	13 ± 1	58.2 ± 11.8
13	5.9	64.4 ± 1.7	55.0 ± 6.6	131 ± 3	BLQ	2.6 ± 0.3	12.7± 0.3	0.7 ± 0.2	70 ± 8	6.8 ± 2.7
14	5.8	22.1 ± 1.2	21.1 ± 2.4	116 ± 7	3.5 ± 0.1	0.9 ± 0.0	2.6 ± 0.3	1.7 ± 0.1	30 ± 3	4.7 ± 3.2
15	5.8	24.5 ± 1.3	33.7 ± 3.7	181 ± 9	2.0 ± 0.1	1.8 ± 0.0	5.1 ± 0.6	2.2 ± 0.0	44 ± 3	4.4 ± 3.2
16	6.1	16.4 ± 0.5	16.3 ± 1.6	120 ± 3	1.7 ± 0.1	0.9 ± 0.1	2.5 ± 0.2	1.5 ± 0.1	22 ± 1	4.3 ± 1.6
17	5.7	23.5 ± 0.9	26.8 ± 1.5	205 ± 14	47.0 ± 2.0	1.8 ± 0.1	3.0 ± 0.2	3.1 ± 0.2	81 ± 2	6.0 ± 0.9
Mean ± SD	5.5 ± 0.9	24.9 ± 18.4	26.2 ± 15.3	136 ± 50	9.9 ± 18.2	1.4 ± 0.8	4.5 ± 3.8	1.8 ± 0.8	42 ± 25	12.9 ± 20.0
Oolong tea										
18	6.1	4.3 ± 0.2	122 ± 6	116 ± 5	112 ± 7	48.4 ± 2.8	13.4 ± 0.9	13.7 ± 0.7	293 ± 32	59.4 ± 6.8
19	6.5	16.3 ± 1.6	113 ± 16	205 ± 2	105 ± 2	49.1 ± 0.4	13.2 ± 1.4	12.7 ± 0.8	242 ± 107	5.0 ± 2.4
20	6.6	5.5 ± 1.1	72 ± 15	78 ± 2	18 ± 6	18.6 ± 2.3	6.3 ± 1.2	5.6 ± 2.1	107 ± 42	5.0 ± 2.1
21	6.3	11.4 ± 0.5	70 ± 3	113 ± 2	51 ± 1	28.5 ± 0.3	6.5 ± 0.5	8.7 ± 0.2	138 ± 49	4.1 ± 1.6
22	6.1	8.3 ± 0.4	41 ± 10	105 ± 2	33 ± 1	16.2 ± 0.1	3.7 ± 0.4	4.1 ± 0.4	82 ± 39	3.8 ± 1.9
23	6.1	11.8 ± 1.2	165 ± 17	199 ± 5	154 ± 8	75.7 ± 1.0	15.9 ± 0.8	18.8 ± 0.8	353 ± 150	4.2 ± 2.3
24	6.3	11.0 ± 1.3	83± 6	173 ± 4	50 ± 3	32.9 ± 1.4	9.7 ± 0.5	10.8 ± 0.6	158 ± 54	5.2 ± 1.3
25	6.4	9.1 ± 0.8	110 ± 7	141 ± 21	83 ± 10	45.1 ± 2.6	10.5 ± 1.7	12.0 ± 1.7	218 ± 65	3.8 ± 2.3
26	6.3	23.1 ± 1.8	19 ± 7	155 ± 5	BLQ	1.0 ± 0.0	4.4 ± 0.7	2.1 ± 0.3	26 ± 7	4.3 ± 1.8
27	6.6	5.3 ± 0.2	147 ± 7	151 ± 8	209 ± 9	32.2 ± 0.7	22.5 ± 1.1	26.0 ± 1.2	426 ± 36	115 ± 6.9
Mean ± SD	6.3 ± 0.2	10.6 ± 5.7	94 ± 46	144 ± 41	90 ± 62	34.8 ± 21.0	10.6 ± 5.8	11.4 ± 7.1	204 ± 126	21.0 ± 37.3

刪除: examined in this study

格式化: 字型: (中文) 新細明體, (中文) 中文 (台灣)

刪除: drink

格式化: 字型: (中文) 新細明體, (中文) 中文 (台灣)

格式化: 字型色彩: 藍色

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BLQ, below the limit of quantification (0.15 μg/mL). Total catechins=EGCG + EGC + GCG + ECG + EC.

Table 5. Concentrations of glucose, sucrose, fructose, and total sugars of tea drinks (g/L)[a]

	D-Glucose	Sucrose	D-Fructose	Total sugars [a]
Green tea drink				
1	-[b]	1.3 ± 0.1	-	1.3 ± 0.1
2	-	1.5 ± 0.1	-	1.5 ± 0.1
3	-	-	-	-
4	-	-	-	-
5	-	4.1 ± 0.0	1.0 ± 0.0	5.1 ± 0.1
6	-	-	-	-
7	-	65.5 ± 0.5	-	65.5 ± 0.5
8	-	39.2 ± 0.1	-	39.2 ± 0.1
9	-	-	-	-
10	-	3.4 ± 0.2	-	3.4 ± 0.2
Black tea drink				
11	-	41.0 ± 0.2	-	41.2 ± 0.0
12	18.1 ± 0.3	43.7 ± 2.4	19.4 ± 2.1	81.2 ± 4.8
13	-	38.1 ± 2.6	-	38.2 ± 2.6
14	-	59.5 ± 4.8	-	59.5 ± 4.8
15	-	68.6 ± 4.4	-	68.6 ± 4.4
16	-	60.3 ± 6.9	-	60.3 ± 6.9
17	-	70.2 ± 1.7	-	70.2 ± 1.7
Oolong tea drink				-
18	-	-	-	-
19	-	3.0 ± 0.8	0.9 ± 0.1	3.9 ± 1.1
20	-	-	-	-
21	-	31.0 ± 4.1	-	31.1 ± 4.1
22	-	-	-	-
23	-	-	-	-
24	-	-	-	-
25	-	-	-	-
26	-	35.6 ± 4.2	-	35.6 ± 4.2
27	-	35.7 ± 4.3	-	35.7 ± 4.3

[a] Total sugars, glucose + sucrose + fructose.

[b] The sugar concentration was below the quantification limit of 0.5 g/L.

Table 6. Linear correlation coefficient (r) between tea components and antioxidant activities of tea drinks examined in this study

	Total phenolics	Ascorbic acid	GA	CA	EGCG	EGC	GCG	ECG	EC	EGCG + GCG	Total catechins
Total tea drinks											
DPPH	0.80	0.28	-0.02	0.13	-0.10	0.82	0.69	0.59	0.80	0.69	0.79
ABTS	0.88	0.30	-0.08	0.16	-0.07	0.87	0.80	0.65	0.76	0.79	0.87
Green tea drinks											
DPPH	0.67	0.20	0.00	0.74	0.39	0.50	0.52	0.17	0.31	0.56	0.64
ABTS	0.74	0.33	0.00	0.78	0.44	0.60	0.75	0.18	0.26	0.67	0.75
Black tea drinks											
DPPH	0.48	-0.07	0.95	0.06	0.00	0.90	0.71	-0.13	0.95	0.00	0.50
ABTS	0.90	-0.37	0.77	0.28	0.18	0.78	0.70	0.00	0.58	0.08	0.69
Oolong tea drinks											
DPPH	0.74	0.20	-0.05	0.32	0.00	0.89	0.66	0.78	0.80	0.78	0.84
ABTS	0.86	0.17	-0.04	0.34	0.00	0.90	0.68	0.73	0.78	0.79	0.84

In the linear correlation analysis, tea components were regarded as X and % of DPPH and ABTS radical scavenging activities as Y.

GA, gallic acid; EGCG, epigallocatechchin gallate; EGC, epigallocatechin; GCG, gallocatechin gallate; ECG, epicatechin gallate; EC, epicatechin;

Total catechins=EGCG + EGC + GCG + ECG + EC.

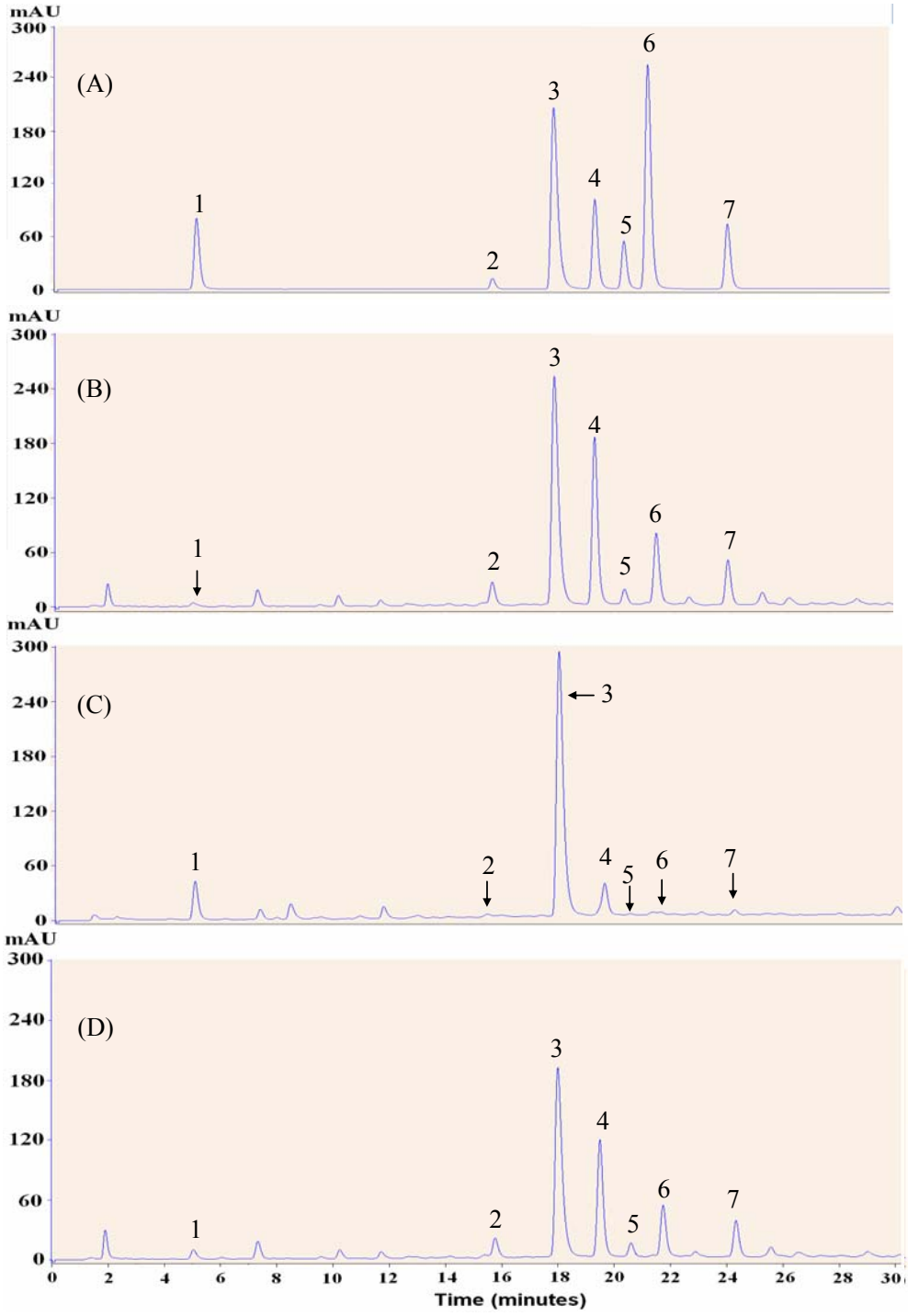

Figure 1. HPLC-UV chromatograms of (A) standards, (B) green tea drink, (C) black tea drink and (D) Oolong tea drink at 280 nm. Peaks: 1, gallic acid (GA); 2, epigallocatechin (EGC); 3, caffeine (CA); 4, epigallocatechin gallate (EGCG); 5, epicatechin (EC); 6, gallocatechin gallate (GCG); 7, epicatechin gallate (ECG).