Distribution of free, esterified, and insoluble bound forms of phenolics in tea seeds and their antioxidant activity

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Abstract In this study, phenolic compounds in their phenolic extract (PE), free phenolic (FP), esterified phenolic (EFP), and insoluble bound phenolic (ISBP) forms were extracted from tea seeds and quantified using HPLC to obtain information on the phenolics that are present in tea seeds along with their antioxidant activities. The total phenolic content of the ISBP fraction was higher than that of the FP and EFP fractions. The phenolic acids in tea seeds exist either in esterified or glycosided forms. Moreover, hydroxycinnamic acid derivatives have been identified as important phenolic acid components in tea seeds. The ISBP fraction was an effective antioxidant based on the results obtained from the *in vitro* assays performed, which include the DPPH and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)-derived nitrogen-centered radical scavenging activities, Fe^{3+} -2,4,6-tripyridyl-s-triazine reducing ability, as well as Cu^{2+} and Fe^{2+} ion chelating effects.

Keywords: tea seed, phenolic compound, antioxidant activity

Introduction

The second most favorite beverage in the world is tea, made from leaves and sprouts of *Camellia sinensis*. As a byproduct of tea cultivation, tea seeds are available in enormous quantities, and approximately 3 million tons of tea is produced and consumed each year (1). In the past, a majority of tea seeds were discarded. Nonetheless, tea seed extract, a byproduct of tea seed processing, has been found to almost completely inactivate the human influenza virus (2), and since then, many studies have focused on the biological activity of tea seed extracts (3–5). In general, tea contains many chemical components that reveal medicinal and pharmacological properties. In particular, biologically active compounds in tea seeds include saponins, flavonoids, vitamins, and oil based materials (6). Several studies have reported the antioxidant activities of plant-derived phenolics (7,8). Phenolic compounds, which are present in every plant, are responsible for the organoleptic properties of foods and beverages derived from plants. Because phenolic compounds affect the quality of food products, it is of considerable importance to be able to analyze them accurately. Recently, various studies have related the exhaustion of polyphenol-rich foods with the prevention of various diseases correlated to aging owing to their antioxidant activities (9).

In fact, the main source of antioxidants in plants is phenolic structured antioxidant compounds found in both free and bound forms. A number of free phenolics are flavonols, whereas mainly

phenolic acids are bound phenolics (10). The free forms of phenolic compounds are uncommon as compared to esters, glycosides, and bound composites (11). Phenolic acid is divided into two main groups: benzoic acid and cinnamic acid. The derivatives of benzoic acid are gallic, protocatechuic, and *p*-hydroxybenzoic acids, whereas the derivatives of cinnamic acid are caffeic, *p*-coumaric, and ferulic acids. Robbins (12) reported that phenolic acid accounts for 30% of the dietary phenolic compounds present in plants as free and bound forms. The bound forms are more commonly found and occur in the form of ester and insoluble bound complexes (13).

Until recently, little research has been reported on phenolic acid and its antioxidant activity in tea seeds. Therefore, to better understand their medicinal properties, it is necessary to conduct an *in vitro* study of the antioxidant activity of phenolic acids according to their different forms present in tea seeds. This study aims to evaluate the distribution of free phenolics (FP), esterified phenolics (EFP), and insoluble bound phenolics (ISBP) in tea seeds along with their antioxidant activities.

Materials and Methods

Chemicals and reagents DPPH radical, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), catechin, ferulic acid, gallic acid, and rutin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Aluminum chloride, anhydrous sodium sulfate,

Folin–Ciocalteu reagent, methanol, *n*-hexane, sodium carbonate, and sodium nitrite were obtained from Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical reagent grade.

Sample preparation Green tea (*Camellia sinensis* L.) seeds collected from the Hadong tea garden (Hadong, Korea) in October 2013 were first de-hulled and washed with water. Then, they were immediately freeze-dried. The resulting lyophilized tea seeds were ground into powder using a grinder and de-oiled via Soxhlet extraction using *n*-hexane.

Isolation of free phenolic compounds Neo *et al.* (14) have previously reported the isolation of FP. Initially, 1 g of tea seeds was weighed and homogenized using a mixture of aqueous methanol and acetone (both at 70%, 1:1 v/v) at room temperature. Then, each homogenized mixture was centrifuged and the supernatant was combined before reducing their volumes by evaporation under vacuum. The pH of the aqueous suspension was also adjusted to 2 and then the aqueous phase was extracted with *n*-hexane to remove other lipid contaminants. The free phenolics in the aqueous phase were extracted using a mixture of diethyl ether–EtOAc (1:1 v/v). The diethyl ether–EtOAc extracts were combined and dried using Na₂SO₄. Next, the drying agent was filtered using Whatman No.1 filter paper and then the filtrate was evaporated to dryness under vacuum at 45°C before re-dissolving the residue using 5 mL of MeOH. The aqueous extract was then combined with the initially obtained cloudy precipitate for the extraction of esterified phenolics.

Isolation of esterified phenolic compounds Neo *et al.* (14) have also reported the isolation of EFP. Initially, esters were directly hydrolyzed using 4 N NaOH at room temperature. Furthermore, the pH of the hydrolyzed solution was adjusted to 2 and *n*-hexane was added to the solution to remove any residual oil. Moreover, the pH-adjusted extract was washed using the diethyl ether–EtOAc mixture. The extracts were combined and evaporated to dryness under vacuum at 45°C, thus leaving behind EFP compounds. These compounds were then re-dissolved in 5 mL of MeOH.

Isolation of insoluble bound phenolic compounds The isolation of ISBP compounds was achieved according to the procedure by Neo *et al.* (14). Initially, the de-oiled tea seeds (obtained after a methanol–acetone extraction) were hydrolyzed using 4 N NaOH at room temperature. The pH of the solution was then acidified to 2 and the solution was centrifuged. The clear supernatant was extracted using *n*-hexane, followed by a diethyl ether–EtOAc, and re-dissolved in 5 mL of MeOH.

Isolation of phenolic extract (PE) compounds Phenolic compounds were extracted from the de-oiled tea seeds according to the procedure by Wang and Helliwell (15). First, 1 g of de-oiled tea seeds was added to 40 mL of 60% EtOH. Subsequently, 5 mL of 6 M HCl was

added to the mixture, and the mixture was refluxed for 2 h. The PE was cooled and filtered, and the final volume was made up to 100 mL using 60% EtOH.

Determination of total phenolic content (TPC) The TPC was colorimetrically estimated using the Folin–Ciocalteu method (16). First, 0.5 mL of Folin–Ciocalteu reagent was added to 0.1 mL of PE followed by the addition of distilled water (7 mL). Second, the mixture was allowed to stand at room temperature for 5 min before adding 1.5 mL of a 7.5% Na₂CO₃ solution. Third, this solution was left at room temperature for 2 h and the absorbance was measured at 765 nm using gallic acid and methanol as the reference standard and blank solution, respectively. All values were expressed as milligrams of gallic acid equivalents (GAE) per 100 g dry matter of the de-oiled tea seeds.

Liquid chromatography analysis of phenolic acids The phenolic acid constituents of the FP, ISBP, EFP, and PE tea seed fractions were determined using a modified method that was originally reported by Mattila and Kumpulainen (17). The extracts were filtered through a 0.45 µm filter and then injected into a HPLC system. The measurements were performed using an Agilent 1260 Infinity Quaternary liquid chromatograph (Hewlett Packard, Wilmington, NC, USA) equipped with a multiple wavelength detector operating at 280 nm. Chromatographic separations were achieved on an Agilent Zorbax RRHD SB-C18 column (2.1 mm i.d.×100 mm, 1.8 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The column temperature and flow rate were set at 30°C and 0.3 mL/min, respectively. Two streams of eluent (A and B) were used to achieve a gradient elution. Solution A comprised water with 0.1% formic acid, whereas solution B comprised acetonitrile with 0.1% formic acid. The following gradient was used: 0% B (0 min), 5% (0–3.5 min), 15% (3.5–7.1 min), 40% (7.1–25 min), 40% (25–26 min), 100% (26–27 min), 100% (27–29 min), and 0% (29–35 min). The standards used were caffeic acid, chlorogenic acid, ferulic acid, gallic acid, *m*-coumaric acid, *o*-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and vanillic acid.

DPPH assay The anti-radical activities of the extracts were estimated according to the procedure by Delgado-Andrade *et al.* (18). First, a 200 µL aliquot of the respective extracts was added to 1 mL of DPPH[•] (74 mg/L) in MeOH. Second, a solution of DPPH[•] prepared daily gave a final absorption of 1.8 AU at 520 nm. Third, the mixture was shaken for 30 min and the absorption was measured at 520 nm. Various concentrations of Trolox solutions were used for calibration (0.15–1.15 mM). The results were expressed as mM equivalents of Trolox (TE)/100 g of the sample.

Ferric reducing ability of plasma (FRAP) assay The ferric-reducing ability of the extracts was evaluated according to the Benzie and Strain method (19). Briefly, 900 µL of FRAP reagent (freshly prepared

and warmed at 37°C) was first mixed with 90 µL of distilled water and 30 µL of the respective extracts or water as an appropriate blank reagent. The final dilution of the reaction mixture's test sample was 1:34. The FRAP reagent contains 2.5 mL of a 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl in addition to 2.5 mL of 20 mM FeCl₃·6H₂O and 25 mL of a 0.3 M acetate buffer (pH 3.6). The absorption was recorded at 595 nm every 15 s using a spectrophotometer (Epoch, Biotech Instruments, Winooski, VT, USA). The reaction was monitored for up to 30 min with the temperature maintained at 37°C. Various concentrations of Trolox solutions were used for calibration. The results were expressed as mM equivalents of TE/100 g of the sample.

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS⁺] assay

The antioxidant capacity was estimated according to the procedure reported by Re *et al.* (20). Briefly, ABTS⁺ was prepared by the reaction between a 7 mM ABTS stock solution and 2.45 mM K₂S₂O₈. The ABTS⁺ solution was diluted with 5 mM PBS buffer (pH 7.4) to obtain an absorbance value of 0.70±0.02 at 730 nm. After adding 10 µL of the respective extracts, Trolox standards were added to 4 mL of the diluted ABTS⁺ solution, and the absorbance was recorded for 20 min using a spectrophotometer (Epoch, Biotech Instruments). Various concentrations of Trolox solutions were used for calibration. The results were expressed as mM equivalents of TE/100 g of the sample.

Metal ion chelating activity The metal ions chelating ability of the extracts was investigated according to the procedures reported by Wang and Xiong (21) and Dinis *et al.* (22). For the Cu²⁺ chelating ability, 1 mL of 2 mM CuSO₄ was mixed with 1 mL of pyridine at pH 7.0 and 20 µL of 0.1% pyrocatechol violet. After adding 1 mL of the respective extracts, the solution's blue color disappeared and this was attributed to the dissociation of Cu²⁺ ions. The absorbance was recorded at 632 nm using a spectrophotometer (Epoch, Biotech Instruments) after 5 min of the reaction. For the Fe²⁺ chelating ability, 100 µL of the extracts was added to 600 µL of distilled water and 100 µL of 0.2 mM FeCl₂·4H₂O. The mixture was left at room temperature for 30 s. The resulting reaction mixture was then added to 200 µL of 1 mM ferrozine, and the absorbance was recorded at 562 nm using a spectrophotometer (Epoch, Biotech Instruments) after a waiting time of 10 min at room temperature. The Cu²⁺ and Fe²⁺ chelating activities were calculated using the following equation:

$$\text{Chelating activity (\%)} = [(A_0 - A_s)/A_0] \times 100\%,$$

where A_0 and A_s are the absorbance values of the control and sample, respectively.

Statistical analysis All of the experimental data were analyzed via the analysis of variance (ANOVA), and the significant differences among means obtained from triplicate analysis at $p < 0.05$ were determined by Duncan's multiple range tests using a statistical analysis software (SPSS 17.0, IBM Inc., North Castle, NY, USA). A

Fig. 1. Total phenolic content of the phenolic extract (PE), free phenolic (FP), esterified phenolic (EFP), and insoluble bound phenolic (ISBP) forms in tea seed.

correlation matrix was used to standardize the variables, which were not measured on the same scale.

Results and Discussion

Total phenolic content The TPC of the extracts, as determined by spectrophotometric analysis, ranged from 1.52 to 38.68 mg GAE per 100 g of dried de-oiled tea seeds (Fig. 1). The TPC of the ISBP fraction was significantly higher ($p < 0.05$) than that of the FP and EFP fractions. The sum of TPC in the FP, ISBP, and EFP fractions was 19.78 mg GAE, whereas that of PE was 38.68±0.48 mg GAE per 100 g of dried de-oiled tea seeds. The sum of the three fractions (FP, ISBP, and EFP) resulted in a lower amount of TPC compared to PE. In this study, two extraction methods were used to gain a better understanding of the phenolic compounds existing in tea seeds. Velioglu *et al.* (23) reported that the antioxidant property of the compounds was well associated with their phenolic content. Phenols exhibit good antioxidant, antimutagenic, and anticancer properties (24). Hence, PE can be considered to comprise both free and bound phenolic compounds in tea seeds obtained via solvent extraction and acid reflux.

Liquid chromatography analysis of phenolic acids The phenolic acid constituents of the FP, ISBP, EFP, and PE tea seed fractions (as quantified by HPLC) are shown in Table 1 and Fig. 2. The FP fraction had the lowest phenolic acid content, whereas higher amounts of phenolic acids were detected in both ISBP and EFP fractions. This suggests that the phenolic acids of tea seeds were either esterified or glycosided. Phenolic acids are generally present in the bound form and are easily changed from one form to another in biological

Table 1. The phenolic acid constituents in the phenolic extract (PE), free phenolic (FP), esterified phenolic (EFP), and insoluble bound phenolic (ISBP) forms in tea seeds¹⁾

	Concentrations (mg/100 g of sample, dried de-oiled tea seeds)				
	PE	FP	ISBP	EFP	Total phenolic acids ³⁾
hydroxybenzoic acid derivatives (HBA)					
<i>p</i> -Hydroxybenzoic acid	-	-	-	-	-
Gallic acid	-	-	3.391±0.001	-	3.391±0.001 ^f
Vanillic acid	8.272±0.020 ^{a2)}	0.394±0.003 ^c	0.399±0.001 ^c	1.339±0.003 ^b	10.404±0.007 ^b
hydroxycinnamic acid derivatives (HCA)					
Chlorogenic acid	0.265±0.016 ^c	0.728±0.019 ^b	4.997±0.030 ^a	0.271±0.001 ^c	6.261±0.017 ^d
Caffeic acid	3.398±0.025 ^b	0.121±0.002 ^c	0.107±0.001 ^c	15.402±0.004 ^a	19.028±0.008 ^a
<i>p</i> -Coumaric acid	0.841±0.063 ^c	1.134±0.005 ^b	2.807±0.002 ^a	-	4.782±0.023 ^e
Ferulic acid	-	-	-	-	-
<i>m</i> -Coumaric acid	7.665±0.007	-	-	-	7.665±0.007 ^c
<i>o</i> -Coumaric acid	-	-	0.451±0.001	-	0.451±0.001 ^g
Total HBA	8.272±0.020 ^a	0.394±0.003 ^d	3.790±0.001 ^b	1.339±0.003 ^c	13.795±0.007
Total HCA	12.169±0.028 ^b	1.983±0.009 ^d	8.362±0.009 ^c	15.673±0.003 ^a	38.187±0.012
Total phenolic acids ⁴⁾	20.441±0.024 ^a	2.377±0.006 ^d	12.152±0.005 ^c	17.012±0.003 ^b	

¹⁾Value are mean±SD (n=5); -: not detected (limit of detection: 0.1 mg/100 g).

^{2)a–g}Different superscripts within a column indicate significant difference at the *p*<0.05 level.

³⁾Total phenolic acids are the sum of individual phenolic acids identified in all four phenolic fractions.

⁴⁾Total phenolic acids are the sum of each phenolic acid in each of the phenolic fraction. PE, phenolic extract; FP, free phenolic; ISBP, insoluble bound phenolic; EFP, esterified phenolic.

processes (25). In this study, the most abundant phenolic acids were caffeic acid (19.03 mg/100 g of dried de-oiled tea seeds), vanillic acid (10.40 mg/100 g of dried de-oiled tea seeds), *m*-coumaric acid (7.67 mg/100 g of dried de-oiled tea seeds), and chlorogenic acid (6.26 mg/100 g of dried de-oiled tea seeds). In addition, hydroxycinnamic acid derivatives have been identified as important phenolic acid components in tea seeds. The sum of the six hydroxycinnamic acids is the highest in the EFP fraction (15.67 mg/100 g of dried de-oiled tea seeds). The total amount of the nine phenolic acids was approximately 20.44, 17.01, 12.15, and 2.38 mg/100 g of dried de-oiled tea seeds in the PE, EFP, ISBP, and FP fractions, respectively. Phenolic acid has been reported to exhibit antioxidant, antimutagenic, antitumor, and anticarcinogenic properties (26). Hence, the ISBP fraction with its higher phenolic acid content indicated stronger antioxidant activities compared to the FP fraction. Hydroxycinnamic acid derivatives generally occur as esters in foods, whereas hydroxybenzoic acid derivatives are mostly present as glucosides (27). The total amounts of hydroxybenzoic acid derivatives in this study were approximately 40, 17, 31, and 8% for the PE, FP, ISBP, and EFP fractions, respectively. Meanwhile, the total amounts of hydroxycinnamic acid derivatives in the PE, FP, ISBP, and EFP fractions were approximately 60, 83, 69, and 92%, respectively. Thus, the result suggested that the tea seed phenolic acids were present in the insoluble bound form.

DPPH radical scavenging activity All four tea seed extracts exhibited antioxidant potential. The antioxidant activities of the extracts were in the order of ISBP>PE>EFP>FP and ranged from 8.55 to 61.68 mM TE as determined by the DPPH assay. In comparison to other fractions, the FP fraction with the lowest TPC exhibited significantly

lower (*p*<0.05) antioxidant activities, as shown in Table 2 and Fig. 1. The high antioxidant activity of the ISBP fraction (at 61.68 mM TE/100 g of dried de-oiled tea seeds) represents the total antioxidant capacity of the dried de-oiled tea seed extract. Meanwhile, the higher free radical scavenging ability of the insoluble bound fraction could be attributed to the fact that the TPC obtained from this particular extract was higher than that of the other polyphenolic extracts, which is line with many earlier reports that correlated the TPC to the antioxidant properties of plants (28,29). In addition, the presence of glycoside in the bound polyphenols may reduce the capacity of the extract to scavenge free radicals.

2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity As shown in Table 2, the ISBP fraction had the highest ABTS activity, which is in agreement with the results from the DPPH and FRAP assays. The ABTS radical scavenging activity of the ISBP fraction was 3640.13 mM TE/100 g of dried de-oiled tea seeds. Quantitatively, the DPPH and ABTS assays gave very different results even though the same trend was observed in both studies. Del Castillo *et al.* (30) have previously reported that stronger responses during the ABTS test could be obtained via aqueous dilution as opposed to using ethanolic dilution, and they attributed this difference to the fact that some of the components contributing to the antioxidant activity in aqueous dilutions are insoluble in ethanol. In this study, the low values obtained can be attributed to the fact that the DPPH assay was conducted in methanolic media. Rufián-Henares and Morales (31) reported that the difference in radical scavenging activity is because of the different reaction media.

Fig. 2. Representative high-performance liquid chromatography separations of phenolic acids from tea seed extracts. A=PE (phenolic extract); B=FP (free phenolic); C=ISBP (insoluble bound phenolic); D=EFP (esterified phenolic) at 280 nm. Peak identification: (1) gallic acid; (2) chlorogenic acid; (3) caffeic acid; (4) *p*-coumaric acid; (5) *m*-coumaric acid; (6) *o*-coumaric acid; and (7) vanillic acid.

Table 2. Antioxidant activity determined by the TEAC_{DPPH}, TEAC_{FRAP}, and TEAC_{ABTS} methods¹⁾

	TEAC _{DPPH}	TEAC _{FRAP}	TEAC _{ABTS}
PE	15.54±0.24 ^{b2)}	145.33±12.03 ^b	760.08±17.54 ^b
FP	8.55±0.20 ^d	62.21±9.94 ^b	302.55±8.43 ^d
ISBP	61.68±0.47 ^a	1249.63±106.91 ^a	3640.13±47.77 ^a
EFP	9.90±0.24 ^c	93.71±12.40 ^b	354.56±16.34 ^c

¹⁾PE, phenolic extract; FP, free phenolic; ISBP, insoluble bound phenolic; EFP, esterified phenolic.

²⁾Data expressed as mM equivalents of Trolox/100 g of dried de-oiled tea seed; ^{a-d}Different superscripts within a column indicate significant difference at the $p<0.05$ level.

Table 3. Metal ions chelating activity of the phenolic extract (PE), free phenolic (FP), esterified phenolic (EFP), and insoluble bound phenolic (ISBP) forms in tea seeds¹⁾

	Cu ²⁺ chelating activity (%)	Fe ²⁺ chelating activity (%)
PE	21.60±0.29 ^{b2)}	20.42±0.76 ^b
FP	16.91±0.36 ^c	3.16±0.84 ^d
ISBP	97.95±0.66 ^a	38.96±1.56 ^a
EFP	20.81±0.30 ^b	11.06±0.80 ^c

¹⁾PE, phenolic extract; FP, free phenolic; ISBP, insoluble bound phenolic; EFP, esterified phenolic.

^{2)a-d}Different superscripts within a column indicate significant difference at the $p<0.05$ level.

Fe³⁺-2,4,6-Tripyridyl-s-triazine reducing ability As shown in Table 2, all four tea seed extracts showed strong Fe³⁺ reducing activities, similar to the results obtained from the DPPH radical scavenging assay. Remarkably, the Trolox equivalent antioxidant capacity–TEAC_{FRAP} values were constantly higher than those obtained for TEAC_{DPPH}. For the Fe³⁺-TPTZ reducing activity, the ISBP fraction demonstrated the highest value at 1249.63 mM TE/100 g of dried de-oiled tea seeds. The reducing power of extracts is typically related with the existence of reducing agents, which impart antioxidant activity by donating a hydrogen atom and thus terminating the free radical chain. The proven reducing ability of tea seed extracts suggests that it could contribute considerably toward the entire antioxidant effect. Moreover, the antioxidant activity of plant extracts is known to exhibit various mechanistic actions such as binding of transition metal ion catalysts, breakdown of peroxides, prevention of chain initiation, reductive ability on metals, and scavenging of radicals (32). Hence, these results indicate that dried de-oiled tea seed extracts can function as an electron donor.

Metal ion chelating activity As shown in Table 3, the extracts exhibited the Cu²⁺ and Fe²⁺ ion chelating activities. The de-oiled tea seeds exhibited a strong chelating effect on Cu²⁺ and Fe²⁺ ions. The ISBP fraction exhibited the highest metal ion chelating activity. Moreover, the chelating capacity for Cu²⁺ ions of all four tea seed extracts was significantly higher than that for the Fe²⁺ ions. The chelating activities of the ISBP fraction were 97.95% and 38.96% for

Table 4. Pearson's correlation coefficients of antioxidant activity, total polyphenol content, and total flavonoid of the phenolic extract (PE), free phenolic (FP), esterified phenolic (EFP), and insoluble bound phenolic (ISBP) forms in tea seeds¹⁾

	PE	FP	ISBP	EFP
DPPH	0.987	0.930	0.966	0.891
FRAP	0.277	0.893	0.999 ^{*2)}	0.877
ABTS	0.929	0.371	0.988	0.999*
Cu ²⁺ chelating	0.970	0.784	0.987	0.930
Fe ²⁺ chelating	0.812	0.786	0.628	1.000**

¹⁾PE, phenolic extract; FP, free phenolic; ISBP, insoluble bound phenolic; EFP, esterified phenolic.

²⁾*, ** significant at $p<0.05$ and 0.01, respectively.

the Cu²⁺ and Fe²⁺ ions, respectively.

Correlation between antioxidant activity and total polyphenol content

A correlation analysis was conducted between the TPC and antioxidant activity (Table 4). For the PE fraction, there is no correlation between the antioxidant activity and TPC. However, a strong positive correlation ($R^2=0.970$ and $R^2=0.812$, respectively) was observed between the TPC and Cu²⁺ as well as Fe²⁺ ion chelation. Similarly, for the FP fraction, there is no correlation between the antioxidant activity and TPC. However, a marginal positive correlation ($R^2=0.784$) was observed between TPC and Cu²⁺ ion chelation. For the ISBP fraction, a strong negative correlation was noticed between FRAP and TPC ($R^2=0.999$, $p<0.05$), whereas a strong positive correlation ($R^2=0.987$) was observed between Cu²⁺ ion chelation and TPC. Moreover, strong positive significant correlations were obtained between TPC and ABTS as well as Fe²⁺ ion chelation ($R^2=0.999$ and $R^2=1.000$, respectively) for the EFP fraction.

In conclusion, this study has revealed new knowledge on the different types of tea seed phenolics and their antioxidant activities. The TPC of the ISBP fraction was higher than that of the FP and EFP fractions. The TPC of PE was higher than that of all three fractions, *i.e.*, FP, ISBP, and EFP. The phenolic acids in tea seeds exist in esterified or glycosided forms. The most abundant phenolic acids identified in this study were caffeic acid, vanillic acid, *m*-coumaric acid, and chlorogenic acid. Moreover, hydroxycinnamic acid derivatives were found to be a major phenolic acid constituent in tea seeds. The ISBP fraction was an effective antioxidant in different *in vitro* assays with respect to the DPPH and ABTS radical scavenging activities, Fe³⁺-TPTZ reducing ability, as well as Cu²⁺ and Fe²⁺ ion chelating effects. Therefore, our results show that tea seeds, a byproduct of tea cultivation, contain abundant phenolic compounds with antioxidant activity.

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Disclosure The authors declare no conflict of interest.

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