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ORIGINAL PAPER

Black tea processing waste as a source of antioxidant and antimicrobial phenolic compounds

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Abstract The objective of this study was to evaluate the value-added potential of black tea processing waste (BTPW) as a source of antioxidant and antimicrobial phenolic compounds. The effects of extraction solvent (water, 50 % ethanol and 80 % ethanol) and sample [different BTPW streams and black tea (BT)] and their interaction on the yield of phenolics (catechins, theaflavins and gallic acid), antioxidant and antimicrobial activity of the extracts were investigated. Total catechin (EC, EGCG, ECG) and theaflavin contents of BTPW samples were in the range of 5.2–6.0 and 11.5–16.0 mg/g DW, respectively. While catechins and theaflavins were recovered quantitatively using aqueous ethanol solvents, only 25–28 % of catechins and 6–7 % of theaflavins could be recovered in water extracts. Antioxidant activities of BTPW extracts, which were in the range of 2.23–3.59 (for DPPH), 0.59–0.92 (for FRAP) and 1.59–2.43 μmol TE/mg extract (for ABTS assay), were comparable to those of BT extracts. BTPW and BT extracts exhibited antimicrobial activity against *S. aureus* (1.26–3.65 mm), *S. flexneri* (1.33–3.89 mm) and *B. cereus* (1.87–3.90 mm); however, inhibition of *C. albicans* was not observed. BTPW can be used as a raw material for the development of value-added products with antioxidant and antimicrobial properties for applications in food, pharmaceutical, cosmetic and agricultural sectors. Aqueous ethanol solvents offer low-cost, non-toxic, green alternatives for the recovery of antioxidant and antibacterial phenolics from BTPW.

Keywords Black tea waste · Extraction of phenolics · Catechins · Theaflavins · Antioxidant · Antimicrobial

Introduction

Black tea (*Camellia sinensis*), one of the most popular drinks worldwide, is considered a rich source of phenolic compounds such as catechins and theaflavins [1, 2]. Health benefits of tea, and in vitro antioxidant and antimicrobial activity of tea extracts have been mainly attributed to the phenolic content of tea [3, 4].

Black tea processing waste (BTPW) contains parts of the tea plant (such as fibrous materials including stems, stalks and dust particles) that are physically removed during different stages of processing mainly due to quality considerations. Although BTPW is produced in high quantities at tea factories and offers an attractive raw material for the recovery of bioactive phenolic compounds, research on its characterization and processing as a phenolic source has been rather limited.

In their study on the antioxidant activity of BTPW and old tea leaves, Farhoosh et al. [5] investigated the effects of methanolic, hot water and ethyl acetate extracts on lipid oxidation and the reducing power of these extracts. BTPW and old tea leaves had comparable stabilization factors. The inhibiting effect of methanolic extracts of BTPW on lipid oxidation (as measured by oxidation rate ratio) was stronger than that of old tea leaves and green tea leaves. Reducing power of the ethyl acetate extracts was highest, while that of the hot water extracts was lowest. Black tea sub-product, a by-product of black tea processing, which is

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used in the production of instant and iced tea, and for blending purposes, had total phenolic content and DPPH radical scavenging activity in the range of 8.3–9.6 g GAE/100 g DW and 16–18.5 g ascorbic acid equivalents/100 g DW, respectively [6]. While antimicrobial activity of black tea has been investigated [3, 4], the antimicrobial properties of BTPW have not been reported. Research on the utilization of BTPW has been largely limited to the recovery of caffeine [7]. Its use in particleboard manufacture was also proposed due to its superior decay resistance, termite resistance similar to bio-based composites and high termite mortality [8].

The aim of this study was to investigate the potential of BTPW as a source of antioxidant and antimicrobial phenolic compounds. For this purpose, the phenolic contents of black tea and two BTPW samples were determined, and the effects of solvent (water, 50 % ethanol and 80 % ethanol) and sample (black tea and BTPW) on total extraction yield, phenolic yield (catechins, theaflavins and gallic acid), antioxidant activity and antimicrobial activity of the extracts were investigated.

Materials and methods

Materials

Black tea (BT, Grade 2) and BTPW (oven waste-OW and grading waste-GW) samples were obtained from the same processing line at a black tea factory in the Black Sea region of Turkey during the second harvest season of 2012. Samples were kept at −20 °C until they were used.

Ethanol (reagent grade, ≥99.8), Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (97 %), ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (≥98 %), potassium persulfate (ACS grade, ≥99 %), DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-Tris (2-pyridyl)-*s*-triazine) (≥99 %), acetic acid (analytical grade), HCl (analytical grade), Na_2CO_3 (ACS grade), Folin–Ciocalteu phenol reagent (2 N), gallic acid (ACS grade, ≥98), EDTA (ethylenediaminetetraacetic acid disodium salt, dihydrate) (ACS grade, ≥99 %), L-ascorbic acid (98 %), (+)-catechin (C) (≥99.0 %), gallic acid monohydrate (≥98.0 %), caffeine (≥99.0 %), (−)-epicatechin (EC) (≥98 %), (−)-epicatechingallate (ECG) (≥98 %), (−)-epigallocatechin (EGC) (≥95 %), (−)-epigallocatechingallate (EGCG) (≥95 %), (−)-catechingallate (CG) (≥98 %), (−)-gallocatechingallate (GCG) (≥98), tea extract from black tea (containing ≥ 80 % theaflavins) and theaflavin standard (>90 %) were obtained from Sigma, USA. Methanol (HPLC grade, VWR, Belgium), $FeCl_3$•$6H_2O$ (ACS reagent, Carlo Erba, Italy), acetonitrile (HPLC grade, JT Bakers, USA) and acetic acid (HPLC grade, Fisher Scientific, UK) were also used. Milli-Q-water from a Millipore Water Purification System (Milli-Q A10, France) was used for all experiments.

Solvent extraction

Samples were extracted in triplicate using water, 50 % ethanol and 80 % ethanol (2 % w/v) at 70 °C for 2 h using a water bath with an orbital shaker operated at 150 rpm. After filtration under vacuum, water extracts were freeze-dried directly. Ethanol extracts were freeze-dried after ethanol was evaporated using a rotary evaporator. All extracts were stored at −20 °C until further analysis. Extractions using 70 % methanol were also carried out in triplicate according to ISO 14502-2:2005 [9] to determine the phenolic content of GW, OW and BT as described in the HPLC analysis section. Total extraction yields were determined gravimetrically as % w/w on a dry weight basis (dwb). Moisture content (% w/w) of the samples was determined according to ISO 1573:1980 [10].

Determination of total phenolic content

Total phenolic content of the extracts was determined using the Folin–Ciocalteu assay according to Singleton et al. [11] as modified by Ainsworth et al. [12] for a microplate reader. Gallic acid (0–0.12 mg/ml) was used as a standard. Sample or gallic acid standard (100 µl) was mixed with 200 µl of 10 % Folin–Ciocalteu reagent in a micro centrifuge tube. Then, 800 µl of 700 mM Na_2CO_3 was added, and the mixture was incubated at room temperature for 2 h. After incubation, 200 µl of mixture was transferred to the well of a microplate. Absorbance readings were made at room temperature at 765 nm using a microplate reader (Thermo Scientific, Multiskan Go, Finland). Results were expressed as milligram gallic acid equivalents (GAE)/g dry weight (DW).

HPLC analysis

Phenolic (catechin, theaflavin and gallic acid) and caffeine contents of the extracts were determined according to ISO 14502-2:2005 [9] using a Thermo Accela HPLC system (Thermo Scientific, England) equipped with Phenomenex Phenyl-Hexyl column (4.6 mm × 250 mm, 5 µm) and a PDA detector. Peaks were identified by comparing their retention times with those of standards and spiked samples. Tea extract from black tea (containing ≥ 80 % theaflavins) was used for the identification of theaflavins. Quantification was done using the external standard method by constructing calibration curves. While individual catechin standards were used for the quantification of catechins, theaflavins were quantified using the standard theaflavin (>90 %). Data were expressed as mg/g DW.

Antioxidant analysis

Antioxidant activity of the extracts was determined using ABTS, DPPH and FRAP assays. Freeze-dried extracts were dissolved in 80 % methanol and were ultrasonicated for 15 min in an ultrasonic bath (Bandelin, Sonorex Digitec, Germany). Seven dilutions of the extracts and the standard (Trolox) were prepared in the concentration range of 0 and 0.06 mg/ml in 80 % methanol. After the antioxidant assays, absorbance versus concentration graphs were plotted and slopes of the linear portions of the graphs were determined. Antioxidant activities of the extracts were then calculated by dividing the slopes of the extracts by the slope of the Trolox standard. Results were expressed as μmol Trolox equivalent (TE)/mg extract.

DPPH radical scavenging activities were determined according to the method of Brand-Williams et al. [13] as modified by Fukumota and Mazza [14]. Sample or Trolox standard (22 μl), and DPPH solution (200 μl, 150 μM in 80 % methanol) were mixed in the well of a microplate. Absorbance readings were made at 30 °C and 515 nm using a microplate reader after 30 min, 3 and 5 h incubation at room temperature in the dark. The time interval giving the highest slope was used for the calculations.

FRAP was determined according to the method described by Benzie and Strain [15] with modifications for use with a microplate reader as described by Güçlü Üstündağ and Mazza [16]. Briefly, FRAP reagent was prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 10 mM TPTZ in 40 mM HCl and 2.5 ml of 20 mM $FeCl_3$ solution. Sample or Trolox standard (50 μl), and FRAP reagent (250 μl) were mixed in the well of a microplate. Absorbance readings were made at 593 nm at room temperature after 5 min of incubation.

ABTS radical scavenging activities were determined according to the method of Re et al. [17] with modifications as described by Güçlü Üstündağ and Mazza [16]. Briefly, 5 ml of 7 mM ABTS solution was reacted with 88 μl of 140 mM potassium persulfate solution. The reaction mixture was allowed to stand for 12–16 h at room temperature in the dark to produce a stable ABTS radical. The absorbance of the ABTS solution was adjusted to 0.70 ± 0.05 at 734 nm with 80 % methanol. Sample or Trolox standard (50 μl), and ABTS solution (250 μl) were mixed in the well of a microplate. Absorbance readings were made using a microplate reader at 30 °C after 5 min of incubation and 10 s of shaking.

Antimicrobial analysis

Important foodborne microorganisms including *Staphylococcus aureus* ATCC 25923, *Shigella flexneri* ATCC 12022, *Bacillus cereus* ATCC 11778 and *Candida albicans* ATCC 10231 were selected to test the antimicrobial activity of the extracts. All strains were obtained from Yeditepe University Food Microbiology Laboratory Collection. The identity of the strains was confirmed by gram staining and biochemical assays. Cultures were stored at −80 °C in the appropriate growth medium containing 30 % glycerol for the bacterial cultures and 10 % glycerol for the yeast culture. Viable counts were performed according to standard methods on Tryptic Soy Agar (TSA, Merck, Germany) at 37 °C for 18–24 h for the bacterial cultures and Sabouraud Dextrose Agar (SDA, Merck, Germany) at 30 °C for 24 h for the yeast culture. Results were expressed in CFU/ml.

The antimicrobial activities of all samples were tested using the agar well diffusion assay. The bacterial and yeast cultures were grown in Tryptic Soy Broth (TSB, Merck, Germany) at 37 °C for 18 h and in Sabouraud Dextrose Broth (SDB) at 30 °C for 24 h, respectively. All grown cultures were centrifuged (Hettich, EBA 20, Germany) at 3500 g for 10 min. The supernatant of each organism was discarded, and pellet was dissolved in saline solution to produce 0.5 McFarland standards, containing approximately 10^8 CFU/ml organisms. Final concentrations of the bacterial and yeast cultures were adjusted to 10^6 and 10^5 CFU/ml, respectively, by streaking onto appropriate agar with sterile cotton swab to generate a lawn of organisms.

Due to low solubility of the extracts in water, the freeze-dried samples were dissolved in 20 % (v/v) dimethyl sulfoxide (DMSO, Merck, Germany) to improve the diffusion of the extracts through agar. The total concentration of the samples was adjusted to 100 mg/ml, and the samples were filtered through 0.45-μm membranes (Sartorius, Germany) prior to analysis.

A sterile glass cylinder of 5 mm diameter was used to create wells. The wells were filled with 100 μl of samples (100 mg/ml). The plates were incubated at 37 °C for 18 h for the bacterial cultures and at 30 °C for 18 h for *C. albicans*. While sterile double-distilled water and 20 % (v/v) DMSO were used as negative controls, 100 μg/ml penicillin/streptomycin (Gibco, UK) and chlorhexidine (DrogSan, Turkey) were used as positive controls. The antimicrobial activity was evaluated by measuring the zone of inhibition around the wells against the tested microorganisms and was expressed in millimeter.

Statistical analysis

Data were expressed as mean ± standard deviation. Statistical analysis was carried out using SPSS Statistics. A one-way ANOVA using the GLM procedure and Tukey's test for multiple comparisons were used to compare the phenolic content of different samples. A two-way ANOVA using GLM including interaction terms was used to determine the effects of sample and solvent on total yield, phenolic yield

Table 1 Phenolic content (mg/g DW) of black tea (BT) and black tea processing waste (OW and GW) determined using ISO method (with 70 % methanol extraction)

Compound	Phenolic content (mg/g DW)[a]		
	Sample[b]		
	OW	GW	BT
Catechins			
GC	ND[c]	ND	ND
EGC	ND	ND	ND
C	ND	ND	ND
EC	4.69 ± 0.09^{b}	3.99 ± 0.21^{b}	5.87 ± 0.45^{a}
EGCG	$1.05 \pm 0.05^{a,b}$	0.91 ± 0.08^{b}	1.11 ± 0.04^{a}
GCG	ND	ND	ND
ECG	0.26 ± 0.02^{a}	0.31 ± 0.02^{a}	0.20 ± 0.02^{b}
Total catechins	6.01 ± 0.14^{b}	5.21 ± 0.28^{b}	7.18 ± 0.50^{a}
Total theaflavins	16.00 ± 0.59^{b}	11.53 ± 0.50^{c}	18.67 ± 0.36^{a}
Gallic acid	0.65 ± 0.02^{b}	0.59 ± 0.03^{b}	1.22 ± 0.08^{a}
Caffeine	16.59 ± 0.50^{b}	16.09 ± 1.29^{b}	20.28 ± 1.14^{a}

[a] Data expressed on a dry weight basis as mean ± SD ($n = 3$)

[b] *OW* oven waste, *GW* grading waste, *BT* black tea

[c] *ND* not detected. Means in the same row that share a letter are not significantly different ($p > 0.05$)

and antioxidant activity of the extracts. When interaction was significant, a simple main effects test was carried out to test the effect of the sample and solvent at each level of the other factor. When interaction was not significant, Tukey's test for multiple comparisons was carried out for each significant factor. For the analysis of antimicrobial activity, a three-factor model including three main effects (microorganism, sample and solvent) and interaction terms was used. Correlations between antioxidant activities and phenolic contents of the extracts were evaluated using Pearson correlation coefficient. p values <0.05 were considered statistically significant in all analyses.

Results and discussion

Phenolic content of black tea and black tea processing waste

Phenolic content of the samples was determined according to the standard ISO method using 70 % methanol. Results were reported on a dry weight basis using the moisture data (5.31 % ± 0.19 for OW, 8.35 % ± 0.08 for GW and 6.14 % ± 0.35 for BT) (Table 1). The BT sample contained 7.18 mg total catechins/g DW, 18.67 mg theaflavins/g DW, 1.22 mg gallic acid/g DW and 20.28 mg caffeine/g DW. While epicatechin (EC, 82 % of total catechins), epigallocatechingallate (EGCG, 15 %) and epicatechingallate (ECG, 3 %) were present, epigallocatechin (EGC), catechin (C), gallocatechin (GC) and gallocatechingallate (GCG) were not detected in the BT sample.

Phenolic content of black tea shows wide variation depending on numerous factors such as variety, geographical origin, agricultural and harvesting practices, harvest season, and processing and analytical methods [18]. Total catechin content of different grades of Turkish black tea (1–7) obtained from the same processing line showed a relatively small variation (18.3–22.5 mg total catechins/g tea). Higher quality grades (1 and 2) with the lowest fiber contents had higher catechin contents. Total theaflavin content was in the range of 1.36–4.17 mg/g, with the highest value obtained for 'grade 2' sample [2]. Total catechin content of Turkish black tea samples obtained from 20 tea plants in the first plucking season was in the range of 4.3–24.2 mg/g [19]. The effects of processing method and plucking season on the catechin and theaflavin contents of Turkish black tea samples (0.53–5.18 mg/g DW and 10.07–14.27 mg/g DW, respectively) were also demonstrated [20].

A much larger variation was observed especially for total catechin content when commercial samples from different geographical regions, factories (processing methods), raw materials, seasons and harvest time were included in the analysis. For example, total catechin content of commercially available black tea samples varied in the range of 5.4–69.5 mg/g DW in the USA [1], 5.6–47.5 mg/g DW in the UK [21], 6.69–37.37 mg/g tea [22] and 11.7–55.3 mg/g [23] in China, and 10.19–41.84 mg/g in Netherlands [24]. Theaflavin content, which was not reported for all samples, varied between 1.17 and 25.1 mg/g [1, 22, 23]. The extent of variation is also apparent in the total catechin contents of black tea samples (294 samples from 17 countries) included in the ISO tea database (0.5–135.6 mg/g) [25].

A similar variation was observed in the catechin profile in the literature. In most studies, EGCG and ECG were observed as the predominant catechins, with C, EC, GC and EGC also present [21, 24]. Samples with GC, C and EC as the predominant catechins were also reported [23]. Turkmen and Velioglu [20] only detected EGCG and ECG in Turkish black tea, whereas EGC and CG were the main catechins of different grades of black tea analyzed by Serpen et al. [2], with EGCG, ECG, EC and C being present to a lesser extent. Turkish black tea samples containing GC as the predominant catechin followed by C, EC and ECG were also reported [19]. Differences in the catechin profile might also arise from differences in plucking standard as specific catechins are distributed to different extents throughout the tea plant [26].

While the total catechin content of BT sample in this study is within the range of values reported for Turkish black tea, it is characterized by a higher total theaflavin and EC content. High EC content can be indicative of the

Table 2 Total extraction (% w/w) and total phenolic yield (mg GAE/g DW) of black tea (BT) and black tea processing waste (OW and GW)

Sample[a]	Extraction solvent	Total extraction yield (% w/w)[b]	Total phenolic yield (mg GAE/g DW)[b]
OW	Water	21.51 ± 0.55	42.00 ± 1.71
	50 % ethanol	29.58 ± 0.19	75.62 ± 4.97
	80 % ethanol	26.18 ± 2.02	71.52 ± 10.94
GW	Water	19.55 ± 1.44	33.43 ± 4.53
	50 % ethanol	24.94 ± 0.59	63.23 ± 1.61
	80 % ethanol	23.63 ± 0.50	57.61 ± 1.65
BT	Water	22.72 ± 1.46	54.94 ± 3.99
	50 % ethanol	33.71 ± 4.67	109.82 ± 14.11
	80 % ethanol	27.45 ± 0.60	83.94 ± 3.02

[a] *OW* oven waste, *GW* grading waste, *BT* black tea

[b] Data expressed on a dry weight basis as mean ± SD ($n = 3$)

extent of oxidation. The presence of EC in higher proportions in black tea compared to tea leaf has been attributed to the participation of the EC quinone in further oxidation reactions as an electron donor during black tea processing, which results in the quinone reverting to EC [27].

In addition to the factors affecting phenolic content of black tea leaves, extraction conditions (extraction solvent, time, solvent: tea ratio, loose tea vs. tea bag) used for the analysis of phenolics should also be considered while comparing the literature values. Water as an extraction solvent and brewing times of 30 s–5 min are commonly used to prepare extracts reflecting tea preparation customs [18]. However, higher phenolic yields could be achieved using aqueous ethanol and methanol, and longer extraction times. The effect of extraction solvent on phenolic yield is discussed in more detail in "Solvent extraction of phenolics" section.

Total catechin, theaflavin, gallic acid and caffeine contents of BTPW samples (OW and GW) were significantly lower than those of BT (Table 1). This is an expected result as waste samples contain fibrous parts of the tea plant (such as stems and stalks) with a lower phenolic content. No significant differences were observed in the composition of the waste samples except for the theaflavin content, which was highest in the OW sample followed by BT and GW. The high theaflavin content of the OW sample, which was collected from the filters of the drying oven and was composed mainly of dust particles, can be attributed to its small particle size. While small particle size can be indicative of a higher degree of leaf disruption (hence oxidation) [18], it might also facilitate the analytical extraction process by decreasing the mass transfer limitation.

Solvent extraction of phenolics

In this study, aqueous ethanol (50 and 80 %) and water were used as extraction solvents for the recovery of phenolic compounds with antioxidant and antimicrobial activity from BT and BTPW. Total extraction yield and phenolic yields of the solvent extracts are given in Tables 2 and 3.

Total extraction yield

The highest total extraction yield was achieved with 50 % ethanol (24.9–33.7 %), followed by 80 % ethanol (23.6–27.5 %) and then water (21.5–22.7 %) for all samples (Table 2). OW and BT had significantly higher total extraction yields than GW. Total extraction yield obtained with water was lower than those of aqueous ethanol solvents at 70 °C. A higher extraction yield can be obtained using water at higher temperatures. For example, extraction of BTPW using water at 80–130 °C resulted in yields of 25–30 % [5]. Total yields of 70 % methanol ISO extracts of OW, GW and BT were 22.37 ± 1.56, 23.73 ± 0.62 and 25.20 ± 0.38 %, respectively.

Yield of phenolics

Yields of total phenolic content obtained using water and aqueous ethanol from BT, OW and GW are given in Table 2. A significant interaction was observed between sample and solvent. Total phenolic yield of water was significantly lower than that of aqueous ethanol solvents for both waste samples. For BT, however, 50 % ethanol had significantly higher total phenolic yield than 80 % ethanol, followed by water. A significant sample effect was observed for aqueous ethanol solvents (i.e., BT > OW > GW) and water (i.e., BT > OW and BT > GW). Total phenolic yields of ethanolic extracts of BT in our study (83.94–109.82 mg GAE/g DW) are consistent with those obtained by Astill et al. [18] (90.3–151.0 mg GAE/g DW), Serpen et al. [2] (75.2–82.9 mg GAE/g tea) and Thea et al. [6] (95–133 mg GAE/g DW) for 70 % methanol. Yields for ethanolic

Table 3 Phenolic yield (mg/g DW) of aqueous ethanol (50 % and 80 % ethanol) and water extracts of black tea (BT) and black tea processing waste (OW and GW)

Compound	Yield (mg/g DW)[a]								
	OW[b]			GW[b]			BT[b]		
	Water	50 % etOH	80 % etOH	Water	50 % etOH	80 % etOH	Water	50 % etOH	80 % etOH
Catechins									
GC	ND[c]	ND	ND	ND	ND	ND	ND	ND	ND
EGC	ND	ND	ND	ND	ND	ND	ND	ND	ND
C	ND	ND	ND	ND	ND	ND	ND	ND	ND
EC	1.15 ± 0.23	3.50 ± 0.96	4.13 ± 0.65	1.19 ± 0.33	3.18 ± 0.69	4.12 ± 0.57	1.56 ± 0.48	6.53 ± 0.67	5.97 ± 1.32
EGCG	0.33 ± 0.02	1.93 ± 0.17	1.60 ± 0.17	0.16 ± 0.14	1.58 ± 0.06	1.57 ± 0.02	0.46 ± 0.10	2.31 ± 0.33	1.73 ± 0.12
GCG	ND	ND	ND	ND	ND	ND	ND	ND	ND
ECG	ND	0.51 ± 0.03	0.49 ± 0.02	ND	0.91 ± 0.10	1.00 ± 0.06	ND	0.70 ± 0.05	0.69 ± 0.14
Total	1.48 ± 0.22	5.94 ± 0.88	6.22 ± 0.72	1.35 ± 0.46	5.67 ± 0.84	6.69 ± 0.65	2.02 ± 0.58	9.55 ± 0.32	8.40 ± 1.54
Total theaflavin	0.95 ± 0.09	10.83 ± 2.26	16.11 ± 1.19	0.69 ± 0.02	6.89 ± 0.69	11.44 ± 1.36	1.37 ± 0.66	13.11 ± 1.06	16.31 ± 1.62
Gallic acid	1.76 ± 0.04	1.80 ± 0.22	1.53 ± 0.13	1.14 ± 0.13	1.21 ± 0.04	1.20 ± 0.10	2.14 ± 0.10	2.33 ± 0.44	2.04 ± 0.11
Caffeine	16.86 ± 0.68	19.36 ± 1.58	20.01 ± 1.34	13.14 ± 1.53	15.25 ± 0.32	16.59 ± 0.49	18.42 ± 1.08	22.73 ± 2.27	20.31 ± 0.77

[a] Data expressed on a dry weight basis as mean ± SD ($n = 3$)

[b] *OW* oven waste, *GW* grading waste, *BT* black tea

[c] *ND* not detected

extraction of BTPW are in the range reported by Thea et al. [6] for 70 % methanolic extracts of black tea sub-product (83–96 mg GAE/g DW).

The yields of individual phenolics recovered from BT and BTPW using water and aqueous ethanol solvents as determined by HPLC are given in Table 3. While 25–28 % of total catechins and 6–7 % of total theaflavins were recovered in water extracts compared to ISO extracts, the recovery values for ethanol extracts were 98–133 % and 87–101 %, respectively.

The interaction term (sample × solvent) was significant only for total catechin and theaflavin yield of the extracts. Total catechin and theaflavin yields obtained using water were significantly lower than those obtained using aqueous ethanol for all samples. A significant difference between 50 and 80 % ethanol extracts was observed only for total theaflavin yield, with 80 % ethanol extracts having significantly higher yields.

Sample effect on total catechin and theaflavin content was significant only for aqueous ethanol solvents. Significantly lower catechin yields were obtained for aqueous ethanol extracts of waste samples. For 80 % ethanol, GW had a significantly lower theaflavin yield than OW and BT. A significant difference was also observed between OW and BT for 50 % ethanol.

Sample had a significant effect on caffeine and gallic acid yield (i.e., BT > OW > GW). Solvent effect was also significant for caffeine such that aqueous ethanol gave higher yields than water; however, no significant solvent effect was observed for gallic acid. The different trends in caffeine and phenolic contents of the extracts can be attributed to distribution of these compounds within the tea plant (resulting in their presence in the waste and black tea samples in varying amounts) and their stability during processing.

Aqueous ethanol and methanol were shown to be much better solvents for theaflavins than water [1, 22]. However, no clear trend could be established for catechins in the literature. In the study of Wang et al. [22], the effects of boiling water (90 °C, 30 min) and different concentrations of ethanol/water mixtures (40–95 %, 80 °C, 20 min) on the extraction efficiency of tea phenolics were investigated. Total catechin and gallic acid yields of water extracts (11.74, 2.61 g/kg DW, respectively) were significantly higher. For total theaflavins, the highest extraction efficiency was obtained with 40–60 % ethanol (4.32–4.42 g/kg DW). The yields of total catechins, gallic acid and theaflavins decreased with increasing ethanol concentration of aqueous ethanol solvents. Similarly, Khokhar and Magnusdottir [21] found boiling water to be a better solvent for catechins and caffeine (5 min extraction) followed by 80 % methanol and 70 % ethanol at room temperature. However, total catechin, theaflavin and caffeine contents of 80 % ethanol extracts (reflux condenser, 3 × extraction, 60 °C, 15 min) were higher than those of water extracts (90 °C, 5 min) for all 32 commercial black tea samples analyzed by Friedman

et al. [1]. Similarly, higher catechin and theaflavin yields were obtained with 80 % cold methanol (14 h) compared to boiling water (10 min); the difference between the caffeine contents was small yet statistically significant (23.33 mg/g for water, 22.18 mg/g for 80 % methanol) [20]. The differences between the observed solvent effects can be attributed to differences between the extraction parameters (such as extraction time, temperature, number of extraction steps and sample–solvent ratio), which show a wide variation as indicated above. Solvent effects on extraction efficiency can be partly explained by solubility considerations. For example, limited water solubility of theaflavins limits their extractability. However, mass transfer aspects, such as interactions with the sample matrix, also need to be considered.

While water extracts contained only EC and EGCG, ECG was also detected in the ethanol extracts. A significant solvent–sample interaction was observed for EC and ECG. EC and ECG yields of water extracts were significantly lower than those of aqueous ethanol extracts. A significant sample effect on EC and ECG yield was observed for aqueous ethanol solvents. Waste samples had significantly lower EC than BT samples had. However, a significantly higher ECG yield was obtained from GW (i.e., GW > BT > OW). Both solvent (i.e., 50 % ethanol > 80 % ethanol > water) and sample effect (i.e., BT > OW > GW) were significant for EGCG.

Epimerization of catechins and degradation of catechins and theaflavins can occur during extraction of tea samples. The extent of degradation is determined by extraction time and temperature. Epimerization compounds (such as C and GCG) were not detected in our study after 2 h extraction at 70 °C. This is an expected result as epimerization reactions of catechins have been reported at temperatures higher than 80 °C in water and tea infusions [28]. Degradation of catechins (29 %) and theaflavins (56 %) was observed at 70 °C after 3 h in water [29]. Degradation did not appear to occur to a significant extent in aqueous ethanol solvents as evidenced by high recovery values obtained in relation to ISO extraction. However, it might have been a factor during water extraction.

Antioxidant activity

Antioxidant activities of BTPW (OW and GW) and BT extracts were determined using three different assays (DPPH, FRAP, ABTS) (Table 4). The results were expressed as w/w extract. Unit conversions were done using yield data where necessary for comparison purposes. For the DPPH assay, only the solvent effect was significant (i.e., 50 % ethanol > 80 % ethanol > water). Comparison and extensive discussion of DPPH radical scavenging activity results are not always possible because of differences in the incubation times and the units used to represent the results. Most of the studies on plant extracts including tea usually performed DPPH assay with 20–60 min of incubation times [30, 31]. However, time needed to complete the reaction between DPPH radical and antioxidant compound depends on the nature of the compound: Compounds such as gallic acid show slow kinetic behavior, and it may take 1–6 h to complete the reaction [13]. In our study, the reaction between DPPH radical and antioxidant compounds continued up to 5 h (data not shown). That may be the reason for lower DPPH scavenging activity of black tea reported by Buratti et al. [32] (20 mg/100 ml) who used a reaction time of 30 min. Another difficulty for the comparison of DPPH radical scavenging activity of black tea is the lack of consistency on units of reported values: Results were reported as EC50, % inhibition [30], antiradical power [33], or in terms of standard compound equivalents such as Trolox [32] or ascorbic acid [34].

Table 4 Antioxidant activity of black tea (BT) and black tea processing waste (OW and GW) extracts

Sample[a]	Extraction solvent	Antioxidant activity (μmol TE/mg extract)[b]		
		DPPH	FRAP	ABTS
OW	Water	2.36 ± 0.26	0.65 ± 0.01	1.59 ± 0.18
	50 % ethanol	3.59 ± 0.38	0.92 ± 0.11	2.38 ± 0.25
	80 % ethanol	3.31 ± 0.41	0.88 ± 0.07	2.37 ± 0.20
GW	Water	2.23 ± 0.27	0.59 ± 0.05	1.73 ± 0.16
	50 % ethanol	3.51 ± 0.15	0.85 ± 0.02	2.17 ± 0.55
	80 % ethanol	3.37 ± 0.40	0.85 ± 0.02	2.43 ± 0.13
BT	Water	2.43 ± 0.24	0.68 ± 0.03	1.77 ± 0.12
	50 % ethanol	3.91 ± 0.37	0.93 ± 0.02	2.36 ± 0.14
	80 % ethanol	3.17 ± 0.25	0.82 ± 0.05	2.08 ± 0.15

[a] *OW* oven waste, *GW* grading waste, *BT* black tea

[b] Data expressed on a dry weight basis as mean ± SD ($n = 3$)

The antioxidant activities of 50 and 80 % ethanol extracts determined by the FRAP assay (0.82–0.93 μmol TE/mg extract) were significantly higher than those of water extracts (0.59–0.68 μmol TE/mg extract). However, there were no significant differences between samples. Since FRAP assay results of black tea were reported in terms of Fe^{2+} (for example; Benzie and Szeto [35]), a direct comparison with the literature values is not possible. Higher FRAP values were observed in our study for BT and BTPW than the FRAP value reported for grapes (1281 μmol TE/100 g) [36].

For ABTS assay, there were also no significant differences between samples, but solvent effect was significant. Aqueous ethanol extracts had significantly higher antioxidant activities than water extracts. There is wide variation in the ABTS values reported for black tea in the literature

Table 5 Pearson's correlation coefficients between the antioxidant activity obtained using different assays (DPPH, ABTS, FRAP), and between antioxidant activity and phenolic content of the extracts

	DPPH	ABTS	FRAP
ABTS	0.878*		
FRAP	0.728*	0.755*	
Total phenolics	0.763*	0.775*	0.609*
Total catechins	0.806*	0.791*	0.675*
Total theaflavins	0.669*	0.750*	0.669*
Gallic acid	−0.476	−0.376	−0.488
Caffeine	−0.359	−0.268	−0.319

* Significant correlation ($p < 0.05$)

due to the differences in extraction parameters [37] or ABTS assay procedures [38], in addition to inherent variations in phenolic content. The ABTS radical scavenging activity of water extract of black tea reported by Dubeau et al. [37] (3.97 µmol TE/L) is lower than the values obtained in our study, possibly due to shorter extraction times (10 min). Liebert et al. [38] obtained higher ABTS values (1.7–3.6 mmol TE/L) for black tea using even shorter extraction times (0.5–10 min). Myoglobin-induced oxidation of ABTS in H_2O_2 used in that study results in faster reaction of antioxidants and thus higher antioxidant activity as noted by Re et al. [17].

Antioxidant activities of solvent extracts determined with DPPH, FRAP and ABTS assays are consistent with total catechin and theaflavin contents determined by HPLC: 50–80 % ethanol extracts contained higher total catechins and theaflavins than water extracts (Table 3). A significant correlation was observed between antioxidant activity and total phenolic, catechin and theaflavin content of the extracts ($r = 0.609$–0.878) (Table 5) indicating the contribution of catechins and theaflavins to the antioxidant activity of the samples.

Antimicrobial activity

Water and aqueous ethanol extracts of BT and BTPW exhibited antimicrobial activity against *S. aureus* (1.26–3.65 mm), *S. flexneri* (1.33–3.89 mm) and *B. cereus* (1.87–3.90 mm). However, inhibition of *C. albicans* was not observed (Table 6).

A significant interaction was observed only between solvent and microorganism. Aqueous ethanol extracts, which had higher phenolic content than water extracts, had significantly higher antimicrobial activities against all tested bacteria. Antimicrobial effects of 50 and 80 % ethanol extracts on *B. cereus* were not significantly different. However, 80 % ethanol extract, which had higher theaflavin content, had significantly higher antimicrobial activity than 50 % ethanol extract against *S. aureus* and *S. flexneri*.

The microorganism effect varied according to the solvent. While 80 % ethanol showed similar activity against all microorganisms, a significant difference was observed between the susceptibility of the microorganisms to 50 % ethanol extracts (i.e., *B. cereus* > *S. aureus* > *S. flexneri*). *S. flexneri* was significantly less susceptible to water extracts than *S. aureus* and *B. cereus*. Almajano et al. [3] also observed high susceptibility of *B. cereus* to tea infusions.

BT and OW extracts had comparable antimicrobial activities, which were significantly higher than those of GW extracts against all bacteria for all solvents. Comparable activities for OW and BT, which had similar theaflavin contents, point to the theaflavin content of the extracts as an important contributor to their antimicrobial activity.

The activities of individual catechins and theaflavins when considered together with the antimicrobial activity of

Table 6 Antimicrobial activity of black tea (BT) and black tea processing waste (OW and GW) extracts

Sample[a]	Extraction solvent	Zone of inhibition (mm)[b]			
		S. aureus	*S. flexneri*	*B. cereus*	*C. albicans*
OW	Water	1.88 ± 0.29	1.57 ± 0.30	2.06 ± 0.57	ND[c]
	50 % ethanol	3.20 ± 0.27	2.93 ± 0.47	3.56 ± 0.47	ND
	80 % ethanol	3.62 ± 0.50	3.89 ± 0.72	3.90 ± 0.68	ND
GW	Water	1.26 ± 0.22	1.33 ± 0.19	1.87 ± 0.17	ND
	50 % ethanol	2.61 ± 0.22	2.12 ± 0.29	3.32 ± 0.29	ND
	80 % ethanol	3.47 ± 0.25	3.19 ± 0.31	3.61 ± 0.24	ND
BT	Water	1.89 ± 0.83	1.37 ± 0.40	1.94 ± 0.31	ND
	50 % ethanol	3.65 ± 0.79	2.57 ± 0.29	3.79 ± 0.22	ND
	80 % ethanol	3.48 ± 0.44	3.43 ± 0.42	3.86 ± 0.39	ND

[a] *OW* oven waste, *GW* grading waste, *BT* black tea

[b] Data expressed as mean ± SD ($n = 3$)

[c] *ND* inhibition not detected

tea infusions/extracts point to an association between catechin and theaflavin content of black tea and its antimicrobial activity [4, 39]. Similar to the findings of this study, the antimicrobial activity of black tea has been shown to vary with the type of the microorganism, the content and composition of the phenolics in extracts, and the extraction solvent used [4, 39, 40]. Black tea extracts have been previously shown to be effective against *S. aureus* and *B. cereus* [3, 4, 40]. Both theaflavins and catechins have been shown to have antifungal activity against *C. albicans* [41]. However, the extracts tested in this study did not have an effect on *C. albicans,* which might be due to the phenolic content of the extracts. Almajano et al. [3] also observed no inhibitory effect of black tea infusion on *C. albicans*.

The results of this study highlight the potential of BTPW, which is of commercial significance due to high amount of production and logistical considerations (such as for waste collection), as raw material for the development of value-added products with antioxidant and antimicrobial properties for applications in food, pharmaceutical, cosmetic and agricultural sectors. Aqueous ethanol solvents offer low-cost, non-toxic, green alternatives for the recovery of antioxidant and antibacterial phenolics from BTPW. BTPW can thus be considered a source of phenolics in addition to caffeine. Further research on the separation of caffeine from phenolics can lead to development of multiple bioactive products from this waste stream.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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