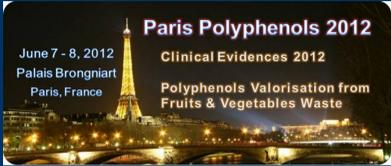


BLACK TEA WASTE AS A SOURCE OF BIOACTIVE PHENOLIC COMPOUNDS: Antioxidant Capacity, Antibacterial Activity and Anticancer Effect



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ABSTRACT

In this study, total phenolic content, antioxidant capacity, antibacterial activity and anticancer effect of water extracts of black tea waste (BTW) were investigated. Black tea wastes were extracted with water using ultrasound at room temperature and using an autoclave at 120 °C for 30 min (5% w/v) in duplicates. Total phenolic content of ultrasonicated black tea, ultrasonicated BTW and autoclaved BTW extracts were determined to be 124, 134 and 123 mg Gallic Acid Equivalents (GAE)/g freeze-dried extract, respectively, using the Folin-Ciocalteu method. BTW and black tea extracts showed similar antioxidant capacity as determined by FRAP (0.186-0.241 μmol Trolox Equivalents (TE)/g freeze-dried extract) and ABTS assays (1.094 to 1.123 μmol Trolox Equivalents (TE)/g freeze-dried extract). The antibacterial activity against four foodborne pathogens (*Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778) was determined using spot-on-lawn method. BTW and black tea extracts showed antibacterial activity against the *E. coli* and *B. cereus* at 400 mg/ml. Anticancer effect of black tea and BTW extracts (1 mg/ml) on Human Colorectal Adenocarcinoma Cancer Cells (Caco-2) was investigated using WST-1 Cell Proliferative Assay. Autoclaved BTW, ultrasonicated BTW and black tea had 91.8%, 83.6% and 85.6% cytotoxicity against Caco-2 cells, respectively. BTW extracts had antioxidant, antimicrobial and anticancer effects similar to that of black tea highlighting the potential of this waste stream for the recovery of bioactive phenolic compounds.

INTRODUCTION

Black tea is a source of bioactive compounds such as catechins, theaflavins and thearubigins, which have health promoting properties related to their antioxidant, antibacterial and anticancer effect.

Black tea production waste (BTW) is a by-product of tea production containing tea fiber and stalk.



Despite its economic potential for the recovery of tea bioactives, BTW is currently being underutilized. Research on utilization BTW has so far been limited to extraction of caffeine (İçen et al. 2009) and determination of total antioxidant capacity (Farhoosh et al., 2007). Therefore, the main objective of this study is to investigate the potential of black tea waste as a source of bioactive phenolic compounds.

MATERIALS AND METHODS

Samples

Black tea waste was obtained from a tea factory in the North Sea region of Turkey.



Extract Preparation

Water extracts of BTW were prepared (5% w/v) in duplicates using

- ✓ ultrasound at room temperature for 30 min: BTW-US
 - ✓ autoclave at 120 °C for 30 min : BTW-AC
- Ultrasonicated black tea extract (room temperature, 30 min) was used as control: BT-US
Extracts were freeze dried before further analysis.

Antioxidant Analysis

Trolox standards were prepared in the concentration range of 0-0.04 mg/ml in 80% methanol. Analyses were carried out in triplicates.

➤ FRAP (Ferric Reducing Ability) assay (Benzi and Strain, 1996)

- ✓ 50 μl of diluted sample (0-1 mg/ml) or Trolox mixed with 250 μl of FRAP reagent
- ✓ Absorbance measured at 593 nm after 5 min incubation using a microplate reader (Thermo Scientific, Multiskan Go, Finland)

➤ ABTS assay (Pellegrini et al., 1999)

- ✓ 50 μl of diluted sample (0-0.1 mg/ml) or Trolox mixed with 250 μl of ABTS solution
- ✓ Absorbance measured at 734 nm after 5 min incubation at 30°C using a microplate reader

Results expressed as $\mu\text{mol TE/g}$ dry extract by dividing the slope of the linear portion of absorbance versus concentration graph of the sample by the slope of the Trolox standard.

Total Phenolic Assay (Ainsworth and Gillespie, 2007)

- ✓ The level of total phenolics was estimated using Folin- Ciocalteu method.
- ✓ Analyses were performed in triplicates.
- ✓ Results expressed as mg GAE/g dry extract

Inhibition of Cancer Cell Proliferation

- ✓ 1.1×10^4 /well Caco-2 cells were cultured in 96 well plate
- ✓ Inoculated with 1 mg/ml samples
- ✓ Incubated for 48 hour at 37°C in 5 % CO₂ incubator
- ✓ Cell proliferative WST-1 reagent added and incubated for 2 h to test the metabolic activity of viable cells

The absorbance of formazan product was determined at 450nm with reference 630 nm using ELISA plate reader (Bio-Tek Elx800, USA)

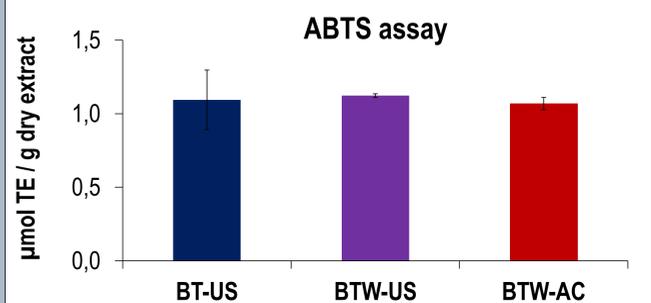
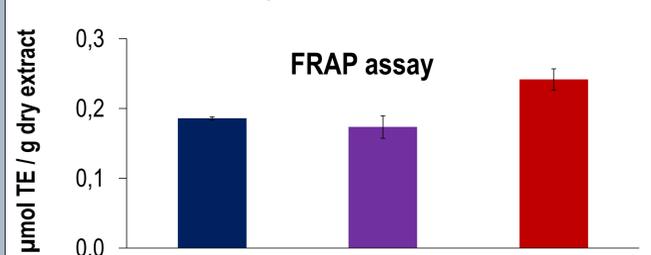
Antibacterial Activity

- ✓ Antimicrobial activity was tested using a concentration of 400 mg/ml of BTW extracts
- ✓ Screened using spot-on-lawn method against
 - *L. monocytogenes* ATCC 15313,
 - *S. aureus* ATCC 25923,
 - *E. coli* ATCC 25922,
 - *B. cereus* ATCC 11778

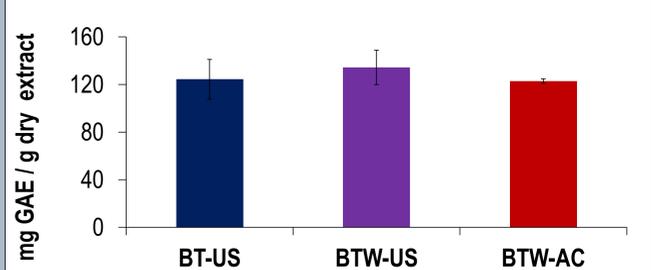


RESULTS AND DISCUSSION

Antioxidant Capacity

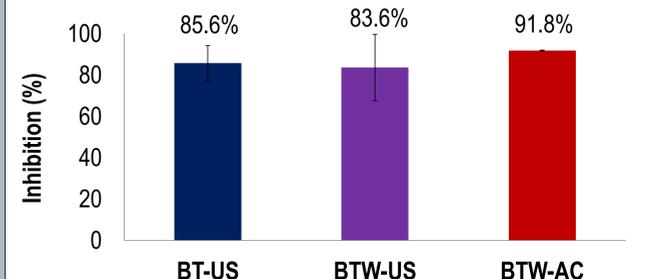


Total Phenolic Content



✓ Antioxidant capacity and total phenolic content of BTW extracts were comparable with those of black tea extract.

Inhibition of Cancer Cell Proliferation



✓ BTW samples inhibited the growth of more than 80% of Caco-2 cancer cells.

Antibacterial Activity

	BT-US	BTW-US	BTW-AC
<i>L. monocytogenes</i>	-	-	-
<i>S. aureus</i>	-	-	-
<i>E. coli</i>	+	+	+
<i>B. cereus</i>	+	+	+

✓ BT-US and BTW extracts showed antibacterial activity against *E. coli* and *B. cereus*.

✓ In conclusion, BTW extracts had antioxidant, antimicrobial and anticancer effects similar to that of black tea highlighting the potential of this waste stream for the recovery of bioactive phenolic compounds.

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