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# Antioxidant and Antibacterial Activities of Various Extracts and Fractions of Fresh Tea Leaves and Green Tea

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**Abstract:** Polyphenol content, polyphenol yield, antioxidant and antibacterial activities of methanol, ethanol and water extracts and their crude, ethyl acetate and water fractions derived from fresh tea leaves (FTL) and green tea (GT) were evaluated. Ethyl acetate fractions of ethanolic extracts contained the highest polyphenol content in FTL and GT samples (680.2 and 560.8 mg gallic acid equivalent $g^{-1}$ dry extract, respectively). For each of 3 extraction solvents, ethyl acetate fractions always had highest polyphenol content, for both teas. Ethyl acetate fractions of water extracts of FTL and GT had highest antioxidant activity (80.8 and 78.6 g ascorbic acid $100g^{-1}$ dry extract). A rather high correlation coefficient ($R^2$ = 0.9376 for FTL and 0.9783 for GT) was obtained between antioxidant activity to total polyphenol content for both tea. Ethyl acetate fractions of extracts showed antibacterial activity on *S. aureus* and *B. cereus*. Crude extract of FTL were also effective on *S. aureus*.

**Key Words:** Tea, extraction, fraction, polyphenols, antioxidant, antibacterial

## Taze Çay Yaprağı ve Yeşil Çayın Ekstrakt ve Fraksiyonlarının Antioksidan ve Antibakteriyel Etkileri

**Öz:** Taze çay yaprağı (FTL) ve yeşil çayın (GT) metanol, etanol ve su ham ekstraktları ile bu ekstraktların etil asetat ve su fraksiyonlarının polifenol içeriği, polifenol verimi, antioksidan ve antimikrobiyel aktiviteleri belirlenmiştir. FTL ve GT örneklerinde etanol ekstraktlarının etil asetat fraksiyonunun en yüksek düzeyde polifenol içerdiği (sırasıyla 680.2 ve 560.8 mg gallik asit eşdeğeri $g^{-1}$) belirlenmiştir. Her üç ekstraksiyon solventinde de etil asetat fraksiyonu en yüksek polifenol içeriğine sahiptir. En yüksek antioksidatif aktiviteyi FTL ve GT'nın su ekstraktlarının etil asetat fraksiyonu göstermiştir (sırasıyla 80.8 ve 78.6 g askorbik asit eşdeğeri 100 $g^{-1}$ kuru ekstrakt). FTL ve GT örneklerinin antioksidan aktivitesi ile toplam fenolik madde miktarı arasında oldukça yüksek (sırasıyla $R^2$ = 0.9376 ve 0.9783) bir korelasyon mevcuttur. Her iki çay örneğinin etil asetat fraksiyonu *S. aureus* ve *B. cereus* üzerinde antibakteriyel etki gösterirken FTL'nin ham ekstraktı *S. aureus* üzerinde etki göstermiştir.

**Anahtar Kelimeler:** Çay, ekstraksiyon, fraksiyon, polifenoller, antioksidan, antibakteriyel

## Introduction

Tea is an important source of dietary polyphenols (Gramza and Korczak, 2005). FTL are rich in polyphenols, particularly (flavan 3-ols) and flavonol glycosides (Clifford et al. 2000). GT is produced when freshly picked leaves are steamed, rolled, dried and fired, therefore, its chemical composition including polyphenols differs very little from that of FTL (Wheeler and Wheeler 2004). Tea flavanols have recently received much attention owing to their various biological activities (Chen et al. 2001). Their beneficial properties are thought to include antioxidant (Navas et al. 2005), antimutagenic (Halder et al. 2005), anticarcinogenic (Zhu et al. 2005) and antibacterial (An et al. 2004) effects. Tea is widely consumed throughout the world not just as a popular beverage but also available in a wide range of food, beverage, toiletry and cosmetic products, since its extracts can be prepared in a variety of physical forms, for example, strong infusions, soft extracts and powders, it is now widely available in a range of food, beverage and toiletry and cosmetic products (Wang et al. 2000). The use of tea extracts in edible oil systems (Navas et al. 2005, Yilmaz 2006) and cooked muscle foods (Nissen et al. 2004) have been reduced the rate of peroxide accumulation, which improves product stability.

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Due to these facts, it would be interesting to optimize an extraction process to obtain maximum yields of these substances, useful as nutritional supports in the prevention of diverse diseases (Pinelo et al. 2004). Amount of total polyphenol from the same plant and their antioxidant and antimicrobial activities may vary widely depending on extraction conditions applied. Farhoosh et al. (2007), found that extraction method was effective on extraction yield and antioxidant activity of old tea leaves and black tea leaves extracts. According to Negi et al. (2005), methanol extract showed the highest antioxidant and antimicrobial activities in sea buckthorn seeds which were extracted with chloroform, ethyl acetate, acetone and methanol.

However, despite the abundant literature about this topic and there have been some reports on extraction conditions of polyphenols from GT (Sharma et al. 2005, Bu-Abbas et al. 1997, Row and Jin 2005) and FTL (Yao et al. 2004), no information is available on the effect of different extraction methods on the polyphenol content, antioxidant and antibacterial activities of FTL and GT. Therefore, the objective of the present study was to determine total polyphenol content, and antioxidant and antibacterial activities of various fractions (ethyl acetate, chloroform and water) derived from FTL and GT extracted by methanol, ethanol and water which were the common extraction solvents for polyphenolic compounds.

## Materials and Methods

**Plant materials:** Samples of FTL were plucked from the orchards of Tea Research Institute, Rize, Turkey and freeze-dried (Labconco FreeZone 6, Maryland-USA) as soon as possible after plucked. GT sample (Çay-Kur brand) was purchased from a local market in Ankara-Turkey. Both tea leaves were ground using a coffee grinder and sieved to obtain 150-300 µm particle size and stored at + 4 °C until used.

**Chemicals:** Absolute ethanol (min 99.8 %), methanol (min 99.9 %) and chloroform (99.0-99.4 %) were analytical grade from Riedel-de Haën (BioChemica Fluka Cheme GmbH Buchs-Switzerland). Ethyl acetate (min 99.8 %) was from Merck (Darmstadt-Germany). Folin-Ciocalteu's reagent and DPPH (2,2-diphenyl-1-picryhydrazyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid and all other chemicals were analytical grade and from Merck.

**Extraction:** Dry ground sample (1 g) was extracted with ethanol, methanol and distilled water to get crude extracts as follows. Water extract of tea samples was obtained as follows: 1 g sample was infused with 50 g freshly boiled distilled water for 10 min in a 1 liter thermos® flask. The infusion was filtered through a plug of cotton wool and rapidly cooled under tap water. The extract was freeze-dried and weighed to calculate the yield.

Methanol and ethanol extracts of the samples were obtained by the following procedure. 1 g sample was extracted with 50 mL methanol or ethanol for 2 h on an orbital shaker at room temperature in the dark. The mixture was filtered through Whatman No.1 and the filtrate was evaporated to dryness under vacuum at 40 °C. The dry extract was weighed and the yield was calculated. The whole process was carried out in triplicate for each solvent.

**Fractionation:** The crude dry extracts were dissolved in 10 mL of distilled water. The water solution was partitioned sequentially with chloroform (to remove caffeine and pigments) and ethyl acetate in the ratio of 1 : 1 (v/v). The solvents were chosen as they are in common use to fractionate tea components (Satoh, 2005). Each liquid-liquid extraction was carried out two times for 10 min by shaking on orbital shaker using a separating funnel and the organic phases were pooled. The combined ethyl acetate phases were dried with anhydrous sodium sulphate and filtered. The chloroform and ethyl acetate phases were then evaporated under vacuum to dryness. The dry residues were weighed to determine the yield and then re-dissolved in 50 % ethanol in water. The remaining aqueous phases were freeze-dried, weighed and re-dissolved in distilled water.

**Determination of extraction yield:** The yield of evaporated/lyophylized dried extracts based on dry weight basis was calculated from equation shown below:

$$\text{Yield (\%)} = (W_1 * 100) / W_2$$

where $W_1$ was the weight of extract after evaporation/lyophylization of solvent and $W_2$ was the dry weight of the tea or tea leaf sample.

**Determination of total polyphenol:** Total polyphenol was determined according to Folin-Ciocalteu's method as explained in detail in Turkmen et al. (2006). Polyphenol yield for each extract was also calculated per gram of dry tea leaf by multiplying the polyphenol content with the respective extraction yield.

**Antioxidant activity:** The antioxidant activity of tea samples (200 µg $mL^{-1}$) was measured by using the DPPH assay (Turkmen et al. 2006). Standard curve of reference antioxidant ascorbic acid (0-150 µg $mL^{-1}$) was assayed under identical conditions for affinity to scavenge DPPH. Antioxidant activity of samples was transformed to ascorbic acid equivalent (AEAA) defined as g of ascorbic acid equivalents per 100 g of dry extract. Higher values of the AEAA are related to higher antioxidant activity of the sample.

**Antibacterial activity:** To determine antibacterial activity, bacterial cultures, namely, *Staphylococcus aureus*, *Listeria monocytogenes* (ATCC 7644), *E. coli* O157:H7, *Hafnia alvei*, *Salmonella enteritidis* (ATCC 13076), *E. coli* Type 1 and *Bacillus cereus* were obtained from Ankara University, Department of Food Engineering, Ankara, Turkey. All test bacteria were grown in Tyriptic Soy Broth (Merck, Germany) at 37 ºC for 18-24 h. Antibacterial activity of tea extracts (2 mg $mL^{-1}$) was determined according to Turkmen et al. 2007).

**Statistical analysis:** All data were expressed as means ± standard deviation of triplicate measurements and analyzed by SPSS for Windows (ver.10.1, Chicago, IL, USA). One-way analysis of variance (ANOVA) and Duncan’s multiple range test were carried out to test any significant differences among various treatments. Values of $P < 0.05$ were considered as significantly different ($\alpha = 0.05$). Correlations between variables were established by regression analysis.

## Results

**Extraction yield and total polyphenol:** The extraction yields (Table 1) for FTL and GT varied from 10.3 to 50.1 % and 1.6 to 32.0 %, respectively. A wide range of the yields among extracts were observed depending on the extraction solvent, type of extract and plant material used. As it can be seen from Table 1, the extraction yields in FTL were higher than those in GT, indicating that FTL extracts contained more substances soluble in solvent used. For FTL, the highest yields (23.03-50.09 %) were achieved by using methanol as an initial extraction solvent, followed by water and ethanol, respectively. In the case of GT, however, extraction yield using three different solvents showed the following order: water > methanol > ethanol and significant differences were found among them. In all cases, the extraction yield of water fractions from crude extracts was higher than that of ethyl acetate fractions and in general, the yield of chloroform fractions was the lowest (data not shown).

Total polyphenol contents of FTL and GT ranged from 155.4 to 680.2 mg GAE $g^{-1}$ dry extract and from 128.6 to 560.8 mg GAE $g^{-1}$ dry extract, respectively, depending on the extract type, extracting solvent used and plant material (Table 1). Chloroform fractions from FTL and GT gave the lowest polyphenol content, less than 1 mg $g^{-1}$ dry material (data not shown). Data shows that ethyl acetate fractions had the highest level of polyphenol (on dry extract basis), followed by crude extracts and water fractions, respectively, for both FTL and GT extracts. In general, for both FTL and GT, polyphenol content of crude extracts and ethyl acetate fractions when used ethanol as an extraction solvent was significantly higher than those when used methanol and water (Table 1). However, the case is different for water fractions. The highest polyphenol content was obtained with methanol as solvent, followed by water and ethanol, respectively. GT extracts showed total polyphenol contents lower than FTL extracts. Polyphenol yield of FTL (18.3-169.5 mg GAE $g^{-1}$ dry leaf) was found to be very much higher than that of GT (5.3-88.5 mg GAE $g^{-1}$ dry leaf) as observed for the extraction yield. Methanol for FTL and water for GT were found to be the most efficient solvents.

**Antioxidant activity:** AEAA values of FTL and GT fractions ranged from 22.2-80.8 g ascorbic acid $100g^{-1}$ extract and 16.1-78.6 g ascorbic acid 100 $g^{-1}$ extract, respectively, at the concentration of 200 µg $mL^{-1}$ (Table 2), depending on crude extract /fraction, extraction solvent and material. The order of AEAA values of both tea leaves were as follows: ethyl acetate fraction > crude extract > water fraction. As it can be seen, ethyl acetate fractions from both FTL and GT clearly showed the greatest antioxidant activity which were correlated with their higher polyphenol content. With respect to extraction solvents used, for example, no significant difference was found between activities of ethyl acetate fractions derived from ethanol and water crude extracts of FTL but in the case of GT, there was a significant difference ($P < 0.05$) between them. Antioxidant activity of FTL extracts was found to be higher than that of GT extracts at the same concentration (200 µg $mL^{-1}$).

Table1. Total polyphenol content (mg GAE $g^{-1}$ dry extract), polyphenol yield (mg GAE $g^{-1}$ dry weight tea) and extraction yield (%) of various fractions of different solvent extracts from fresh tea leaves and green tea.

| Sample | Parameters in fractions of main extraction solvents | Main extraction solvents | | |
|---|---|---|---|---|
| | | Methanol | Ethanol | Water |
| Fresh tea leaves | *Polyphenol content* | | | |
| | Crude extract | 338.4 ± 6.14[a*] | 355.2 ± 2.21[a] | 351.2 ± 11.54[a] |
| | Ethyl acetate fraction | 523.7 ± 31.92[a] | 680.2 ± 4.44[b] | 615.8 ± 5.98[b] |
| | Water fraction | 192.2 ± 2.88[b] | 155.4 ± 3.18[b] | 183.3 ± 4.99[b] |
| | *Polyphenol yield* | | | |
| | Crude extract | 169.5 ± 3.55[c] | 78.8 ± 0.63[a] | 133.6 ± 2.16[b] |
| | Ethyl acetate fraction | 119.9 ± 1.80[c] | 60.6 ± 1.81[a] | 93.2 ± 2.08[b] |
| | Water fraction | 43.7 ± 1.93[c] | 18.3 ± 0.49[a] | 38.0 ± 0.70[b] |
| | *Extraction yield* | | | |
| | Crude extract | 50.1 ± 0.23 | 22.5 ± 0.94 | 39.4 ± 1.34 |
| | Ethyl acetate fraction | 23.3 ± 1.20 | 10.3 ± 0.23 | 13.5 ± 1.13 |
| | Water fraction | 24.1 ± 1.71 | 10.5 ± 0.52 | 24.3 ± 0.26 |
| Green tea | *Polyphenol content* | | | |
| | Crude extract | 283. 6 ± 3.96[b] | 276.5 ± 11.70[b] | 204.4 ± 3.02[a] |
| | Ethyl acetate fraction | 481.8 ± 9.48[a] | 560.8 ± 11.99[b] | 470.2 ± 3.68[a] |
| | Water fraction | 161.4 ± 2.84[b] | 128.6 ± 1.92[a] | 155.9 ± 4.05[b] |
| | *Polyphenol yield* | | | |
| | Crude extract | 64.9 ± 3.14[b] | 11.8 ± 0.29[a] | 88.5 ± 3.19[c] |
| | Ethyl acetate fraction | 42.1 ± 1.43[b] | 7.4 ± 0.15[a] | 49.6 ± 0.56[c] |
| | Water fraction | 22.4 ± 0.50[b] | 5.3 ± 0.21[a] | 32.7 ± 1.47[c] |
| | *Extraction yield* | | | |
| | Crude extract | 22.1 ± 1.06 | 5.8 ± 0.22 | 32.0 ± 0.47 |
| | Ethyl acetate fraction | 8.6 ± 0.57 | 1.6 ± 0.07 | 8.6 ± 0.03 |
| | Water fraction | 13.8 ± 0.20 | 3.3 ± 0.29 | 24.1 ± 0.70 |

*Means sharing the different letters in rows are significantly different at P< 0.05.

Table 2. Antioxidant activity (g AEAA $100g^{-1}$ dry extract) of various fractions of different solvent extracts from fresh tea leaves and green tea.

| Sample | Fractions of main extraction solvents | Main extraction solvents | | |
|---|---|---|---|---|
| | | Methanol | Ethanol | Water |
| Fresh tea leaves | Crude | 52.0 ± 1.76[a*] | 52.1 ± 1.72[a] | 59.8 ± 2.02[b] |
| | Ethyl acetate fraction | 74.1 ± 2.19[a] | 79.3 ± 0.92[b] | 80.8 ± 0.13[b] |
| | Water fraction | 32.0 ± 2.42[b] | 30.7 ± 2.14[b] | 22.2 ± 0.75[a] |
| Green tea | Crude | 44.3 ± 0.08[b] | 30.5 ± 0.46[a] | 32.5 ± 1.17[a] |
| | Ethyl acetate fraction | 70.8 ± 2.28[a] | 64.8 ± 1.76[a] | 78.6 ± 1.66[b] |
| | Water fraction | 23.3 ± 0.77[b] | 21.8 ± 0.69[b] | 16.1 ± 0.66[a] |

*Means sharing the different letter in rows are significantly different at P< 0.05

**Antibacterial activity:** The results of the antibacterial activity of tea extracts are presented in Table 3. In addition, solvent used as control had no inhibitory effect on the tested bacteria. Regardless of tea samples, the water fractions of the crude extracts did not exhibit antibacterial activity against all of the bacteria tested. However, in both FTL and GT, ethyl acetate fractions and crude extracts were found to have activity against only two bacteria, namely *S. aureus* and *B. cereus*, depending on the extract type. In FTL, *S. aureus* was inhibited by both ethyl acetate fractions and crude extracts but *B. cereus* was inhibited by only ethyl acetate fractions. In the case of GT, only ethyl acetate fractions exhibited antibacterial activity against these two bacteria but crude extracts obtained using water had also potential action against *S. aureus*. As can be seen, in both FTL and GT, ethyl acetate fractions generally possessed more activity than crude extracts, which is in accordance with the results of antioxidant activity. This may be attributed to their high concentrations of polyphenols. With respect to extraction solvent used, for FTL extracts, ethanol, methanol and water gave similar efficiency. In the case of GT extracts, water was found to be more efficient than other two. The results have shown that of three gram-positive bacteria, *S. aureus* was found to be more sensitive than *B. cereus.* According to data obtained, FTL extracts proved to have more activity against test bacteria than those of GT extracts which contained lower total polyphenol content. Thus, it can be concluded that antibacterial activity of tea extracts is related to the polyphenol content. Thus, it can be concluded that antibacterial activity of tea extracts is related to the polyphenol content.

Table 3. Antibacterial activity of various fractions of different solvent extracts from fresh tea leaves and green tea

| Bacterium | Fraction | Fresh tea leaves<br>Diameter of inhibited zone (mm) | | | Green tea<br>Diameter of inhibited zone (mm) | | |
|---|---|---|---|---|---|---|---|
| | | *Methanol* | *Ethanol* | *Water* | *Methanol* | *Ethanol* | *Water* |
| *S. aureus* | Crude | 12.0 ± 0.00 | 12.0 ± 0.00 | 11.5 ± 0.00 | nzd* | nzd | 10.0 ± 0.00 |
| | Ethyl acetate | 13.0 ± 1.00 | 15.0 ± 0.00 | 15.3 ± 0.33 | 10.0 ± 0.00 | 11.0 ± 0.52 | 14.8 ± 0.60 |
| | Water | nzd | nzd | nzd | nzd | nzd | nzd |
| *H. alvei* | Crude | nzd | nzd | nzd | nzd | nzd | nzd |
| | Ethyl acetate | nzd | nzd | nzd | nzd | nzd | nzd |
| | Water | nzd | nzd | nzd | nzd | nzd | nzd |
| *S. enteritidis* | Crude | nzd | nzd | nzd | nzd | nzd | nzd |
| | Ethyl acetate | nzd | nzd | nzd | nzd | nzd | nzd |
| | Water | nzd | nzd | nzd | nzd | nzd | nzd |
| *L. monocytogenes* | Crude | nzd | nzd | nzd | nzd | nzd | nzd |
| | Ethyl acetate | nzd | nzd | nzd | nzd | nzd | nzd |
| | Water | nzd | nzd | nzd | nzd | nzd | nzd |
| *B. cereus* | Crude | nzd | nzd | nzd | nzd | nzd | nzd |
| | Ethyl acetate | 8.0 ± 0.00 | 8.7 ± 0.33 | 8.5 ± 0.29 | 7.0 ± 0.00 | 8.2 ± 0.17 | 8.3 ± 0.17 |
| | Water | nzd | nzd | nzd | nzd | nzd | nzd |
| *E. coli* O157:H7 | Crude | nzd | nzd | nzd | nzd | nzd | nzd |
| | Ethyl acetate | nzd | nzd | nzd | nzd | nzd | nzd |
| | Water | nzd | nzd | nzd | nzd | nzd | nzd |
| *E. coli* | Crude | nzd | nzd | nzd | nzd | nzd | nzd |
| | Ethyl acetate | nzd | nzd | nzd | nzd | nzd | nzd |
| | Water | nzd | nzd | nzd | nzd | nzd | nzd |

nzd: no zone detected

**Discussion**

**Extraction yield and total polyphenol:** The extraction yields in FTL were higher than those in GT, indicating that FTL extracts contained more substances soluble in solvent used (Table 1). Similarly, Farhoosh et al. (2007) reported that significant differences were found between the extraction yields of fresh tea leaves and black tea wastes. For FTL, the highest yields (23.03-50.09 %) were achieved by using methanol as an initial extraction solvent, followed by water and ethanol, respectively. Similar findings were reported for buckwheat (Sun and Ho, 2005). Farhoosh et al. (2007) applied the ratios of 25 : 1 and 20:1 for water and methanol extracts, respectively, (50 : 1 in our study). Besides the ratio of solvent-solid, other factors such as tea leaf variety and polyphenol composition of the leaf may have also affected extraction yield of the leaves. On the other hand, Yao et al. (2004) reported that methanol was the most efficient solvent than boiling water for the extraction of polyphenols from FTL in the ratio of about 60:1 (solvent:solid), which is in agreement with our present data.

Chloroform fractions from FTL and GT gave the lowest polyphenol content, less than 1 mg $g^{-1}$ dry material (data not shown), which is in agreement with the results from green tea (Bu-Abbas et al. 1997) and warmwood (Canadanovic-Brunet et al. 2005) and no further work was carried out on these fractions. The ethyl acetate fractions had the highest level of polyphenol for both extracts (Table 1). This can be attributed to the fact that ethyl acetate selectively removed and concentrated more apolar compounds from tea such as the catechins and theaflavins (Larger et al. 1998). Crude and ethyl acetate fractions of ethanolic extracts contained higher total polyphenols as compared to others. This can be due to variation in affinity of the extraction solvents for tea leaves constituents in terms of their different extraction conditions such as polarity of extracting solvents and temperature (Farhoosh et al. 2007). The lower polyphenol content of GT extracts may be due to thermal destruction of polyphenols during heat treatment to inactivate polyphenol oxidase enzyme despite the fact that the content and composition of tea polyphenols are strongly influenced by various factors such as variations in leaf variety, harvesting season, climate, processing method and analytical method (Gramza and Korczak 2005; Luximon-Ramma et al. 2005; Wheeler and Wheeler, 2004). Chan et al. (2007), reported that total polyphenol of methanol extract from green tea was significantly lower than that of fresh leaves. We have found that methanol was the most efficient solvent for both teas. Similar observations were reported for pine sawdust and almond hulls by Pinelo et al. (2004) who suggested that chemical characteristics of the solvent and diverse structure and composition of the natural products ensure that each material-solvent system shows different behavior. The literature findings are in accordance with our data. No correlation was found between the total polyphenol yield and the total polyphenol content for both FTL and GT, confirming the results of previous studies (Pinelo et al. 2004; Jayaprakasha et al. 2003). This is also in accordance with the findings by Sun and Ho (2005) who found that methanol extract of buckwheat gave a higher yield than the ethanol extract although both extracts showed similar total phenolic contents.

**Antioxidant activity:** The highest antioxidant activity of ethyl acetate fractions (Table 2) can be attributed to the fact that there are more antioxidant components present in these extracts which could react rapidly with DPPH radicals and reduce most of DPPH radical molecules (Canadanovic-Brunet et al. 2005). The results showed that the activities of ethyl acetate fractions of water extracts from FTL and GT were comparable to the reference antioxidant, ascorbic acid. The most potent activity of ethyl acetate soluble fraction has been reported for different plants by various studies (Canadanovic-Brunet et al. 2005; Farhoosh et al. 2007). Limited literature is available about comparison of antioxidant activities of different extracts from the same plants. However, it is not difficult to find reports for other plants. In the study carried out by Yu et al. (2005), total antioxidant activity (TAA) values of methanol and ethanol extracts from directly peeled peanut skin were similar but for roasted peanut skin the TAA of ethanol extract was higher than that of methanol extract. A similar observation was also reported for pine sawdust and almond hull extracts (Pinelo et al. 2004). Although FTL and GT extracts have the same polyphenols profile (Wheeler and Wheeler, 2004), antioxidant activity of FTL extracts were found higher than the other, this difference can be result of higher polyphenol concentrations of FTL extracts. According to the results from this study, FTL may be considered as better potential source of antioxidant as compared to GT. Crude extracts and fractions with high antioxidant activities showed high polyphenol contents as well and linear correlation ($R^2$ = 0.9376 for FTL; $R^2$ = 0,9783 for GT) was found between them (Figure 1), confirming that polyphenols are likely to contribute to the antioxidant activity of these plant extracts (Negi et al. 2005; Yu et al. 2005).

**Antibacterial activity:** *S. aureus*, which was found more sensitive than *B. cereus* (Table 3), is widely used in the antimicrobial testing of plant extracts and other compounds because of its medical significance in causing opportunistic infections and

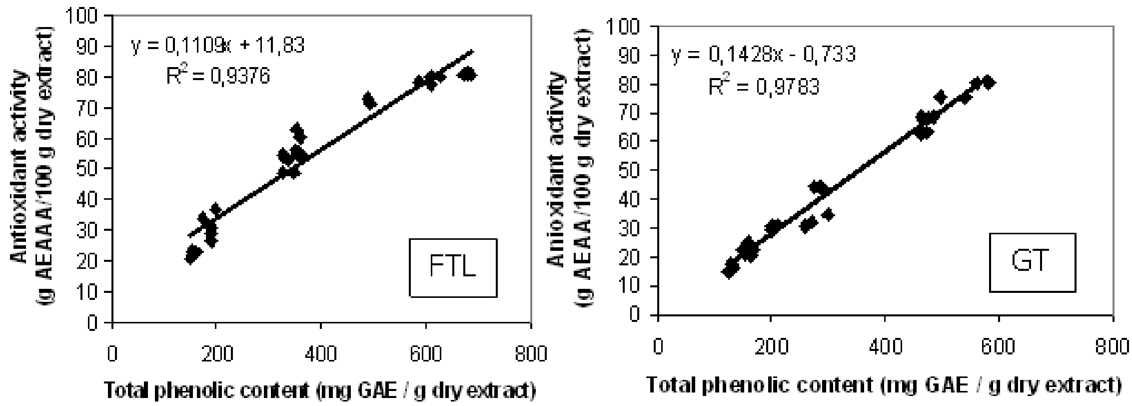


Figure 1. Correlation between total phenolic content and antioxidant activity for fresh tea leaves (FTL) and green tea (GT)

food-borne disease (Dupont et al. 2006). Wu et al. (2007) reported that water extracts of different teas including green tea had antimicrobial activity against *S. aureus* and *B. subtilis* at final concentration of 2 mg $mL^{-1}$ but no activity against *E. coli*, one of the gram-negative bacteria, confirming our results. The higher resistance of gram-negative bacteria is attributed to the presence of lipopolysaccharides in their outer membranes (Alzoreky and Nakahara, 2003; Negi et al. 2005). It can be concluded that stronger antibacterial activity of FTL extracts (as compared to GT extracts) is related to the polyphenol content (Table 1). Similarly, Chou et al. (1999) found that the antimicrobial activity of unfermented teas was stronger than fermented ones with fewer amounts of catechins.

## Conclusions

Extraction yield, total polyphenol, antioxidant and antibacterial activities of FTL and GT varied significantly, depending on the extracting solvent, extract type and plant material used. For the extraction and polyphenol yields of FTL and GT, the most efficient solvents were found to be methanol and water, respectively, while, in general, the highest total polyphenol concentrations of both FTL and GT were achieved by ethanol. Among tea extracts ethyl acetate fractions had the highest total polyphenol concentrations and exhibited the best antioxidant and antibacterial activities. Water fractions were the least efficient in terms of properties measured. Total polyphenol content of tea extracts correlated well with their antioxidant activities. According to the results from this study, FTL extracts might be more suitable as functional ingredients in the cosmetic as also in food industry because of their higher polyphenol yield, antioxidant and antibacterial activities.

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