

Total Phenolic Contents and Antioxidant Activities of Different Herbal Tea Extracts from Turkey

Ferda Eser

Amasya University, Suluova Vocational Schools, Suluova, Amasya, Turkey

Abstract: The aim of the present study is to determine the total phenolic contents (TPC), DPPH radical scavenging activities, and reducing power of commercially consumed herbal teas including sage tea (ST), blackberry tea (BT), apple tea (AT), Echinacea tea (ET), lime tea (LT), rosehip tea (RT), and green tea (GT) in Turkey. For this purpose, various solvents were used for extraction of the tea samples and the results were compared in terms of total phenolic contents and antioxidant activities, as well. Hot water is found as the best solvent for the extraction of total phenolics from ST, BT, AT, and RT, respectively. On the other hand, the highest TPC values are obtained with the extraction of ET, LT, and GT in the presence of aqueous acetone (50%) solvent system. Generally all samples gave high DPPH radical scavenging activities depending on their extraction solvents. The highest radical scavenging activity was found as 94.92 ± 0.01 % for ST in ethanol, while the lowest activity was obtained as 5.98 ± 0.14 % for BT with water extract. Ferric reducing antioxidant assay results are closed each other and the highest activity (0.27 ± 0.74 mM Trolox) was observed in the ethanol extract of ST. Antioxidant activities and TPC of the herbal tea samples varied depending on the extraction solvent. Water or aqueous organic solvent extracts of sage tea and green tea samples gave high TPC values and antioxidant activities.

Key words: *Herbal tea, DPPH radical scavenging activity, Total phenolic, Reducing power*

INTRODUCTION

Tea is one of the most consumed beverages all over the world [1] and different forms of tea including fruits, fruit mixtures etc. are consumed mostly infusion of dried herbs or parts of plant species such as leaves, flowers, seeds, fruits, and roots in warm or hot water [2]. Recent studies showed that these herbal teas exhibit biological properties such as reducing the risk of chronic diseases [3] having anticarcinogenic, antiatherogenic, chemopreventive, antimicrobial, and antioxidant effects [4]. In recent years, a substantial body of evidence has indicated an important role of free radicals due to the ability to retard oxidative degradation of lipids and thereby prevent many of diseases such as cirrhosis cancer, arteriosclerosis, rheumatoid arthritis, and as well as in degenerative processes associated with aging [5-7]. It is known that phenolic constituents are responsible from the antioxidant activity of plant materials due to

their redox properties in the way of absorbing and neutralizing free radicals [8].

Many investigators have studied different forms of tea samples because of their health-related properties or bioactivities such as antimicrobial and antioxidant [9-11]. Generally, the studies are focused on the antioxidant properties of green and black teas [12-14]. In another study, various leafy herbal tea (LHT) extracts, including rosemary, persimmon leaf, green tea, peppermint, black tea, mate tea, mulberry leaf, lotus leaf, lemongrass, rooibos, and bamboo leaf were studied in terms of their antioxidant and antimicrobial activities [15].

The interest in herbs is increasing day by day because of their strong antioxidant property which facilitates to enhance human health by the way of nutrition. Thus, in this study, 7 different herbal tea samples that believed to have many beneficial effects and mostly consumed by Turkish people are investigated for their total phenolic

Corresponding Author: Ferda Eser, Amasya University, Suluova Vocational Schools, Suluova, Amasya, Turkey, Tel:+905052128408

contents, DPPH radical scavenging activities, and the ferric reducing antioxidant power, as well. Different solvent and solvent systems were used for the herbal tea extraction processes in order to compare the results in terms of phenolic contents and antioxidant activities. The current study can contribute to a better understanding of the health promoting and nutritional properties of mostly consumed herbal teas in Turkey.

EXPERIMENT

Herbal Teas and Preparation of Extracts

Herbal teas including sage tea (*Salvia officinalis*), blackberry tea (*Rubus procerus*), apple tea (*Malus domestica*), Echinacea tea (*Echinacea angustifolia*), lime tea (*Tilia platyphyllo*), rosehip tea (*Rosa canina*), and green tea (*Camellia sinensis*) were purchased from local market, Amasya, Turkey.

Extraction of the tea samples was performed using boiling water, aqueous MeOH (50%), EtOH, aqueous EtOH (50%), acetone, and aqueous acetone (50%), respectively. Boiling water was used in order to obtain similar conditions during the preparation of tea for consumption. Other solvents were used for the determination of the difference in terms of phenolic contents and antioxidant activities, as well. 1 g of each herb was infused into 10 ml of solvent for 5 min and filtered through no.1 Whatman filter paper. The filtrates were directly used for the analyses.

Folin-Ciocalteu Assay for Total Phenolics

Each sample (50 μ l) was dissolved in distilled water (950 μ l) and sodium carbonate (2%, 2.5 ml) was added to the mixture. After addition of the Folin-Ciocalteu reagent (500 μ l), test tubes were vortexed for 5 seconds. The samples were incubated in dark for 40 min. At the end of the time, absorbances were recorded at 725 nm. Gallic acid was used as standard, and total phenolic content (TPC) of the samples was expressed as gallic acid equivalent (GAE).

1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) Free Radical Scavenging Activity

The free radical scavenging activity of the herbal tea samples were carried out according to the method of Blois [16] with little modifications. The method was discussed comprehensively elsewhere [17] that DPPH radical absorbs at 517 nm, on the other hand its absorption decreases in the presence of an antioxidant or a radical species [17]. Shortly, sample solutions (0.2 ml)

were mixed with ethanol (3 ml). Solution of DPPH in ethanol (1 mM, 375 μ l) was added to the mixture. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. Then the absorbance was measured at 517 nm using a spectrophotometer (Thermo Scientific, Ceneys 10S UV-VIS spectrophotometer). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent DPPH radical scavenging effect was calculated using the following equation:

$$\text{DPPH radical scavenging effect (\%)} \\ = [100 - (A_0 - A_1/A_0) \times 100] \quad (1)$$

where A_0 was the absorbance of negative control reaction and A_1 was the absorbance in the presence of the sample of herbal tea extracts or standards as positive control.

All measurements were performed in triplicate.

Ferric Reducing Antioxidant Power Assay (FRAP)

Ferric reducing antioxidant assay was carried out according to Zhu et al. [18] with slight modification. Phosphate buffer (pH 7.5, 0.2 M, 0.2 ml) solution was added to the each of herbal tea extracts (0.2 ml). After the addition of potassium ferric cyanide (1%, 0.2 ml), the mixture was incubated at 50°C for 20 min. Thereafter, trichloroacetic acid (10%, 0.2 ml) was added to the mixture and vortexed for 5 sec. Distilled water (0.8 ml) and FeCl₃ (0.1%, 0.2 ml) were added and the absorbances were measured 655 nm. The reducing capacity of the herbal tea samples was calculated with reference to the reaction signal given by a Trolox™ solution. FRAP values were expressed as mM Trolox™.

RESULTS AND DISCUSSION

Some compounds give an electron or hydrogen atom to DPPH radical that results decolorization of purple colored solution of DPPH in methanol. The importance of radical scavenging activity is due to the harmful effects of free radicals to human body which present in biological systems and foods, as well. Formation of large amount of free radicals cause fast oxidation of lipids in nourishments thus decrease their quality. The method is preferred because of the fact that it can apply in a short time and large number of samples can be analyzed in a short period of time. Additionally, it is possible to detect natural products at low concentrations [19]. In addition, the method has been used extensively in the prediction of antioxidant

activities due to its relatively short time required analysis.

Many studies were conducted on antioxidant activity of herbal teas [15, 20-21] and our findings are parallel to the previous reports [22,-23]. Previous studies revealed that there is a linear relationship between total phenolic content and antioxidant activity [24-26]. Due to the presence of hydroxyl groups, phenols have the ability to destroy radicals. Formation of stable phenoxyl radical occurs after giving of hydrogen atoms of phenols to radicals. Therefore, determination of the phenolic contents of plant samples is important in the prediction of the antioxidant activity [27-29]. Usually, DPPH radical scavenging activity depends on the structures of flavonoids and their arrangements of hydroxyl groups. It is reported that, hydroxyl groups at B ring which are substituted at 3,4- position (i.e., *ortho* 3, 4,-), hydroxyl groups at *meta* 5, 7 position at ring A, have high radical scavenging activity It has been further reported that no single compound was able to react with all kinds of radicals [30-31].

FRAP is a simple, fast, and cheap method that used for the determination of hydrophilic and lipophilic antioxidants [32]. For the prediction of the antioxidant activity of a sample, reducing power may serve as an indicator. This reducing power was observed according to the ferric ions (Fe^{3+}) - the ferrous ions (Fe^{2+}) transformation [33].

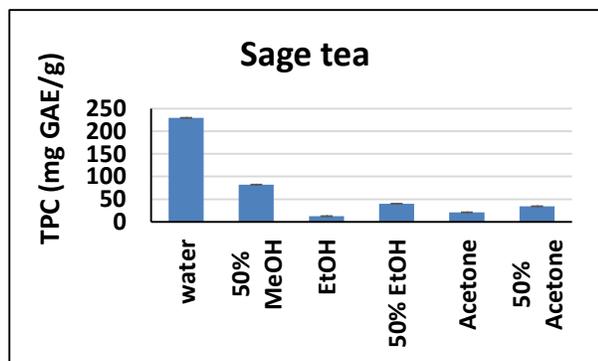


Figure 1 Total phenolic contents of different solvent extracts of sage tea

Figure 1 indicates the total phenolic contents of the different solvent extracts of sage tea. As seen from figure 1, it is clear that hot water extract of sage tea has the highest phenolic content. The high total phenolic content (TPC) in sage tea water extract (229.21 mg GAE/g) indicates that the sample contains more water-soluble phenolic compounds. Especially, phenolic acids

are more water-soluble than other organic solvents [34]. The lowest TPC is obtained in the presence of EtOH solvent. The phenolic contents of other extracts of sage tea samples decrease in the order of water (A) > MeOH 50% (B) > EtOH 50% (D) > Acetone 50% (F) > Acetone (E) > EtOH (C).

Figure 2 Total phenolic contents of different solvent extracts of blackberry tea

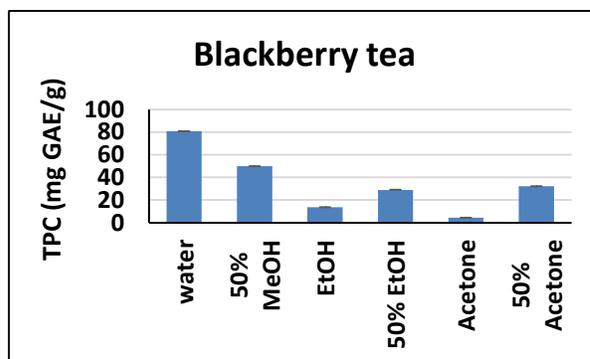
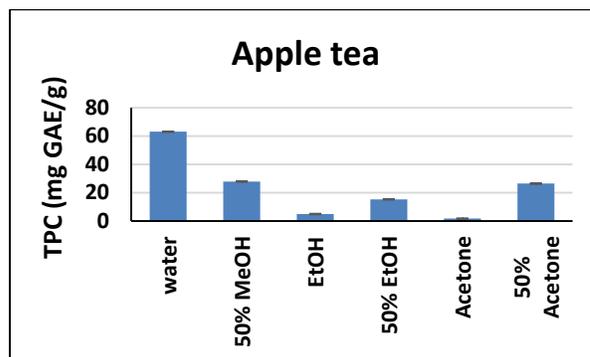


Figure 3 Total phenolic contents of different solvent extracts of apple tea



Similar results of TPC in terms of solvent effect for sage tea are obtained for blackberry and apple tea samples, respectively. The highest TPC is obtained with water extracts of blackberry tea (BT) and apple tea (AT) with the values of 80.77 mg GAE/g and 63.06 mg GAE/g, respectively. Acetone extracts of both BT and AT samples have lowest TPC. Generally, aqueous solvents gave higher total phenolic values than their absolute organic solvents (Figure 2, Figure 3).

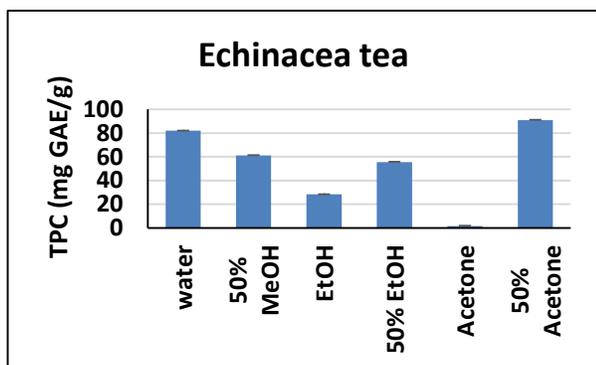


Figure 4 Total phenolic contents of different solvent extracts of Echinacea tea

Highest TPC value (90.96 mg GAE/g) was obtained with acetone (50%) for Echinacea tea (Figure 4). It is clear that, water-soluble phenolic compounds are higher than absolute organic solvents. TPC of ET decreased as such: F > A > B > D > C > E (Figure 4).

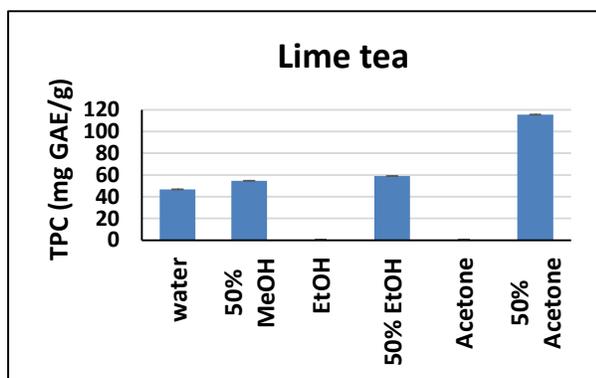


Figure 5 Total phenolic contents of different solvent extracts of lime tea

Figure 5 depict that acetone (50%) extract of LT contain higher amount of phenolics (115.44 mg GAE/g) as compared to others. It is reported that, acetone-water solvent system is effective for the extraction of tannins [35]. Furthermore, the solubility of phenolic compounds is higher in alcohols and acetone due to their intermediate polarity nature rather than more polar (e.g. water) solvents [36]. Previous studies revealed that, acetone-water solvent system is able to extract high levels of phenolic compounds from other plants [37-38].

The use of acetone-water mixture as solvent for food products is generally regarded as safe [39-40].

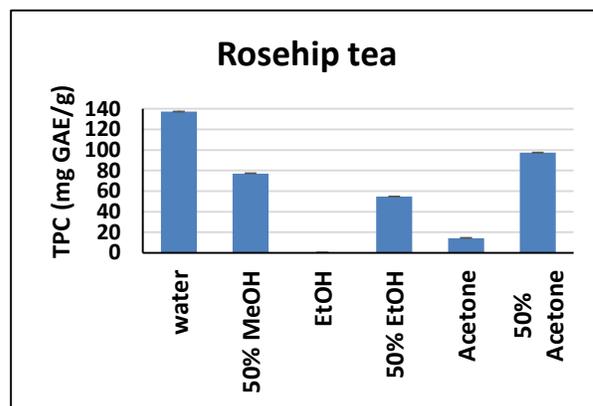


Figure 6 Total phenolic contents of different solvent extracts of rosehip tea

Water extract of rosehip tea has the highest phenolic content (137.36 mg GAE/g) followed by acetone 50% (97.36 mg GAE/g) and MeOH 50% (77.14 mg GAE/g), respectively (Figure 6). The results from our study were similar to the previous reports for total phenolic content in *Rosa canina* L. from Bulgaria [41].

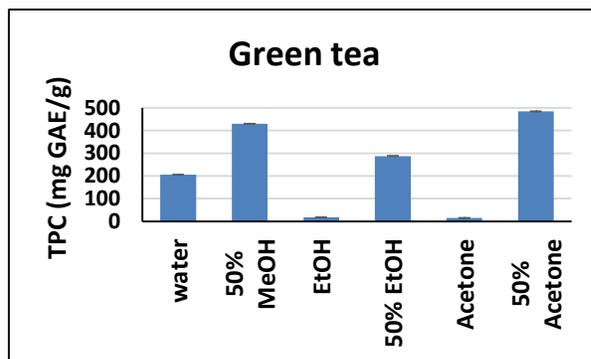


Figure 7 Total phenolic contents of different solvent extracts of green tea

Aqueous organic solvents are better than either water or absolute organic solvents for the extraction of phenolic compounds from green tea. Highest TPC (484.40 mg GAE/g) is obtained with acetone (50%), followed by MeOH 50% (429.99 mg GAE/g), and EtOH 50% (287.51 mg GAE/g), respectively (Figure 7). The findings are parallel with the study of Do et al. [42].

Comparison of TPC of water extracts for different herbal tea samples is given in figure 8. ST has the highest phenolic content value (229.21 mg GAE/g) while LT has the lowest value (46.62 mg GAE/g). TPC values of the samples decreases in the order of: ST (229.21 mg GAE/g) > GT (205.99 mg GAE/g) > RT (137.36 mg GAE/g) > ET (81.96 mg GAE/g) > BT

(80.77 mg GAE/g) > AT (63.07 mg GAE/g) > LT (46.62 mg GAE/g), respectively.

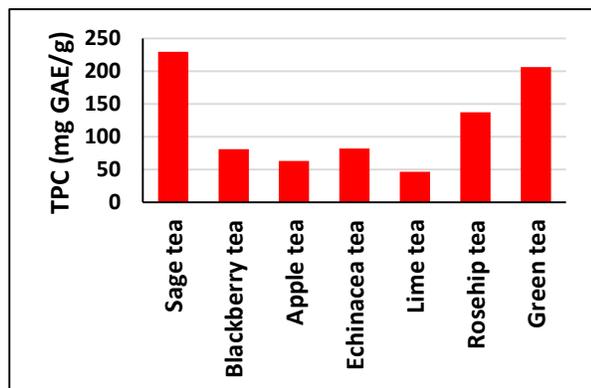


Figure 8 Total phenolic contents of water extracts of herbal tea samples

Different classes of phenols can be extracted from plants with various solvents and solvent mixtures depending on their polarities [43]. Due to the fact that each plant contains different types and amount of phenolic compounds. Therefore, determination of the ideal solvent is an important step for the extraction of the targeted compounds [44-45].

DPPH and FRAP assay results are given in Table 1. Inhibition of DPPH radicals by ST extracts is ranged from 71.51% in the acetone extract to 94.92% in ethanol extract. Although ethanol extract of ST has low TPC value (12.29 mg GAE/g), the highest DPPH and FRAP activity results are obtained with EtOH extract. This can be explained by the chemical structures of the phenolics which can be extracted with organic solvents. In another words, phenolics of ST which have lower polarities are more effective in the scavenging of DPPH radicals and reducing ferric ions, as well. High inhibition values are obtained for GT, as well. DPPH radical scavenging activity ranged from 70.99% to 89.49% in the presence of acetone and ethanol solvents for GT, respectively. Effect of solvent type on DPPH scavenging activity is more significant for other herbal tea samples under studied. For instance, DPPH activity ranged from 5.98% in the water extract to 91.67% in EtOH (50%) extract. A solvent may be efficient on one plant and less efficient on another. The phenomena may be explained by the fact that the recovery of antioxidant compounds such as polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction. Although FRAP assay results are closed each other, the highest value

(0.27 ± 0.74 mM Trolox™) is obtained for ST with EtOH (Table 1).

Table 1 DPPH and FRAP assay results of herbal tea samples; ST: Sage tea, BT: Blackberry tea, AT: Apple tea, ET: Echinacea tea, LT: Lime tea, RT: Rosehip tea, GT: Green tea, A: Water, B: MeOH (50%), C: EtOH, D: EtOH (50%), E: Acetone, F: Acetone (50%)

Herbal teas	Extract solvent	DPPH (%)	FRAP (mM Trolox™)
ST	A	76.97 ± 0.02	0.13 ± 0.03
	B	82.31 ± 0.01	0.18 ± 0.11
	C	94.92 ± 0.01	0.27 ± 0.74
	D	87.06 ± 0.01	0.18 ± 0.04
	E	71.51 ± 0.03	0.17 ± 0.04
	F	84.58 ± 0.02	0.17 ± 0.33
BT	A	5.98 ± 0.14	0.11 ± 0.02
	B	78.90 ± 0.01	0.13 ± 0.02
	C	37.72 ± 0.02	0.15 ± 0.05
	D	91.67 ± 0.00	0.14 ± 0.04
	E	24.73 ± 0.02	0.16 ± 0.02
	F	89.62 ± 0.01	0.15 ± 0.05
AT	A	58.69 ± 0.08	0.12 ± 0.09
	B	85.60 ± 0.01	0.13 ± 0.05
	C	38.87 ± 0.01	0.15 ± 0.05
	D	92.05 ± 0.01	0.15 ± 0.04
	E	25.46 ± 0.02	0.16 ± 0.03
	F	92.01 ± 0.01	0.15 ± 0.07
ET	A	67.7 ± 0.01	0.11 ± 0.07
	B	88.47 ± 0.01	0.14 ± 0.05
	C	54.16 ± 0.04	0.15 ± 0.04
	D	88.47 ± 0.01	0.16 ± 0.08
	E	56.30 ± 0.09	0.17 ± 0.01
	F	86.03 ± 0.00	0.17 ± 0.02
LT	A	78.00 ± 0.02	0.13 ± 0.03
	B	85.65 ± 0.01	0.14 ± 0.04
	C	19.48 ± 0.02	0.15 ± 0.03
	D	86.46 ± 0.00	0.15 ± 0.04
	E	35.37 ± 0.02	0.16 ± 0.03
	F	85.56 ± 0.00	0.15 ± 0.09
RT	A	8.07 ± 0.02	0.11 ± 0.03
	B	26.74 ± 0.13	0.13 ± 0.01
	C	47.24 ± 0.03	0.15 ± 0.01
	D	70.52 ± 0.02	0.14 ± 0.04
	E	14.35 ± 0.03	0.16 ± 0.09
	F	61.55 ± 0.03	0.14 ± 0.05
GT	A	80.26 ± 0.02	0.12 ± 0.14
	B	86.12 ± 0.00	0.14 ± 0.04
	C	89.49 ± 0.02	0.15 ± 0.01
	D	85.69 ± 0.00	0.16 ± 0.05
	E	70.99 ± 0.06	0.15 ± 0.11
	F	80.78 ± 0.01	0.17 ± 0.03

CONCLUSION

The most commonly consumed 7 herbal teas were investigated for their DPPH radical scavenging properties, ferric ions reducing power, and total phenolic contents. Water and aqueous organic solvents are better for the extraction of herbal tea samples. Although considerable results were obtained for all samples depending on extraction solvent, highest values were obtained for sage and green tea samples, respectively. The herbal tea infusions analyzed in this study are generally presented high antioxidant activity in terms of free radical scavenging, thus indicating possible benefits to human health when present in the diet.

In the light of the obtained results, sage tea, Echinacea tea, lime tea and green tea can be consumed for their free radical scavenging abilities which prevented aging. Consequently, the radical scavenging activity of herbal tea differs depending on the constituent of functional compounds. Outstanding of the present study reveal that, other antioxidant activity tests and qualitative phenolic compound analysis must be done for evaluation of whole herbal tea samples.

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