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Research article

Analysis of antioxidant capacity, total phenolic and total flavonoid contents in boric acid applied *Camellia sinensis* L.

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Abstract

Consumption of *Camellia sinensis* L. (tea), a popular beverage, is very common today. In addition to its consumption as a beverage, it is suggested that adding tea to other foods can increase their antioxidant activities. It is known that boric acid used as an insecticide, herbicide, and fungicide, has antioxidant and anti-inflammatory effects. This study aimed to determine the antioxidant capacity (AC), total phenolic content (TPC), and total flavonoid content (TFC) of extracts prepared in different solvents of tea grown in soil treated with boric acid. The area in Rize/Türkiye was divided into 4 groups. No application was made to the control group (B0 group). Boric acid prepared in sodium tetraborate buffer was applied to the other three areas as a single dose at concentrations of 100 (group B1), 300 (group B3), and 500 (group B5) mg m^{-2}. The obtained tea leaves were ground and infused in water, 20% ethanol, and 50% ethanol by the classical infusion method. The extracts obtained after infusion were analyzed for TPC, TFC, and AC. It was determined that ethanol (20% and 50%) was better solvent than water in terms of TPC, TFC, and AC. Although the TPC of extracts prepared in water of tea leaves grown in soils where different doses of boric acid were applied did not change, differences were observed in their flavonoid contents and antioxidant capacities. These results indicated that boric acid should be at a certain dose to improve the quality of the tea plant. In addition, different solvents can be used to reveal more of the tea content.

Keywords: *Antioxidant capacity; boric acid; C. sinensis; flavonoid content; green tea; phenolic content*

1. Introduction

Camellia sinensis L. (tea) is a plant greatly cultivated in tropical and subtropical regions and is the most widely consumed beverage after water due to its cooling, revitalizing, and mild stimulant effects containing highly bioactive compounds beneficial to health. These bioactive compounds in the tea plant include alkaloids, flavonoids, triterpenoids, caffeine, steroids, saponins, oils, carotene, essential oils, and vitamins (Ramlah, 2017). In addition, tea has bioactive properties such as antioxidative, anti-bacterial, anti-viral, and anti-inflammatory. Tea is also greatly used as a component of folk medicines (Hayat et al., 2015; Zhao et al., 2022).

Nowadays, the use of tea has started to change and a lot of research and development has been done on food products enriched with tea powder, including sponge cake (Ahmed et al., 2023), biscuits (Phongnarisorn et al., 2018), bread (Ning et al., 2017), dry noodles (Yu et al., 2020) and ice cream products (Baruah et al., 2012). The addition of green tea powder to food products can enhance antioxidant activity and significantly reduce peroxide production during product storage (Ning et al., 2017). Phenolic compounds, flavonoids, and antioxidants are the dominant constituents of tea leaves, accounting for 30% of their dry weight. Antioxidants protect living organisms from uncontrolled production of reactive oxygen species and accompanying protein, lipid, and DNA damage (Srinivasan et al., 2007; Juan et al., 2021). Antioxidant compounds such as polyphenols, phenolic acids, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, and/or lipid peroxide and thus inhibit oxidative mechanisms leading to degenerative

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diseases (Wu et al., 2011). Due to its diverse composition and benefits, tea can be used in different sectors, most notably the food processing sector. Several substances can be added to improve the color, taste, shape, and texture of tea and to extend its shelf life, in addition, adding green tea powder to baked goods is also beneficial for health (Goh et al., 2015; Mashkour et al., 2022). According to the study by Owuor et al. (2008), compounds that have beneficial health effects in tea are affected by many factors such as variety, harvest season, plant age, climate, environmental conditions, and processing conditions. Catechins and epigallocatechin gallate are the most abundant phenolic and flavonoid compounds in tea, and thanks to these compounds, tea shows antioxidant activity (Liang et al., 2024; Ntamo et al., 2024).

Boron is one of the essential microelements in plant growth. In addition, it has many different functions such as tissue differentiation, vegetative growth, membrane integrity, regulation of enzymatic reactions, sugar transport, and nucleic acid synthesis (Camacho-Cristobal et al., 2008; Koshiba et al., 2009; Ahmad et al., 2012; Pereira et al., 2021). Boron, one of the important elements involved in the concentration and metabolism of phenolic compounds in plants, should be present in soil and irrigation water under natural conditions. Deficiency or toxicity of boron in the form of boric acid in the soil in agricultural areas affects product yield and quality. Moreover, boron deficiency leads to oxidative stress and cell death. There is a decrease in antioxidant activity in the roots and leaves of plants with boron deficiency. Research has shown that high boron concentration increases antioxidant enzyme activity and thus protects against oxidative stress (Balci and Taban, 2023).

In this study, it was aimed to explore the concentrations of the TPC, TFC, and AC of the extracts obtained by infusing tea leaves grown in soils treated with different concentrations of boric acid in different solvents. Thus, this study aims to increase the antioxidant properties of tea leaves by improving crop quality during tea cultivation and to maximize the beneficial effects of natural antioxidant intake with consumption.

2. Materials and methods

2.1. Collection of experimental material

Fresh leaves of *C. sinensis* were collected from the Caykur trial garden located in the Rize/Cayeli district. The plot was divided into four groups. Each group consisted of five different areas ($10m^2$). While no application was made to the fields in the control (Boron 0) group, boric acid prepared in sodium tetraborate buffer at 3 different concentrations (Boric acid 1 (B1)-100 mg m^{-2}, Boric acid 3 (B3)-300 mg m^{-2}, and Boric acid 5 (B5)-500 mg m^{-2}) was applied to the soil of the other three groups. Harvested tea plant leaves were dried at room temperature (RT) for a week, ground with a grinding machine, and stored at room temperature (RT).

2.2. The preparation of C. sinensis leaf extract

For the extracts to be used in the study, the classical infusion method was used for the ground tea samples. In the classical infusion method, water, 20% ethanol, and 50% ethanol were used as solvents for each experimental group. To infuse 5 g of tea leaves, 250 mL of liquid was used at 30°C. The extracts obtained were cooled to RT and centrifuged at 4000 g for 15 min and then, the supernatants were used for TPC, TFC, and AC analysis.

2.3. Total phenolic content (TPC) analysis

Quantification of TPC was carried out by spectrophotometric method. For the detection of the concentration of TPC, 1500 µL Folin-Ciocalteu reagent (2 N, diluted 10 times) was added to the tubes containing 300 µL blank, standard compound gallic acid (GA) and tea leaf extract, and the solutions to be mixed thoroughly were kept in the dark at RT for 3 min. Then, 1.2 mL of 7.5% (w/v) Na_2CO_3 was added to the extract, standard, and blank tubes mixed thoroughly, and incubated for 90 min at RT and in the dark. After incubation, absorbances were read at 765 nm. The concentration of TPC in the extracts was calculated using the standard calibration chart created with OD765 values of different GA concentrations (Singleton and Rossi, 1965). The TPC concentration was expressed as µg GA equivalent/mL.

2.4. Total flavonoid content (TFC) analysis

The TFC of tea leaf extract was analyzed by the $AlCl_3$ colorimetric method (Zhishen et al., 1999). In the method, 4% NaOH, 10% $AlCl_3$, and 5% $NaNO_2$ solutions were used. Quercetin was used to prepare the calibration curve. For flavonoid determination, 4 mL distilled water, 1 mL standard quercetin solutions, and tea leaf extract were added to the tubes, and 0.3 mL 5% $NaNO_3$ was added and incubated for 5 min at RT. After incubation, 0.3 mL of 10% $AlCl_3$ was added to the tubes and kept in the dark and at RT for 5 min. Then, 2 mL of 4% NaOH and 2.4 mL of dH_2O were added to the tubes and absorbance measurements were performed at 415 nm. The concentration of total flavonoids in the extracts was calculated by using the standard calibration graph of quercetin.

2.5. Determination of antioxidant capacity (AC)

The total AC of tea extracts was detected according to Prieto's method (Prieto et al., 1999). 0.4 mL of tea leaf extracts were taken and 4 mL of phosphomolybdenum solution (obtained by mixing 0.6 M H_2SO_4, 28 mM $Na_2HPO_4.12H_2O$ and 4 mM ammonium molybdate, 25 mL each) was added, the analysis tubes were well closed with leak-proof caps, vortexed and incubated in a water bath at 95°C for 90 min. Subsequently, the samples were rapidly cooled, and the absorbance values of the green color formed after the reaction were determined at 695 nm. The ascorbic acid standard solution was prepared for the calculation. AC was presented as ascorbic acid equivalent (mg AAE/g) dry extract.

2.6. Statistical analysis

GraphPad Prism Version 9.0 statistical software was used for data analysis. The results are presented as mean ± SD. Data distributions were based on the Shapiro-Wilk test. ANOVA and Tukey's post hoc tests were used for analyses. P value less than 0.05 was accepted as significant.

3. Results

3.1. Total phenolic content (TPC)

There was no difference in the TPC of the extracts obtained

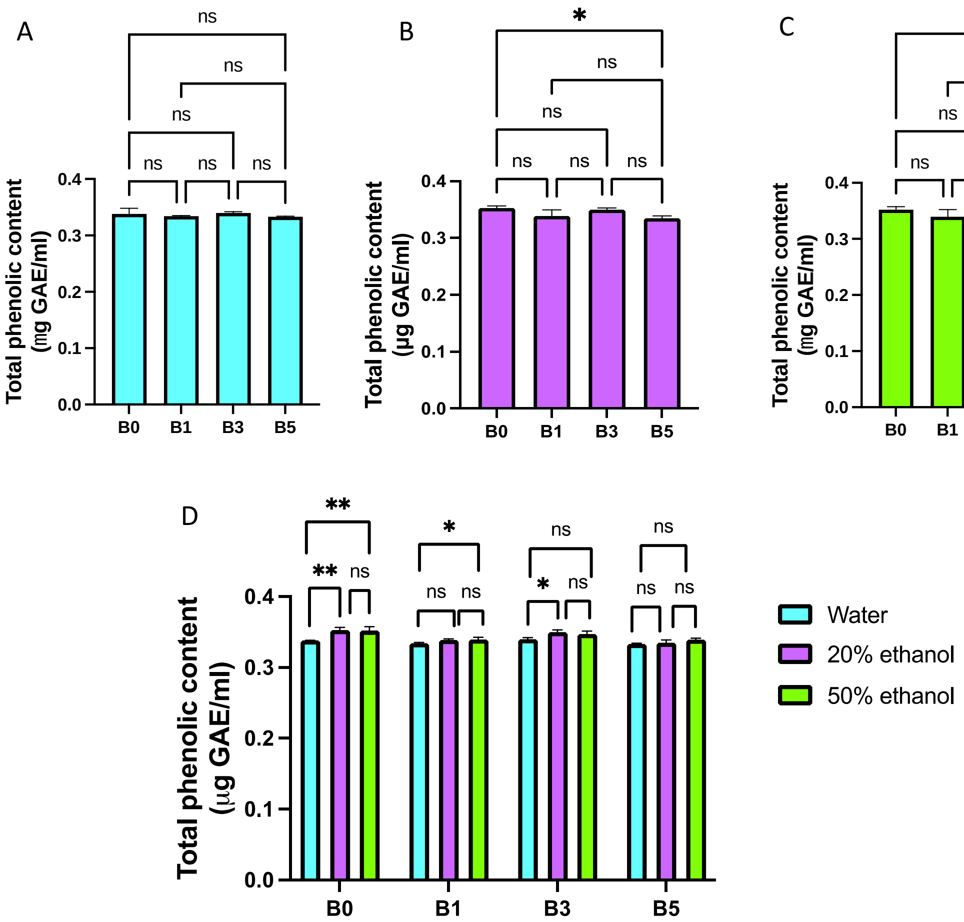

Fig. 1. Effects of 0 mg m^{-2} (B0), 100 mg m^{-2} (B1), 300 mg m^{-2} (B3), 500 mg m^{-2} (B5) boric acid treatments on TPC. Tea extract prepared with water (A), tea extract prepared with 20% ethanol (B), tea extract prepared with 50% ethanol (C), comparison of total phenolic compounds of different solvents (D). *$p < 0.05$, **$p < 0.01$, ns; non-significant.

from water infusion of tea leaves grown in soils with different doses of boric acid (Fig.1A). The concentration of TPC in the extract prepared with 20% ethanol of tea leaves collected from the B1 and B3 groups did not show any change compared to the B0 group. The concentration of TPC in the extract prepared with 20% ethanol of tea leaves in the B5 group was significantly decreased compared to the B0 ($p < 0.05$, Fig. 1B). Similar to the tea extracts prepared with water, no difference was observed in the TPC concentration of the tea extracts prepared in 50% ethanol among the B0, B1, B3, and B5 groups (Fig. 1C).

The TPC concentration of the extracts obtained by infusing tea leaves grown in soil treated with boric acid at different concentrations in different solvents is shown in Fig. 1D. It was determined that the TPC concentration of tea extracts obtained by brewing in 20% and 50% ethanol of tea leaves grown in non-boric acid treated soils was significantly increased compared to tea extracts prepared with water ($p < 0.01$ for both). The TPC concentration of the extracts prepared with 50% ethanol of the teas grown in group B1 was higher than the extracts prepared with water ($p < 0.05$). The TPC concentration of the extracts prepared with 20% ethanol of the teas grown in the B3 group was significantly higher than the extracts prepared in water ($p < 0.05$). However, there was no difference in the amount of TPC between the extracts prepared with different solvents of the B3 group.

3.2. Total flavonoid content (TFC)

The TFC of tea extracts prepared with water was significantly lower in the B1 and B5 groups compared with the B0 group ($p < 0.001$ and $p < 0.0001$, respectively, Fig. 2A). When the B1 and B5 groups were compared with the B3 group, the TFC of tea extracts prepared with water was lower in the B1 and B5 groups ($p < 0.0001$ for both). Similar to water, the TFC of tea extracts prepared with 20% ethanol was lower in the B1 and B5 groups compared to the B0 group ($p < 0.01$ for both). The TFC in the B3 group was significantly higher compared to the B1 and B5 groups ($p < 0.05$ and $p < 0.01$, respectively, Fig. 2B). The TFC of tea extracts prepared with 50% ethanol was significantly decreased in the B1, B3, and B5 groups compared to the B0 group ($p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively, Fig. 2C).

The TFC of the extracts obtained from tea leaves grown in soil treated with boric acid at different concentrations and infused in different solvents are presented in Fig. 2D. The TFC of the tea extracts obtained by brewing the tea leaves collected from the B0 group in 20% and 50% ethanol were significantly increased compared to the tea extracts prepared with water ($p < 0.01$ for both). However, there was no difference between 20% and 50% ethanol extracts. The TFC in both 20% and 50% ethanol extracts of teas grown in group B1 were higher than those prepared with water ($p < 0.001$ and $p < 0.0001$, respectively). In the B1 group, the TFC of tea extracts prepared in 50% ethanol were higher than those prepared in 20% ethanol ($p < 0.001$). The TFC of tea extracts prepared in 20% and 50% ethanol was significantly higher than those prepared in water ($p < 0.05$ for both). Similarly, the TFC of extracts prepared in water of teas grown in group B5 was lower than those prepared in 20%

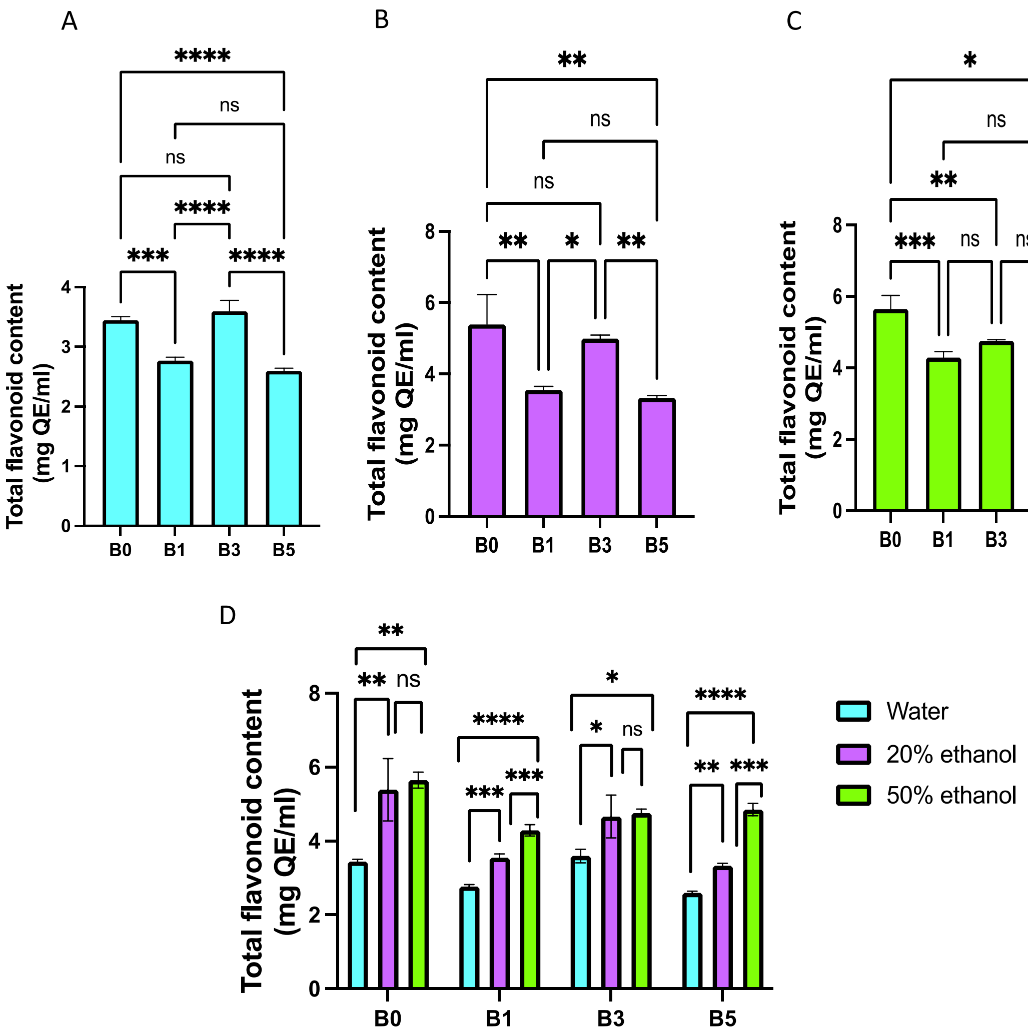

Fig. 2. Effects of 0 mg m^{-2} (B0), 100 mg m^{-2} (B1), 300 mg m^{-2} (B3), 500 mg m^{-2} (B5) boric acid treatments on the TFC in tea extract prepared with water (A), 20% ethanol (B), and 50% ethanol (C). Comparison of TFC in different solvents (D). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns; non-significant.

and 50% ethanol ($p < 0.01$ and $p < 0.0001$, respectively). In the B3 group, the TFC of tea extracts prepared with 50% ethanol was higher than those prepared with 20% ethanol ($p < 0.001$).

3.3. The antioxidant capacity (AC)

When the AC in the tea extracts prepared with water was compared, it was observed that the AC in the B1 and B5 groups was significantly lower compared to the B0 group ($p < 0.001$, $p < 0.0001$, respectively, Fig. 3A). However, the AC elevated in the B3 group compared to the B0 group ($p < 0.01$, Fig. 3A). Moreover, the AC in the B3 group was higher compared to the B1 and B5 groups ($p < 0.0001$ for both, Fig. 3A). The AC in the tea extracts prepared in 20% ethanol was similar to the brewed tea extracts, and the AC was lower in the B1 and B5 groups compared with the B0 group ($p < 0.0001$ for both, Fig. 3B). The AC in the tea extracts prepared with 50% ethanol in the B0 group was higher than in the B1, B3, and B5 groups ($p < 0.0001$, $p < 0.05$, and $p < 0.001$, respectively, Fig. 3B). The AC in the B1 and B5 groups was significantly reduced compared to the B3 group ($p < 0.01$ for both, Fig. 3C).

As the AC in the extracts prepared with different solvents of tea leaves grown in boron-free soils was compared, it was found that the AC of the extracts prepared in 20% and 50% ethanol were higher than those prepared in water ($p < 0.001$ for both, Fig. 3D). In the B1 group, the AC in the tea extracts brewed in 20% and 50% ethanol was significantly increased compared to the extract prepared in water ($p < 0.05$ and $p < 0.01$, respectively, Fig. 3D). Similarly, in group B3, the AC in tea extracts prepared in 20% and 50% ethanol was higher than the tea extract prepared in water ($p < 0.01$ for both, Fig. 3D). In group B5, the AC in the tea extract brewed in 20% ethanol was similar to that of extract brewed in water. However, the AC in the extract dissolved in 50% ethanol was higher than the tea extracts brewed in water and 20% ethanol ($p < 0.001$ for both, Fig. 3D).

4. Discussion

In this study, TPC, flavonoid content, and AC in the extracts prepared in water, 20% and 50% ethanol solvents from tea leaves grown in soil treated with different concentrations of boric acid were evaluated.

Tea has economic and social importance for many countries around the world. Consumption of tea, which is considered the second most consumed beverage in the world after water, is known to have beneficial effects on various human diseases such as cardiovascular and liver diseases (Keller and Wallace, 2021; Li et al., 2022). Tea is composed of polyphenols, minerals, caffeine, and trace amounts of amino acids, vitamins,

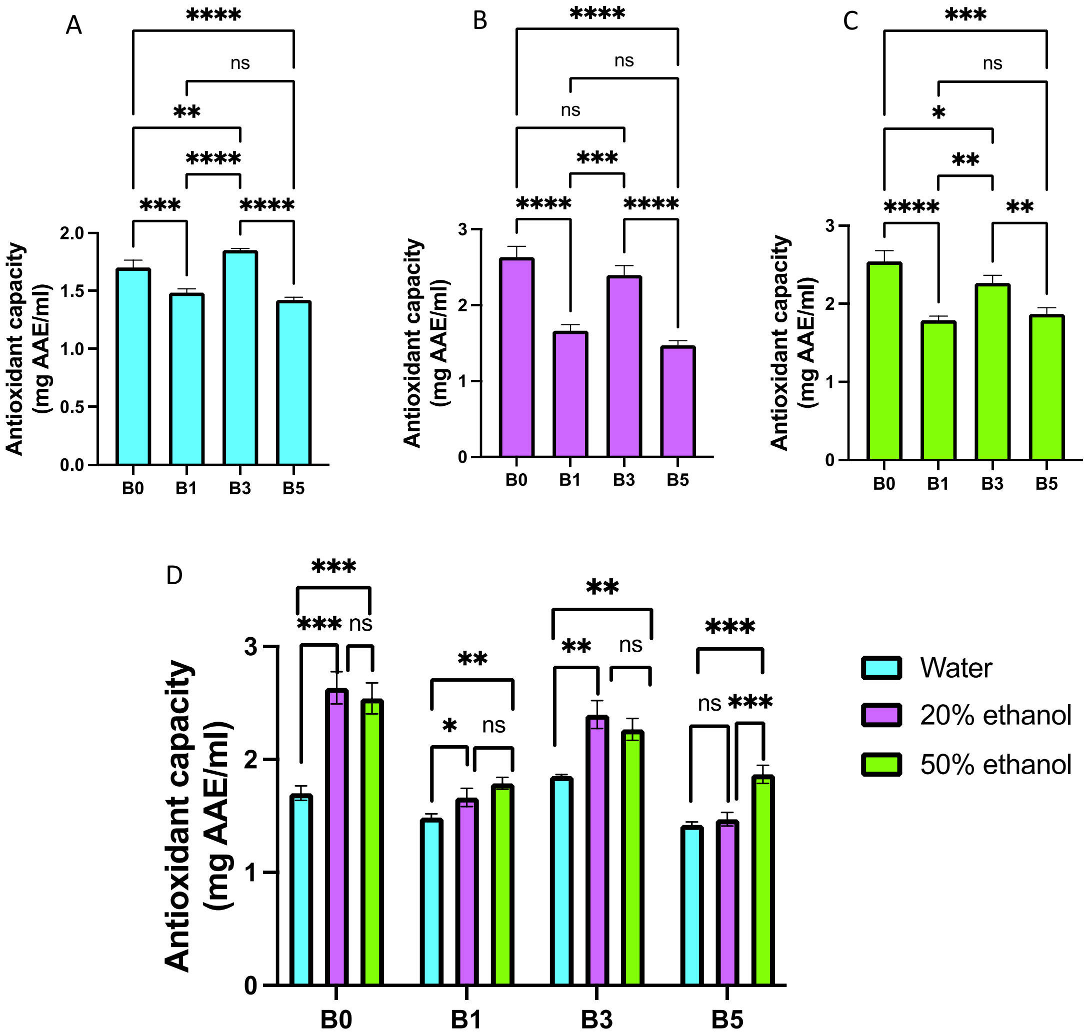

Fig. 3. Effects of 0 mg m^{-2} (B0), 100 mg m^{-2} (B1), 300 mg m^{-2} (B3), 500 mg m^{-2} (B5) boric acid treatments on the AC in tea extract prepared with water (A), 20% ethanol (B), and 50% ethanol (C). Comparison of AC in different solvents (D). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns; non-significant.

and carbohydrates, and the composition of tea varies depending on the fermentation process used for its production. Green tea has been noticed to be richer in antioxidants compared with other types of tea (Prasanth et al., 2019). The health benefits of green tea are associated with its high catechin level. In addition, the application of micronutrients such as boron, manganese, and zinc are important in increasing the yield of tea (Karak et al., 2017; Kumar, 2017).

It has been suggested that boron deficiency affects the growth of tea as well as its quality (Baruah et al., 2011). According to Gohain et al. (2000), foliar boric acid application had little effect on tea quality. Similarly, Hajiboland et al. (2013) reported that tea plant has a high tolerance to boron deficiency. In this study, it was determined that there was no difference in the concentration of TPC in the extracts prepared with water, 20%, and 50% ethanol from tea leaves grown in boric acid-treated soils. There was a decrease in the concentration of TPC in the extracts prepared with 20% ethanol from tea leaves grown in the B5 group. While the TFC level of the extracts prepared with water and 20% ethanol from tea leaves grown in the B0 and B3 groups were similar, a decrease was observed in the B1 and B5 groups compared to B0. The TFC level in the tea extract prepared with 50% ethanol was the highest in the B0 group. When the AC in the tea extracts was compared, the AC in the extract brewed in water was the highest in the B3 group. Also, the AC of the extracts prepared in 20% and 50% ethanol was the highest in the B0 group. While the application of boric acid to the soil does not affect the TPC concentration in tea leaves grown in this soil, its effect on the TFC concentration and AC varies depending on the dose of boric acid.

In studies on plant extracts, it was determined that TPC, TFC, and antioxidant activity values differed depending on solvents. It was emphasized that different solvents should be used for each plant (Coklar and Akbulut, 2016; Bursal et al., 2021; Yolci et al., 2022). Cabrera et al. (2021) examined green tea extracts prepared with synthetic fresh water, mineral salt solution, sodium bicarbonate solution, and deionized water at five different hardness levels to evaluate the effect of water hardness on caffeine and catechin content. As a result, they found that total catechin yield decreased as water hardness increased. Catechins, which have strong antioxidant properties, are polyphenol compounds that are important components of tea leaves as in many plants (Bae et al., 2020).

In the study by Baskaya Sezer (2023) on the phenolic contents of blackberry (*Rubus plicatus* L.) extracts prepared with different solvents (water, ethanol, methanol, and the forms of these solvents with acetic acid (1%) or hydrochloric acid (1%) added), it was reported that the highest phenolic content was found in samples extracted with ethanol and methanol with hydrochloric acid added. In another study, the TPC of yellow and blue poppy (*Papaver somniferum* L.) seed extracts prepared with different solvents such as ethanol, methanol, purified water, and acetone were analyzed. For yellow and blue poppy, the highest concentration of phenolic content was reported in the extract prepared with ethanol. The lowest concentration of phenolic content was found in the extract obtained with acetone for yellow poppy and in the extract prepared with methanol for blue poppy (Buran et al., 2022). In this study, it was determined that the TPC in the tea leaves extracts prepared in 20% and 50% ethanol were higher than extracts prepared in water. The TPC in tea leaf extract obtained from boric acid-treated soils was found to be higher only in the B1 and B3 groups in ethanol than in water. No difference was observed in terms of TPC in the extracts of tea leaves obtained from group B5 prepared in solvents such as water and ethanol. It can be said that solvent and boric acid affect the increase of TPC for the tea plant. On the other hand, high boric acid application to the soil may negatively affect the TPC of the tea plant.

Yolci et al. (2022) investigated the effect of dissolving the flowers of the plant called safflower or false saffron (*Carthamus tinctorius* L.) in different solvents such as water, methanol, acetone, and ethanol on the total concentrations of phenolic and flavonoid substances, and total antioxidant activity changes in flowers. For safflower flowers, water was reported to be the most suitable solvent for the highest TPC and antioxidant activity, while methanol was the most suitable solvent for TFC. In another study, extraction solvents were tested for six plant species grown in Sudan and the findings showed that ethanolic (50% ethanol, and 70% ethanol) and acetone extracts were rich in phenolic and flavonoid compounds and thus may contribute to high antioxidant activity (Dirar et al., 2019). In this study, when the TFC and AC of tea extracts brewed in water, 20% and 50% ethanol were compared, it was determined that the extracts brewed in ethanol had higher flavonoid content and AC than water. Thus, it can be said that changing the solvent of tea, which is consumed with water in daily life, as in other plants, can improve its quality.

As a result, TPC, TFC, and antioxidant activity in the green tea extracts may vary depending on the solvents they are prepared. It was detected that especially ethanol is a better solvent for the tea plant than water. According to that boric acid application can improve the green tea quality provided that it is at certain doses, while high doses may hurt the contrary.

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Conflict of interest: The author declares that she has no conflict of interest.

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