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ORIGINAL ARTICLE

# A New Substrate and Nitrogen Source for Traditional Kombucha Beverage: *Stevia rebaudiana* Leaves

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**Abstract**
Recently, the use of different herbal products as carbon sources instead of black and green tea in the preparation of traditional kombucha has been investigated. In this study, functional kombucha was prepared by adding *Stevia rebaudiana Bertoni* leaves, which have special organoleptic properties, to kombucha medium, and some properties of the beverage were analyzed. Tea blends were determined as 100% green tea (control = C), 75% green tea (GT) + 25% Stevia (ST), 50% GT + 50% ST, and 100% ST. On the 15th day of fermentation, gluconic acid (43.12 ± 0.01 g/L) was detected as dominant organic acid in GT75 + ST25 samples compared to group C ($p < 0.05$). According to physicochemical parameters that determine the drinkability properties of prepared teas, the best results were in GT25 + ST75 compared to group C ($p < 0.05$). It proved that the highest activity was in GT25 + ST75 on the 10th day in the groups that applied different antioxidant tests (DPPH, MCA, and CUPRAC). The antimicrobial activities of kombucha at 25, 50, 75, and 100% concentrations of GT and ST reached the highest levels in the GT25 + ST75 group in samples after 10 days of fermentation for all selected microorganisms. The results prove that GT25 + ST75 kombucha is a functional product with high drinkability on the 10th day of fermentation and also more beneficial for health due to the phenolic compounds from both green tea and Stevia. *Stevia rebaudiana* leaves can be suggested that be used as a new substrate and nitrogen source for kombucha production.

**Keywords** Kombucha tea · *Stevia rebaudiana Bertoni* · Organic acids · Antioxidant activity · Antimicrobial activity

## Introduction

Changing social dynamics also changed people's eating habits. Increasing health problems caused importance of healthy nutrition to increase and people to turn on natural and functional foods. Traditionally prepared fermented products form the basis of natural and functional

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foods. Recently, scientists have been conducting intensive scientific studies to elucidate effective compounds that add functionality to traditional foods. One of these traditional fermented beverages is a microorganism culture medium known as kombucha [1]. Kombucha is a beverage obtained as a result of fermentation of various teas (especially black, green and oolong tea), sugar, and symbiotic bacteria and yeast culture (SCOBY) [2]. Tea has a long history for human history. Today, it is consumed as the most popular beverage after water in many countries of the world. When the general content profile of tea is examined, it contains polyphenols, flavonoids (theaflavins and thearubigins), catechins, tannins, and essential oil. Green tea (GT) is considered a suitable medium for fermentation as it contains quercetin, kaempferol, and myricetin flavonoids, proteins, and amino acids in addition to other tea components [3]. Tea polyphenols have been the subject of many scientific studies as they have important effects in the prevention and treatment of various diseases [4–6]. This beverage, also called tea-fungus, is consumed in different countries of the world due to its health benefits [7]. While yeasts in the culture ferment tea sugar to produce ethanol, bacteria oxidize the alcohol formed to form acetic acid [1]. In addition to acetic acid, organic acids such as gluconic, lactic, malic, citric, and tartaric are also formed [2, 8]. Some research data have revealed that various herbal products can be used as substrates in the preparation of kombucha [9, 10]. However, every herbal product with tea potential is not considered a good nitrogen source because it does not contain purine derivatives. Cvetkovic [10] showed *Echinacea purpurea L.* and *Satureja montana L.* as alternatives to tea in kombucha production and reported that fermentation was achieved with less chemicals. Similarly, it was stated that *Melissa officinalis L.* can be used as a good nitrogen source for fermentation [11]. Today, researchers have aimed to add more functionality to the symbiotic culture by using many herbal substrates (fruit, wine, milk, herbal teas, etc.) other than sweetened black tea in the preparation of kombucha [12]. In this study, different concentrations of *Stevia rebaudiana* leaves were added to the microbial culture medium as a substrate for kombucha prepared with green tea.

*Stevia rebaudiana Bertoni* is a plant from the Asteraceae family that has been used as a traditional sweetener for many years by the Indians living in South America [13]. The high sweetness and calorie-free nature of the plant is attributed to diterpenic structures known as steviol glycosides [14]. It has been reported that these compounds contain a large amount of important phytochemical components (flavonoids, alkaloids, hydroxycinnamic acid, vitamins, phytosterols, essential oils, etc.) in Stevia leaves [15]. Water-soluble steviol glycosides are also important as they are approximately 300 times sweeter than sucrose and contain low-calorie (close to zero calories) sugar [14]. It has been reported that the steviosides in Stevia show therapeutic effects in the prevention of various diseases such as cardiovascular diseases, diabetes mellitus, arthritis, obesity, and neural disorders [13]. This study was carried out to determine the physicochemical, biochemical, and microbiological properties of kombucha samples produced at 25 °C at different rates (25, 50, 75, and 100%) and fermentation times (0, 5, 10, and 15 days). In addition, the use of tea made from stevia leaves as a nitrogen source or substrate was investigated instead of black and green tea used in kombucha fermentation. One of the main objectives of the study was to produce a more functional drink with a different taste thanks to the steviol glycosides of kombucha prepared with Stevia leaves.

## Material and Methods

### Stevia and Green Tea Samples

*Stevia rebaudiana* leaves used in tea making were purchased from a local spice producer (Arifoglu Biomedical Cosmetics Food Industry and Trade Inc.). Green tea leaves were purchased from products that were produced organically and packaged sterile by the Caykur company.

### Preparation of Kombucha Culture

Kombucha cultures (SCOBY) were obtained from a company named Shaman's Secret operating in the province of Istanbul/Turkey. The cultures taken were kept in a sterile environment. The SCOBY was activated for 14 days in a climate cabinet at 25 °C. In the study, using two different substrates, different concentrations of kombucha tea were used. These were 100% green tea (GT), 75% GT + 25% stevia (ST), 50% GT + 50% ST, and 100% ST. For kombucha, 7 g per L of each sample was weighed on a precision scale and placed in the strainer used for brewing tea. Seventy grams of sucrose per liter was then weighed. Prepared sterile water (3 L) was taken into a clean container and boiled. During boiling, sucrose was added to the water and boiled for 5 min until completely dissolved. Then, green tea was dipped into boiled sugar water and applied to infusion for 6 min. After the infusion, the prepared tea was cooled to 25–30 °C at room temperature and placed in jars. Finally, a culture of kombucha was placed for 10% inoculation. Within the scope of the study, 1 L sample was drawn from the jars at the relevant times for the 0th, 5th, 10th, and 15th days of fermentation. The samples were prepared for freezing by transferring them to clean and sterilized containers by filtering with a nuclear funnel and Whatman paper using a vacuum pump. It was placed in containers and quickly moved to − 80 °C and frozen for 72 h. After freezing, powder forms were prepared by lyophilizing (24 h). These stock samples were stored at − 20 °C until use. Lyophilization process was carried out with Telstar LyoQuest brand device. After lyophilization, kombucha samples were pulverized and stored at − 20 °C until used (Figs. 1, 2, 3, 4, and 5).

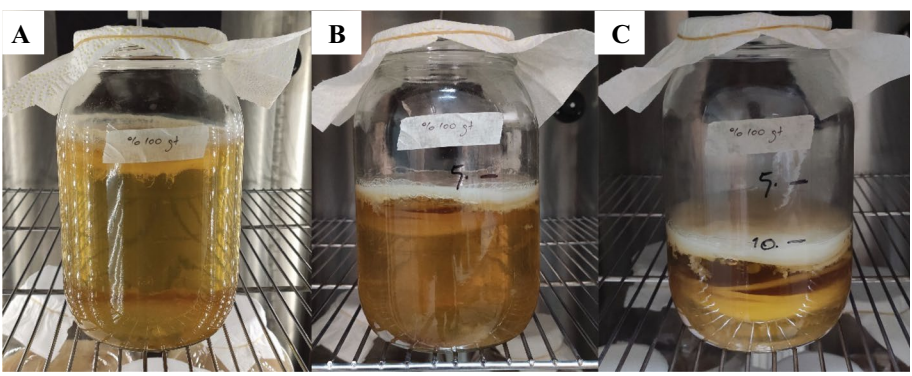


**Fig. 1** GT100: **A** 5th day, **B** 10th day, and **C** 15th day of fermentation

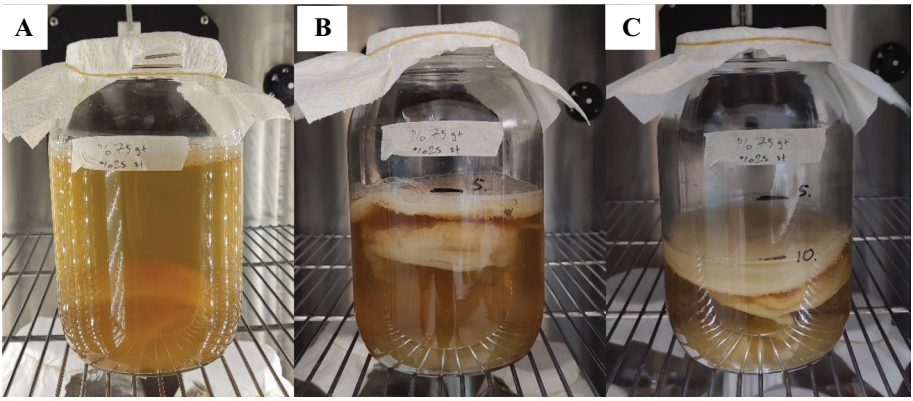


**Fig. 2** GT75 + ST25: **A** 5th day, **B** 10th day, and **C** 15th day of fermentation

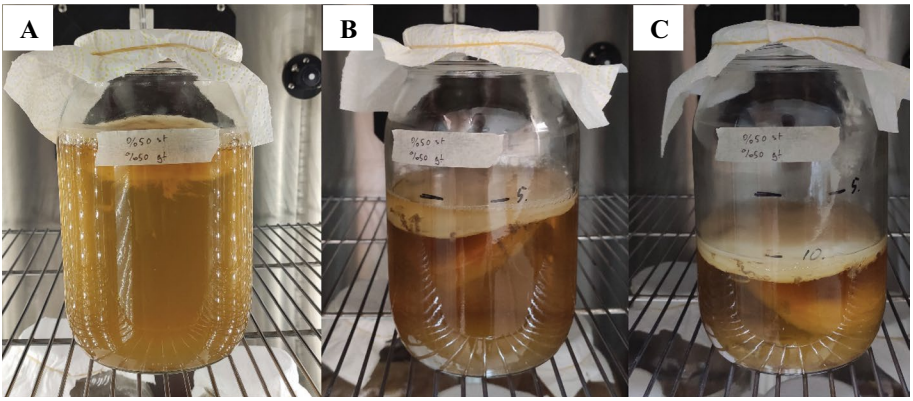


**Fig. 3** GT50 + ST50: **A** 5th day, **B** 10th day, and **C** 15th day of fermentation

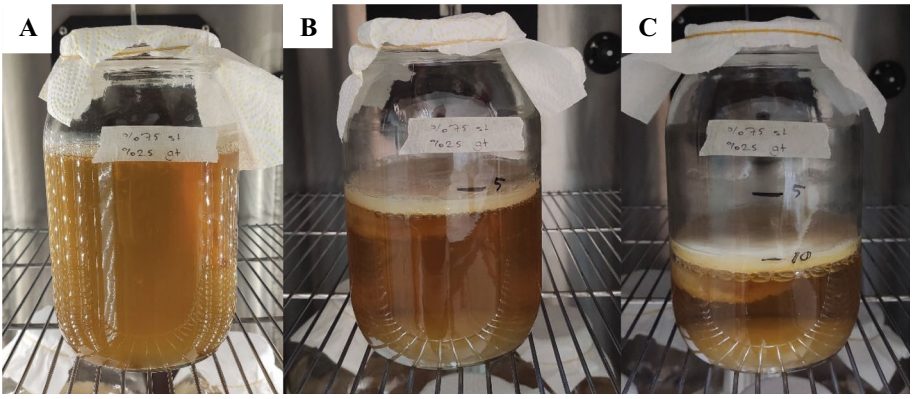


**Fig. 4** GT25 + ST75: **A** 5th day, **B** 10th day, and **C** 15th day of fermentation

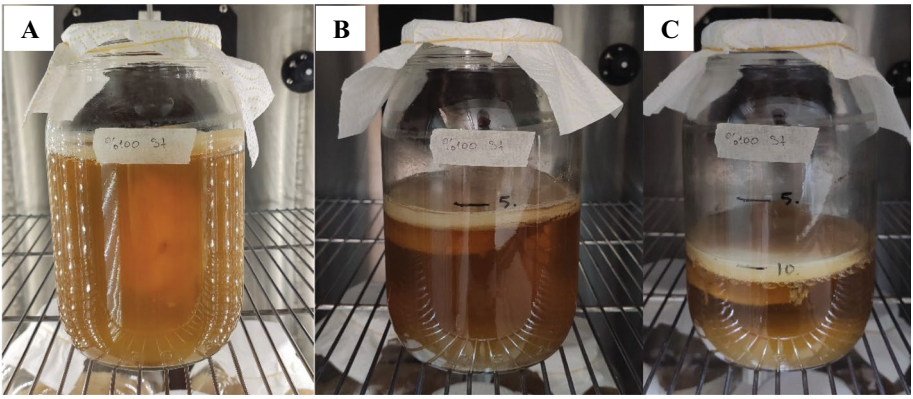


**Fig. 5** ST100: **A** 5th day, **B** 10th day, and **C** 15th day of fermentation

## pH, TA, and Brix

pH levels were measured with a Mettler Toledo (Model: Seven Easy GMBH 8603, Switzerland) brand pH meter and °Brix (°B) using a refractometer (Atago, PAL-3, Japan). For the determination of titratable acidity (TA), sodium hydroxide and phenolphthalein solutions were used for the standard, and the results were given by acetic acid g/L.

## DPPH Radical Scavenging Activity

Free-radical scavenging activities of the solutions were determined using 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical. Brand-Williams et al. [16]'s study was carried out by making some changes required by the experimental conditions. One hundred fifty microliters of sample at 1 mg/mL concentrations was pipetted into the test tubes, and 100 µL of 1 mM DPPH solution was added to it, and incubated for 30 min at room temperature in a light-free environment. After incubation, absorbance at 517 nm was recorded against a blank containing only methanol. Ascorbic acid standard was used as control. The standard was prepared at 1 mg/mL concentrations of ascorbic acid with distilled water. Studies were carried out in 3 parallels. The reduced absorbance gave the remaining free radical scavenging activity.

## Cuprac Assay

For this study, 500 µL of $CuCl_2$ solution and 500 µL of $C_2H_7NO_2$ (1 M pH: 7.0) solution were put into test tubes. Five hundred microliters of neocuproin ($C_{14}H_{12}N_2$) ($7.5 \times 10^{-3}$ M) solution was added to each tube. One hundred microliters of lyophilisate solution at a concentration of 1 mg/mL was placed on it and made up to 550 µL with distilled water. For the blank samples, distilled water was substituted for the extract. It was incubated for 30 min at room temperature and in a water bath (50 ºC). The absorbance at 450 nm was read against the blank sample, and ascorbic acid was used as a standard [9].

### Metal Chelating Activity

The metal chelating activity (MCA) of the lyophilized sprout extracts was determined [17] according to the method by Dinis et al.. The stock solution was prepared as 800 µL extract + 100 µL solution (2 mM $FeCl_2$). To initiate reaction, 400 µL (5 mM) of ferrozine was added and incubated at room temperature for 10 min. Absorbance values were measured spectrophotometrically at 562 nm. Results were expressed as mg EDTA equivalent/100 g extract.

### Total Phenolic Compounds

Total phenol content was adapted according to the method of Singleton and Rossi [18] using the Folin-Ciocalteau reagent. This method is based on the formation of blue-colored compounds by transporting electrons from phenolic compounds to phosphomolybdic and phosphotungstic acids in alkaline medium. In kombucha lyophilisates prepared at 1 mg/mL, 0.1 mL was taken and added to 1 mL of Folin-Ciocalteau solution (10 times diluted) and incubated for 5 min. One milliliter of sodium bicarbonate (7.5%) solution was added to it. Samples were read absorbance at 765 nm after 90-min incubation. Results were calculated according to gallic acid standards.

### Determination of Organic Acids by HPLC

D-Glucuronic acid, D-gluconic acid, acetic acid, ascorbic acid, and succinic acid were determined using high-performance liquid chromatography (HPLC, VARIAN, Pro Star, USA). In this method, 5-µL samples were first passed through a membrane filter (0.45 µM), and 10-µL filter sample was injected into HPLC. Aminex® HPX-87H was used as the ion exclusion column, and 0.01 N $H_2SO_4$ (pH 2.34) was used for the mobile phase. The flow rate (0.5 mL/min) and the temperature of the column were fixed at 25 °C. The measurement was determined at 210 nm with the Diode Array Detector (DAD 3262).

### Microbiological Quality and Safety Assessment

The antimicrobial activity of the lyophilisate containing fermented metabolites was investigated against some important pathogenic bacteria (*Escherichia coli* O157:H7-ATCC 35150, *Salmonella enterica subsp. enterica* ATCC13311, *Staphylococcus aureus* MRSA ATCC 43300, *Pseudomonas aeruginosa* ATCC 27809, *Listeria monocytogenes*). For this purpose, pathogen strains grown in Brain Heart Infusion liquid medium at 37 °C for 18 h were inoculated ($10^7$ kob/mL) into 5-mL semi-solid medium and spread on solid agar surface containing the same medium. One hundred microliters of lyophilisate (1 mg/mL) containing fermented metabolites was transferred to 6-mm-sized wells drilled on the solid agar surface. Zones of inhibition around the wells were measured in mm after 24 h of incubation at 37 °C.

### Calculation of Statistical Data

SPSS version 21 (Chicago, IL, USA) software was used for statistical analysis of the obtained data. All results are given as means (SEM) obtained from three independent experiments ($n \geq 3$). At least three replications were made for each sample in the study. Variability between means of results was determined by ANOVA and Tukey's multiplex analysis of variance. Significance levels were considered statistically significant for $p \leq 0.05$.

## Results and Discussion

### Evaluation of pH, TA, and Brix Analyses

The pH values of GT100 (C), GT25 + ST75, GT50 + ST50, GT75 + ST25, and ST100 concentrations used in kombucha production at 25 °C and on the 0th, 5th, 10th, and 15th days of fermentation are given in Table 1. There was a decrease in pH levels in all infusion groups due to the increase in fermentation time ($p < 0.05$). It is thought that the main

**Table 1** Physicochemical changes in different fermentation times of kombucha prepared with GT and ST leaves at different concentrations

| Groups | FT (day) | Physicochemical parameters | | |
|---|---|---|---|---|
| | | pH | TA (g/100 mL) | °Bx (%) |
| GT100 | 0 | 3.46 ± 0.06a | 0.20 ± 0.04e | 7.82 ± 0.02b |
| | 5 | 3.14 ± 0.02b | 0.29 ± 0.09d | 7.65 ± 0.01cd |
| | 10 | 2.97 ± 0.04bc | 0.35 ± 0.10cd | 7.71 ± 0.03bc |
| | 15 | 2.99 ± 0.01bc | 0.71 ± 0.05bc | 7.53 ± 0.01cd |
| GT25+ST75 | 0 | 3.29 ± 0.03a | 0.17 ± 0.09e | 7.76 ± 0.04bc |
| | 5 | 3.13 ± 0.03b | 0.24 ± 0.07d | 7.71 ± 0.0bc |
| | 10 | 2.99 ± 0.03bc | 0.33 ± 0.06cd | 7.51 ± 0.02d |
| | 15 | 2.97 ± 0.04bc | 0.62 ± 0.05c | 7.09 ± 0.03e |
| GT50+ST50 | 0 | 3.14 ± 0.04bc | 0.21 ± 0.09e | 7.54 ± 0.01cd |
| | 5 | 3.11 ± 0.03b | 0.27 ± 0.10d | 7.43 ± 0.01d |
| | 10 | 2.96 ± 0.01bc | 0.38 ± 0.09cd | 7.61 ± 0.02b |
| | 15 | 2.93 ± 0.04bc | 0.73 ± 006bc | 7.17 ± 0.03e |
| GT75+ST25 | 0 | 3.07 ± 0.01b | 0.28 ± 0.07d | 7.67 ± 0.02b |
| | 5 | 2.99 ± 0.02bc | 0.32 ± 0.11cd | 7.58 ± 0.06cd |
| | 10 | 2.79 ± 0.03c | 0.43 ± 0.08cd | 7.47 ± 0.04d |
| | 15 | 2.81 ± 0.04c | 0.87 ± 0.06bc | 7.29 ± 0.04de |
| ST100 | 0 | 2.69 ± 0.02c | 0.67 ± 0.01c | 7.91 ± 0.02a |
| | 5 | 2.57 ± 0.03d | 0.81 ± 0.10bc | 7.89 ± 0.05a |
| | 10 | 2.39 ± 0.06e | 1.11 ± 0.07b | 7.84 ± 0.03b |
| | 15 | 2.44 ± 0.05e | 1.94 ± 0.08a | 7.79 ± 0.04bc |

Values followed by different lowercase letters within the same column are significantly different ($p < 0.05$) ($n = 3 \pm$ SD)

*TA* titratable acidity (acetic acid g/100 mL)

reason for the decrease in pH levels in kombucha samples during fermentation is due to the increase in organic acid content. The research results of Sievers et al. [19], Yavari et al. [20], and Ayed and Hamdi [21] were in line with our data. One of the factors that can contribute or prevent the development of some types of microorganisms in foods is the $H^+$ concentration in the environment. Acetic acid bacteria tolerate the pH value of the medium in the range of 3.6–6.3, and a pH of not less than 5.4 facilitates their growth. The optimum pH for yeasts is in the range of 4.5–6.5, depending on the species and species [22]. The pH value of GT75 + ST25 kombucha was 2.79 ± 0.03 and 2.81 ± 0.04 in 10 and 15 days of storage, respectively. The pH levels in all groups on the 5th–10th days of fermentation decreased due to the increase in organic acid production. However, this value caused a slight increase and fluctuation in GT75 + ST25 and ST100 between 10 and 15 days of storage (Table 1). In previous studies, it was revealed that some molecules produced as a result of microbial activities, depending on the liquid concentration in the fermentation medium, can cause fluctuations in pH by showing a buffering effect [23, 24]. Gradual decreases in pH were exhibited in association with the increased fermentation process (from 5th to 15th days) due to the buffering properties of kombucha infusions. Tanticharakunsiri et al. [25] reported that when they mixed oolong tea, mint, and oolong tea + mint mixtures they added to kombucha consortium, the pH decreased rapidly within the first 7 days of fermentation, and then a little more after the 7th day. Tamer et al. [26] reported that the cinnamon infusion after fermentation had the highest pH value of the teas they prepared with black and green tea infusion sweetened to kombucha culture, as well as some medicinal plant infusions (linden, lemon balm, sage, echinacea, mint, and cinnamon). In another study, the initial pH of kombucha teas prepared with white, green, black, and red tea was reported between 5.34 and 6.53. It was stated that it decreased to 2.31–2.53 levels on the 14th day of fermentation [27]. In this study, pH values of 15th day were determined at level of 2.44–2.97 in the drinkable acidity range. It should not be forgotten that beverages consumed at very low pH may cause adverse effects on digestive system. For this reason, amount of consumption of beverage obtained is as important as fermentation period of kombucha.

The TA of tea infusions in all groups ranged from 0.29 ± 0.09 to 1.94 ± 0.08 g/100 mL. On the 15th day of fermentation, there was a time-dependent increase in TA (Table 1). At the end of fermentation, the highest values were determined as 1.11 ± 0.07 and 1.94 ± 0.08 g/100 mL in ST100 samples on the 10th and 15th days, respectively (Table 1). The lowest TA levels were 0.24 ± 0.07 and 0.33 ± 0.06 in GT25 + ST75 on the 5th and 10th days, respectively. Yıkmış and Tuğgüm [28] determined the TA values of 50% mixtures of kombucha and purple basil as 8.40 ± 0.00 after 30 days of fermentation and 8.24 ± 0.14 for 75% mixture. They also explained that TA had similar results throughout the entire storage except the first day between the kombucha and the 50% mix. They said that after the 20th day of fermentation, TA did not show a significant difference on the 10th and 20th days. In some studies, sensory analysis data revealed that the formation of an extremely acidic taste is not preferred by the consumer. It is known that when TA reaches 0.25 g/100 mL and lower levels, it makes it difficult to drink kombucha [3]. Tanticharakunsiri et al. [25] reported that after fermentation with kombucha consortium, the TA content of oolong tea, mint, and oolong tea + mint mixtures showed a gradual increase from 0 to 21 days of fermentation for all kombucha groups. On the 21st day of fermentation, the highest TA was found in oolong tea infusion (10.1 ± 0.51 g/L) and the lowest in mint infusion (2.82 ± 0.06 g/L). Velicanski et al. [11] expressed the changes in pH and TA levels of traditional kombucha prepared with *Melissa officinalis* as the plant providing sufficient nitrogen for the development of SCOBY. In some studies, the incompatibilities in pH and TA are explained

by the buffer capacity of the fermentation medium, that is, the carbon dioxide released during fermentation decomposes the water solution and produces amphiprotic hydrocarbonate anion ($HCO_3^-$). It is thought that these anions may react with hydrogen ions originating from organic acids in the fermentation medium and may have contributed to the buffering capacity of the system by preventing pH changes [10]. Such trends in pH and TA parameters are typical in kombucha fermentation and have been observed by some authors [24, 29]. Although an increase in TA was observed throughout fermentation, some authors reported a limited increase in acetic acid concentration during a prolonged cultivation (60 days) [23]. In the light of this information, TA has been used instead of pH for the critical parameter that determines the termination process of kombucha fermentation in some studies.

It was determined that °Bx of all kombucha samples varied between 7.09 ± 0.03 and 7.89 ± 0.03 (Table 1). The lowest °Bx values in all tea applications occurred on the 15th day (Table 1). Yeasts convert glucose and fructose into ethanol in the kombucha medium. Acetic acid bacteria, on the other hand, use ethanol in the environment to produce gluconic acid [30, 31]. As yeasts and bacteria metabolize sucrose during fermentation, reductions in °Bx of the infusion groups occurred after a while (Table 1). Compared to GT100 (C), the °Bx value on the 15th day of GT25 + ST75 is 7.09 ± 0.03. Yıkmış and Tuğgüm [28] explained the °Bx results of purple basil added to the kombucha medium as 8.50 ± 0.00, the highest °Bx value during 30-day storage, and 7.07 ± 0.06, the lowest in the purple basil group. [26] stated that kombucha prepared with sweetened black and green tea infusion and some medicinal plant infusions decreased the amount of sugar due to microorganism activities after fermentation, and there was a decrease in °Bx in relation to this situation.

## Measurement of Total Phenolic Content and Antioxidant Activities

TPC in the samples taken on the 0th, 5th, 10th, and 15th days of fermentation in GT, ST, and GT + ST kombucha teas prepared at different concentrations are shown in Table 2. TPC amounts in all kombucha infusions were given as gallic acid equivalents. In the results obtained, the highest TPC was calculated as 595 ± 0.65 mg GAE/L at the end of the 10th day fermentation of GT25 + ST75 infusion. The highest antioxidant capacities were determined as 0.947 ± 028 mmol Trolox/g by DPPH (85.85 ± 0.48%), MCA (1.107 ± 0.43), and CUPRAC method at the end of the 10th day fermentation of GT25 + ST75 infusion. In the study, GT100 kombucha was determined as the control group. The changes in the other kombucha samples were compared with the GT100 group and evaluated. At the end of the 10th day of fermentation of kombucha samples, there was a 26.6% increase in GT25 + ST75 kombucha compared to GT100 group ($p < 0.05$). At the end of the 10th day of fermentation, the change in antioxidant capacity was found to be 5.19% in DPPH, 14.58% in MCA, and 20% in CUPRAC in GT25 + ST75 group ($p < 0.05$). Ayed and Hamdi [21] reported that the total phenolic content of kombucha prepared with cactus pear juice increased by 23% after 6 days of fermentation. The same researchers said that the antioxidant activity of the kombucha drink they produced, determined by the DPPH and ABTS tests, increased by 81% and 65%, respectively. Abuduaibifu and Tamer [32] also stated that after the addition of black and red goji berry to kombucha prepared with black tea, after 48 h of fermentation, the highest amount of phenolic substances was found in black tea, black goji berry, and red goji berry kombucha, respectively. According to the DPPH method, they reported the antioxidant activities of black tea, red goji berry, and black goji berry infusions as 80.21 ± 0.24, 74.30 ± 2.98, and 80.84 ± 0.85 µmol Trolox/g, respectively.

**Table 2** Changes in antioxidant capacity and total phenolic content in different fermentation times of kombucha prepared with GT and ST leaves at different concentrations

| Groups | FT (day) | Antioxidant parameters | | | |
|---|---|---|---|---|---|
| | | TPC (mg GAE/L) | DPPH (% inhibition) | MCA (% inhibition) | CUPRAC (mmol Trolox/g) |
| GT100 | 0 | 425 ± 0.45c | 75.22 ± 0.41bc | 0.775 ± 0.16d | 0.635 ± 0.72d |
| | 5 | 420 ± 0.24c | 73.85 ± 0.83bc | 0.765 ± 0.14d | 0.687 ± 0.46cd |
| | 10 | 470 ± 0.37bc | 77.97 ± 0.71b | 0.964 ± 0.21b | 0.758 ± 0.39bc |
| | 15 | 488 ± 0.45b | 81,73 ± 0.28ab | 0.806 ± 0.19cd | 0.801 ± 0.21b |
| GT25 + ST75 | 0 | 481 ± 0.21b | 74.56 ± 0.92bc | 0.814 ± 0.12cd | 0.756 ± 0.61bc |
| | 5 | 545 ± 0.41ab | 77.61 ± 0.37b | 0.854 ± 0.32c | 0.833 ± 0.26b |
| | 10 | 595 ± 0.65a | 85.85 ± 0.48a | 1.107 ± 0.43a | 0.947 ± 0.28a |
| | 15 | 367 ± 0.59d | 81.91 ± 0.63ab | 0.746 ± 0.36d | 0.904 ± 0.31ab |
| GT50 + ST50 | 0 | 405 ± 0.68cd | 67.34 ± 0.72e | 0.791 ± 0.72cd | 0.727 ± 0.34c |
| | 5 | 423 ± 0.84c | 75.64 ± 0.54bc | 0.794 ± 0.61cd | 0.735 ± 0.22c |
| | 10 | 474 ± 0.73bc | 79.76 ± 0.44b | 0.847 ± 0.82c | 0.806 ± 0.68b |
| | 15 | 364 ± 0.94d | 83.52 ± 0.31a | 0.714 ± 0.72d | 0.849 ± 0.43b |
| GT75 + ST25 | 0 | 398 ± 0.29cd | 71.22 ± 0.48bc | 0.743 ± 0.86d | 0.637 ± 0.12d |
| | 5 | 421 ± 0.38c | 72.41 ± 0.64bc | 0.762 ± 0.52d | 0.696 ± 0.61cd |
| | 10 | 471 ± 0.74bc | 76.53 ± 0.39b | 0.961 ± 0.61b | 0.767 ± 0.53bc |
| | 15 | 298 ± 0.82e | 80.29 ± 0.74ab | 0.668 ± 0.36e | 0.810 ± 0.74b |
| ST100 | 0 | 307 ± 0.53e | 74.72 ± 0.33bc | 0.682 ± 0.64e | 0.605 ± 0.08e |
| | 5 | 319 ± 0.47de | 65.43 ± 0.80e | 0.713 ± 0.61d | 0.610 ± 0.33e |
| | 10 | 339 ± 0.87de | 69.55 ± 0.63cd | 0.912 ± 0.71b | 0.681 ± 0.61cd |
| | 15 | 363 ± 0.28d | 73.31 ± 0.71bc | 0.717 ± 0.55d | 0.725 ± 0.64c |

Values followed by different lowercase letters within the same column are significantly different ($p < 0.05$) ($n = 3 \pm$ SD)

They also calculated that after 48 h of fermentation, antioxidant activities increased by 17.18% (black tea), 24.78% (red goji berry kombucha), and 16.96% (black goji berry kombucha) in the samples examined with the DPPH test. According to CUPRAC analysis, antioxidant activities were reported as 53.71 ± 1.60, 0.29 ± 0.20, and 0.65 ± 0.21 µmol Trolox/g, respectively. In another study, Gaggìa et al. [33] determined antioxidant activities of kombucha prepared with green tea, black tea, and rooibos leaves in the samples taken after 7 days of fermentation with the DPPH test at 1.30, 0.87, and 0.45 mmol TE/g dry weight, respectively. During fermentation, various microorganisms such as acetic acid bacteria and yeasts in the kombucha consortium can degrade the released enzymes and polyphenolic compounds into smaller molecules with high antioxidant activity [34, 35]. As explained by many researchers doing research in this field, fermentation contributes significantly to the conversion of complex compounds into simpler forms [36–38]. On the 5th day of fermentation, TPC levels did not show a statistically significant difference in GT100 and ST kombuchas at different concentrations ($p > 0.05$). In our results, TPC levels were statistically decreased in all groups on the 15th day of fermentation compared to the samples in GT100 ($p < 0.05$). Yıkmış and Tuğgüm [28] also measured TPC and AA (DPPH and CUPRAC) at the end of 1-, 10-, 20-, and 30-day fermentation periods of their blends of purple basil kombucha at 50% and 75%. They reported that after the 30th day of fermentation, the total amount of phenolic substances in all samples decreased compared to the 1st day. They found that the amount of phenolic compounds in the kombucha mixture at 50% concentration decreased significantly in the samples at all fermentation times, and even there was no significant difference in the amount of TPC on the 10th and 20th days. In the studies supporting our results, it was also explained by some researchers that the amount of TPC in kombucha prepared with different plant extracts increased linearly during fermentation, reached the highest value at the last stage of fermentation, and then decreased [39–41]. It has been reported that there is an increase in the amount of phenolic substances in kombucha samples as a result of the breakdown of polyphenols with large molecular weights by enzymes in yeast and bacteria at certain times of fermentation [42]. It has been explained that decreases in the amount of TPC during certain periods of fermentation and storage processes may be due to changes in the polymerization of phenolic substances [43]. The variety and amount of phenolic compounds in kombucha infusions can be affected by various factors such as the type used in tea preparation, age of the plant leaves, ripening time, geographical features, production methods, hydrolysis, isomerization, and polymerization [11]. Another important result determined in the current study was that kombucha prepared from stevia leaves at different concentrations had higher TPC and AA levels than both GT100 and ST100 ($p < 0.05$). Teabucha teas with *Echinacea purpurea* L. and *Satureja montana* were reported to show higher TPC and AA than traditional kombucha tea [10]. On the 10th day of fermentation, it was determined that the antioxidant effect of kombucha produced from green tea and Stevia leaves at other concentrations was lower than that of group C, except for GT25 + ST75 kombucha samples. It is thought that this may be due to the fact that the catechins in green tea are suppressed by the antioxidant molecules in Stevia. Tamer et al. [26] also stated that mint infusion in kombucha they produced with infusions of different plant extracts was more effective in terms of TPC and AA than linden, lemon balm, sage, echinacea, and cinnamon. Before fermentation, they found TPC as 1145.74 ± 18.89 mg GAE/100 mL and AA as 78.67 ± 0.66, 150.28 ± 5.32, and 87.04 ± 2.77 µmol TE/g by DPPH, FRAP, and CUPRAC methods, respectively. They reported that after fermentation, TPC content increased to 1865.28 ± 44.76 mg GAE/100 mL, and AA increased to 89.65 ± 5.63, 223.85 ± 5.79, and 135.38 ± 1.50 µmol TE/g, respectively, with the same methods determined. Another AA determination method in our study was MCA.

This method is used to inhibit the destructive effects of free heavy metals. On the 10th day of fermentation, there was a 44.71% increase in GT25 + ST75 kombucha compared to control (GT100) samples in MCA. However, in other concentration groups, the ability of kombucha to bind ferrous ions decreased. This may indicate that the polymeric catechins in green tea may have undergone depolymerization during the fermentation process, resulting in the release of smaller molecules that exert higher antioxidant potential. These research data also revealed that there were increases and decreases in the values determined by different AA determination methods in kombucha infusions as a result of the fermentation times on the 10th and 15th days. Similarly, Vohra et al. [44] observed decreases in the DPPH free radical scavenging power of kombucha prepared using different carbon sources (black and green tea, jiggery, and Kelulut honey) for 7, 14, 28, and 60 days and a prolonged fermentation time except for the green tea + jaggery combination on the 7th day of fermentation in all of the samples. Another group of researchers found fluctuations in antioxidant activity (DPPH) during the butterfly pea kombucha fermentation process. In their study, they determined that AA increased significantly on day 4 of fermentation and decreased over the next 4 days. They explained that after the 8th day of fermentation, the AA gradually increased again, and the activity remained stable until the 20th day of fermentation [45]. It is thought that the differences between the values determined in the fermentation times may be due to changes in the substrate and production procedure and temperature differences. Also, another reason for this fluctuation may be the presence of certain antioxidant compounds (flavonoids, tannins, and phenols) found in stevia leaves, as well as strong microbial activities that degrade polyphenols during fermentation.

## Determination of Organic Acid Content

Organic acid contents of kombucha prepared with green tea and Stevia were determined by HPLC method and are shown in Table 3. The results obtained showed that all kombucha infusions contained glucuronic acid, gluconic acid, acetic acid, ascorbic acid, and succinic acid (Fig. 6). All determined organic acid types were determined at the highest rates in GT100 samples. The lowest organic acid content was in GT25 + ST75 and ST100 groups. Shahbazi et al. [46] also determined the highest organic acid content as acetic acid in kombucha prepared with cinnamon, cardamom, or Shirazi thyme. Among them, cinnamon kombucha was declared to have the highest acetic acid content. Chen and Liu [23] fermented kombucha prepared with tea mushroom samples in Taiwan for 60 days. At the

**Table 3** Changes in the basic organic acid content of kombucha produced with GT and ST at different fermentation times

| Groups | Organic acid contents (g/L) | | | | |
|---|---|---|---|---|---|
| | Glucuronic acid | Gluconic acid | Ascorbic acid | Acetic acid | Succinic acid |
| GT100 | 1.41 ± 0.01a | 44.24 ± 0.01a | 1.72 ± 0.01 a | 12.47 ± 0.01 a | 3.24 ± 0.01 a |
| GT25 + ST75 | 1.28 ± 0.01bc | 41.75 ± 0.01bc | 1.36 ± 0.01bc | 11.39 ± 0.01c | 2.89 ± 0.01bc |
| GT50 + ST50 | 1.37 ± 0.01b | 42.24 ± 0.01b | 1.41 ± 0.01bc | 11.84 ± 0.01b | 2.98 ± 0.01b |
| GT75 + ST25 | 1.39 ± 0.01ab | 43.12 ± 0.01ab | 1.55 ± 0.01b | 12.04 ± 0.01ab | 3.05 ± 0.01ab |
| ST100 | 1.25 ± 0.01c | 38.89 ± 0.01c | 1.32 ± 0.01c | 11.88 ± 0.01b | 2.78 ± 0.01c |

Values expressed with different lowercase letters in the same column indicate statistically significant difference ($p < 0.05$) ($n = 3 \pm$ SD)

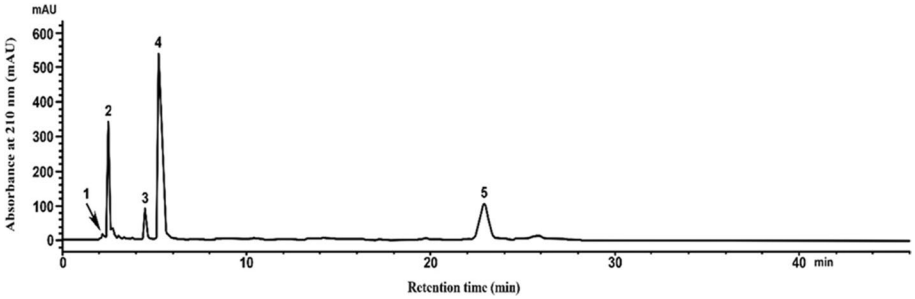


**Fig. 6** HPLC chromatograms at 210 nm of kombucha prepared GT (100%) in 15-day fermentation at 25 °C. Peak (1) glucuronic acid; peak (2) gluconic acid; peak (3) acetic acid; peak (4) ascorbic acid; peak (5) succinic acid, respectively

end of this period, they reported acetic acid concentration as 8 mg/L. Jayabalan, et al. [47] studied the changes in organic acid content of kombucha produced with green tea during fermentation. Their results revealed that the highest amount of acetic acid (9.5 mg/L) in green tea was on day 15 of fermentation. The other highly concentrated organic acid found in the kombucha infusion was glucuronic acid. While this organic acid was at very low rates at the beginning of fermentation, it was calculated at 839.06–1158.2 mg/L levels at the end of fermentation. The bioactive role of glucuronic acid in kombucha is attributed to the detoxification of cells [48]. In another study, Yang et al. [49] infused the symbiotic bacteria gluconacetobacter sp. A4 and 10 different types of lactic acid bacteria from kefir into kombucha. They reported that the highest levels of glucuronic acid (5 mg/L) were in black tea. They also measured that there was no citric acid in the early stages of fermentation, but at concentrations of 39.22–471.58 mg/L as the fermentation process progressed. Malbasa et al. [50] used molasses as a source of organic matter for the kombucha fermentation medium and reported that the presence of L-lactic acid increased during fermentation. The presence of malic (89–276 mg/L) and oxalic acid (8.4–24.8 mg/L) was also determined in kombucha samples at the end of fermentation. Tamer et al. [26] in kombucha culture prepared with herbal teas (linden, lemon balm, sage, echinacea, mint, and cinnamon), D-glucuronic acid (235.623 ± 2.49 mg/L), D-gluconic acid (1045.57 ± 0.76 mg/L), lactic acid (706.183 ± 63.82 mg/L), and acetic acid before fermentation acid (6546.86 ± 81.34 mg/L) were determined. It was reported that after fermentation of these organic acids, D-gluconic acid, lactic acid, and acetic acid levels reached 237.52 ± 2.35 mg/L, 257.08 ± 18.22 mg/L, and 1776.31 ± 3.70 mg/L, respectively. It was determined that D-glucuronic acid increased from 34.22 ± 3.77 to 37.71 ± 1.30 mg/L before and after fermentation, respectively. In Shahbazi et al. [46], although D-glucuronic acid as an organic acid was not initially detected in green tea kombucha fermented at 28 °C for 16 days, it was observed to increase to 839.06 ± 94.13 mg/L after fermentation. It was explained that lactic acid levels increased from 48.35 ± 3.21 to 145.71 ± 17.76 mg/L, and acetic acid levels increased from 944.54 ± 81.12 to 2395.64 ± 189.12 mg/L during fermentation. It is thought that the differences between this organic acid diversity and levels arise from fermentation time, temperature, and substrate sources. The highest D-glucuronic acid (61.92 ± 0.19 mg/L) and lactic acid (915.07 ± 14.61 mg/L) contents were found in linden kombucha and the highest D-gluconic acid (298.74 ± 2.68) and acetic acid (1776.31 ± 3.70) in lemon balm kombucha samples, respectively. Jayabalan et al. [47] reported the initial presence of only D-glucuronic acid as organic acid during fermentation of green and black tea kombucha at

24 °C for 18 days. At the end of fermentation, lactic and acetic acid were also observed in kombucha infusions. Chakravorty et al. [40] reported that the amount of acetic acid, the most characteristic organic acid in kombucha, reached 0.65 ± 0.06 g/L at the beginning and 16.57 ± 0.09 g/L after 21 days of fermentation. They also stated that the gluconic acid content determined after fermentation increased from 0.33 ± 0.03 to 7.36 ± 0.87 g/L. Studies have revealed the difference in organic acid diversity and concentrations in kombucha infusions. It is thought that these differences may be caused by factors such as inoculum source, sugar and tea concentrations, fermentation time, and temperature [31]. It has been announced by different researchers that acetic, glucuronic, gluconic, tartaric, malic, citric, lactic, succinic, and malonic acids have been determined as organic acid content in studies on kombucha to date [40, 41, 47, 50]. Jakubczyk et al. [27] determined a sudden increase in organic acidity in the first 7 days of fermentation in kombucha they prepared with different teas (black, green, white, red). In the analyses performed on the 14th day of fermentation, green tea (9147.40 mg/L), white tea (9132.20 mg/L), black tea (9083.03 mg/L), and red tea (9071.02 mg/L) content from highest to lowest acetic acid content, respectively, were calculated. The pH of the kombucha consortium and the presence of some organic acids in the environment are effective in determining the microorganism growth and therefore the chemical content of the beverage [33]. Low pH and high acidity not only promote the growth of microbes that can colonize this formation, but also provide a protection against the growth of unwanted and harmful microorganisms.

## Microbiological Assessment

The antimicrobial activities on some food pathogens by disc diffusion method in kombucha samples prepared from different concentrations of green tea and stevia leaves are shown in Table 4. In the current study, it was determined that the antimicrobial activity of kombucha tea prepared with GT100 alone was lower than tea prepared with different concentrations of ST (Table 4). It has been observed that the fermentation times have a direct effect on the antimicrobial activities of the prepared kombucha. We can say that kombucha has an inhibitory effect on different bacterial strains from the 5th to the 15th day of fermentation in all groups. When all tea infusions were compared, the highest antimicrobial activities were GT75 + ST25 (22.72 mm) and GT25 + ST75 (22.01 mm) for *Escherichia coli*, GT100 (23.26 mm); GT75 + ST25 (23.32 mm), GT75 + ST25 (21.75 mm), and GT25 + ST75 (21.47 mm) on *Staphylococcus aureus*; GT75 + ST25 (21.70 mm) and GT50 + ST50 (21.28 mm) on *Pseudomonas aeruginosa* GT100 (23.42 mm); and GT75 + ST25 (22.84 mm) for *Listeria monocytogenesis.* The bacterial strains identified as test organisms in the study were chosen because these strains are the most common potential sources of contamination from the environment during kombucha production. Akarca and Tomar [51], total aerobic mesophilic bacteria, and yeast/mold counts and *Lactococcus/Streptococcus* counts of kombucha teas produced with red carrot (*Daucus carota L.*), red beet (*Beta vulgaris L.*), and purple cabbage (*Brassica oleracea L.*) vegetables, species of bacteria decreased, On the other hand, it was determined that the numbers of osmophilic yeast and acetic and lactic acid bacteria increased. They said that this decrease may be due to the increase in the number of acetic and lactic acid bacteria during the fermentation period and the increase in the concentration of organic acids in the environment. Ayed et al. [30] reported that the numbers of osmophilic yeast, acetic acid, and lactic acid bacteria increased in kombucha samples during the fermentation period at different temperatures. The results of this study show parallelism with

**Table 4** Microbiological analyses in different fermentation times of kombucha prepared with GT and ST leaves at different concentrations

| Groups | FT (day) | Microbiological strains (diameter of inhibition zone mm) | | | | |
|---|---|---|---|---|---|---|
| | | *Escherichia coli* | *Salmonella enterica* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Listeria monocytogenes* |
| GT100 | 0 | 10.45 ± 0.68c | 10.49 ± 0.57c | 10.45 ± 0.10c | 10.45 ± 0.28c | 12.18 ± 0.60c |
| | 5 | 10.12 ± 0.28c | 11.41 ± 0.45c | 11.65 ± 0.54c | 10.71 ± 0.10c | 12.08 ± 0.75c |
| | 10 | 16.25 ± 0.25b | 15.22 ± 0.58b | 14.54 ± 0.67b | 15.18 ± 0.26b | 17.05 ± 0.63b |
| | 15 | 21.29 ± 1.39a | 23.26 ± 0.53a | 20.54 ± 0.38a | 21.22 ± 0.74a | 23.42 ± 1.42a |
| GT25+ST75 | 0 | 10.98 ± 0.58c | 10.77 ± 0.17c | 10.08 ± 0.84c | 10.89 ± 0.76c | 11.08 ± 0.22c |
| | 5 | 11.54 ± 0.65c | 10.11 ± 0.85c | 10.38 ± 0.17c | 10.26 ± 0.66c | 11.27 ± 0.38c |
| | 10 | 17.27 ± 0.98b | 16.18 ± 1.06b | 14.65 ± 0.83b | 15.36 ± 0.37b | 16.94 ± 0.42b |
| | 15 | 22.01 ± 1.03a | 22.43 ± 1.27a | 21.47 ± 1.16a | 20.54 ± 0.33a | 22.45 ± 0.37a |
| GT50+ST50 | 0 | 10.78 ± 0.72c | 10.97 ± 0.53c | 10.19 ± 1.23c | 11.26 ± 0.49c | 11.56 ± 0.28c |
| | 5 | 12.01 ± 0.83c | 11.53 ± 0.73c | 10.11 ± 1.42c | 11.33 ± 0.92c | 12.39 ± 0.80c |
| | 10 | 17.63 ± 0.48b | 16.47 ± 0.70b | 15.17 ± 1.22b | 16.26 ± 0.69b | 16.64 ± 0.84b |
| | 15 | 21.31 ± 0.97a | 22.32 ± 0.19a | 20.28 ± 0.98a | 21.28 ± 0.22a | 22.75 ± 1.24a |
| GT75+ST25 | 0 | 11.33 ± 0.88c | 12.90 ± 0.33c | 11.62 ± 0.63c | 11.06 ± 0.37c | 11.44 ± 0.55c |
| | 5 | 12.11 ± 0.29c | 12.75 ± 0.83c | 11.53 ± 0.48c | 11.33 ± 1.02c | 12.39 ± 0.33c |
| | 10 | 16.63 ± 1.54b | 17.27 ± 1.63b | 15.48 ± 0.19b | 15.39 ± 0.86b | 17.48 ± 0.84b |
| | 15 | 22.72 ± 0.74a | 23.32 ± 1.69a | 21.75 ± 0.85a | 21.70 ± 1.33a | 22.84 ± 1.01a |
| ST100 | 0 | 10.28 ± 0.09c | 10.22 ± 0.79c | 10.72 ± 0.83c | 10.52 ± 0.91c | 10.25 ± 0.48c |
| | 5 | 12.29 ± 0.10c | 10.10 ± 0.05c | 11.55 ± 0.44c | 10.53 ± 1.09c | 10.39 ± 0.74c |
| | 10 | 16.32 ± 0.29b | 16.87 ± 0.38b | 15.43 ± 0.39b | 15.20 ± 0.95b | 16.33 ± 0.85b |
| | 15 | 21.43 ± 0.38a | 22.48 ± 0.74a | 19.04 ± 1.63a | 20.19 ± 0.39a | 21.64 ± 1.53a |

Values expressed with different lowercase letters in the same column indicate statistically significant difference ($p < 0.05$) ($n = 3 \pm$ SD)

our research findings. On the 5th day of fermentation, it was observed that the increase in inhibition zone diameters was low in all microorganism strains, especially in GT100. This is thought to be due to the sufficient amount of fermentable carbohydrates in the kombucha consortium and the environmental conditions being favorable for the growth of gram (+) and gram (−) microorganisms. In our current study, it was determined that the inhibition zone diameters increased and the antibacterial activity increased on the 10th and 15th days of fermentation. We can say that this increase is due to the suppression of the growth of bacterial strains determined due to the increase in the acidity of the environment and the number of other fermentative microorganisms. Delik et al. [52], the antibacterial effect of kombucha fermented with endemic and medically important *Origanum bilgeri* on the 0th, 7th and 14th fermentation days was compared. On the 7th day of fermentation, only kombucha with *Origanum bilgeri* added antibacterial effect and inhibition zone was observed in petri dishes inoculated with *K. pneumoniae* and *S. epidermidis*. At the end of 14 days, kombucha with added *Origanum bilgeri* showed antibacterial activity against 5 bacterial strains used in the study, except *E. coli*. Traditional kombucha did not show any effect only against *S. aureus* and *P. aeruginosa* at the end of the 14th day. Similarly, Sözmen et al. [53] and Köse et al. [54] stated that it has a strong antibacterial effect in the studies they carried out using essential oils of *Origanum bilgeri*. Cardoso et al. [55] stated that after 10 days of fermentation, kombucha was effective against *S. aureus* and *L. monocytogenes* at a minimum inhibitory concentration of 250 µL/mL, but had no antibacterial effect against *E. coli* and Salmonella. It is thought that these differences in antibacterial effects of kombucha are primarily caused by antibacterial substances produced in different concentrations and varieties in fermentations as a result of the differences in the bacterial strains used, kombucha cultures, fermentation times, the amount of sugar used, or the fermentation temperatures. The antimicrobial activity of kombucha tea is attributed to the low pH value, especially due to the acetic acid concentration formed in the environment. Acetic acid concentration (1 g/L) can inhibit pathogen and spore-forming bacterial strains [56]. Acetic and other organic acids may affect antimicrobial activity through cytoplasmic acidification and accumulation of dissociated acid anion to toxic levels [11]. Watawana et al. [57] explained the presence of an antibiotic substance with antimicrobial activity in kombucha. In the results of Battikh et al. [39]'s research, antimicrobial activity was determined against *E. coli* ATCC 35218, and the inhibition zone was similar to the 5th day fermentation samples of our study. Velicanski et al. [11] determined the antimicrobial activity of lemon balm kombucha beverage for *E. coli* with an inhibition zone of 11.3 mm. This result revealed that the kombucha tea beverage showed good antimicrobial activity and increased its potential to be used as a stronger antimicrobial agent when prepared with herbal products. Shahbazi, et al. [46] reported that kombucha samples containing cinnamon exhibited higher antimicrobial activity on *E. coli*, *S. typhimurium*, and *S. aureus*. In the study by Neffe-Skocińska et al. [8], fermentation was carried out at 20, 25, and 30 °C for 10 days in the production of kombucha in which green tea, black tea, and sucrose were used as substrates. The highest acetic acid bacteria count (7.61 log CFU/mL) was reached after 10 days of fermentation at 25 °C. The data of these researchers support our study results. In our current study results, there was an increase in organic acid content, especially in acetic acid, after the 10th day. The antibacterial activity results of GT100 prepared as different TA and control samples and kombucha samples at other GT and ST concentrations confirm the previous assumptions that acetic acid is the dominant active ingredient of kombucha [10, 24]. On the 15th day of fermentation, higher inhibition zones were observed in all groups as the acidity of the beverage increased

(Table 4). This proves that the intensity of kombucha's antimicrobial activity is directly related to the concentration of acetic acid. Acetic acid and other organic acids show their effects on antimicrobial activity mainly by two main mechanisms. One of them is cytoplasmic acidification and the other is the accumulation of dissociated acid anion at toxic levels [58]. Studies on the antimicrobial activity of kombucha made from sweetened black tea, green tea, and salted tea have also revealed that acetic acid is the predominant organic acid [10, 24]. The present results prove to us that the type of tea used for SCOBY inoculation is not very important for the antimicrobial activity of kombucha. The bacteriostatic activity of GT100 and GT75 + ST25 kombucha against the three Gram-negative strains (*E. coli*, *S. enterica*, and *P. aeruginosa*) identified for testing was determined by the presence of some other antimicrobial components in the kombucha beverage other than acetic acid, possibly by tannins from the tea or by SCOBY. It can be explained by the presence of some metabolites produced. This activity can be explained by the presence of steviol glycosides, the predominant phenolic component in *Stevia rebaudiana*, because the soluble phenolic agents showing their antimicrobial effects by causing hyperacidification in the plasma membrane of the microorganism, and potentially the structure of $H^+$-ATPase, which is required for ATP synthesis, may be damaged [11].

## Conclusion

Kombucha beverage produced with *Stevia rebaudiana Bertoni* leaves was tried for the first time in this study. In order to determine the usability of stevia leaves as an alternative substrate instead of black and green tea used as a substrate in traditional kombucha production, the physicochemical, antioxidant, and microbiological properties of kombuchas consisting of green tea and stevia at various concentrations were compared. As a result of the analyses made in the prepared kombuchas, the samples obtained on the 10th of fermentation at GT25 + ST75 concentration showed organic acid composition diversity and high antioxidant and antimicrobial activities. The present study supported the hypothesis of defining a functional beverage by preparing kombucha from medicinal plants with tea potential. In addition to the high compatibility of the newly developed functional beverage products in terms of flavor, at the same time, their more advanced bioactivities and health benefits compared to traditional kombucha tea can be considered promising in industrial production. More in vivo research is needed to prove the effects of kombucha prepared with Stevia leaves on human health.

**Author Contribution** The author carried out all the stages of the research himself.

**Availability of Data and Materials** The data used to support the research findings are available from corresponding author.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

**Competing Interests** The author declares no competing interests.

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