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Comparison of Biological Activities and Bioactive Components of Seed, Leaf, and Blossom Parts of *Camellia sinensis* (L.) Kuntze and Commercial Black Tea

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Summary: In this study, bioactive properties and GC-MS analyses of the *Camellia sinensis* (seed, leaf, and blossom parts) and commercial black tea extracts were compared. The total phenolic content (TPC) and antioxidant and antimicrobial activities of extracts were determined. The gas Chromatography-Mass Spectrometry (GC-MS) technique was used to identify the major bioactive compounds in extracts. The TPC and antioxidant activity values of the alcohol extract prepared from the leaf part of the *C. sinensis* are higher than the other extracts. There was a strong correlation between total phenolic content and antioxidant amount among the ethanol extracts of *C. sinensis.* In antimicrobial evaluation, the ethanol extracts showed more activity; the ethanolic extract of *C. sinensis* seeds was the most effective. GC-MS results indicated various organic compounds in the *C. sinensis* extracts, mainly saturated and unsaturated aromatic esters, aromatic alcohols, some cyclic structures, aromatic amine, and boranic esters with different therapeutic activities. The black tea extracts exhibited a more straightforward variety of bio components as aromatic esters and boranic ester. According to the obtained results, *C. sinensis* and black tea extracts would exert several beneficial effects by their biological activities thanks to the possible synergistic effect of chemical contents detected by GC-MS analysis. However, GC-MS results indicated that the black tea sample had much fewer bioconstituents than fresh *C. sinensis* plant samples.

Keywords: Disk Diffusion Test; DPPH; Folin-Ciocalteu Method; FRAP; GC-MS.

Introduction

Tea is produced from an infusion of flavorful leaves, and that has been known for centuries as one of the most widely consumed beverages globally for its desirable aroma, taste, and putative positive physiological functions [1-2]. Plant of tea has two botanical varieties: *Camellia assamica* (L.) and *C. sinensis* (L). There are three different tea types: the fermented black tea, which is the most consumed form in Turkey, the semi-fermented oolong tea, and the non-fermented white, yellow, and green tea, depending on the manufacturing process [3].

Tea production in Turkey is carried out in the North-Eastern Anatolia Region, starting from the west Georgian border until the Ordu province [4]. Due to the ecological conditions, tea harvest, and dry tea production in Turkey last 5-6 months, it is done 9-11 months globally. Rize province, where Turkey's first tea factory is taking first place in Turkey's tea production.

The chemical substance of tea can be variable as well as a flavor because of particular fermentation processing. Ahmed et al. (2019) reported that tea leaves' composition varies depending on climatological, cultural, and genetic factors [5].

Tea contains many chemicals that have positive health effects that arise mainly from the tea's phenolic substances from the heart to the skin also especially; many studies also emphasize oral health and the anti-carcinogenic effects of tea [6-7]. Because of its medicinal properties, tea has been analyzed by phytochemical screening and revealed alkaloids, saponins, tannins, catechins, and polyphenols. With the revealing of the effects of phenolics on health, tea has gained a functional beverage feature. Tea made from the leaves of plant *C. sinensis* has been demonstrated to have favorable properties include antioxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic, and antibacterial activity against many pathogens [8-10].

The sensitivity of bacterial strains to the tea extract is related to differences in cell wall components [11]. The antimicrobial activity of catechins is associated with membrane-dependent cellular processes [12]. Tea polyphenols, which are constituents of tea extracts, have previously been

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reported to have antibacterial activities against phytopathogenic, human and animal disease-related, and foodborne bacteria [13-15].

Total phenolic content and antioxidant and antimicrobial activity values vary depending on the preferred extraction conditions in studies with plants. Farhoosh et al. (2007) found that the extraction method virtually affected extraction yield and antioxidant activity of old tea and black tea leaf extracts [16]. Various drying and extraction methods and fermentation processes also affect the extraction yield, TPC, antioxidant and antimicrobial activity of tea [17-19]. There were studies related to the chemical composition of *C. sinensis* leaves or tea extracts [18-20]. However, as far as we know, the variation of chemical content present in different parts of *C. sinensis* from Turkey has not been reported in the literature.

The present study is designed to check the comparison of antimicrobial, antioxidant activity, and the determination of bioactive components of water and ethanol extracts of *C. sinensis* (L.) and commercial black tea. The fact that extracts of different plant parts prepared in different solvents were tested separates the study from studies in the literature.

Experimental

Chemicals and equipments

All chemicals were analytical grade and purchased from Sigma-Aldrich® (Germany)**,** and deionized water was used for all the performed analyses (MP Minipure dest up, Turkey). All media, blank discs, and antibiotic discs were purchased from Oxoid.

Collection of samples

Seeds, leaves (from young shoots), and blossoms of *C. sinensis* (L.) were collected in May 2012 from the Çamlıhemşin/Rize province of Turkey. To identify these specimens, the pictures and descriptions of the Flora of Turkey book were instructive [21]. The voucher specimen (OMUB 8818 and OMUB 8819) was deposited at the Herbarium of the Department of Biology, Ondokuz Mayıs University, Samsun, Turkey. The plant samples of black tea were obtained from local markets.

Preparation of plant extracts

Fresh tea leaves, blossoms, and seeds were washed under running distilled water. After drying at shade, they were cut into pieces and grinded to a powdery form using pestle and mortar. The powder samples of each part were stored in airtight plastic tubes. Water and ethanol extracts were prepared by mixing 10.0 g powdered plant material with 500 mL of sterile distilled water or ethanol in a round bottom flask on a rotary shaker (Remi rotary shaker RS-12) at room temperature [22-23]. The extracts were kept at 4°C for five days. Both water and ethanol extracts were then filtered through a muslin cloth for coarse residue and filtered through Whatman No.1 filter paper, and then the solvents were removed through an evaporator. The crude extracts were stored at -20°C until used.

Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu procedure using gallic acid as standard [24]. The color intensity that develops in proportion to the phenolic content was recorded spectrophotometrically at 760 nm. The total phenolic content of each extract was calculated as gallic acid equivalent (mg GAE/g sample) using a standard graphic drawn for gallic acid in the concentration range between 0.015 and 0.5 mg/mL (r^2 = 0.9952).

Antioxidant activity assays

DPPH radical scavenging assay

2,2'-diphenyl-1-picrylhydrazyl (DPPH) is used to evaluate a mixture or standard's antioxidant activity. For this purpose, the DPPH solution was mixed with a series of varying quantities of each sample to be tested, and the mixtures were kept in the dark at room temperature for 30 minutes. At the end of the incubation, the resultant color intensity was measured at 517 nm. The rate of radical scavenging was calculated for each different concentration using the equation below. A_{blank} is the absorbance value obtained by performing the same processes with the solvent used to prepare the extract instead of the sample.

$$\text{Scavenging rate (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100$$

After calculating the scavenging rates for each concentration, a graph was drawn between the concentration vs. scavenging ratios. Using this graph, the extract concentration that was sufficient to scavenge 50% of the radicals in the medium was calculated and expressed as SC_{50} (mg/ml) [25].

Ferric reducing antioxidant power (FRAP)

The antioxidant activities of the extracts prepared from *C. sinensis* and commercial black tea were determined by the FRAP assay described by Benzie and Strain (1996) with minor modifications [26]. This

method's principle is based on reducing a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its colored ferrous form (Fe^{2+}-TPTZ) in the presence of antioxidants. The FRAP reagent was freshly prepared to contain 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 300 mM acetate buffer (pH 3.6). The appropriate amount was mixed with a sufficient amount of the sample to be tested, and after the incubation duration, increases in absorbance were monitored at 593 nm. A standard calibration graph was created using $FeSO_4.7H_2O$, and FRAP values of samples were presented as µmol $FeSO_4$/g sample.

Antimicrobial assay

Antibacterial and antifungal activities were measured using disk diffusion methods on agar plates [27]. We selected the tested bacteria from Gram-positive and Gram-negative ones that CLSI recommended. Four Gram-positive bacteria (*Bacillus cereus* ATCC®10876^T, *Clostridium perfringens* ATCC®313124^T, *Listeria monocytogenes* ATCC®7677^T, *Staphylococcus aureus* ATCC®25923^T) and six Gram-negative bacteria (*Escherichia coli* ATCC®25922^T, *Pseudomonas aeruginosa* ATCC®27853^T, *Salmonella enterica* subsp. *enterica* serovar Typhi ATCC®14028^T, *Klebsiella pneumoniae* ATCC®13883^T, *Shigella sonnei* ATCC®25931^T, *Yersinia enterocolitica* ATCC®27729^T) and two fungal type strains (*Aspergillus niger* ATCC®9642^T, *Candida albicans* ATCC®10231^T) were obtained from ATCC (American Type Culture Collection). Ampicillin (AM10), Cefazolin (KZ30), Nystatin (NY100), and 70% ethanol were used as control groups. Test procedures were realized by CLSI standard procedures [28-29]. Fresh bacterial and fungal cultures were prepared, and then bacterial and fungal density was adjusted by a densitometer (Biosan, Latvia). 100 µL 0.5 McFarland bacteria and 1 McFarland fungi were spread by sterile swap at least three different ways. In 30 minutes, sterile 6 mm blank discs were placed on sterile 20 mL Mueller-Hinton Agar plates and loaded 50 µL extract solution. Inhibition zones were determined after appropriate incubation temperature (37°C for bacterial strains and 30°C for fungal strains) and time (24 hours for bacteria 48 hours for fungi). All tests were performed in triplicate.

GC-MS analysis

GC-MS analysis of the *C. sinensis* seed, leaf, and blossom extracts (for water and ethanol) were carried out using GC-MS (Hewlett Packard 5890 Series II GC Plus-Hewlett Packard 5971 Series MS) equipped with a column (Innowax 19091N-136, 60 m×0.250 mm i.d.; film thickness 0.25 µm). GC-MS program was applied as follows: The initial oven temperature was 70 °C and then increased to 240 °C by raising 5 °C per min. The carrier gas was helium (flow rate: 0.77 ml/min). For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was operated, and the detector temperature was 280 °C. The extracts were injected into GC-MS in the splitless mode by resolving the extracts in absolute ethanol.

Statistical analyses

SPSS software for Windows version 18 (Chicago, IL, USA, 2009) was used for statistical evaluation [30]. Results were represented as Average±SE. Categorical variables not fitting a normal distribution were compared using non-parametric tests, i.e., the Kruskal–Wallis test for multiple variables and the Mann–Whitney *U*-test for single variables, coefficient; $p<0.05$ was considered significant. The Spearman rank correlations are also presented.

Results and Discussion

Total Phenolic Contents and Antioxidant Activities of Tea Samples

Phenolic compounds are ubiquitous microconstituents of plants and considerable phytochemicals of plant extracts. They are an integral part of the human diet; being present in plant foods, including beverages, are the primary sources of antioxidant capacity [31].

Depending on the differences in tea processing, the number of bioactive components in their composition may differ [10]. Therefore, the investigated samples' total phenolic contents (TPC) were determined by the Folin–Ciocalteu (F–C) method. The order of TPC values was as follows: *C. sinensis* leaf (E=Ethanol)> *C. sinensis* seed (W=Water)> *C. sinensis* leaf (W)> commercial black tea (W)> *C. sinensis* blossom (W) *C. sinensis* seed (E)> *C. sinensis* blossom (E)> commercial black tea (E). In particular, *C. sinensis* leaf exhibited approximately 15 times higher content of phenolic compounds than black tea (W). TPC values are generally significantly higher in water extracts, except for the ethanol extract of the leaf part (Table-1). In the case of TPC, there was a significant difference between the values obtained for water and ethanol extracts (Mann-Whitney $U=36.000$; $Z=-2.078$ $p=0.038$), while this difference is not seen in the FRAP and DPPH results ($U_{FRAP}=54.000$; $Z=-1.040$ $p=0.298$; $U_{DPPH}=45.000$; $Z=-1.560$ $p=0.119$). In addition, negative correlations were calculated between the TPC values, depending on the solvent (($rS=-0.433$, $p=0.05$) and plant part ($rS=-0.485$, $p=0.05$) differences.

Table-1: Total phenolic content and antioxidant activity in various *C. Sinensis* (L.) parts and commercial black tea extracts.

Sample	Solvent	TPC (mgGAE/g)	FRAP (µmolFeSO$_4$.7H$_2$O/g)	DPPH-SC$_{50}$ (mg/mL)
***C. sinensis* seed**	**water**	**4.453±0.006**	**4.856±0.004**	**1.754±0.147**
	ethanol	**0.598±0.001**	**1.62±0.002**	**2.644±0.145**
***C. sinensis* leaf**	**water**	**3.977±0.002**	**0.450±0.012**	**3.522±0.006**
	ethanol	**15.600±0.001**	**6.125±0.011**	**1.158±0.141**
***C. sinensis* blossom**	**water**	**0.736±0.014**	**1.112±0.010**	**1.983±0.266**
	ethanol	**0,140±0,009**	**0.802±0.009**	**2.446±0.102**
Commercial black tea	**water**	**1.186±0.017**	**6.312±0.003**	**0.925±0.235**
	ethanol	**0.110±0.021**	**0.155 ±0.003**	**19.318±0.008**

Values are means ± SE (n: 3)

Furthermore, as is known, phenolics are antioxidants that can show their effects in different ways. For this reason, the antioxidant activity of the samples was also evaluated. Antioxidant activity was measured according to two different methods, and there is a fairly high correlation between the results obtained by these two methods. However, when all tested extracts were taken into account, no regular relationship was found between the calculated total phenolic acid values and the mentioned antioxidant activity values. As a result, we can say that each phenolic does not contribute to the antioxidant effect, or there are no phenolics suitable for the test methods' mechanism.

Additionally, the correlation between TPC and DPPH scavenging activity values was analyzed by non-parametric correlation analysis. Ethanol extracts displayed the strongest DPPH-scavenging activities and the most precious total phenolic content among all tested extracts. In the case of ethanol extracts of *C. sinensis*, a strong negative correlation (r_S=-0.763, p=0.001) was observed between TPC and DPPH, whereas such a correlation was observed between water extracts. Similar strong negative correlations were reported by Fidrianny et al. (2015-2018) [32-33].

The correlation between TPC and FRAP was also analyzed by non-parametric correlation analysis. Ethanol extracts displayed the strongest antioxidant activity and the most precious total phenolic content among all tested extracts. There was a powerful positive correlation between TPC and FRAP among the ethanol extracts of *C. sinensis* (r_S=0.949, p=0.001) but was not the same among the water extracts. Similar strong positive correlations were reported by Gan et al. (2017) and Casagrande et al. (2018) between the level of phenolic compounds and FRAP values [34-35]. The value of the Spearman test is 0.00, which shows that the correlation between FRAP and DPPH is significant and worth assessing. Value of Spearman correlation is –0.919 (r_S= -0.919, p<0.01). The negative mark shows that the correlation is reciprocal between the FRAP and DPPH. The value 0.919 shows that the correlation's strength is very strong because it approaches the value of one. Tlili and Sarıkürkçü (2020) also examined the bioactive components and antioxidant effects of water extracts of 5 different medicinal plants purchased from a local shop selling medicinal and aromatic herbs in Isparta Province [36]. One of the most interesting results of this study reported in the literature is that the *C. sinensis* species contains a significantly higher amount of gallic acid among these five plants. Therefore, a high TPC value as gallic acid equivalent may be an expected result. Yaylacı Karahalil and Can (2019) concentrated exclusively on the *C. sinensis* blossom and detected significant antioxidant and antimicrobial activity based on the intense presence of gallic acid and catechin [18].

Health benefits of tea such as antioxidant, anti-inflammatory, immunostimulating, antitumor, hypoglycemic, obesity, and anti-allergic activities are attributed to its chemical composition of catechins, polysaccharides, proteins, amino acids, and saponins. The potential use of tea flowers in food and medicine is guaranteed, as its aqueous extracts have also been demonstrated to be safe for animals. The composition and concentration of metabolites, known as the source of the beneficial properties of tea, vary according to the varieties and different development periods of the flora. It has been shown that the antioxidant activity of tea flowers increases from the moment of budding and reaches a maximum at the 3rd stage when the petals begin to split and reach a minimum when they are in full bloom [37]. In addition, it is a well-known fact that different parts of all herbal sources may differ in terms of component content, and the extraction of these components may be at different degrees under different conditions. Therefore, in this study, the antioxidant activity of the extracts of different parts of the tea plant prepared with two different solvents and the total phenolic content, which can be considered as the primary source of this activity, was tried to be determined. Thus, unlike many studies in the literature, it aims to bring innovation to the literature. Luo et al. (2020) studied the feasibility of improving the extraction yield of green tea antioxidant polyphenols with a combination of ultrasound-assisted extraction and deep eutectic solvents [38]. Since it is the most preferred beverage, a new one is added to the studies on the biological effects of tea every day. One of them is related to pu-erh tea. It has been reported that the plant *Camellia sinensis* var. *assamica* obtained from the Yunnan province of China has antioxidant effects as well as cytotoxic, antimicrobial, and antihemolytic activities. On the other hand, it has been demonstrated that it has inhibitory properties on α amylase and glucosidase [39].

Table-2: Inhibition Zone Diameter (IZD) of water and ethanol extracts of *C. sinensis* (L.) parts and commercial black tea. The results are expressed as Mean±SE of three determinations.

Extracts and control groups	Solvents	*Escherichia coli* ATCC®25922^T	*Pseudomonas aeruginosa* ATCC®27853^T	*Salmonella enterica* subsp. *enterica* serovar Typhi ATCC®14028^T	*Klebsiella pneumoniae* ATCC®13883^T	*Shigella sonnei* ATCC®25931^T	*Yersinia enterocolitica* ATCC®27729^T	*Bacillus cereus* ATCC®10876^T	*Clostridium perfringens* ATCC®313124^T	*Listeria monocytogenes* ATCC®7677^T	*Staphylococcus aureus* ATCC®25923^T	*Aspergillus niger* ATCC®9642^T	*Candida albicans* ATCC®10231^T
C. sinensis seed	water	7.33±0.57	6.00±0.00	6.00±0.00	6.33±0.46	6.23±0.38	7.33±1.26	12.15±1.82	9.56±1.27	6.33± 0.57	7.33±0.57	6.00±0.00	16.26±1.82
	ethanol	13.33±0.57	14.66±2.12	15.33±0.67	17.02±0.57	14.96±0.75	16.26±1.53	14.46±0.57	6.66±0.57	16.05±1.26	15.16±0.57	13.36±2.46	10.00±0.20
C. sinensis	water	10.33±1.02	13.33±2.46	6.33±0.57	6.33±0,47	6.33 ±0.27	7.00±1.02	8.23±0.57	9.08±1.02	9.06±1.82	6.33±0.31	9.18±0.57	6.33±0.57
leaves	ethanol	11.33±0.57	12.00±1.00	6.66±0.57	6.00±0.00	14.36±0.33	12.16±0.57	13.13±2.46	10.33±1.04	12.46±1.24	10.23±0.57	12.71±0.57	17.00±0.00
C. sinensis	water	6.00±0.00	7.66±0.57	6.00±0.00	6.66±0.57	6.66±0.57	6.66±0.57	8.00±0.00	9.33±0.57	10.28±0.57	7.14±1.26	9.56±1.02	11.56±0.57
blossom	ethanol	12.66 ±2.31	16.33±0.57	12.66±0.57	15.33±0.57	14.33±2.82	11.33±0.57	15.11±2.12	6.33±1.26	11.04±2.12	8.36±0.57	16.13±1.14	8.34±0.57
Commercial	water	8.00±1.00	13.66±0.57	11.66±1.26	6.33±0.34	7.33±1.82	6.33±0.57	9.33±1.02	6.33±0.33	10.66±0.57	16.61±1.33	8.00±0.00	6.08±1.26
black tea	ethanol	12.00±1.00	13.66±0.57	11.66±0.57	7.33±0.57	10.33±0.57	6.00±0.00	9.24±0.57	12.33±1.02	10.52±1.02	17.00±1.00	8.03±0.57	12.03±0.57
AM10	water	40.67± 1.15	30.66±1.15	35.33±1.15	40.33±0.57	44.33±2.12	26.66±0.57	26.00±1.00	44.66± 2.36	29.66±0.52	26.33±1.52	6.00±0.00	34.00 ±1.00
KZ30	water	47.66±0.46	29.33 ±0.87	43.66±2.53	44.33± 1.52	43.66±0.57	34.33 ±0.57	14.66±0.85	43.33 ± 3.12	30.66± 1.15	23.66± 0.57	6.00±0.00	35.33 ± 1.15
NY100	water	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	15.66±0.57	15.33±0.57
70% ethanol		6.00±0.00	6.00±0.00	6.00 ±0.00	6.00±0.00	6.00±0.00	6.00 ±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Antimicrobial assay

The extract samples were tested for antimicrobial activity against a total of twelve pathogenic microorganisms, including Gram-positive and Gram-negative bacteria and fungal strains. The standard antibiotic disk of AM10, KZ30, and NY100 was used for comparison purposes. The eight different extracts of tea showed moderate antibacterial and antifungal activity against some of the test organisms. The data obtained from the antimicrobial activity assay measured in terms of the inhibition zone diameter in mm are shown in Table-2. The extracts and antimicrobial agents used in this study were statistically tested with the Kruskal-Wallis test. The Kruskal-Wallis test results indicated statistically significant differences in at least two tested extracts and AM10, KZ30, and NY100 ($p<0.005$). The synthetic antibiotics exhibited the highest antimicrobial activity (AM10, KZ30, and NY100) used as a positive control.

Based on SPSS analysis, there was a significant difference ($p<0.05$) between *K. pneumoniae* and *P. aeroginosa* inhibition zone diameters (*Mann Whitney-U*=165.000;z=-2.606*; p=0.009*) and *C. sinensis* seed (E) and *C. sinensis* leaf (W) extracts were significantly differed from other extracts (x^2(10, n= 396)=202.564; ($p<0.05$)). On the other hand, there was not a significant difference between inhibition zone diameters and organism type (Gram-positive bacteria, Gram-negative bacteria, and fungi) (x^2(2, n= 288)=0.957; *p=0.619*).

From Table-2, it is observed that the seed ethanol extract of *C. sinensis* showed moderate antimicrobial activity against some test organisms, namely *E. coli, P. aeruginosa, S. enterica* subsp. *enterica* serovar Typhi, *K. pneumoniae*, *S. sonnei, Y. enterocolitica, B. cereus*, *L. monocytogenes, S. aureus*, and *C. albicans*. However, it did not show activity against *C. perfringens*. Vice versa, The *C. sinensis* seed water extract showed antifungal activity against *C. albicans*. While the ethanolic extract of *C. sinensis* fresh leaves showed antimicrobial activity against *C. albicans* and *S. sonnei*, the water extract of *C. sinensis* fresh leaves did not. Also, the ethanolic extract of *C. sinensis* blossom showed antibacterial activity against *P. aeruginosa, K. pneumoniae*, and *B. cereus*, and antifungal activity to *A. niger*. We could say the water extract of *C. sinensis* blossoms did not show any antimicrobial activity to the test organisms (inhibition zone diameters are almost 10 mm and below). The Black tea ethanolic extract showed moderate antimicrobial activity against *E. coli*, *P. aeruginosa, S. enterica* subsp. *enterica* serovar Typhi, *C. perfringens, S. aureus*, and *C. albicans.* Based on Kruskal-Wallis analysis, there was a significant difference ($p<0.05$) between inhibition zone diameters and extract solvent type (*U*=0.000; *p*=0.000; *z*=-4.157; *r*=-0.848).

It can be seen from the results in Table-2 that almost all bacteria showed more sensitivity to commercial antibiotics than the extracts in this study. However, it was not the same for fungi and yeast. Water extract of *C. sinensis* seed and ethanolic extract of *C. sinensis* leaves showed more inhibition effect to *C. albicans*. The bigger inhibition zone diameter was recorded for ethanolic extract of *C. sinensis* blossoms. Here we show that different parts and different solvents of *C. sinensis* have a different antimicrobial effects.

Ulusoy et al. (2010) found that antimicrobial activity was not linearly correlated with total phenolic compounds or antioxidant activity [40]. However, there was a statistical relationship between TPC and antimicrobial activity and antioxidant activity in the present study, but no linear correlation with antimicrobial and antioxidant activity ($p>0.05$). The value of significance of the Spearman test is 0.031, which shows that the correlation between the TPC and antimicrobial activity is moderately significant. Value of Spearman correlation is -0.442 (r_S= -0.442, $p<0.05$). The negative mark shows that the correlation is reciprocal between the TPC and antimicrobial activity. Disk diffusion values obtained from Gram-positive bacteria and fungi have not shown linear correlations with TPC, while disk diffusion values for Gram-negative bacteria were moderate ($0.581 \leq r \leq 0.659, P < 0.05$). Todorovic et al. (2017) reported no linear correlation between TPC and Gram-positive bacteria but a strong linear correlation between TPC and Gram-negative bacteria [41]. Last decade several researchers focused on the effects of tea on gut microbiota [42-44] To the reports, polyphenols from *C. sinensis* may inhibit pathogenic bacteria and stimulate probiotic bacteria [45]. So, it is expected that different parts of *C. sinensis*, which have polyphenols in different quantities, must have a different antibacterial effect.

GC-MS analysis

Plants are rich in secondary metabolites with different bioactive properties, and these metabolites have a variety of structural rearrangements. GC-MS technique provides the analysis of esters, carboxylic acids, aldehydes, ketones, alcohols, terpenes, etc., and is a valuable tool for analyzing bioactive natural compounds of plants [46]. In this study, three different parts of *C. sinensis* (L.) from Rize city, Çamlıhemşin province of Turkey, and commercial black tea were screened for their phytoconstituents using the GC-MS method. Water and ethanol extracts of *C. sinensis* (L.) seed, leaf, and blossom parts were used for the analysis.

Table-3: GC-MS analysis results for the *C. sinensis* (L.) seed extract.

C. sinensis seed extract							
Extracts/ Peaks[1]	**t_R[2]**	**Area[3] (%)**	**Compound class**	**Extracts/ Peaks**	**t_R**	**Area (%)**	**Compound class**
Water extract				***Ethanol extract***			
1	**32.08**	**39.61**	**Aromatic ester**	**1**	**32.16**	**27.45**	**Aromatic ester**
2	**30.56**	**20.32**	**″**	**2**	**32.15**	**9.17**	**″**
3	**28.40**	**18.95**	**″**	**3**	**31.94**	**3.31**	**″**
4	**32.30**	**7.01**	**″**	**4**	**31.59**	**2.15**	**Ester**
5	**33.07**	**0.84**	**Boranic ester**	**5**	**44.98**	**5.80**	**Aromatic unsaturated ester**
6	**19.14**	**0.79**	**Aromatic ester**	**6**	**28.48**	**21.16**	**Aromatic ester**
7	**38.29**	**0.78**	**″**	**7**	**30.83**	**2.10**	**Cyclic alcohol**
8	**33.97**	**0.57**	**″**	**8**	**31.76**	**1.66**	**Aromatic ester**
9	**14.17**	**0.56**	**Ester**	**9**	**31.00**	**1.19**	**″**
10	**20.89**	**0.54**	**″**	**10**	**31.45**	**1.13**	**″**
11	**32.85**	**0.50**	**Aromatic/ aliphatic ether**	**11**	**32.98**	**1.11**	**Unsaturated cyclic substituted ester**
				12	**30.62**	**1.06**	**Unsaturated cyclic substituted ester**
				13	**31.26**	**1.03**	**Cyclic borane**
				14	**31.64**	**0.89**	**Aromatic amine**
				15	**31.34**	**0.85**	**″**
				16	**30.39**	**0.82**	**Aromatic ester**
				17	**33.25**	**0.77**	**Aromatic substituted alkane**
				18	**32.82**	**0.76**	**Aromatic amine**
				19	**34.00**	**0.76**	**Alcohol**
				20	**33.49**	**0.74**	**Aromatic ester**
				21	**33.18**	**0.72**	**Aromatic amine**
				22	**31.14**	**0.69**	**Aromatic ester**
				23	**30.33**	**0.69**	**Cyclic compound**
				24	**31.06**	**0.66**	**Aromatic ester**
				25	**33.38**	**0.66**	**″**
				26	**38.36**	**0.66**	**″**
				27	**47.25**	**0.62**	**″**
				28	**43.71**	**0.58**	**″**
				29	**33.08**	**0.55**	**Aromatic amine**
				30	**32.90**	**0.54**	**″**
				31	**31.18**	**0.51**	**″**

Table-4: GC-MS analysis results for the *C. sinensis* (L.) leaf extract.

C. sinensis leaf extract							
Extracts/ Peaks[1]	**t_R[2]**	**Area[3] (%)**	**Compound class**	**Extracts/ Peaks**	**t_R**	**Area (%)**	**Compound class**
Water extract				***Ethanol extract***			
1	**32.12**	**35.03**	**Aromatic ester**	**1**	**32.13**	**49.92**	**Aromatic ester**
2	**28.46**	**18.84**	**″**	**2**	**28.46**	**24.0**	**″**
3	**32.33**	**10.89**	**″**	**3**	**32.33**	**17.82**	**″**
4	**31.48**	**7.23**	**″**	**4**	**32.90**	**2.46**	**″**
5	**20.07**	**4.79**	**Aromatic alcohol**	**5**	**32.83**	**1.09**	**″**
6	**32.66**	**3.35**	**Aromatic ester**	**6**	**33.14**	**0.94**	**Boranic ester**
7	**32.99**	**1.97**	**Aromatic unsaturated ester**	**7**	**38.36**	**0.63**	**Aromatic ester**
8	**33.13**	**1.52**	**Cyclic diketone**				
9	**33.65**	**0.92**	**Aromatic ester**				
10	**34.01**	**0.83**	**Aromatic alcohol**				
11	**33.36**	**0.82**	**Cyclic compound**				
12	**31.90**	**0.80**	**Aromatic ester**				
13	**32.85**	**0.78**	**″**				
14	**33.46**	**0.70**	**″**				
15	**33.54**	**0.64**	**″**				
16	**34.30**	**0.61**	**″**				
17	**38.05**	**0.52**	**Aromatic ester**				
18	**31.76**	**0.50**	**Unsaturated ester**				

Table-5: GC-MS analysis results for the *C. sinensis* (L.) blossom extract.

***C. sinensis* blossom extract**							
Extracts/ Peaks[1]	**t_R[2]**	**Area[3] (%)**	**Compound class**	**Extracts/ Peaks**	**t_R**	**Area (%)**	**Compound class**
Water extract				***Ethanol extract***			
1	**32.15**	**40.15**	**Aromatic ester**	**1**	**32.96**	**60.71**	**Aromatic ester**
2	**28.48**	**18.17**	**Alkane**	**2**	**32.76**	**15.81**	**″**
3	**32.35**	**10.79**	**Aromatic ester**	**3**	**28.49**	**15.58**	**″**
4	**11.97**	**3.50**	**Unsaturated ester**	**4**	**32.82**	**1.42**	**″**
5	**28.77**	**2.24**	**Aromatic unsaturated ester**	**5**	**32.90**	**1.34**	**″**
6	**32.96**	**2.05**	**Aromatic ester**	**6**	**33.15**	**1.04**	**Boranic ester**
7	**12.66**	**1.52**	**Aldehyde**	**7**	**33.05**	**0.99**	**Aromatic ester**
8	**32.83**	**1.35**	**Aromatic ester**	**8**	**38.37**	**0.52**	**″**
9	**33.50**	**1.10**	**Cyclic compound**				
10	**10.35**	**0.95**	**Unsaturated ester**				
11	**33.12**	**0.95**	**″**				
12	**33.26**	**0.84**	**Aromatic substituted alkane**				
13	**40.91**	**0.77**	**Ester**				
14	**33.19**	**0.69**	**Aromatic ester**				
15	**29.99**	**0.67**	**Aromatic alcohol**				
16	**33.50**	**0.66**	**Alcohol**				
17	**38.37**	**0.60**	**Aromatic ester**				
18	**33.36**	**0.55**	**Alkane**				
19	**33.60**	**0.54**	**″**				
20	**34.37**	**0.52**	**Cyclic compound**				
21	**34.49**	**0.50**	**Alkene**				

Table-6: GC-MS analysis results for the commercial black tea extract.

Commercial black tea extract							
Extracts/ Peaks[1]	**t_R[2]**	**Area[3] (%)**	**Compound class**	**Extracts/ Peaks**	**t_R**	**Area (%)**	**Compound class**
Water extract				***Ethanol extract***			
1	**31.81**	**17.44**	**Aromatic ester**	**1**	**32.09**	**61.39**	**Aromatic ester**
2	**32.14**	**13.89**	**″**	**2**	**28.42**	**17.26**	**″**
3	**28.49**	**10.24**	**″**	**3**	**32.30**	**13.12**	**″**
4	**32.36**	**6.85**	**″**	**4**	**32.72**	**2.62**	**″**
5	**25.29**	**3.28**	**″**	**5**	**32.94**	**1.62**	**″**
6	**33.15**	**1.63**	**Boranic ester**	**6**	**33.07**	**0.81**	**Aromatic ketone**
7	**27.62**	**1.61**	**Aromatic ester**				
8	**30.64**	**1.54**	**″**				
9	**31.53**	**1.48**	**″**				
10	**30.81**	**1.30**	**″**				
11	**30.21**	**1.20**	**″**				
12	**31.44**	**1.17**	**″**				
13	**31.26**	**1.10**	**″**				
14	**31.09**	**0.98**	**″**				
15	**31.36**	**0.98**	**″**				
16	**30.90**	**0.92**	**″**				
17	**30.99**	**0.86**	**″**				
18	**30.38**	**0.85**	**″**				
19	**31.17**	**0.78**	**″**				
20	**32.98**	**0.76**	**″**				
21	**30.43**	**0.67**	**″**				

[1]Samples: *Camellia sinensis* (L.); Commercial black tea, [2]t_R: Retention time (in minutes); [3]The peaks amounting to at least 0,5 % of the total compounds were taken into account

Table 3-6 shows the results of GC-MS analysis (Figure S1-S8). The compounds were determined by comparing their molecular weights and molecular fragmentations with spectra from the libraries of Wiley and Aromsa. The relative amount (%) of each component expresses a comparison of its average peak area to the total areas. The results indicated that *C. sinensis* (L.) had 11; 31 (seed), 18; 7 (leaf), and 21; 8 (blossom), and the commercial black tea had 21; 6 different phytoconstituents in water and methanol extracts, respectively. The peaks amounting to at least 0.5 % of the total compounds were taken into account. According to the peak areas of the samples' total ion chromatograms, the first five compounds are aromatic esters for tea seed water extract, aromatic esters and aromatic unsaturated ester for tea seed ethanol extract, aromatic esters and aromatic alcohol for tea leaf water extract, aromatic esters for tea leaf alcohol extract, aromatic esters, alkane, unsaturated ester and aromatic unsaturated ester for tea blossom water extract, aromatic esters for tea blossom alcohol extract, and aromatic esters for commercial black tea water and ethanol extracts.

The results indicated more phytocomponents in water extracts in the form in which the tea is

consumed than ethanol extracts except for *C. sinensis* (L.) seeds extract. Organic structures detected in the *C. sinensis* (L.) samples are esters, unsaturated esters, aromatic esters, aromatic unsaturated esters, alcohol, aromatic alcohols, cyclic alcohols, cyclic diketones, unsaturated cyclic substituted esters, aromatic amine, alkane, aromatic substituted alkane, aldehydes, alkene, aromatic, aliphatic ether, and boranic ester. In contrast, in the black tea extracts, only aromatic esters and boranic ester were detected. The most common compounds of *C. sinensis* (L.) and the commercial black tea extracts are 1,2-benzenedicarboxylic acid, dimethyl ester (**1**) as an aromatic ester, and the most noticeable compounds are boranic ester as lactic acid n-butyl boronate (**2**) and cyclic compounds such as 1,3,5,7-tetramethyladamantane (**3**) and 1,3-cyclohexanedione (**4**) (Fig. 1). The least common structures in the *C. sinensis* (L.) extracts are aromatic amine, aromatic, aliphatic ether, and alkane. The commercial black tea water and ethanol extracts had only two different organic compound classes: aromatic esters and boranic ester. These results indicated that unprocessed fresh tea *C. sinensis* (L.) contains many more phytocomponents than processed tea, such as commercial black tea.

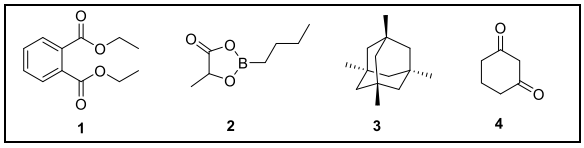
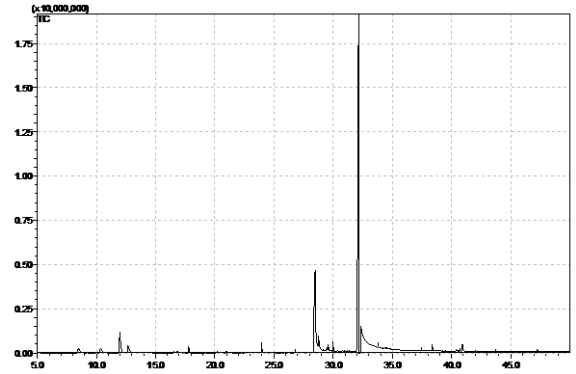
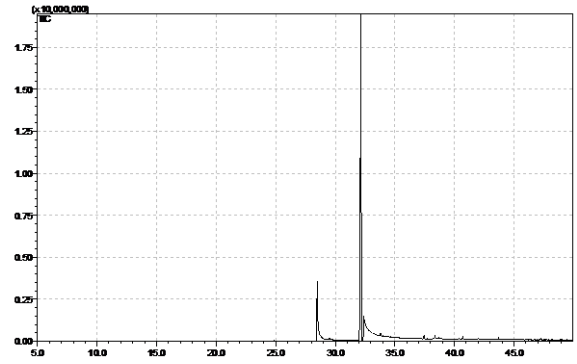


Fig. 1: Some featured organic structures in *C. sinensis* (L.) and commercial black tea extracts. **1**: 1,2-Benzenedicarboxylic acid, dimethyl ester; **2**: Lactic acid n-butyl boronate; **3**: 1,3,5,7-Tetramethyladamantane; **4**: 1,3-Cyclohexanedione

Boranic ester (boronate) was determined as one of the biocomponents of the *C. sinensis* (L.) and commercial black tea. Boron in the form of boric acid or borates is an essential element for the plant's growth. Some evidence indicates the importance of boron on humans and animals because it is probably an essential micronutrient [47, 48]. As representative examples, total ion chromatograms of green tea blossom water and ethanol extracts can be seen below (Fig. 2).



(a)



(b)

Fig. 2: Total ion chromatograms of the *C.sinensis* (l.) blossom's water (**a**) and ethanol extracts (**b**) [x: Retention time (min); y: signal intensity

Conclusion

As a conclusion of this study, it can be said that the extracts prepared with different solvents of different parts of the *C. sinensis* and black tea sample (Rize province Çamlıhemşin region) differ. The total phenolic content and antioxidant activity value of the alcohol extract prepared from the leaf part of the *C. sinensis* sample is higher than the other extracts tested. To our observations, we can say *C. sinensis* seed, leaf, and blossom possess moderate antimicrobial activity against several bacteria and fungi strains. In antimicrobial evaluation, the ethanol extracts showed more antibacterial and antifungal activity; the ethanolic extract of *C. sinensis* seeds was the most effective. The GC-MS analysis results showed different peaks determining esters as the principal compound and aromatic, aliphatic ether as the least common compound. The results could be concluded that *C. sinensis* (L.) seed, leaf, and blossom parts extracts have various bioactive compounds. Among

them, *C. sinensis* (L.) seeds ethanol extract had the most phytoconstituents, while *C. sinensis* (L.) leaves extract had the least number of phytoconstituents. Also, the GC-MS study results revealed that all parts of *C. sinensis* (L.) have more bio components with different organic functionality than commercial black tea. Therefore, it could be stated that the unprocessed fresh tea plant has a more nutritious content. Therefore, this research has once again demonstrated that *C. sinensis* (L.) has pharmaceutical importance. The natural floral odor and lovely color of the tea blossom extracts have shown that the fresh tea plant is a natural material that can be used in the cosmetic industry.

References

1. P. Muniandy, A. B. Shori, A. S. Baba, Influence of green, white, and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage, *Food Packag. Shelf Life*, **8**, 1 (2016).
2. F. Hajiaghaalipour, J. Sanusi, M. S. Kanthimathi, Temperature and time of steeping affect the antioxidant properties of white, green, and black tea infusions, *J. Food Sci.*, **81,** 246 (2016).
3. G. Chen, Q. Yuan, M. Saeeduddin, S. Ou, X. Zeng, H. Ye, Recent advances in tea polysaccharides: Extraction, purification, physicochemical characterization and bioactivities, *Carbohydr. Polym.*, **153**, 663 (2016).
4. F. Seyis, E. Yurteri, A. Ozcan, Y. Savsatli, Organic tea production and tea breeding in Turkey: challenges and possibilities, *Ekin J.*, **4**, 60 (2018).
5. S. Ahmed, T. Griffin, D. Kraner, K. Schaffner, D. Sharma, M. Hazel, A. L. Leitch, C. M. Orians, W. Han, J. R. Stepp, A. Robbat, C. Matyas, C. Long, D. Xue, R. F. Houser, S. B. Cash, Environmental Factors Variably Impact Tea Secondary Metabolites in the Context of Climate Change: A Systematic Review, *Front. Plant. Sci.*, **10**, 939 (2019).
6. E. Polat, A. Karaman, S. Öğüt, The effect of tea on cardiovascular diseases, *ADÜ Ziraat Derg.*, **17,** 131 (2020).
7. A. Y. Balappanavar, *In Tea Chemistry and pharmacology, Tea and Oral Health. In Tea-Chemistry and Pharmacology*, IntechOpen, London, p. 1 (2020).
8. L. Armstrong, M. A. Vieira do Carmo, Y. Wu, L. A. Esmerino, L. Azevedo, L. Zhang, D. Granato, Optimizing the extraction of bioactive compounds from puerhtea (*Camellia sinensis* var. assamica) and evaluation of antioxidant, cytotoxic, antimicrobial, antihemolytic, and inhibition of α-amylase and α-glucosidase activities, *Food Res. Int.*, **137**, 1 (2020).
9. P. Goswami, C. Kalita, A. C. Bhuyan, Antibacterial Activity of Black Tea Extract against *S. mutans, S. aureus, L. acidophilus, Klebsiella* and *E. coli*, *J. Evol. Med. Dent. Sci.*, **9**, 18 (2020).
10. C. Elmas, C. Gezer, Composition and Health Effect of Tea Plant (*Camellia sinensis*), *Akademik Gıda*, **17**, 417 (2019).
11. L. Bouarab-Chibane, V. Forquet, P. Lantéri, Y. Clément, L. Léonard-Akkari, N. Oulahal, P. Degraeve, C. Bordes, Antibacterial properties of polyphenols: characterization and QSAR (Quantitative structure–activity relationship) models, *Front. Microbiol.*, **10,** 829 (2019).
12. I. Górniak, R. Bartoszewski, J. Króliczewski, Comprehensive review of antimicrobial activities of plant flavonoids, *Phytochem. Rev.*, **18**, 241 (2019).
13. Z. Chen, X. Lv, M. Zhao, P. Zhang, X. Ren, X. Mei, Encapsulation of green tea polyphenol by pH responsive, antibacterial, alginate microgels used for minimally invasive treatment of bone infection, *Colloids Surf. B.*, **170**, 648 (2018).
14. N. Zhang, W. Liu, K. Qian, In vitro antibacterial effect of tea polyphenols combined with common antibiotics on multidrug-resistant *Klebsiella pneumoniae*, *Minerva Med.*, **111**, 536 (2020).
15. M. Efenberger-Szmechtyk, A. Nowak, A. Czyzowska, Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products, *Crit. Rev. Food Sci. Nutr.*, **61**, 149 (2021).
16. R. Farhoosh, G. A. Golmovahhed and M. H. H. Khodaparast, Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.), *Food Chem.*, **100**, 231 (2007).
17. M. M. M. Bindes, V. L. Cardoso, M. H. M. Reis, D. C. Boffito, Maximisation of the polyphenols extraction yield from green tea leaves and sequential clarification, *J. Food Eng.*, **241**, 97 (2019).
18. F. Yaylacı Karahalil, Z. Can, Investigation of Biochemical Usefulness of Tea (*Camellia sinensis*) Flower, *J. Apitherapy Natur.*, **2**, 21 (2019).
19. E. Yurteri, A. Özcan, F. Seyis, Effects of Organic Fertilizer and Drying Methods on Total Phenolic Content and Antioxidant Capacity of Organic White Tea (*Camellia sinensis* (L.) Kuntze), *TURJAF*, **7**, 1641 (2019).
20. K. Yazici, B. Goksu, Effects of kaolin (M-99-099) application on antioxidant and phenolic

compounds in tea leaves (*Camellia sinensis* LO Kuntze), *Biochem. Genet.*, **55,** 367 (2017).
21. P. H. Davis, *Flora of Turkey and the East Aegean Islands*, University Press, Edinburgh p. 223 (1966-1968).
22. M. C. H. Mohan, S. M. Rao, and P. Kumari, Antimicrobial Activity of Selected Indian Medicinal Plants, *J. Microbiol. Biotech. Env. Sci*, **11**, 355 (2009).
23. A. Yildirim, A. Mavi, M. Oktay, A. A. Kara, O. F. Algur, V. Bilaloglu, Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf Ex DC), Sage (*Salvia triloba* L.), and Black Tea (*Camellia sinensis*) Extracts, *J. Agric. Food Chem.*, **48**, 5030 (2000).
24. V. L. Singleton, J. A. Rossi, Colorimetry of total phenolics with phosphomolibdic-phosphotungtic acid reagents, *Am. J. Enol. Vitic.*, **16**, 144 (1965).
25. A. Priftis, D. Stagos, K. Konstantınopoulos, C. Tsıtsımpıkou, D. A. Spandıdos, A. M. Tsatsakıs, M. N. Tzatzarakis, D. Kouretas, Comparison of antioxidant activity between green and roasted coffee beans using molecular methods, *Mol. Med. Rep.*, **12**, 7293 (2015).
26. I. F. Benzie, J. J. Strain The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Anal. Biochem.*, **239**, 70 (1996).
27. M. Balouiri, M. Sadiki, S. K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, *JPA*, **6**, 71 (2016).
28. *Performance Standards for Antimicrobial Susceptibility Testing*, 30th Edition *CLSI supplement M100*, Clinical and Laboratory Standards Institute, Wayne, PA. USA, p. 23 (2020).
29. *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 13th Edition. *CLSI standard M02*, Clinical and Laboratory Standards Institute, Wayne, PA. USA, p. 15 (2018).
30. SPSS Inc. PASW statistics for windows, version 18.0. Chicago, IL: SPSS Inc., (2009).
31. Y. Aguilera, M. A. Martin-Cabrejas, E. G. de Mejia, Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: their role in prevention of chronic diseases, *Phytochem. Rev.*, **15**, 405 (2016).
32. I. Fidrianny, A. Rizkiya, K. Ruslan, Antioxidant activities of various fruit extracts from three solanum sp. using DPPH and ABTS method and correlation with phenolic, flavonoid and carotenoid content, *J. Chem. Pharm. Res.* **7**, 666 (2015).
33. I. Fidrianny, H. Suhendy, M. Insanu, Correlation of phytochemical content with antioxidant potential of various sweet potato (*Ipomoea batatas*) in West Java, Indonesia, *Asian Pacific J. Trop. Biomed.*, **8**, 25 (2018).
34. J. Gan, Y. Feng, Z. He, X. Li, H. Zhang, Correlations between antioxidant activity and alkaloids and phenols of maca (*Lepidium meyenii*), *J. Food Qual.*, 1 (2017).
35. M. Casagrande, J. Zanela, A. W. Júnior, C. Busso, J. Wouk, G. Iurckevicz, P. F. Montanher, F. Yamashita, C. R. M. Malfatti, Influence of time, temperature and solvent on the extraction of bioactive compounds of *Baccharis dracunculifolia*: In vitro antioxidant activity, antimicrobial potential, and phenolic compound quantification, *Ind. Crops. Prod.*, **125**, 207 (2018).
36. N. Tlili, C. Sarikürkçü, Bioactive compounds profile, enzyme inhibitory and antioxidant activities of water extracts from five selected medicinal plants, *Ind. Crops. Prod.*, 151, 1 (2020).
37. D. Chen, G. Chen, Y. Sun, X. Zeng, & H. Ye, Physiological genetics, chemical composition, health benefits and toxicology of tea (*Camellia sinensis* L.) flower: A review, *Food Res. Int.*, 109584 (2020).
38. Q. Luo, J. R. Zhang, H. B. Li, D. T. Wu, F. Geng, H. Corke, X.-L. Wei & R. Y. Gan, Green extraction of antioxidant polyphenols from green tea (*Camellia sinensis*), *Antioxidants*, **9**(9), 785 (2020).
39. L. Armstrong, M. A. V. do Carmo, Y. Wu, L. A. Esmerino, L. Azevedo, L. Zhang & D. Granato, Optimizing the extraction of bioactive compounds from pu-erh tea (*Camellia sinensis* var. *assamica*) and evaluation of antioxidant, cytotoxic, antimicrobial, antihemolytic, and inhibition of α-amylase and α-glucosidase activities, *Food Res. Int.*, **137**, 109430 (2020).
40. E. Ulusoy, S. Kolayli, A. O. Sarikaya, Antioxidant and antimicrobial activity of different floral origin honeys from Turkiye, *J. Food Biochem.*, **34**, 321 (2010).
41. V. Todorovic, M. Milenkovic, B. Vidovic, Z. Todorovic, S. Sobajic, Correlation between antimicrobial, antioxidant activity, and polyphenols of alkalized/nonalkalized cocoa powders, *J. Food Sci.*, **82**, 1020 (2017).
42. L. Wang, B. Zeng, X. Zhang, Z. Liao, L. Gu, Z. Liu, Q. Zhong, H. Wei, X. Fang, The effect of green tea polyphenols on gut microbial diversity and fat deposition in C57BL/6J HFA mice, *Food Funct.*, **7**, 4956–4966 (2016).
43. L. Wang, B. Zeng, Z. Liu, Z. Liao, Q. Zhong, L. Gu, H. Wei, X. Fang, Green tea polyphenols modulate colonic microbiota diversity and lipid

metabolism in high-fat diet treated HFA mice, *J. Food Sci*., **83**(3), 864-873 (2018).

44. H.-H. Zhang, J. Liu, Y. J. Lv, Y. L. Jiang, J. X. Pan, Y. J. Zhu, M-G. Huang, & S. K. Zhang, Changes in intestinal microbiota of type 2 diabetes in mice in response to dietary supplementation with instant tea or matcha, *Can. J. Diabetes*, **44**(1), 44-52 (2020).
45. M. A. S. Khairudin, A. M. Mhd Jalil & N. Hussin, Effects of Polyphenols in Tea (*Camellia sinensis* sp.) on the Modulation of Gut Microbiota in Human Trials and Animal Studies, *Gastroenterol. Insights*, **12**(2), 202-216 (2021).
46. M. J. Andreea, M. Culea, C. Gherman, O. Cozar, Characterization of some plant extracts by GC–MS, *Nucl. Instrum. Methods Phys. Res. B-beam Interactions with Materials and Atoms*, **267**, 338 (2009).
47. I. Donoiu, C. Militaru, O. Obleagă, J. M. Hunter, J. Neamțu, A. Biță, I. R. Scorei, O. C. Rogoveanu, Effects of boron-containing compounds on cardiovascular disease risk factors–a review, *J. Trace. Elem. Med. Biol.*, **50**, 47 (2018).
48. S. A. Abdelnour, M. E. Abd El-Hack, A. A. Swelum, A. Perillo, C. Losacco, The vital roles of boron in animal health and production: A comprehensive review, *J. Trace. Elem. Med. Biol*., **50**, 296 (2018).