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Antimicrobial and Antioxidant Activity of Different Herbal Tea Combinations

Tuğba DEMİR[1] Sema AĞAOĞLU[1]

[1] *Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Cumhuriyet, Sivas, Turkey.*

Correspondence: *Tuğba Demir (tugba@cumhuriyet.edu.tr)* Received: 19.06.2020 Accepted: 17.10.2020

ABSTRACT

Objective: Today, the use of components obtained from plant extracts is rapidly increasing, especially in the pharmaceutical industry. Eight different plants, which are used as winter tea and are frequently consumed among herbal teas, were selected in the study. The aim of study was to investigate the antimicrobial and antioxidant activities of teas obtained from medicinal and aromatic plants such as Linden, Ginger, Cinnamon, Sage, Daisy, Turmeric, Clove and Rosehip. Five different pathogens (*Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa)* were selected from common disease-causing pathogens.

Materials and Methods: A total of 21 combinations were made for each plant. Disc diffusion and Minimum inhibition concentration methods were used to determine antimicrobial activity. DPPH (2,2 Difenil-1-Pikrohidrozil) method was used to determine antioxidant activity. The amount of total phenolic and tannins contents contained of herbal teas were also determined using the Folin-Ciocalteu reagent (FCR) method.

Results: The highest value among the antimicrobial activities of herbal teas (triple combination) was measured against *E. faecalis* (25.11 mm). The herbal combination with the highest value measured was found in the ginger+cinnamon+clove group. The highest antioxidant value was measured in this mixture (36.8 mg/mL).

Conclusion: Because some plants have more bioavailability, these benefits can be suppressed in a mixture. When determining these mixtures, the consumption will be more beneficial for public health, given the recommendations of researchers and experts.

Keywords: *Herbal tea, Antimicrobial, Antioxidant, Pathogen, Public health*

INTRODUCTION

Medical plants are used effectively in the treatment of diseases from past to present. In recent years, the acceptance of traditional medicine as an alternative healthcare has led researchers to investigate the bioactive properties and bioavailability of these plants. Plant originated medicines continue to be important resources to combat serious health problems, especially in developing countries (Mothana *et al*., 2010).

According to the report of the World Health Organization (WHO), about 60-80% of the world population benefits from traditional medicinal plants in the treatment of common diseases (Schuster and Wolber, 2010; WHO, 2013).

Today, the rapid increase of infectious diseases and the development of resistance against existing drugs by pathogens microorganisms lead to an increase in new and improved potential searches against bacterial and viral infections (Gibbons, 2004). Despite the latest developments in drug combinations developed using different technologies; it is supported by research that plant-based compounds can still be important drug sources for humans (Salim *et al*., 2008).

Plant-derived antimicrobials have a long history of research among new therapeutic agents. Plants can constantly interact with free radicals, with external environmental factors that can rapidly change metabolism and potentially cause harm. Thus, they support anabolic reactions in the body by combating harmful free radicals that may occur in metabolism. Plants develop alternative defense strategies by creating various chemical metabolites to overcome stress conditions that may occur with their unique metabolic interactions. Therefore, the use of plants in both traditional and modern health systems is highly preferred by researchers (Avila *et al*., 2008).

Various traditionally used medicinal plants have been involved in many studies in terms of their various biological activities and bioavailability using in vivo and in vitro study models. It is in the literature that extracts obtained from plants that have therapeutic properties and the phytochemicals isolated from them are preferred in developing modern medical practices (Abdalla *et al*., 2013). The phytochemicals that plants contain in their structure provide significant benefits on the immune system. In addition, when more than one phytochemical is combined, the bioactive property increases. Thus, with these compounds in a single plant structure, it can show antimicrobial, antifungal, antidiabetic, anti-inflammatory, anticancer and antiviral properties (Puangpronpitag and Sittiwet, 2009; Pundir *et al*., 2010; Sasidharan and Menon, 2010; Gupta *et al*., 2015; İlkimen and Gülbandılar, 2018; Demir *et al*., 2019; Vatlak *et al*., 2019; Rovna *et al*., 2020). This study was carried out to determine the antimicrobial and antioxidant properties of some herbal tea combinations.

MATERIALS and METHODS

Preparation of Plant Extracts

The plants used in the study were obtained from local sales outles of Sivas province. Linden, sage, daisy and rosehip were dried in an oven (40°C). While cloves are ground into powder, cinnamon, ginger and turmeric are taken as direct powder. In addition, it was brought to the same particle size by mixing (milling) the plants in triple combinations (56 combinations).

Table 1. Herbal teas and combinations

	A:Linden	B:Ginger	C:Cinnamon	D:Sage	E:Daisy	F:Turmeric	G:Clove	H:Rosehip
1	**ABC**	**BCD**	**CDE**	**DEF**	**EFG**	**FGH**	ABG	ABH
2	**ABD**	**BCE**	**CDF**	**DEG**	**EFH**	ABF	ACG	ACH
3	**ABE**	**BCF**	**CDG**	**DEH**	**EGH**	ACF	ADG	ADH
4	**ABF**	**BCG**	**CDH**	**DFG**	ABE	ADF	AEG	AEH
5	**ABG**	**BCH**	**CEF**	**DFH**	ACE	AEF	AFG	AFH
6	**ABH**	**BDE**	**CEG**	**DGH**	AEF	AFG	AGH	AGH
7	**ACD**	**BDF**	**CEH**	ABD	AEG	AFH	BCG	BCH
8	**ACE**	**BDG**	**CFG**	ADE	AEH	BCF	BDG	BDH
9	**ACF**	**BDH**	**CFH**	ADF	BDE	BDF	BEG	BEH
10	**ACG**	**BEF**	**CGH**	ADG	BEF	BEF	BFG	BFH
11	**ACH**	**BEG**	ABC	ADH	BEG	BFG	BGH	BGH
12	**ADE**	**BEH**	ACD	ACD	BEH	BFH	CDG	CDH
13	**ADF**	**BFG**	ACE	BCD	CDE	CDF	CEG	CEH
14	**ADG**	**BFH**	ACF	BDE	CEF	CEF	CFG	CFH
15	**ADH**	**BGH**	ACG	BDF	CEG	CFG	CGH	CGH
16	**AEF**	ABC	ACH	BDG	CEH	CFH	DEG	DEH
17	**AEG**	ABD	BCD	BDH	DEF	DEF	DFG	DFH
18	**AEH**	ABE	BCE	CDE	DEG	DFG	DGH	DGH
19	**AFG**	ABF	BCF	CDF	DEH	DFH	EFG	EFH
20	**AFH**	ABG	BCG	CDG	BCE	EFG	EGH	EGH
21	**AGH**	ABH	BCH	CDH	ADE	EFH	FGH	FGH

The herbal tea combinations analyzed are shown in bold.

Herbal teas were stored at +4°C until analysis. Ground plants were mixed with water in a ratio of 10:1 (ml/g). Then it was extracted in a shaking water bath at 80°C. At the end of the extraction period, the samples were centrifuged (10 min at 5000 rpm). The combinations of herbal teas and mixtures are shown in Table 1.

Antimicrobial activity

Microorganisms (*Staphylococcus aureus* (ATCC 29213), *Streptococcus pyogenes* (ATCC 19615), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) used in antimicrobial activity were obtained from Sivas Cumhuriyet University Research Hospital Microbiology Laboratory. All chemicals used as analytical standard were obtained from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Merck KGaA, Darmstadt, Germany).

Preparation of bacterial cultures

Nutrient Agar was used as medium for bacterial cultures. Sterilized media were poured into petri dishes and incubated at 37ºC for 20 hours in order to grow bacterial cultures. Nutrient Broth was used to determine the minimum inhibition concentration (Ebrahimabadi *et al*., 2010).

Disc diffusion method

Bacterial cultures produced in solid media of microorganisms were used. Serum was suspended in physiological and dilutions of 10^8 cfu/ml were prepared by comparison with a 0.5 McFarland turbidity tube. 100 µl of cultivation was made to the Mueller Hinton Agar (MHA, Merck; 70191, Germany) medium from bacterial dilution. Then incubated at 37 ºC in the over for 18-24 hours. Inhibition zones formed at the end of the incubation were measured (Ebrahimabadi *et al*., 2010).

Determination of minimum inhibition concentration (MIC)

Bacterial cultures were incubated at Nutrient Broth (Merck; 70122, Germany) at 37 °C for 24 hours. The inoculum suspension was prepared. MIC analysis of the prepared herbal teas was determined by the macrobroth dilution method. 25 µl (10^8 cfu/ml) of each bacterial culture was taken into the test tubes with 3 ml Mueller Hinton Broth (MHB, Merck; 70192, Germany) and herbal tea (starting from 25 mg/ml to a concentration of 0.78 mg/ml). It was then incubated at 37°C for 24 hours. The lowest dilution concentration in the tubes where bacterial growth was not seen at the end of the incubation was determined as the MIC value (Oskay *et al*., 2007).

Determination of total phenolic compound content

The total phenolic compound content was determined by the Folin-Ciocalteu method. The results were expressed as g gallic acid equivalent/kg of tea leaves (g GAE/kg). Measurements were conducted in triplicates (Radhakrishnan *et al*., 2014).

Determination of tannin content

The tannin content was determined by a method described by Nakamura *et al*. (2003). The tannin content was calculated from a calibration curve using a catechin as standard and expressed as g catechin equivalents/kg of tea leaves. Measurements were conducted in triplicates.

Antioxidant activity

Free radical screening of each herbal tea extract with DPPH (2,2 Difenil-1-Pikrohidrozil) radical sweep was performed and the results were given as total antioxidants. DPPH solution in methanol was prepared just before starting the analysis. Then 3 mL of this solution was mixed with 100 µl of herbal tea extracts. The samples were incubated at 37°C for 20 minutes in water bath and their absorbance was measured (515 nm). An empty cuvette containing 100 µl of methanol in DPPH solution was prepared and its absorbance was recorded. All experiments were done in three replicates. Antioxidant activity was calculated using the formula below (Brand *et al*., 1995).

% inhibition: [(AB-AE)/AB] × 100

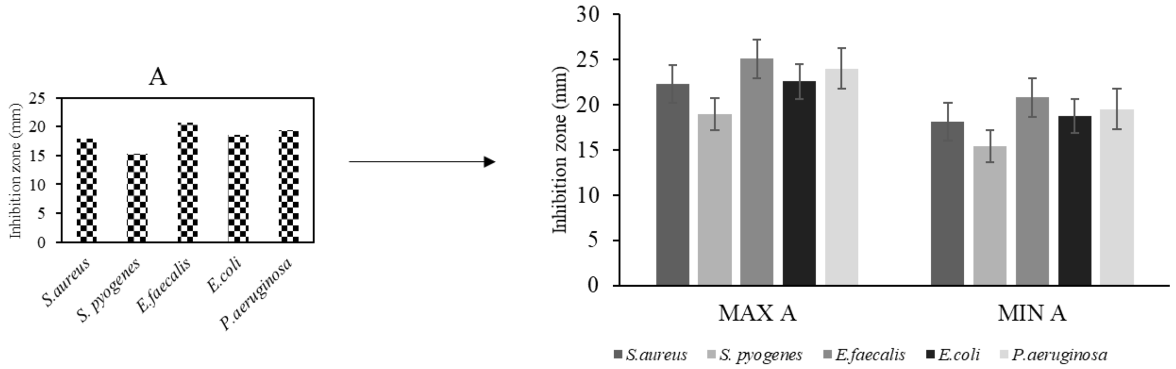
AB: Absorbance of the blank sample and AE: Absorbance of the herbal tea extract.

Statistical analysis

The results are given as three repeat means (±) standard deviation. SPSS was used to analyze statistical computer program results (IBM SPSS Statistics 22, Inc., Chicago, IL, USA). The variance analyzes of the results were made (ANOVA) and the differences were evaluated statistically in 95% confidence interval by Duncan multiple comparison test.

RESULTS

In this study, eight different plants which are used as winter tea and are frequently consumed among herbal teas were selected. Five different pathogens were selected from common disease-causing pathogens. A total of 21 combinations were made for each plant and the antimicrobial activity of these 21 combinations on each pathogen was determined using disc diffusion and minimum inhibition concentration methods. The antimicrobial effects of herbal teas used in our study on five different pathogens are given in Figure 1-8. When the graphs are examined, inhibition zones measured by disc diffusion method different antimicrobial activities in the combination (triple; 21 combinations) of each plant are also shown comparatively. Maximum (MAX: Highest in 21 combinations) and minimum (MIN: Lowest in 21 combinations) values are given in Figure 1-8.



MAX: Highest in 21 combinations, **MIN**: Lowest in 21 combinations

Figure 1. Antimicrobial activities of linden tea measured by disc diffusion method against different microorganisms **MAX**: Highest in 21 combinations, **MİN**: Lowest in 21 combinations

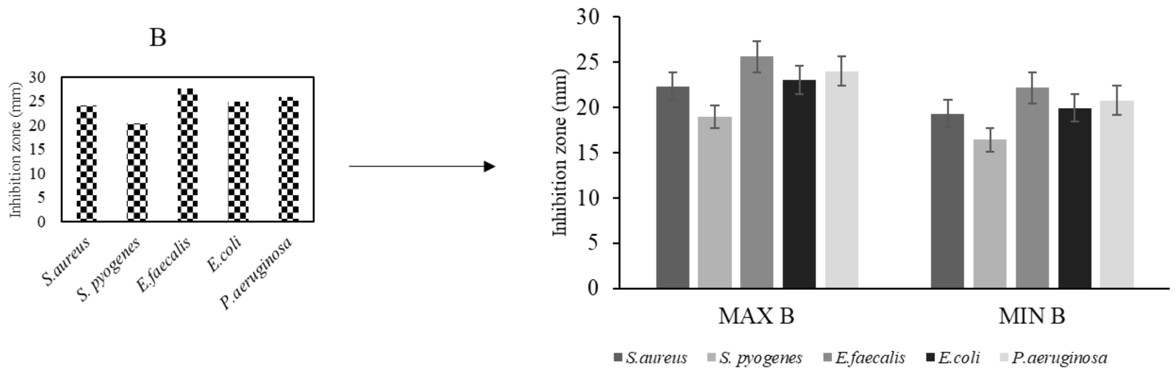


Figure 2. Antimicrobial activities of ginger tea measured by disc diffusion method against different microorganisms.

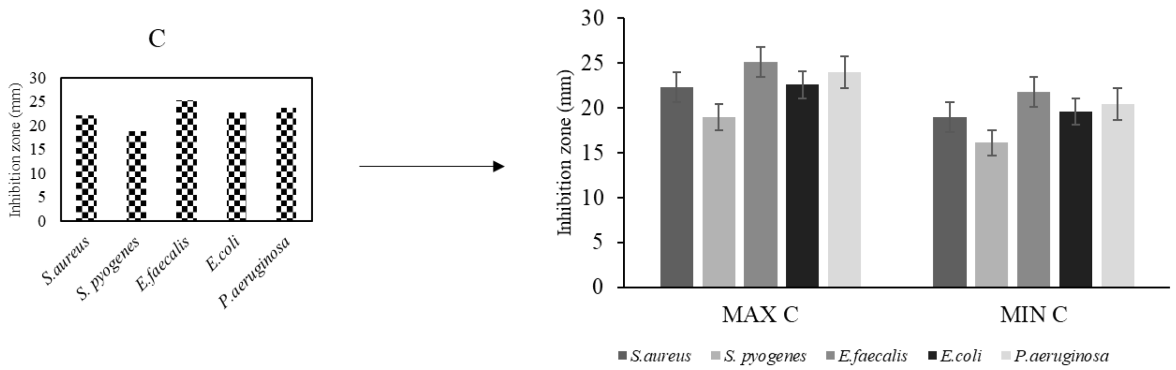


Figure 3. Antimicrobial activities of cinnamon tea measured by disc diffusion method against different microorganisms.

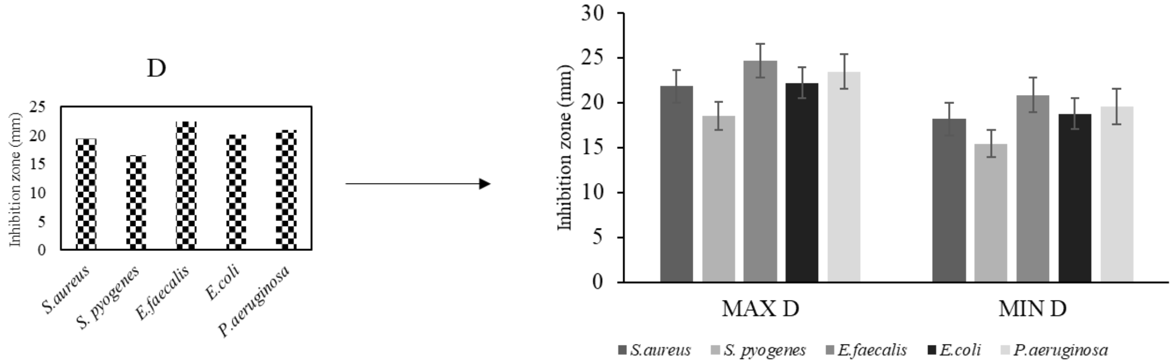


Figure 4. Antimicrobial activities of sage tea measured by disc diffusion method against different microorganisms.

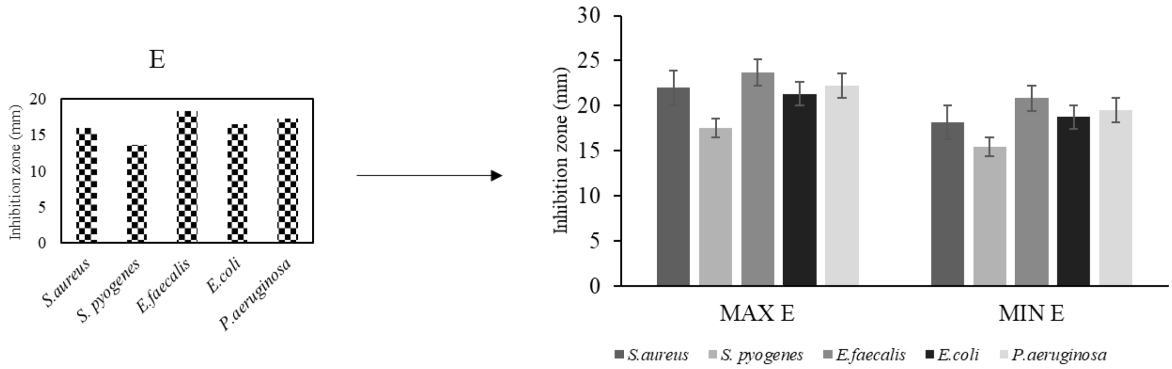


Figure 5. Antimicrobial activities of daisy tea measured by disc diffusion method against different microorganisms.

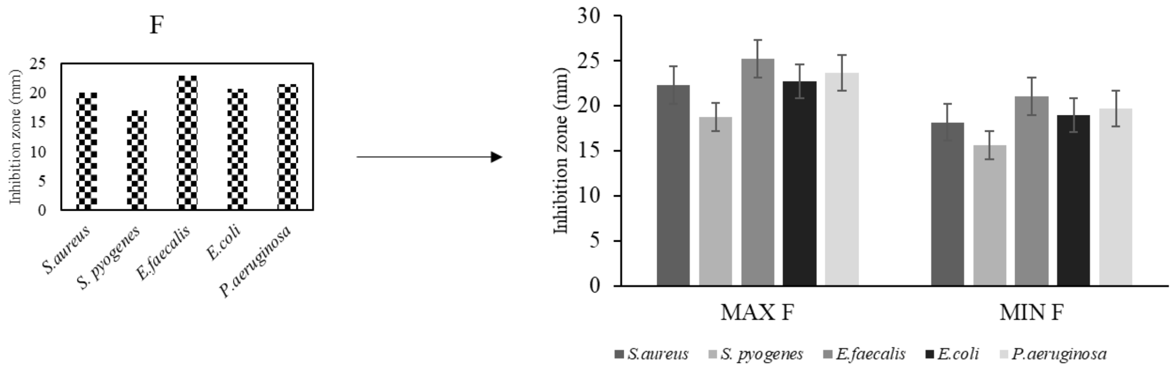


Figure 6. Antimicrobial activities of turmeric tea measured by disc diffusion method against different microorganisms.

When the antimicrobial activity results were evaluated, the extract (single combination), which acts on both gram positive and Gram negative bacteria, was measured as ginger (26 mm) extract. Daisy extract showed the lowest inhibition against gram positive bacteria *S. pyogenes* (14 mm). All extracts showed the highest inhibition on *E. faecalis*. Extracts in which *E.coli*, the fecal contamination indicator, were the most resistant were ginger (23 mm) and cinnamon (23 mm). When the synergistic effects were evaluated, eight combinations (ACG, BCG, CAG, DBG, EBC, FBC, GBC and HBC) showed a positive synergistic effect (Table 2).

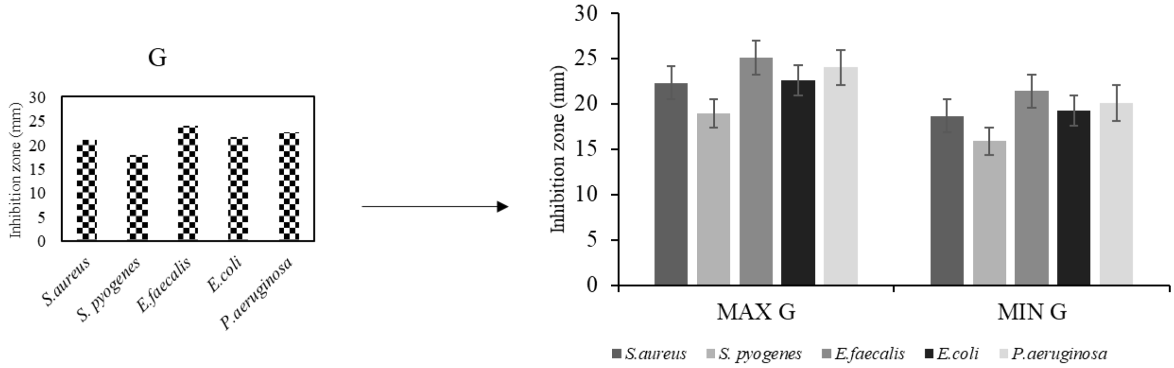


Figure 7. Antimicrobial activities of clove tea measured by disc diffusion method against different microorganisms.

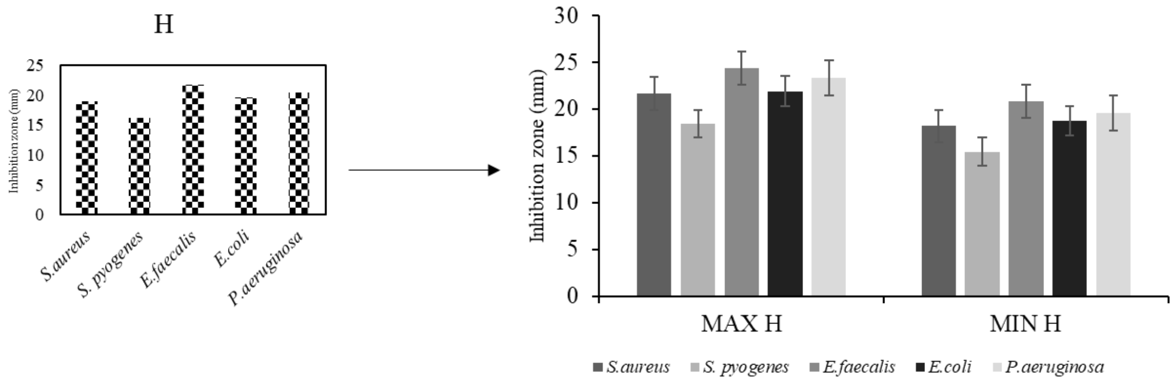


Figure 8. Antimicrobial activities of rosehip tea measured by disc diffusion method against different microorganisms.

Table 2. Positive and negative synergistic effects of herbal tea combinations

Herbal tea	Single highest (İnhibition zone mm)	Synergy (+)↑	Combination	Single lowest (İnhibition zone mm)	Synergy (-)↓	Combination
A	19.00 *	24.01	ACG	15.50 ***	15.44	AEH
B	25.25 **	25.62	BCG	20.40 ***	16.43	BAE
C	25.00 **	25.11	CAG	18.70 ***	16.15	CEH
D	22.38 **	24.60	DBG	16.58 ***	15.44	DEH
E	18.36 **	23.70	EBC	13.60 ***	15.44	EAH
F	22.95 **	25.25	FBC	17.00 ***	15.58	FEH
G	24.11 **	25.11	GBC	17.85 ***	15.87	GEH
H	21.80 **	24.35	HBC	16.15 ***	15.44	HAE

*: *P. aeruginosa*; **: *E. faecalis* ; ***: *S. Pyogenes*

MIC results obtained, disc diffusion results are in line with the results, and the lowest concentration in which each plant extract is effective on the same bacterial group was determined (Table 3). The minimum inhibition concentration of ginger extract was seen in containing *S. pyogenes* with <0.78 mg/mL.

In the literature, the pharmacological effects of plants have been mentioned in many studies and it has been stated that the expected benefits of the mixtures are at different levels. In this study, apart from each plant itself, triple combinations were made with 7 different plants and 21 different combinations. While some extracts retained their inhibition when it was mixed, some of them overcame the effect. Some of the plant combinations could not show their main inhibiting ability in mixtures of combinations. When the study results were evaluated, the most resistant group against the microorganisms from the triple combinations of herbal teas was the ginger + cinnamon + clove (BCG) group. Antimicrobial activity results of this group are 22.33 mm, 18.98 mm, 25.11 mm, 22.6 mm, 24.01 mm against *S. aureus, S. pyogenes, E. faecalis, E. coli, P. aeruginosa* pathogens, respectively. Chloramphenicol, the standard antibiotic used in the disc diffusion method, showed an inhibition zone of 30-35 mm against these pathogens. Within this study limits, BCG group data is close to the standard antibiotic value. Therefore, this group in the study is thought to be a strong antimicrobial agent.

Table 3. MIC values measured against different microorganisms

	S. aureus	*S. pyogenes*	*E. faecalis*	*E. coli*	*P. aeruginosa*
A	>6.25^{b}	12.50^{c}	<3.125^{a}	6.25^{b}	>6.25^{b}
B	>1.56^{b}	<3.125^{c}	>0.78^{a}	<0.78^{a}	<0.78^{a}
C	3.125^{b}	6.25^{c}	>0.78^{a}	<3.125^{b}	1.56^{a}
D	<12.50^{b}	12.50^{b}	<3.125^{a}	3.125^{a}	3.125^{a}
E	12.50^{b}	>12.50^{b}	6.25^{a}	12.50^{b}	6.25^{a}
F	<3.125^{a}	6.25^{b}	>3.125^{a}	3.125^{a}	3.125^{a}
G	3.125^{b}	6.25^{c}	>1.56^{a}	<3.125^{b}	>3.125^{b}
H	6.25^{b}	12.50^{c}	3.125^{a}	>6.25^{b}	3.125^{a}

$^{a, b, c}$ Averages marked with different letters in the same line are statistically different from each other according to Duncan test ($p<0.05$), Chloramphenicol was used as a control (MIC value;> 0,78 mg/ml)

The fact that the antimicrobial activity shown by an extract alone could not show the same level (zone) in the plant mixture made can be evaluated in the sense that the antimicrobial mechanism of this plant does not work at all performance. Plants show the antimicrobial mechanism of action together with the mechanism of action of volatile compounds such as alkaloids and terpenoids.

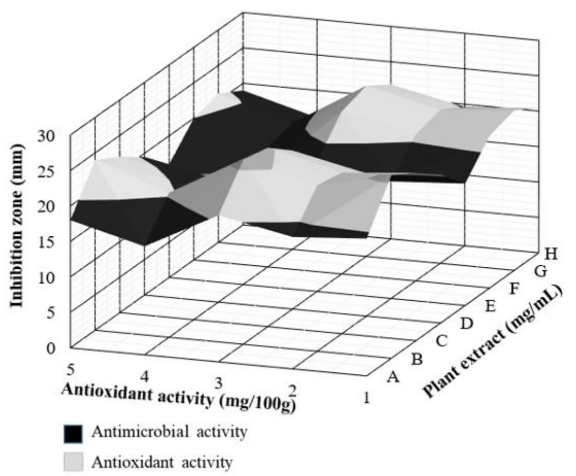


Figure 9. The relationship between antioxidant properties and antimicrobial activities of herbal tea extracts (There was a significant correlation; R^2> 0.87)

Although the chemical reaction caused by the molecular weight of alkaloids in the mixtures made suppressed the antimicrobial effect of the plant, other antioxidant mechanism of action still exists. The chemical composition of herbal teas is shown in Table 4.

Table 4. Chemical composition of herbal teas

	Total Polyphenols content (mg/100 g)	Tannin contents (mg/100 g)	Total antioxidant content (mg/mL)
A	9.2±1.01	0.28±2.11	27.7±1.13
B	15.6±1.88	0.31±1.18	34.9±2.58
C	12.7±0.81	0.25±1.05	36.8±3.05
D	9.3±0.78	0.28±3.08	23.7±0.08
E	7.2±2.07	0.29±2.18	22.4±0.90
F	13.4±1.19	0.44±1.09	38.9±1.18
G	11.8±3.09	0.32±0.44	31.3±3.05
H	11.9±2.08	0.34±0.87	34.1±1.58

The phenolic and flavonoid compounds released by the extraction step were tasked with destroying the cell wall of the pathogen with different defense systems and exhibited different levels of antimicrobial action. In addition, in our study, the total phenolic contents of the herbal teas and the contents of the total tannins were measured and recorded. When these results were evaluated, the highest polyphenols content showed ginger (B: 15.6 mg/100g), followed by turmeric (F: 13.4 mg/100g) (Table 4). The relationship between antioxidant activities and antimicrobial activities of extracts is presented in Figure 9. A significant correlation was determined between the antimicrobial activity of all herbal teas examined against different microorganisms and total polyphenol contents. The highest antioxidant value was measured in BCG mixture (36.8 mg/mL).

DISCUSSION

Plant polyphenols are aromatic hydroxylated compounds that are the most potent and therapeutically useful bioactive substances (Apak *et al.*, 2007). In this study, the difference in the all between formulizations of antimicrobial activity concurs with the results of studies that reported that the secondary metabolites may vary between plants of the same species and of different species (Achakzai *et al.*, 2009). In a study examining the antimicrobial properties of herbal extracts, the highest antibacterial activity of linden extract was measured against to gram negative bacteria *P. aeruginosa* used with disc diffusion method (Vatlak *et al.*, 2019). In our study, the inhibition zone measured as a result of the antimicrobial activity of linden measured using disc diffusion method is 19 mm (*P. aeruginosa*). When the results are evaluated, it is thought that this situation may be effective on the microorganism load and extraction solution and extraction time.

Tshivhandekano *et al.* (2014) were investigated the chemical composition and antimicrobial activities of different herbal teas and the synergistic effects of combined herbal teas. The antimicrobial activity exhibited by some extracts alone differed in the combined extracts. In this study, positive and negative synergistic effects of combinations of herbal teas were investigated. The single highest and lowest antimicrobial activities of herbal teas were evaluated. The highest and lowest antimicrobial activities were then recorded in combination. When comparing the most inhibited bacteria groups at the lowest concentration, gram positive bacteria were more inhibited than gram negative bacteria. In a different study, ginger reported that the minimum inhibition concentration on *S. pyogenes* was measured between 1.25 mg/mL and 2.50 mg/mL. Results of conducted the study by Onianwah and Stanley (2018) were found to be higher compared to our study findings. The MIC results in Table 3 are statistically different from each other according to the Duncan test, with the mean letters marked in different letters on the same line ($P < 0.05$). Antioxidants play an important role in neutralizing oxidative damage caused by free radicals in tissue fluids (Saleh *et al.* 2010). Our study results show that F>C>B plants have significantly high antioxidant activity content. The primary source of total antioxidant activity of these plants is thought to be total polyphenols. Therefore, the significantly higher antioxidant activity found in these plants may be associated with the high content of polyphenols analyzed. Composition of plant bioactive compounds has also been reported to play a role in the antimicrobial activity. Phytochemicals have an important place in plant bioactive components (Güzel and Akpınar, 2017). There are many phytochemical agents that plants naturally possess in their structure. These components, in addition to antioxidant and antimicrobial activity, have antidiabetes, anti-inflammatory and cytotoxic effects (Demir *et al.* 2019). In recent years, research has deepened to make use of plant phytochemicals in antiviral agents. In addition, bacterial inhibition may also vary according to plant extract; the solvent is used for extraction and the organism tested. Polyphenols content are related to the antibacterial activity of tea extracts. Our results indicated that bitki çaylarının had significantly high polyphenols content.

CONCLUSION

Plants that are frequently consumed by the people (winter tea) have been the subject of research in this study. Therapeutic properties of herbal teas that have for public health continue to be the subject of new studies day by day. Unconscious diversification of herbal tea blends may not always be right. Because some plants have more bioavailability, these benefits can be suppressed in a mixture. When determining these mixtures, the consumption will be more beneficial for public health, given the recommendations of researchers and experts.

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