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Preferred medicinal plants during the COVID-19 pandemic period: Microbiological quality of herbal teas obtained from these plants

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ABSTRACT: Although COVID-19 disease has lost its former severity in the period from its emergence in 2019 until today, it still maintains its importance. In this study, it is aimed to determine the plants preferred before and during the COVID-19 pandemic and to determine the microbiological quality of these plants. The information form prepared by ourselves was applied to the local herbalists and market employees in Istanbul in order to determine the plants used in the period and before, and to measure their knowledge about the transportation and storage conditions of the plants. It has been determined that the plants whose use has increased compared to before the pandemic process are turmeric (*Curcuma longa* L.), ginger (*Zingiber officinale* L.), galangal (*Alpinia galanga* (L.) Sw.), thyme (*Thymus vulgaris* L.) and olive (*Olea europaea* L.) leaves, and they are mostly consumed in the form of tea. Plants were obtained from randomly selected herbalists and markets in open and closed forms, and herb and tea forms were subjected to microbiological analysis using the spread plate method. According to the results of the study, it is important that pathogenic bacteria such as *Klebsiella pneumoniae, Bacillus cereus, Escherichia coli, Yersinia* spp. were not detected in the herbs and teas. As a result of the analysis, it was determined that the microbial load of the herb and tea forms of the closed samples taken from the markets was higher than that of the open samples taken from the herbalists, and that the microbial load was largely controlled after the brewing process (infusion-decoction). In addition, the importance of maintaining the water temperature in the brewing methods of teas has been demonstrated.

KEYWORDS: COVID-19; herbal tea; medicinal plants; microbiological contamination; microbiologic quality; pathogenic microorganism.

1. INTRODUCTION

Coronavirus disease is a contagious epidemic disease that first emerged in Wuhan, China in December 2019 and caused serious deaths by affecting the whole world. It was described as a pandemic by the World Health Organization (WHO) in March 2020 [1]. The novel coronavirus disease is caused by the Sars-CoV-2 virus, from the coronavirus family, which includes viruses that usually cause respiratory infections, such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) [2]. It was initially detected in humans in seafood and animal markets in China, and then spread from person to person and spread to other provinces of the people's Republic of China and other world countries [3]. It continues to threaten all humanity by spreading very quickly with the globalization of the world. Although it is known that it mostly affects individuals with weakened immune systems, chronic diseases and the elderly, it has been noted that the disease can cause serious damage to other individuals as well. The most common clinical symptoms are fever, cough, shortness of breath, fatigue and muscle pain [4]. In severe cases, pneumonia, severe respiratory failure and death may develop [5]. The current treatment scheme determined by the FDA (Food and Drug Administration) for COVID-19 includes certain antiviral drugs and monoclonal antibodies. These antiviral drugs are ritonavir, remdesivir and molnupiravir, while monoclonal antibodies are bebtelovimab and tocilizumab [6]. In addition to these drugs, patients are also in search of supportive

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treatment methods. Studies so far have shown that medicinal plants with anti-inflammatory, antiviral, immune system strengthening and cough suppressant effects can be used in addition to treatment to reduce the symptoms of the disease [7,8]. In China, the country where the disease first appeared, it has been suggested to clinically investigate the potential of using herbal products to reduce the symptoms of the COVID-19 pandemic [9]. Some plants, which are known to have cough suppressant, respiratory tract relief, breathing and immune system strengthening effects, and especially prepared as tea, are used among the people to reduce the symptoms of COVID-19.

It is known that herbal teas, which are increasingly used in our country and in the world and accepted as "natural" and "harmless" by consumers, are exposed to various sources and various chemical and biological pollutions such as microorganisms, mycotoxins, pesticides and heavy metals [10,11]. Microbial contamination of the herbal material used in tea making can occur before harvest through soil, water, fertilizer, sewage, animal waste and residues, as well as during production stages such as harvesting, drying, classification, grinding, processing, packaging and storage. Therefore, the storage conditions in herbal teas and the preparation of herbal teas are important. While most microorganisms and pathogens can be relatively reduced when tea is prepared with boiling water, bacterial spores belonging to the Bacillaceae family show resistance even to the infusion method in which heat treatment is applied. In addition, herbal teas prepared by maceration, which is a method that does not apply thermal processing, can contain a significant amount of microorganisms. In a study conducted in Turkey, it was revealed that participants who stated that they consumed herbal tea generally bought herbal teas from pharmacies and markets [12]. It is often not possible to determine whether medicinal herbal teas sold in inappropriate packaging and storage conditions in various places other than pharmacies (herbalists, markets, spice shops etc.) are microbiologically suitable. Plants and herbal teas that are not properly controlled for quality carry a risk in terms of microbiology. In this study, it was aimed to determine the microbial quality of these medicinal plants by determining the most preferred plants by the public during the pandemic period.

2. RESULTS AND DISCUSSION

2.1. Evaluation of the results of information form

The answers given to the questions in the information form applied to the herbalist and market employees in the study are given below in graphs and tables. According to the answers given to questions 1 and 2, the ratios of the plants preferred before and during the pandemic are given in Figure 1.

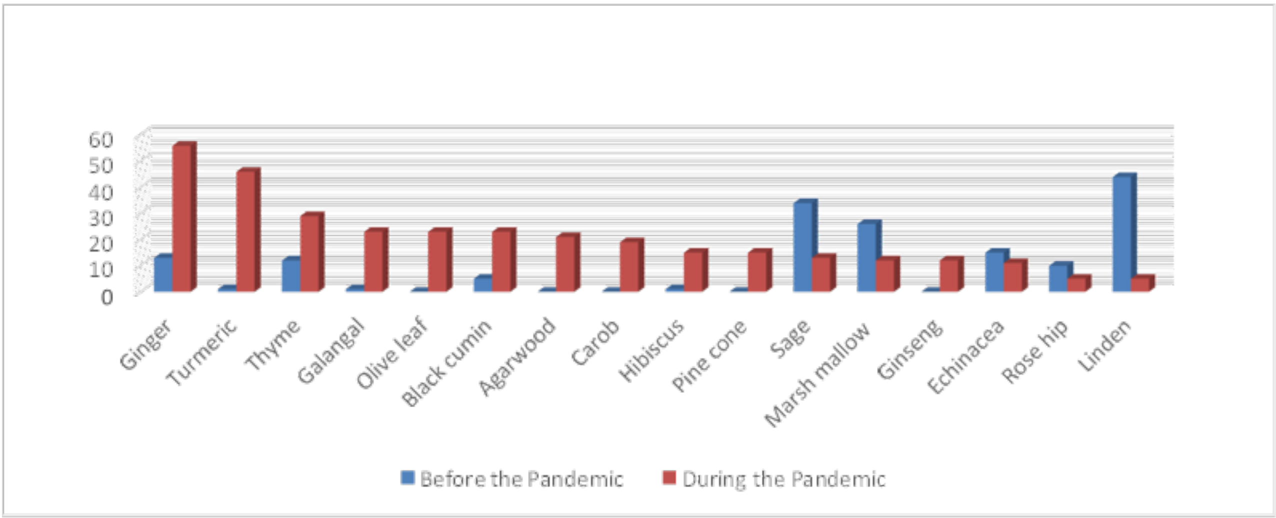

Figure 1. Preference rates of plants before and during COVID-19

According to Figure 1, it was observed that the usage rates of some plants that were frequently used before the pandemic decreased while some increased. Accordingly, it was noted that the preference rates of linden, sage and marsh mallow decreased significantly, while the preference rates of ginger, turmeric, thyme, galangal, olive leaf, black cumin, agarwood, carob, pine cone and hibiscus increased. While plants were generally used to reduce the symptoms of the common cold before the pandemic, it can be said that plants that strengthen the immune system started to be preferred in order to prevent COVID-19 during the pandemic period [13-18]. Therefore, plants such as linden, sage and marsh mallow were replaced by other plants such as ginger, turmeric and thyme. Although it was not preferred before the pandemic, olive leaf was the plant with the highest preference rate during the pandemic. It is known that the plant has antiviral effect on various viruses [19]. However, there is no definite information that it has an effect against COVID-19 yet. Although there is no scientific proof of the intense interest in this plant, it is thought that the reason is that it is frequently mentioned in the media. It is concluded from Table 1 that the majority of the individuals buy plants for protection purposes; mixed, in tea form on the recommendation of the seller.

While it was determined that the majority of herbalists and market employees (percentage added) were not informed about the storage conditions of the supplier during the delivery of the plants to the seller, it was found that they kept the plants in a cool environment and did not encounter any deterioration in the plants. When degradation was encountered, it was stated that 15% used the method of sieving, 9% returned, 9% exterminated and 8% sorted.

As a result, it has been determined that while the public trusts the sellers in the selection of plants to be used in the process of both protection and treatment against infectious diseases, unfortunately, herbalists and those working in this position do not have enough information about how microbial spoilage can occur and what conditions the plants are exposed to during the process of obtaining the plants they sell. For this reason, it is thought that criteria should be established to show how plants should be protected and preserved against microbial spoilage during the process of harvesting, obtaining products from plants, transportation, storage and delivery to herbalists, and both suppliers and herbalists should be trained on these criteria.

Table 1. Questions of information form and response rates

Questions Applied to Herbalist and Market Employees	Given Answers	* % ratio
During COVID-19, is the public demanding plants to treat symptoms or to prevent disease?	Prevention	56
	Treatment	7
	Prevention and treatment	37
During COVID-19, is the public deciding which plants to buy based on your advice or on their own demand?	Their own demand	25
	Advice	63
	Their own demand and advice	12
In which forms are plants requested from you?	Tea	66
	Paste	47
	Oil	23
	Extraction	7
	Capsule	6
	Molasses	8
	Syrup	6
	Powder	6
Are the plants requested from you in a mixture or as a single variety?	Mixture	60
	Single variety	27
	Mixture and single variety	13
What procedure do you follow when you detect degradation in plants?	No degradation detected	59
	Sieving	15
	Return	9
	Extermination	9
	Sorting	8
Can you give information about the storage conditions of the plants you have?	Cool dark and dry place	7
	Cool environment	48
	Cool and dark place	33
	Cool and dark place with a closed package	4
	Cool and dry place	8

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Table 1. Questions of information form and response rates (Continuation)

Under what conditions do the suppliers of your products store these plants and how do they procure them?	No idea	52
	Closed box	4
	Packaged at the appropriate temperature	13
	The supplier itself (does not buy the product from anyoneelse)	31
How did you gain your knowledge about the effects of the plants you offer to the public?	Research	8
	Experience	69
	Experience and research	13
	Education	10

* Since the total number of herbalists and markets is 100, the answers given are given directly as percentages.

2.2. Microbiological analysis results

Packaged and open forms of each plant examined in the study were procured. Tea was obtained from each plant by appropriate brewing method. Teas and plants were analyzed separately and the analysis results are given in Table 2. Leaves, stems, flowers, seeds, fruits, peels and roots of plants are used as tea. A large number of microorganisms can be found in plants, especially in areas with high soil contact. Since decontamination other than washing is not applied during the collection, processing and preparation of herbal teas, the possibility of microbial contamination during these processes is quite high. As a matter of fact, considering the connection of the plants examined in the study with the soil, it is observed that the microbial load density is higher in plants such as ginger, turmeric, galangal, which have a subsoil body or high contact with the soil, while the microbial load is reduced or even almost non-existent in plants with reduced connection to the soil such as thyme and olive leaves. When the samples were evaluated for the presence of pathogenic microorganisms, *Salmonella, Shigella* and *Staphylococcus aureus* were not detected (Table 2).

When the microorganism loads obtained in the study were analyzed on plant basis, only TMAB load was determined in olive leaf and the load was 10^2 CFU/g; TMAB load and TMAF loads were determined as 10^3 CFU/g in thyme. No growth of other bacterial groups was observed. These values are within the acceptable microbiological limit ranges in the WHO (World Health Organization) guidelines for evaluating the quality of herbal medicines and the Turkish Food Codex [20,21]. Thyme and olive leaves are known to have antimicrobial effects against many bacteria, viruses, yeasts and fungi [22-24]. It can be said that the limited amount of microbial load detected in both plant varieties is due to this. Growth was detected in the plant samples and it was observed that the bacterial and fungal load was under control in the tea forms. It can be said that this control is due to the temperature of the water used during brewing. The temperature of the boiling water controlled the growth of the vegetative microorganisms. In a study conducted by Ting et al. (2013), it was stated that microbial growth was present in plants and mixtures used as traditional medicine, but the growth decreased after boiling [25].

When the results of the samples of galangal and ginger plants were evaluated, TMAB, TMAF, aerobic spore and Enterobacteriaceae bacterial loads were detected in both plants. When the plants were evaluated in terms of TMAB load, which is a hygiene indicator, 10^5 CFU/g was detected in packaged samples, no microorganisms were detected in ginger plant in open samples, while 10^4 CFU/g bacterial load was detected in open galangal plant [26,27]. It was observed that the values were within the limit values according to the acceptable microbiological limits in the "Guidelines for the Evaluation of the Quality of Herbal Medicines" of WHO and Turkish Food Codex [20,21,28]. In previous studies, it has been stated that microbial contamination of packaged products offered for sale is generally less than that of products offered for sale openly [29,34]. However, in this study, it is quite remarkable that the packaged plants contained a higher bacterial load compared to the plants sold in the open. This situation is thought to be caused by an advanced contamination during harvesting, drying, storage, transportation as well as packaging in the factories of the purchased brand. It shows that companies should comply with the hygiene conditions during storage, processing and packaging of plants and that there should be a standard in these processes.

When ginger and galangal plants were evaluated in terms of Enterobacteriaceae bacterial load, bacterial load ranging from 10^3 CFU/g to 10^7 CFU/g in plant samples and from 10^2 CFU/ml to 10^5 CFU/ml in tea samples were detected. When the data of the ginger plant purchased in packaged form were examined, it was observed that the bacterial load in the sample obtained from the market was not in compliance with the Turkish Food Codex, but when it became tea, the bacterial load decreased, but still remained within the limit according to the Codex. It was observed that the presence of bacteria detected in both plant and tea samples in the open form bought from herbalists remained within the limit values according to the 2009 and 2011 Turkish Food Codex [21,28].

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Table 2. Microbial load of plants (CFU/g) and teas (CFU/ml)

Type of Bacteria Examined	Sample Form	Thyme		Ginger		Turmeric		Olive Leaf		Galangal	
		Market*	Herbalist**	Market*	Herbalist**	Market*	Herbalist**	Market*	Herbalist**	Market*	Herbalist**
TMAB	Plant	1,.0x10³	7.2x10³	2.2x10⁷	-	-	-	3.2x10²	4x10²	7.8x10⁷	3.1x10⁴
	Tea	-	-	3.1x10⁷	-	-	-	-	-	1.5x10⁴	2.1x10⁶
Enterobacteriaceae	Plant	-	-	3x10⁴	1.1x10³	-	-	-	-	2.3x10⁷	1.3x10⁷
	Tea	-	-	8.7x10³	4.5x10³	-	-	-	-	5x10²	4.4x10⁷
Aerobic Spore	Plant	-	-	-	1x10⁴	3.5x10²	1.5x10⁴	-	-	-	-
	Tea	-	-	-	3.5x10⁴	3.5x10²	2.5x10²	-	-	-	-
TMAF	Plant	-	3.9x10³	3.0x10³	-	3.4x10⁴	-	-	-	8.4x10⁴	2.7x10⁴
	Tea	-	-	8.5x10²	-	4.7x10³	-	-	-	-	1.5x10³
Salmonella, Shigella	Plant	-	-	-	-	-	-	-	-	-	-
	Tea	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	Plant	-	-	-	-	-	-	-	-	-	-
	Tea	-	-	-	-	-	-	-	-	-	-

* Packed form

** Opened form

\- No growth

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When galangal plant was evaluated, it was observed that the bacterial load of the packaged sample was higher than the open sample and both values did not comply with the Codex values. In the tea forms, it was observed that the number of bacteria (CFU/ml) decreased in the tea obtained from the plant sold in packaged form compared to the other samples. It might be that the galangal taken from the market is in root form and the decoction method is used in tea brewing. With the decoction method, the brewing water must be heated to 100 °C, that is, boiled. As a matter of fact, the galangal sample taken from the herbalist was not boiled, but brewed. As a result, the final temperature of the tea was measured as 83°C. This result showed that more positive results can be obtained in bacterial control by using the decoction method in the brewing of teas. If tea is to be obtained from plants using the infusion method, it is important to ensure that the container used for brewing has features that minimize heat loss during the process. When galangal and ginger plants were evaluated in terms of aerobic spore bacterial load, it was observed that there was no reproduction in galangal plant; in ginger plant, 10^4 spores/g were observed in plant and tea samples taken from the herbalist. While brewing plants with hot water can control pathogenic bacteria, it does not eliminate the risk of spore bacteria. The presence of spore bacteria indicates the presence of pathogenic bacteria especially belonging to Clostridium and Bacillus genera. Although the presence of *Bacillus cereus* has been demonstrated in some studies [30], one isolate was identified as *Bacillus cereus* in this study.

When the samples were evaluated in terms of Total Mesophilic Aerobic Fungi (TMAF) load, it was observed that the data of galangal plant was at the limit and ginger plant was below the limit according to the Turkish Food Codex; it was observed that fungal growth could be controlled by tea production in both plants. In studies conducted throughout the world and Turkey, it is stated that fungal (yeast-mold) growth is above 80% on average and that these contaminations mostly occur during the arrival stages of the plants to the buyer [31-34].

When the results of the turmeric plant were analyzed in terms of the presence of aerobic spores, it was observed that both open and packaged samples contained spore loads between 10^2 spores/gr and 10^4 spores/g, and the spore load in the open sample was higher. When evaluated in terms of TMAF load, 3.4×10^4 CFU/g was found in the sample taken from the market and it was observed that the number decreased to 4.7×10^3 CFU/ml upon obtaining tea from the plant by brewing method. As a result, it can be stated that the microbial load in the plant was controlled by brewing.

During the enumeration phase of the study, different bacterial colonies were selected and isolated. The isolated colonies were purified and each strain was phenotypically identified according to Bergey's Manual of Systematic Bacteriology [35,36]. The identified isolates (genus and species) are given in Table 3. Of the isolation, 9 Gram positive and 13 Gram negative bacterial species were isolated. It was observed that 41% of the identified isolates belonged to the genus *Bacillus* spp. and 59% to the family Enterobacteriaceae. The presence of pathogenic bacteria such as *Klebsiella pneumoniae, Bacillus cereus, Escherichia coli, Yersinia* spp. among the identified isolates is also remarkable. Similar studies in Turkey and in the world were examined. In the study conducted by Akduman and Omurtag Korkmaz (2020), *Cronobacter sakazakii* was detected in herbal teas subjected to microbiological examination. In none of the samples *Salmonella* spp. not detected [29]. Vural et al. (2020) in the study of medicinal herbal teas, *Escherichia coli* and *Salmonella* spp. detected, but *Staphylococcus aureus* and *Bacillus cereus* were not detected [34]. Adounkpe et al. (2017) identified *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Aspergillus niger, Aspergillus flavus, Penicillium expansum* and *Fusarium solanii* in herbal teas [37].

Table 3. Phenotypic identification of bacteria

Isolate Number	Identified Genus/Species	Isolate Number	Identified Genus/Species
1	*Obesumbacterium proteus*	12	*Serratia marcescens*
2	*Yersinia* spp	13	*Bacillus* spp.
3	*Enterobacter* spp.	14	*Obesumbacterium proteus*
4	*Bacillus* spp.	15	*Enterobacter cloacae*
5	*Bacillus globisporus*	16	*Klebsiella pneumoniae*
6	*Bacillus marinus*	17	*Pseudomonas spp.*
7	*Bacillus marinus*	18	Unidentified

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8	*Bacillus* spp.	19	*Enterobacter cloacae*
9	*Bacillus marinus*	20	*Yersinia* spp
10	*Obesumbacterium proteus*	21	*Bacillus cereus*
11	*Klebsiella pneumoniae*	22	*Escherichia coli*

3. CONCLUSION

As a result, it is known that there is an unconscious demand for consumption of herbal products among the public both to protect against infections and to alleviate symptoms during the infection process. In this direction, in our study, it was determined that the first 5 teas preferred and consumed by the public during COVID-19 pandemic were thyme, ginger, turmeric, olive leaf and galangal. Consumers mostly procure these plants from herbalists. It was also observed that the herbalists lacked information about the microbial degradation development of the plants used in tea production and how the plants are exposed to contamination during the supply process. It is anticipated that this situation can be addressed by implementing necessary arrangements and organizing training sessions for herbalists and consumers. If herbal products are not inspected, the risk of microbial contamination is undeniably high in stages such as collection, drying and storage of drugs. In the current study, the samples were analyzed for Total Mesophilic Aerobic Bacteria (TMAB), Total Aerobic Spore Bacteria, Total Mesophilic Aerobic Fungi (TMAF), Staphylococcus aureus and *Salmonella* sp. as pathogenic microorganisms using spread plate counting method. In terms of bacterial diversity, the highest diversity was observed in ginger plants, while the lowest diversity was observed in olive leaves. *Staphylococcus aureus* and *Salmonella* sp. were not detected, but pathogenic bacteria such as *Klebsiella pneumoniae, Bacillus cereus, Escherichia coli, Yersinia* sp. were identified. These bacteria are among the pathogenic bacteria that may threaten public health. It may be recommended to repeat such studies on more samples in order to identify other pathogens for public health. In addition, the study revealed that the bacterial load of plants with underground stems was higher and it was determined that attention should be paid to maintaining the water temperature in the methods to be used in the production of teas. It is also understood that especially infants, patients and people with immune deficiency should pay attention to the temperature of the brewing water they use and the brewing time.

4. MATERIALS AND METHODS

The study was conducted between August 2021 and March 2022 in Istanbul, Turkey. The study consisted of three stages: determining the plants preferred during the pandemic period, purchasing the plants and brewing tea from them, and assessing the microbiological quality of the obtained teas.

4.1. Determination of plants

The randomly selected herbalists or markets located within the borders of Istanbul province formed the universe of the study. A 10-question information form (Table 4), which was created by ourselves in order to determine the medicinal plants that were frequently preferred by the public during the COVID-19 pandemic period and whose use increased during the period, was applied to the selected herbalists or market employees (n=100 people). The information forms applied to identify the plants were filled using face-to-face interview technique. All tables and graphics obtained from the information form used were prepared in the Microsoft Office Excel program.

Table 4. Questions of information form

	Questions Directed to Local Herbalists and Market Employees
1	Which plants were you selling against respiratory infections before COVID-19?
2	Which plants did you start selling frequently during the COVID-19 process?
3	During COVID-19, is the public demanding plants to treat symptoms or to prevent disease?
4	During COVID-19, is the public deciding which plants to buy based on your advice or on their own demand?
5	In which forms are plants requested from you?

6	Are the plants requested from you in a mixture or as a single variety?
7	What procedure do you follow when you detect degradation in plants?
8	Can you give information about the storage conditions of the plants you have?
9	Under what conditions do the suppliers of your products store these plants and how do they procure them?
10	How did you gain your knowledge about the effects of the plants you offer to the public?

4.2. Procurement of plants and preparation of teas

According to the results of the information form, leaves of turmeric (*Curcuma longa* L.), ginger (*Zingiber officinale* L.), galangal (*Alpinia galanga* (L.) Sw.), thyme (*Thymus vulgaris* L.) and olive (*Olea europaea* L.) leaves were determined as the top 5 most preferred plants during the COVID- 19 pandemic. Both open and closed forms of plants were obtained from randomly selected herbalists and markets. Samples from markets were taken in closed form, and samples from herbalists were taken in open form. The list of plants collected in the study is given in Table 5. It was also reported that the selected plants are frequently used in tea form by the public. Therefore, herbal teas were obtained from each of the plants according to the brewing methods (i.e. infusion and decoction) practiced by people at home.

Table 5. Table of samples labelling

From Herbalist	Sample Number	From Market	Sample Number
Ginger	1	Ginger	2
Galangal	3	Galangal	4
Turmeric	5	Turmeric	6
Thyme	7	Thyme	8
Olive Leaf	9	Olive Leaf	10

4.3. Microbiological analysis

Microbiological analysis of both pre-brewing (dry forms) and post-brewing (infusion- decoction) tea forms of the plants selected in the study were carried out.

4.3.1. Microbiological analysis before brewing

In the study, samples obtained from herbalists and market were examined for Total Mesophilic Aerobic Bacteria (TMAB), Total Mesophilic Aerobic Fungi (TMAF), aerobic spores, Staphylococcus aureus, Salmonella Shigella and Enterobacteriaceae bacterial loads before brewing (powder, root forms) using spread plate method. Dilutions of up to 10^{-10} were obtained from the samples using peptone dilutions under aseptic conditions and cultivation was performed. Plate Count Agar (PCA), Potato Dextrose Agar (PDA), Baird Parker Agar (BPA), Salmonella Shigella Agar (SSA) and Eosin Methylene Blue Agar (EMB) were used for the cultures of the respective microorganism. The dilutions prepared for the count of aerobic spore-forming bacteria were then kept in a 80°C water bath for 1 minute and then inoculated into the medium. The inoculated petri dishes were incubated at 37°C for 18-24 hours (for bacteria), +4°C for 7 days (for spores) and 27°C for 18-24 hours (for fungi), respectively. After incubation, the results were obtained in CFU/g by counting the colonies growing on the petri dishes. Colonies that differed from each other according to their colony morphology were isolated using the streak plate method [38-42].

4.3.2. Microbiological analysis after brewing

Herbal teas are prepared by infusion or decoction method according to their properties. Galangal, which is obtained from the market, is brewed with the decoction method due to its underground stem form. The infusion method was used for brewing other plants. In both methods 5 g of herbal tea was added to 100 ml of water (5%). Tap water was used in the brewing process. For control and comparison purposes, the temperatures of the water before brewing and the tea at the time of brewing were measured with a thermometer (Table 6). The microbial loads of the teas obtained were examined in terms of the same microorganisms using the same method as before brewing.

Table 6. Temperatures of the brewing water and teas

Sample	Water Temperature Used for Brewing	Tea Temperature	Sample	Water Temperature Used for Brewing	Tea Temperature
1	99 °C	63 °C	6	100 °C	82 °C
2	99 °C	75 °C	7	100 °C	74 °C
3	99 °C	83 °C	8	100 °C	64 °C
4	22 °C (Decoction)	100 °C	9	99 °C	75 °C
5	100 °C	77 °C	10	99 °C	70 °C

In the study, the bacteria with different colony morphology were purified using the streak plate method. This method involves transferring the sample taken from the culture medium with loop to sterile medium. Sample transfer is carried out by drawing lines with a loop in each compartment of the petri dish, which is considered as four compartments. Gram staining, IMVIC tests (Indole Test, Methyl Red (MR) Test, Voges – Proskauer (VP) Test, Citrate Utilization Test (simply Citrate Test)), carbohydrate tests, mobility test, H_2S and urease tests were applied to the obtained isolates [41,42].

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