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Original Research Article

Does Daily Consumed Herbal Tea Have an Inhibitory Effect on Dental Plaque Formation?

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ABSTRACT

In this study, developmental conditions of some bacteria responsible for the formation of dental plaque on teeth were identified, levels of dental plaque formation were determined, and the antimicrobial effect of some herbal extracts on the biofilm producing bacteria was investigated. For this purpose, 9 dental bacterial strains and *Streptococcus mutans* ATCC 25175 selected as standard were allowed to create biofilm on dental composite material. In later stages, the bacterial numbers on the restorative material were determined. Then, inhibitory effects of green tea, black tea, linden, sage, ginger and cinnamon extracts, which are often consumed in daily life, on bacteria responsible for dental plaque formation was examined. To the results, almost all of the isolates and *S.mutans* found to form biofilm in the range of 3.62 to 5.05 log CFU/mm^2 on the dental composite resin surface while the highest attachment was determined as 4.71 log CFU/mm^2 for *S. mutans*. Also, all of the herbal extracts showed antimicrobial activity at different levels. Besides, the dental strains were identified as different species of *Enterobacter, Halanaerobacter, Klebsiella, Staphylococcus, Streptococcus, Thermoproteus* genera. Finally, it was found that cinnamon, ginger and green tea presented the highest inhibitory effect among plant extracts used in the study.

Keywords: Antimicrobial activity, Biofilm, Dental plaque, Herbal extracts, *Streptococcus mutans*

INTRODUCTION

Dental caries and periodontal diseases are generally not considered as important diseases by humans however, they are serious health problems considering their prevalence and the high number of untreated cases (Berger *et al.* 2018; Brown *et al.* 2019). According to the World Health Organization's report published in 2003, oral health services are not adequately offered in developing countries (Peterson 2003). The reason for this can be explained with; "the cost of treatment for oral and dental health is high" and "these treatments are known as fourth most expensive treatments in developed countries". In this situation, it is more valuable to prevent the diseases as well as curing the diseases worldwide. The first problem that associates with oral health is dental caries and the first stage of dental caries is pathogenesis which will form with dental plaque formation (Zhou *et al.* 2016). Dental plaque is one of the best-defined biofilm structures. In this form approximately 1000 different microorganism species can be found. Among these microorganisms while some of them can be reported as useful for human health, most of them show pathogenic properties (Sands *et al.* 2017; Wróblewska *et al.* 2015). *Streptococcus mutans* is the best-known cariogenic bacterium involved in dental biofilm formation (Brown *et al.* 2019; Niu *et al.* 2020; Pinna *et al.* 2017). Biofilm structure provides some advantages to bacteria such as ease of access to nutrients and protection from environmental conditions (antimicrobial agents, adverse pH and osmotic pressure) (Gün and Ekinci 2009; Tremblay *et al.* 2015). Biofilm formation begins with the attachment of bacteria on a surface. In dental plaque formation, bacteria adhere to the pellicle layer formed on the tooth surface immediately after oral cleansing. The first attached bacteria are *Streptococcus*, *Lactobacillus* and *Actinomyces*, which are known as early plaque bacteria (Bowen *et al.* 2018). The shared feature of these bacteria are Gram positivity and the ability to adhere to tooth enamel. Gram negative bacteria such as *Prevotella* spp, *Porphyromonas* spp, *Neisseria* and

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Capnocytophaga species cling to tissues such as gingival glands and cause gingivitis since to they grow optimally in anaerobic condition (Pinna *et al.* 2017).

Since ancient times, herbal extracts have been used to traditionally cure many diseases. The fact that microorganisms have not developed an acquired resistance against herbal extracts make the herbs of interest to scientists (Bazargani and Rohloff 2016; Park and Yoon 2018). Some of the herbals used in this study and which have very frequent consumption in human daily diet are briefly given below.

Green tea and black tea are the most consumed drinks in the world among the herbal teas. Green tea is a good antioxidant, and is anticariogenic and antimutagenic due to the fact that high polyphenol contents like catechins (George *et al.* 2017; Goenka *et al.* 2013). Black tea is the fermented form of green tea. During the fermentation process polyphenols in green tea are oxidized and the anticariogenic property of black tea originates from the components such as teaphilin, tearubin and fluoride (Moynihan and Petersen 2004).

For a long time, sage tea has been widely used in the fields of cosmetics and medicine. Sage is one of the medicinal plants used in the treatment of fever, rheumatism, sexual problems and unbalanced nervous conditions. It is known that phenolic compounds such as carnosic acid and mannol in the structure have antimicrobial effects (Bozin *et al.* 2007; Moreira *et al.* 2013).

Ginger root tubers are a herb used as a spice and medicine. Gingerol, paradol, shogaol and zingeron which are found in the structure of ginger show antibacterial property (Karna *et al.* 2012).

Cinnamon is a plant with strong antimicrobial properties thanks to its essential oils such as sinnemalaldehyde, eugenol and phenolic compounds such as vanylic, caffeic, gallic, ferulic, p-coumaric acid. Since ancient times, cinnamon has been used both as medicine and spice in the kitchens (Nabavi *et al.* 2015).

Leaves and flowers of linden are boiled and consumed as tea. Farnesol found in linden is recorded as the agent to prevent biofilm formation (Alves *et al.* 2013).

The main factors causing the formation of dental caries are genetic factors, daily diet and mouth flora (Huang *et al.* 2011). Foods consumed during the day can both support and prevent tooth decay formation. Several studies have shown that some of the carbohydrates, especially saccharose, cause tooth decay (Moynihan and Petersen 2004; Wu *et al.* 2020). It is predicted that dental caries can be prevented by increasing the consumption of foods that prevent dental biofilm formation. In this respect, herbal extracts are thought to inhibit the formation of dental biofilm by their antimicrobial properties and the current study was focused on the inhibitory effect of daily consumed herbal tea on dental plaque formation.

MATERIALS AND METHODS

In the current study, nine bacteria [seven Gram negative (311, 711, 712, 811, 921, 1021, 1022) and two Gram positive (721, 911)] isolated from tooth surfaces (Gunes Altuntas 2013) and determined as biofilm producers were used as main material (Güneş Altuntaş *et al.* 2013). *Streptococcus mutans* ATCC 25175 used as the standard strain in the assays. The dental composite material with a diameter of 4 mm and a height of 2 mm produced by the Department of Restorative Dentistry of the Dentistry Faculty of Ankara University, used in biofilm formation experiments. Leaves of black tea, green tea, linden, cinnamon and ginger were supplied from several herbalist in Ankara, Turkey.

The potential of biofilm formation on restorative filling materials by bacteria

Determination of biofilm formation potential of the bacteria on the restorative material was done by the method of Adetunji and Odetokun (2012) with little modifications. The strains were cultivated in TSB (Tryptic Soy Broth, Merck) with 1% inoculation rate at 37ºC for 24 hours. The 1 mL portion of active cultures were added to the 24 well plate after adjusting to $O.D._{600}$: 0.4 (6 log CFU/mL). The restorative dental composites were then put into the wells and to leave at 37ºC for 24 hours in order to follow biofilm formation.

At the end of the incubation period the composites were carefully taken out from the wells and washed with sterile distilled water to remove unattached bacteria. Afterward the composites were placed into PBS and

mixed well to detach bacteria into the solution. Serial dilutions were prepared and cultivated on TSA. Then, the colony forming unit were calculated (Halkman and Ayhan 2005).

The area (A) of the dental composite was calculated by using the cylinder surface area formule given below; (r:2 mm, h:2 mm, π:3)

$$A = 2\pi r^2 + 2\pi r.h.$$

Determination of the effect of temperature and pH on biofilm formation

The temperature and pH of intra-oral can be changed under different conditions. The study conducted on this subject reported that pH was calculated 7.3 (±0.4) in the day, 7.0 (±0.5) in the sleep state, and 6.6. (± 0.5) in the case of inhalation. The temperature of intra-oral can be 33.1 (±5.2) in the day time and 33.3 (±6.1) in the sleep state (Choi et al. 2016). Besides, the growth temperatures of oral bacteria and *S. mutans* is declared between 30-47ºC and the optimum growth temperature was reported as 37ºC (Ma and Marquis 1997). Therefore, the temperature degrees were chosen as 33 and 37ºC while pH values were as 6.5 and 7.2 in this study. For this purpose, the strains were inoculated the petri dishes containing TSA adjusted pH 6.5 and 7.2. Then, they incubated at 33 and 37ºC for 24 hours.

Preparing the herbal extracts

Total polyphenol extraction from herbals with hot water

The total polyphenol extraction was performed with the method of Kim *et al.* (2011). The 4 g portions of the herbal tea samples were mixed with 40 mL distilled water. The mixture were heated to 85-95ºC with water bath for 3 hours. At the end of this step the extracts were filtered through Whatman 125 mm paper.

Total polyphenol extraction from herbals with ethanol

Ethanol extraction was performed as Babu *et al.* (2003) offered. 4 g of each sample was added to 40 mL ethanol and mixed for 4 hours without heating. The filtrate was obtained by using Whatman 125 mm paper and then this step was repeated with the herbal residue. The ethanol solvent was removed from the extract with Büchi Rotavapor R-200.

The antimicrobial activity of herbal extracts on dental plaque bacteria

The antimicrobial activity of strains was tested by agar spot and well diffusion assays (Lucke and Schillinger 1989). In order to apply agar spot test, Tryptic Soy Agar (TSA) plates were spreaded with 8 mL soft-agar (TSB with 0.75 agar) containing 100 µL overnight culture of bacteria and then 10 µL extracts were spotted on the agar. After incubation at 37ºC for 24 hours, diameters of the whole inhibition zones were measured. For well diffusion assay, similar to agar spot assay, the bacteria were spreaded on the plates and then wells of 8 mm diameter were cut on TSA plates, and 50 µL portions from the extracts were placed into the wells. Diameters of the whole inhibition zones were measured after incubation at 30°C for 24 h. Water and ethanol (96%) were also used as controls.

The molecular identification of the isolates

The molecular identification step of isolates was performed by the 16S rDNA gene region sequence analysis method (Sanger *et al.* 1977). Isolation of bacterial DNA was the first step of the experiment, Qiagen DNeasy Blood and Tissue Kit was used for this aim. After DNA isolation, DNAs were checked on a 1% agarose gel and then, measurements were made on the NanoDrop ND-1000 spectrophotometer to determine purity and quantity values. The 16S region of the template DNA was amplified in PCR followed by sequencing of the base sequence. Reagents used in PCR; 10 pmol of forward primer (2 µL), 10 pmol of reverse primer (2 µL), 2.5 mM total dNTP (1 µL), 0.5 unit Go Taq DNA Polymerase (0.6 µL) (Promega), 25 mM $MgCl_2$ (2.4 µL), and 10 µL buffer were prepared in a total of 50 µL of PCR mixture as 5 × buffer. The Forward primer (27F) sequence was; AGA GTT TGA TCM TGG CTC AG and Reverse primer (907R) sequence was CCG TCA ATT CCT TTG AGT TT in PCR step which

were offered by Beasley and Saris (2004). After amplification, the visible DNA bands on agarose gel were cut and purified using the Promega Wizard DNA Purification Kit.

Purified PCR products were taken into AB Gene Plate. After sealing thoroughly with sealer, the denaturation was carried out at 94°C for 4 minutes. This suspension was immediately centrifuged at 1500 rpm for 1 minute by immediately cooling on ice to provide single stranded DNA. Finally, pure DNA samples were subjected to the sequence PCR step. Agencourt CleanSEQ kit was used as a PCR cleansing kit. DNA sequence analysis was performed by Beckman Coulter CEQ 8000 automated sequence analysis. The results were evaluated by NCBI database.

Statistical analysis

Data that collected in the context of the study were statistically analysed by Minitab and IBM SPSS Software (Version 2.1.). Results were double checked with these programmes by statistical one-way analysis of variance (ANOVA). Also, the statistically significant differences between the values were determined by Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Evaluation of Biofilm Formation on the Composites at Different Temperatures and pH

Table 1 shows the biofilm capability of 9 dental isolates and *S. mutans* standard strain incubated with dental composite resin discs at 33, 37°C and 6.5, 7.2 pH conditions. It is clearly seen that all the strains showed biofilm formation on the composites in test temperatures and pH values which changed between 3.62-5.05 log CFU/mm^2 except the isolate 921. Also, biofilm capability of the strains by various temperature and pH values were found as significant ($p<0.05$). The isolate numbered 921 was not shown any biofilm capability at 37°C and 7.2 pH. According to the Table 1, 33°C and 7.2 pH conditions were shown the highest biofilm capability conditions among them for almost all strains. However, the strains numbered as 711, 721 and *S. mutans* standard strain were shown the highest biofilm formation at 37°C and 7.2 pH conditions as 4.87, 4.63 and 4.71 log CFU/mm^2, respectively.

Table 1 The biofilm formation of dental isolates (log CFU/mm^2) at different temperatures and pH values.

Strain number	33ºC / 6.5 pH	33ºC / 7.2 pH	37ºC / 6.5 pH	37ºC / 7.2 pH
921	3.78±0.04^c	4.79±0.01^a	4.50±0.21^b	nd
911	4.10±0.18^d	5.05±0.58^a	4.54±0.15^b	4.40±0.61^c
711	3.62±0.31^d	4.72±0.06^b	4.44±0.06^c	4.87±0.08^a
712	4.55±0.12^c	4.74±0.09^a	4.54±00^d	4.67±0.08^b
721	4.13±0.58^d	4.58±0.01^b	4.46±0.15^c	4.63±0.10^a
311	3.70±0.09^d	4.63±00^a	4.23±0.03^c	4.36±0.65^b
811	4.56±0.22^c	4.83±0.11^a	4.55±0.05^d	4.75±0.08^b
1021	3.92±0.40^d	4.78±0.11^a	4.34±00^b	4.21±0.95^c
1022	4.95±0.13^b	5.01±0.30^a	4.68±0.06^c	4.67±0.19^d
S. mutans	4.19±0.87^d	4.63±0.20^b	4.49±00^b	4.71±0.09^a

*Values followed by the different small letters in rows are significantly different ($p < 0.05$)
**nd: not determined

The study conducted by Gungor *et al.* (2013), the level of binding *S. mutans* on permanent teeth, removed during periodontal and orthodontic treatment, was searched. After incubation, the presence of artificial saliva the bacterial adhesion was found as 5.87 log CFU / mm^2, and in the absence of saliva, the value was calculated as 4.71 log CFU / mm^2. Whereas the methods used in the study is differ from the current study, it can be reported that the main results have similarities.

The optimum growth temperature of *S.mutans* and other dental microorganisms involved in the formation of biofilm is 37ºC **(**Ma and Marquis 1997). Oral temperature is generally measured as 33,1 °C (± 5,2) and 33,3 °C (± 6,1), and oral pH is between 7,3 (± 0,4) and 6,6 (± 0,5) (Choi *et al.* 2016). The model designed as in vitro oral conditions; these temperatures and pH values were selected to show dental plaque formation. 33ºC and 7.2 pH are the temperature and pH calculated in the mouth when people are awake during the day (Choi *et al.* 2016). 4.58- 5.05 log CFU/mm^2 are the determined levels for bacterial binding at these conditions in the study. Similarly,

but with minor differences the bacterial adhesion was detected between 4.23 – 4.68 log CFU/mm^2 at 37ºC and 6.5 pH. *S. mutans* numbers were 4.63 CFU/mm^2 and 4.49 CFU/mm^2 respectively. As a result of these observations, the maximum bacterial binding was determined at 33ºC with 7.2 pH.

In fact, most of the bacteria attached to the surface of teeth are aerobic bacteria (Poulsen 1999). However, anaerobic bacteria are mostly involved in biofilms in gingival groove (Wroblewska *et al.* 2015). The bacteria isolated from the tooth surface and determined as biofilm producers were included to study and the experiments were performed at aerobic conditions. For this reason, the bacteria that are considered to be aerobics are more compatible with the literature studies showing maximum biofilm formation at dailytime temperature and pH of oral.

S.mutans is able to attach to the surface of the filler material at 37ºC and 7.2 pH conditions than the other temperature and pH values. *S.mutans* maintains its vitality under acidic conditions like pH 5.5 and causes caries. Some strains can survive even at pH 3 (Liu *et al.* 2014). The optimum growth conditions of *S.mutans* are known as 7.5 pH and 37°C (Welin-Neilands and Svensater 2007). Therefore, the presence of biofilms at pH 37°C and 7.2 pH conditions is consistent with the available data. The obtained results are shown in Table 1.

It was determined that bacteria formed biofilm on dental composite resin surface at 37°C and 7.2 pH conditions in the mouth during a day. Another point to note is that the number of biofilm-forming bacteria on the surface of the filler material is higher at neutral pH conditions. While during sleeping, pH reaches to more acidic conditions such as pH 6.5. As a result of the study, it was observed that the bacteria were kept to the surface of the filling material at the lowest level in case of breathing in the mouth.

Evaluation of inhibitory effect of herbal extracts on bacteria

Agar spot and well diffusion methods were used to determine the inhibitory effects of the herbal extracts. In the experiments conducted with the agar spot method, the results were not obtained due to the dissemination of the extract dropped on the petri dish surface and not forming a smooth zone. Thus, the results obtained with the well diffusion method were evaluated and showed in Table 2.

It is clearly seen that in Table 2 ethanol extracts of the herbs showed more strong antimicrobial activity than hot water extracts. Besides, each herbal tea obtained different extract solutions was shown significantly various inhibiton effects on the bacteria ($p<0.05$). To the table, ethanol extracts of sage affected on all strains and the zones were measured between 3.5-9 mm. However, there was not observed any inhibition effects for the hot water extraction of sage. Similarly, inhibition zone formation was not seen in experiments with water extract of black tea whereas ethanol extract of black tea showed antibacterial effect on the strains 311, 712, 721, 921, 1021 and on S.mutans with 3.5-4.5 mm diameters. Likewise, ethanol extracts of ginger showed inhibitory effects on all strains changed from 8.5 to 11.5 mm, when hot water extracts affected just on 712, 811 and S. mutans at rougly 3.5, 4 and 7.5 mm, respecively.

On the other hand, ethanol extracts of green tea had not any antimicrobial effects on the isolates numbered 911, 711 and 311 observed the effects for hot water extracts ones. Besides, both extracts of green tea had not any inhibition effect on S. mutans.

According to the results, both extracts of ginger and cinnamon demonstrated the highest effect on S. mutans. It is known that herbal extracts have antimicrobial effecs on dental strains like S. mutans (George et al. 2017; M. Kim et al. 2020; Li et al. 2019; Limsong et al. 2004; Park and Yoon 2018; Pavlović et al. 2020; Shagana and Geetha 2017; Tatekalva et al.2021). In the literature, antimicrobial activity of herbs were shown commonly using ethanol extracts (Limsong et al. 2004; Pavlović et al. 2020). The reason of that, it has been noted strong antibacterial effect is observed when the extraction is performed with solvents such as dichloroethane, ethanol, methanol and n-hexane solvents instead of water (Beheshtirouy et al. 2015; Moreira et al. 2013). Besides it is known that, antimicrobial activity and antioxidant activity of herbs are related with the used extraction solvents and methods (Sepahpour et al. 2018; Turkmen et al. 2007). In the current study, this information was supported.

Antimicrobial activity of linden tea from Serbia on 23 different test microorganisms including S. mutans was investigated in part of the study by Pavlović et al. (2020). They obtained the extracts by following these steps. First of all, herb dusts ultrasonicated for 30 min with 50 mL of 80 % ethanolic solution in water (80:20, v/v). Then, supernatants were filtered, evaporated to dryness (45 °C), and diluted with 5 mL of methanol. Finally, the extracts in methanolic solution were filtered before analysis. According to their results by well-diffusion method; clear zones were measured in range from 12 to 15 mm on S. mutans. The results were higher than the current study. The reason of that using a different exraction method containing methanol which is known showing strong antimicrobial activitiy using for plant extraction. Besides, the results about inhibition effects of linden on S .mutans in the current study indicated that when compared to hot water solvent, ethanol was inadequate to collect antimicrobial substances from linden.

Table 2 The antimicrobial activity of the plant extracts on dental isolates (zone diameters were given in mm)*.

Hot Water Extracts						
Strain number	**Ginger**	**Green Tea**	**Linden**	**Cinnamon**	**Sage**	**Black Tea**
921	0^{C}	3.5 ± 4.95^{AB}	0^{B}	3.5 ± 4.95^{AB}	0	0
	0^{C}	4 ± 5.65^{AB}	0^{B}	7 ± 0^{AB}	0	0
911	0^{C}	4 ± 5.65^{A}	0^{B}	4 ± 5.65^{AB}	0	0
	0^{C}	4.5 ± 6.36^{A}	0^{B}	8 ± 0^{AB}	0	0
711	0^{C}	4 ± 5.65^{A}	0^{B}	4.5 ± 6.36^{AB}	0	0
	0^{C}	4.5 ± 6.36^{A}	0^{B}	8 ± 0^{AB}	0	0
712	0^{C}	3.5 ± 4.95^{AB}	0^{B}	4 ± 5.65^{B}	0	0
	3.5 ± 4.95^{BC}	0^{B}	0^{B}	3.5 ± 4.95^{B}	0	0
721	0^{C}	0^{B}	3.5 ± 4.94^{A}	4 ± 5.65^{AB}	0	0
	0^{C}	5 ± 7.07^{AB}	4 ± 5.65^{A}	7.5 ± 0.71^{AB}	0	0
311	0^{C}	3.5 ± 4.94^{AB}	0^{B}	4.5 ± 6.36^{B}	0	0
	0^{C}	3.5 ± 4.95^{AB}	0^{B}	3.5 ± 4.95^{B}	0	0
811	4 ± 5.66^{AB}	0^{AB}	0^{B}	7.5 ± 0.71^{A}	0	0
	3.5 ± 4.95^{AB}	4 ± 5.65^{AB}	0^{B}	8 ± 1.41^{A}	0	0
1021	0^{C}	4 ± 5.65^{A}	0^{B}	3.5 ± 4.95^{AB}	0	0
	0^{C}	4 ± 5.65^{A}	0^{B}	6 ± 0^{AB}	0	0
1022	0^{C}	4 ± 5.65^{A}	0^{B}	4 ± 5.65^{B}	0	0
	0^{C}	4.5 ± 6.36^{A}	0^{B}	3.5 ± 4.95^{B}	0	0
S. mutans	7.5 ± 0.71^{A}	0^{B}	0^{B}	7.5 ± 0.71^{A}	0	0
	3.5 ± 4.95^{A}	0^{B}	0^{B}	7.5 ± 0.71^{A}	0	0
Ethanol Extracts						
Strain number	**Ginger**	**Green Tea**	**Linden**	**Cinnamon**	**Sage**	**Black Tea**
921	11 ± 3.53^{AB}	4.5 ± 6.36^{AB}	0^{B}	10 ± 0^{AB}	5 ± 7.07^{C}	4 ± 5.65^{A}
	9.5 ± 0.7^{AB}	4 ± 5.65^{AB}	0^{B}	13 ± 0^{AB}	0^{C}	4 ± 5.66^{A}
911	8.5 ± 0.7^{C}	0^{D}	0^{B}	9.5 ± 0.7^{B}	8.5 ± 2.12^{A}	0^{B}
	8.5 ± 2.12^{C}	0^{D}	0^{B}	11.5 ± 2.12^{B}	8.5 ± 0.7^{A}	0^{B}
711	10.5 ± 0.7^{BC}	0^{D}	0^{B}	12.5 ± 2.12^{B}	9 ± 1.41^{A}	0^{B}
	8.5 ± 0.7^{BC}	0^{D}	0^{B}	9.5 ± 2.12^{B}	7.5 ± 0.71^{A}	0^{B}
712	9 ± 0^{BC}	4 ± 5.65^{ABC}	0^{B}	11.5 ± 0.7^{AB}	8.5 ± 0.7^{AB}	3.5 ± 4.95^{A}
	9.5 ± 0.7^{BC}	4 ± 5.66^{ABC}	0^{B}	11.5 ± 2.12^{AB}	4.5 ± 6.36^{AB}	4 ± 5.65^{A}
721	10 ± 1.41^{ABC}	4 ± 5.67^{BC}	0^{A}	12 ± 2.82^{AB}	8 ± 1.41^{A}	4 ± 5.65^{AB}
	10 ± 1.42^{ABC}	3.5 ± 4.95^{BC}	4.5 ± 6.36^{A}	11 ± 1.41^{AB}	9 ± 1.41^{A}	0^{AB}
311	9.5 ± 2.12^{BC}	0^{D}	0^{B}	10.5 ± 2.12^{B}	4 ± 5.65^{BC}	3.5 ± 4.95^{AB}
	9.5 ± 0.7^{BC}	0^{D}	0^{B}	10.5 ± 2.13^{B}	3.5 ± 4.95^{BC}	0^{AB}
811	9 ± 1.41^{ABC}	3.5 ± 4.95^{BC}	0^{B}	12 ± 2.82^{AB}	4 ± 5.65^{BC}	0^{B}
	11 ± 2.82^{ABC}	4 ± 5.67^{BC}	0^{B}	12 ± 1.41^{AB}	4.5 ± 6.36^{BC}	0^{B}
1021	10.5 ± 0.7^{ABC}	3.5 ± 4.95^{C}	0^{B}	11 ± 1.41^{AB}	8.5 ± 0.7^{A}	4 ± 5.67^{AB}
	9 ± 0^{ABC}	3.5 ± 4.95^{C}	0^{B}	11.5 ± 0.7^{AB}	8 ± 0^{A}	0^{AB}
1022	9 ± 0^{C}	5 ± 7.07^{A}	0^{B}	10.5 ± 0.7^{B}	8 ± 0^{A}	0^{B}
	8.5 ± 0.7^{C}	4 ± 5.65^{A}	0^{B}	10.5 ± 0.8^{B}	7.5 ± 0.71^{A}	0^{B}
S. mutans	11 ± 1.41^{A}	0^{D}	4 ± 5.65^{A}	12.5 ± 2.120^{A}	4.5 ± 6.36^{BC}	0^{AB}
	11.5 ± 0.7^{A}	0^{D}	0^{A}	14 ± 1.41^{A}	4.5 ± 6.36^{BC}	4.5 ± 6.36^{AB}

*All values seen on the table were calculated from 2 parallel results and the replications were given with separately.
**Values followed by the different capital letters in columns are significantly different ($p < 0.05$)

As regards the study of Nomura *et al.* (2020) about investigation of the inhibitory effect of a commercial mouth rinse and each of its major components (chlorhexidine gluconate, ethanol, and green tea extract) on multiple species of oral bacteria (6 different *S. mutans* strains, 2 *S. sobrinus* strains, *S. oralis*, *S. gordonii*, *S. mitis* and *S. salivarius* strains); there was not observed any inhibition effects of green tea extracts on test microorganisms. Thus, it was similar to the results of current study about inhibition effects of green tea extracts on *S. mutans*. Additionally, the current study is important due to the shown inhibition effects of water and ethanol extracts of green tea on different dental isolates. Thus, water extract ones were generally more effectvive than ethanol ones. It could be better for daily consuming of green tea due to consuming with hot water.

Another study about investigation antimicrobial activity of herbal teas on *S. mutans* was done with water and ethanol extracts of green tea, black tea and oolong tea by George *et al.* (2017). Then, the results were compared with 0.2% chlorhexidine. To the results; inhibition zones of the water extracts of green tea, black tea, oolong tea and chlorhexidine were measured as 16.33, 10.33, 19.66 and 22 mm, respectively; when they were found as 14, 9, 20.66 and 22 mm, respectively for ethanol extracts of tea. The results were similar to for green tea with the current study in terms of obtaining higher inhibition effect by the water extract than the ethanol extract. Higher antimicrobial activity of green tea water extracts on dental strains could result from that 30-40% of polyphenols in green tea are water soluble (Archana and Abraham 2011). Then, it is known that polyphenols one of the important bioactive compunds in teas (black, green, oolong) and also teas are rich in catechins that are high antimicrobial activity due to damaged the bacterial cytoplasmic membrane (George *et al.* 2017; Goenka *et al.* 2013).

In the literature; teas especially green tea was commonly studied as herbal extracts in antimicrobial activity searches on dental isolates due to the fact that, most consumed 2nd drink in the World, high antimicrobial and anticariogenic character cause of polyphenol contents and other health benefits (Archana and Abraham 2011; Barroso *et al.* 2018; George *et al.* 2017).

Tatekalva *et al.* (2021) searched antimicrobial potential of aloe vera extract, black tea extract and coriander oil against *S. mutans* and *Enterococcus faecalis*. Then, water was used for extraction of the herbs. In accordance with the results, 21.67 and 24.33 mm zone diameters were measured for 50 and 100 µL black tea extracts, respectively. The black tea extracts were prepared filtered heating at 70°C for 5 minutes 10 g of black tea powder and 100 ml distilled water mixture and then it was again heated for 10 minutes. The results were shown difference with the current study. The difference should come from the using lower heat treatment in a short time in mentioned study due to the fact that, antimicrobial activity of herbs like tea leaves related with extraction solutions and extraction process (Turkmen *et al.* 2007). However, there should need more study with different extraction solutions for various bacteria to determine the optimum extraction conditions of black tea for showing antimicrobial activity.

In the current study, both extracts of cinnamon showed inhibition effects on all the test strains even ethanol extract ones more effective. Besides, the highest effect was detected on *S. mutans* for both extract solution treatments. Similarly, the study of Bersy *et al.* (2021) indicated that, cinnamon extracts (with water) were shown antimicrobial activity on *S. mutans* as much as chlorhexidine. The result is important due to antibacterial agents like chlorhexidine used in the oral diseases treatment and care cause staining of teeth and toxicity (Tawab *et al.* 2020). Besides, the study of Kim and Park (2016) showed that cinnamon extract could be used as a natural antibacterial agent for oral pathogens. Glucosyltransferase adhesive ability, acid-production suppression effect, and antibacterial activity on *S. mutans* and *S. sanguinis* were measured to identify the anti-caries effect of cinnamon extract. According to results of the study, minimum inhibition concentration of the cinnamon ethanol extracts on the oral pathogens was found 4 mg/mL. Thus, antimicrobial activity results of cinnamon extracts on oral pathogens in the current study was in support of the literature.

Antimicrobial activity of sage (*Salvia*) is known (Mendes *et al.* 2020; Pirtarighat *et al.* 2019). The study of Mendes *et al.* (2020) indicated that, inhibition effects of Salvia officinalis (crude extracts, dichloromethane-soluble fractions, subfractions, manool, salvigenin, and viridiflorol) were found on various periodontal pathogens (*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum* and *Prevotella melaninogenica*). In the current study, ethanol extracts of the sage showed strong antimicrobial activity on all of the strain even not to seen in water extracts. Therefore, ethanol could be better solvent for releasing antimicrobial compounds of the sage.

According to the study of Hussein (2019), both aqueous and ethanolic extracts of ginger rhizome showed antimicrobial activity against vancomycin-resistant *Staphylococcus aureus* with 15 and 30 mm, respectively. It is similar to the current study in terms of higher inhibition effect of the ethanol extract of ginger than the water one. Another study was done by Babaeekhou and Ghane (2020) about investigation antimicrobial activity of n-hexane, ethyl acetate, methanol, and aqueous extracts of ginger on *S. mutans* and *S. sobrinu*. Similarly in the end of the study, all of the extracts exhibited antimicrobial activity against the oral pathogens especially, ethyl acetate and methanol extracts had the highest activity while, n-hexane and aqueous extracts the lowest. Also, the study indicated that content of polyphenols, glycosylated flavonoids, steroids etc. of the ginger provide enzyme inactivation that are found in cell memmbrane of bacteria and responsible for bacteria cell adhesion. Thus, antimicrobial and antibiofilm activities of ginger are supported (Babaeekhou and Ghane 2020).

Molecular identification of the isolates

The strains were identified with 16S rDNA sequencing method offered by Sanger and Nicklen (1977). According to the results obtained from NCBI nucleotide sequencing database and shown in Table 3; the isolates numbered as 311, 811, 921 and 1022 were similar to *Enterobacter ludwigii/cloacaea* with 97 or 98% similarity. The other isolates numbered 711, 712, 721, 911 and 1021 were found as *Halanaerobacter salinarius*, *E.ludwigii*/*Klebsiella oxytoca*, *Staphylococcus epidermidis*, *S. gordonii* and *Thermoproteus uzoniensis*/*Bacteriovorax marinus*, respectively. This broad spectrum of species has been explained by many researchers which confirms the idea that many different species live together in teeth surfaces (Bowen *et al.* 2018; Gün and Ekinci 2009; Pinna *et al.* 2017; Wroblewska *et al.* 2015).

Table 3 The 16S rDNA sequencing results of the strains obtained from NCBI database.

Strain Number	Identification Result	Similarity Rate
921	*Enterobacter ludwigii/cloacaea*	%97
911	*Streptococcus gordonii*	%99
711	*Halanaerobacter salinarius*	%100
712	*E.ludwigii/Klebsiella oxytoca*	%99
721	*Staphylococcus epidermidis*	%99
311	*Enterobacter ludwigii/cloacaea*	%97
811	*Enterobacter ludwigii/cloacaea*	%98
1021	*Thermoproteus uzoniensis / Bacteriovorax marinus*	%100
1022	*Enterobacter ludwigii / cloacaea*	%98

CONCLUSIONS

In vitro model adopted in the first stage of this study, the lowest, highest and optimum pH and temperature conditions were determined in the intra-oral environment and attachment of dental bacteria to the dental composite surface was investigated. The investigated dental strains in this study were found belong to different genera and biofilm formation levels were significantly changed by condition. According to the results, the level of adhesion of the bacteria to the dental composite resin surface was found to be close to the level of attachment to the tooth surface. The main reason for the failure of composite resin treatments is the secondary caries formed in the cavity due to polymerization shrinkage between the cavity and the restorative material. In this study bacterial adhesion and biofilm formation on the low shrink restorative material showed that secondary caries may be encountered, and consequently restorative treatment will result in failure.

Also, all herbal tea extracts were showed inhibition effects in different degrees while mostly the highest effect obtained from ethanol extracts except cinnamon. This suggests that polyphenols and some essential oils, which have antimicrobial activity in plants, cannot be sufficiently extracted by hot water extraction. Moreover, using of plant extracts in mouthwash and toothpaste may interfere with the development of antimicrobial resistance of microorganisms, instead of chemical and synthetic antimicrobial agents. Additionally, the synergetic effects of the combined use of herbs with inhibitory effects during the study may be the subject of another study.

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