Journal of Research in Medical and Dental Science
2018, Volume 6, Issue 5, Page No: 361-364

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

A Comparative Study on Antimicrobial Effect of Iranian Green Tea and Hibiscus Tea on Growth of Oral Cariogenic Bacteria *Streptococcus mutans PTCC 1683*

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ABSTRACT

Tooth caries is a major health problem today. Tea with its polyphenolic compounds can show its anticariogenic effects by affecting the main Cariogenic bacteria in the oral cavity. The present study tended to examine the antimicrobial effect of two Iranian teas Camellia sinensis and Hibiscus Sabdariffa in comparison with chlorhexidine on Iranian type of Cariogenic Streptococcus mutans Bacteria. For this purpose, ethanolic extract of plants was first processed; then, the impregnated disk was placed on the bacterial culture medium and diameter of inhibition zones was calculated. After three iterations, the results showed the means 17, 14.16 and 16.83 for zone of inhibition diameter of green tee, hibiscus tea, and control chlorhexidine 0.2%, respectively. Moreover, ANOVA test showed a significant difference between zones of inhibition diameter of Hibiscus Sabdariffa and Camellia sinensis. Multiple comparison tests showed no significant difference between zones of inhibition diameter of these two teas and chlorohexidine control. Examining minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of both hibiscus and green teas also showed that MBC was present in 12.5 mg/ml green tea and 50 mg/ml hibiscus tea and MIC was present in 6.25 mg/ml green tea and 25 mg/ml hibiscus tea. According to findings, it is concluded that anti-microbial effects of Iranian green tea are higher than Iranian hibiscus tea. There is also no significant difference between antimicrobial effects of these three compounds. Accordingly, it is suggested to consider anti cariogenic effect of Iranian green tea and Iranian hibiscus tea and their daily intake.

Key words: Anticariogenic, Tooth decay, Antimicrobial effect, Tea

HOW TO CITE THIS ARTICLE: AmirSina Golmohammadi*, A comparative study on antimicrobial effect of iranian green tea and hibiscus tea on growth of oral cariogenic bacteria S*treptococcus mutans PTCC 1683*, J Res Med Dent Sci, 2018, 6 (5):361-364

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Received: 06/10/2018
Accepted: 29/10/2018

INTRODUCTION

Dental caries is one of the biggest and most important Oral health problems in children and adults today. Among various factors of Caries, Cariogenic bacteria play a significant role. One of the most important bacteria affecting the Caries process is *Streptococcus mutans*. This bacterium is known as primary bacterial cause of dental Caries [1]. *Mutans* starts a tooth Caries process by forming a biofilm layer on tooth surface and forming a microbial plaque [2]. An attempt has been made to identify antibacterial compounds which reduce the amount of these bacteria in oral cavity. Meanwhile, tea is known as an effective compound [3]. Tea with its polyphenolic compounds has known antimicrobial role. Most known teas, including green tea, originate from *Camellia Sinensis*. Hibiscus tea also originates from *Hibiscus sabdariffa*. Studies in Iran show high levels of DMFT index. DMFT index is referred to the total number of Decayed, Missed and Filled teeth that an individual can have. In 2006, a study was conducted on 8301 men and women in Iran; these people had an average of 2.7 ± 2.6 teeth with caries [4]. A study on 3000 first-degree secondary students in Mazandaran province showed that these children had an average of 2.93 ± 4.98 decayed, missed or filled teeth [5]. This indicates the need to pay more attention to oral health in Iran. Using healthy snacks, such as tea, can help reduce these indicators. A study conducted on children has shown that children who took 1 to 3 glasses of tea daily had a lower Caries index than children who took 1-2 glasses a week [6]. In another study, children who took gums containing polyphenol for 24 months had lower increased DMFT than the group that did not take them [7]. Drinking tea in Iran is commonly associated with sweetening compounds such as sugar, chocolate, which may reduce the anti-cariogenic effects of tea. More studies to evaluate the effect of concomitant tea

consumption with sweet compounds compared to tea consumption without these compounds and its effects on dental caries can be remarkable.

MATERIALS AND METHODS

Herbal material

The dried and fresh leaves of *Hibiscus sabdariffa* and Camellia sinensis were taken from a local market. These leaves lacked any chemical processing and were made by Iranian tea makers.

Microorganism

First, *Streptococcus mutans PTCC 1683* was taken from the Iranian Research Organization for Science and Technology (IROST). The bacteria were cultured in a selective culture medium.

Herbal extract

Using the Maceration technique, 10 g powder of both plants dissolved in 100 mg ethanolic solvent. The solution was filtered using Whatman No. 1 filter paper and 100 mg/ml solution prepared. Final powder Solved with DMSO solvent and sterilized under UV rays. The entire process of herbal extract is based on similar studies [8,9].

Disc diffusion test

Antimicrobial activity of ethanolic extract of both hibiscus and green tea was investigated using Kirby-Bauer test and Disk diffusion technique. Microbial suspension was processed using McFarland 0.5 standard and cultured on Agar Muller Hinton medium. The disks impregnated with extract of both plants and the control disks impregnated with chlorohexidine 0.2% were transferred to culture media. The experiment was iterated three times; three disks were placed in each medium.

All three culture media were placed in incubator at 37°C for 1 day. Finally, zone of inhibition of each disk was measured in millimeters [8].

MIC and MBC of green and hibiscus teas

Using Muller Hinton Broth, herbal extracts were made at concentrations 1:2 (50 mg/ml) and 1:4 (25 mg/ml) and 1:8 (12.5 mg/ml) and 1:16 (6.25 mg/ml) and 1:32 (3.125 mg/ml). Then, 100 µl microbial suspensions made by standard McFarland 0.5 were transferred to all test tubes. A test tube containing control hibiscus tea, one containing control *Camellia sinensis* and one for control microbial suspension made. This test was also performed for *Hibiscus sabdariffa* and the test tubes were placed in an incubator at 37°C for 24 hours.

After 1 day, contents of all 13 test tubes were separately cultured on 13 Muller Hinton Broth medium. The culture media were incubated at 37°C for 1 day. The results were evaluated in the form of growth or non-growth of bacterial colonies on culture media and MBC and MIC were determined for both plants [8].

RESULTS

Descriptive results

According to Table 1, zone of inhibition for *Camellia sinensis* was recorded 18, 16 and 17 mm in culture media No. 1, 2 and 3, respectively. Zone of inhibition for *Hibiscus sabdariffa* was recorded at 15, 12.5 and 15 mm in culture media, respectively. These data for control chlorohexidine were recorded at 17, 17.5 and 16 mm.

The study of media cultured after diluting the plant extract and adding microbial compound showed that MBC was observed for hibiscus tea at a concentration of 1:2 (50 mg/ml) and for green tea at a concentration of 1:8 (12.5 mg/ml). MIC was also observed for hibiscus tea at a concentration of 1:4 (25 mg/ml) and green tea at a concentration of 1:16 (6.25 mg/ml) (Table 2).

Table 1: Zone of inhibition diameter of plants by culture media

Studied plants	Medium 1 (mm)	Medium 2 (mm)	Medium 3 (mm)	Mean (mm)
Camellia sinensis	18	16	17	17
Hibiscus sabdariffa	15	12.5	15	14.16
Control chlorohexidine	17	w17.5	16	16.83

Table 2: MBC and MIC for the studied plants

Studied plants	MBC	MIC
Camellia sinensis	1:8 (12.5 mg/ml)	1:16 (6.25 mg/ml)
Hibiscus sabdariffa	1:2 (50 mg/ml)	1:4 (25 mg/ml)

Inferential results could be derived from Table 3 and Table 4. In Table 3, As P<0.05 in ANOVA, there is a significant difference in zone of inhibition diameter between two groups. By multiple comparison tests between *Camellia sinensis* and *Hibiscus sabdariffa*, a significant difference was found in zone of inhibition diameter between two plants, while there was a significant difference in zone of inhibition diameter between these two teas and control Chlorohexidine (Table 4).

Table 3: ANOVA of the difference in zone of inhibition diameter between two plants

		Sum of square	Df	Mean of square	F-value	P
Zone of inhibition	Between groups	167.15	2	7.583	6.205	0.035
	Within groups	333.7	6	1.222		
	Sum	5.22	8			

Table 4: Multiple comparison test between green tea and hibiscus test (Tukeys test)

Studied sample	Studied sample	Mean difference	P	Result
Camellia sinensis	*Hibiscus sabdariffa*	2.83333	0.046	Significant
	Control Chlorohexidine	0.16667	0.981	None Significant

Hibiscus sabdariffa	*Camellia sinensis*	-2.83333	0.046	Significant
	Chlorohexidine control	-2.66667	0.058	None Significant
Chlorohexidine control	*Camellia sinensis*	-0.16667	0.981	None Significant
	Hibiscus sabdariffa	2.66667	0.058	None Significant

DISCUSSION

Antimicrobial effects of tea have been confirmed by many oral hygiene studies. Since tea is a major snack in Iran, the importance of microbiological studies of tea should not be overlooked in the field of dental Caries. The present study tends to investigate the antimicrobial properties of Iranian green tea and hibiscus tea on the most important oral and dental Caries bacterium-*Streptococcus mutans*, compared to chlorhexidine 0.2%. The results showed that the mean zone of inhibition diameter of green growth (17 mm) was slightly higher than that of 0.2% Chlorohexidine mouth wash (16.83 mm). In their study, George et al., showed that the mean length of inhibition zone was 14 mm in green tea and 22 mm in chlorohexidine. In this study, unlike the present study, there was a statistically significant difference between green tea and domestic mouthwash chlorohexidine [10]. In another study in Iran, the results were similar to the present study. Ranjbar et al. claimed that there is no significant difference in diameter of inhibition zone of green tea compared to chlorohexidine mouthwash (P=0.305) [11]. This difference in diameter of inhibition zone of green tea and the significant level can be attributed to the difference in polyphenol content of the teas used in these studies or process of samples. Al-Hashimi in his study on *Hibiscus sabdariffa* calculated the diameter of inhibition zone of alcoholic extract (30 mm). This rate was 14.16 mm in this study [12]. Iranian indigenous teas and Iranian type of *Streptococcus mutans* used in this study as an innovation can explain the difference in results of this study and other studies. The test results also showed that green tea at low concentrations also has its own bactericidal effect. MBC was observed in 1:8 (12.5 mg/ml) concentration of green tea, while it was observed for hibiscus tea in a higher concentration (1:2 (50 mg/ml)). The minimum concentration in which green tea could inhibit bacterial growth (MIC) was 1:16 (6.25 mg/ml), which was observed for *Hibiscus sabdariffa* at a concentration of 1:4 (25 mg/ml). In a study in Portugal on compound of two green tea and black tea, MBC was reported at 12.5 mg/ml, similar to the present study, while MIC was slightly different [13]. Sulistyani et al., reported MBC for hibiscus tea at 57.6 mg/ml and MIC at 7.2 mg/ml, while above study reported MBC and MIC at 150 mg/ml and 25 mg/ml, respectively [14]. Differences in polyphenol content of two plants can be attributed to the difference in their antimicrobial properties. Epigallocatechin and Epigallocatechin-3-Gallate are known as the most active type of polyphenols in green tea [14]. Epigallocatechin-3-Gallate exerts its antibacterial effect by binding to peptidoglycan of bacterial wall [15]. Since the *Streptococcus mutans* cell wall is rich in peptidoglycan [16], the antimicrobial effect of green tea on this bacterium is explained in this way. On the other hand, studies have shown the effect of green tea polyphenol on bacterial membrane [17]. It has been observed that the polyphenol present in green tea has been able to damage cell membrane of *E. coli* by altering the gene expression [18]. Antibacterial compounds of hibiscus tea also include polyphenolic compounds. These compounds interfere with functioning of cell membrane of the bacteria [19]. Antibacterial properties of this plant have been compared with Streptomycin; it has been effective against *E. coli* in contrast to streptomycin [20]. In addition to its anticariogenic role, the polyphenol content in tea has a significant effect on *Enterococcus faecalis*, an important bacterium in endodontic infections and has shown its antimicrobial effect [21]. Properties of hibiscus tea are also not limited to antibacterial compounds. It has been shown in a study that this herbal compound is also effective on *Candida albicans*, the most important oral and dental fungus [22]. All of the above points to the fact that these two teas are not only effective in anticariogenic aspect, but also in other oral hygiene areas.

CONCLUSION

Considering the acceptable results of green tea samples compared to chlorhexidine control, as well as anticariogenic role of hibiscus tea against *Streptococcus mutans*, it is suggested to use these herbal compounds based on their anticariogenic properties. Green tea is suggested in areas where this plant is common and easily found, including Iran. Compounds containing polyphenol, such as gums, toothpastes and mouthwashes can be used more extensively. Effective advertising can be planned for this herbal compound through mass media. Moreover, the benefits of these two teas can be noted in textbooks. School advisers need to play an effective role in introducing these two teas to students. Since green tea is a product of northern Iran and hibiscus tea is a product of the southern regions of Iran, the government can support the agricultural sector and direct the agricultural industry towards production of these valuable products.

POTENTIAL PROBLEMS

This study was done without any problems at the writing and testing stages.

ACKNOWLEDGEMENT

The authors appreciate Dr. Zohreh Gholizadeh Siahmazgi and the knowledge-based research center of Varna Paya Project Maham (VPPM) for their assistance in carrying out the laboratory and biochemical steps of this study and Dr. Badri Abbasi, Assistant Professor of Higher Edu-

cation Management at Free Islamic University, for technical and statistical editing of this study.

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