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Black and green tea antimicrobial effect on Mutans streptococci and Lactobacilli

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Abstract

Background Dental caries continues to be a major public health problem in many countries. The aetiology of dental caries is multifactorial and it is widely accepted that Mutans streptococci and Lactobacilli play major role in the formation of dental caries through adhering to the tooth surface and producing acid from dietary sucrose.

Objectives Finding affordable natural product with anti-cariogenic potential will have a great impact on dental caries management worldwide. There is some published evidence supporting the antibacterial activity of green tea (GT) but studies of black tea (BT) are scanty. This preliminary study aimed to test the activity of aqueous and alcoholic extracts of BT and GT in comparison with sodium fluoride, chlorhexidine and distilled water, on clinical isolates of Mutans Streptococci (MS) and Lactobacilli (LB). **Materials and Methods** In the in-vitro study, MS and LB sensitivity and viable counts were tested with different concentrations of tea extracts. The minimum bactericidal concentration (MBC) of the tea extract, and the time required for killing MS and LB in the presence of tea extracts were determined. An in-vivo study of sequential saliva samples collected from 30 volunteers after rinsing with the aqueous extracts was conducted, to investigate the longevity of tea extract as an antibacterial agent in the mouth. **Results** MS and LB counts were reduced with tea aqueous extract progressively with time. The largest inhibition zone was produced by 50% GT aqueous extract. LB least viable count was recorded at 30% BT aqueous extract. MBC was 35% and 30% for BT and GT extracts respectively. The maximum reduction of MS and LB viable counts was at 30 and 15 minutes respectively, after rinsing with 50% GT aqueous extract. **Conclusion** Tea extract has the potential to reduce MS and LB viable

counts, and can be incorporated in the dental products as an effective anti-cariogenic agent.

Keywords: Mutans streptococci, Lactobacilli, black tea, green tea, antibacterial.

Introduction

Dental caries continues to be a major public health problem in many countries (Bagramian et al., 2009). The aetiology of dental caries is multifactorial and is challenging to manage despite of having innovative approaches in the 21st century. It is widely accepted that Mutans streptococci (MS) and Lactobacilli (LB) play major role in the formation of dental caries through adhering to the tooth surface and producing acid from dietary sucrose. Recently, several studies have explored the potential effect of the natural products to prevent dental caries, including tea (Awadalla et al., 2011, Antonio et al., 2012). Tea, as one of the most popular, affordable and widely available drinks worldwide, would be an efficient and convenient public health intervention, if proved to have an anti-cariogenic activity.

Green tea (GT) has been shown to have a direct bactericidal effect on S mutans via one of its components, catechins (Neturi et al., 2014, Rasheed and Haider, 1998), however the concentration at which the tea would have an inhibitory effect has not been clearly reported (Rasheed and Haider, 1998, Anita et al., 2014, Hegde and Kamath, 2017). GT has also been shown to interact with bacteria and inhibit its adherence to teeth (Otake et al., 1991). Therefore, there is some evidence that GT has an anti-cariogenic effect in humans (Hirasawa et al., 2006, Carvalho et al., 2016, Thomas et al., 2016), however, the evidence for the anti-cariogenic effect of BT is lacking. The aims of this study are to investigate the antibacterial effect of aqueous and alcoholic GT and BT extracts, in comparison with known antimicrobial agents; sodium fluoride (NaF) and chlorhexidine gluconate (CHX) in-vitro and also to assess the aqueous extract of BT and GT as a mouthwash for adults in inhibiting cariogenic bacteria in-vivo.

Materials and methods

The study was conducted according to the Iraqi national standard operative procedures (SOP) for microbiology. An ethical approval for the study in accordance with the Declaration of Helsinki was obtained from the Faculty of Dentistry local Ethics Committee, Baghdad University. Following the ethical approval, verbal consent was obtained from each participant.

Preparation of stock culture

Twenty isolates of MS and LB were obtained under standardised conditions from 20 healthy dental students (13 male and seven female, age 21-23 years), with at least one tooth with dental caries. Participants were asked to refrain from eating and smoking for at least one hr and were given a piece of sugar-free (Samara) natural gum (0.4 - 0.5g) to chew for five min, then saliva was collected in a sterilised screw capped bottles (Hirasawa et al., 2006). The samples were homogenised and diluted with normal saline. A 0.1 ml of two dilutions; 10⁻² and 10⁻³ were cultured in duplicates on glucose-yeast extract-acetic acid agar (Rogosa agar, Merck, Germany) to isolate the LB (Rogosa et al., 1951), and on Mitis Salivarius Bacitracin Agar (MSB) (Difco, MD, USA) to isolate MS (Gold et al., 1973). The plates were incubated aerobically for 48 hrs at 37°C. Colonies were examined directly and under dissecting microscope (magnification x15). Gram staining, catalase production and carbohydrate (mannitol) fermentation tests were conducted on MS and LB, in addition the spore forming and motility tests for LB were assessed to confirm bacterial identification (Koneman E. W. et al., 1992, Krasnow, 2009). MS and LB were purified in same environment and same culture media. MS plates were incubated anaerobically for 48 hrs at 37°C followed by incubation aerobically for 24 hrs at 37°C whilst LB plates were incubated aerobically for 72 hrs. (Nolte, 1982). One colony from each type of bacteria was transferred to 10 ml of sterilised Trypton Soya Broth (TSB) (Oxoid, UK) and incubated for 24 hrs aerobically at 37°C to prepare the stock culture.

Preparation of tea extracts

A 100 grams of commercially available grounded loose dried leaves of 100% BT (Lipton Yellow Label, Sri Lanka) or 100% GT (Twinings, UK) was soaked in 500 ml of distilled boiled water to prepare the aqueous solution, and in 96% ethanol alcohol to prepare the alcohol extraction (Cowan, 1999). Concentrations of 10%, 20%, 30%, and 50% were prepared and filtered by millipore filter size 0.22µm (Merck, Germany).

In-vitro experiments

The effect of the tea aqueous extract on viable counts

The effect of different concentrations of BT and GT aqueous extract on the growth of MS and LB was tested by using agar dilution method and compared to control plates. Final concentrations of 1%, 5%, 10%, 20%, and 30% of BT and GT aqueous extracts were obtained in the Rogosa and MSB agar plates. A suspension of 0.1 ml from dilutions 10⁻², 10⁻⁴ of MS and 10⁻², 10⁻³ of LB, were spread in duplicates onto the tea MSB and Rogosa agar plates respectively. MSB plates were incubated at 37°C for 48

hrs anaerobically, followed by 24 hrs of aerobic incubation and Rogosa plates were incubated at 37°C for 48 hrs aerobically and the colony-forming units per ml (CFU/ml) were counted. This procedure was duplicated on control plates without any aqueous tea extract

The inhibitory effect of the tea extract on MS and LB compared with CHX and NaF.

The MBC of BT and GT aqueous and alcoholic extract was determined by preparing solution of 1%, 5%, 10%, 20%, 30%, 35% and 50% of BT and GT separately, inoculated with 0.1 ml of activated isolates of MS and LB and incubated at 37°C for 24 hrs. The positive control for this experiment was TSB with bacterial inoculum only and the negative control bottles contained TSB and BT or GT extract, without bacterial inoculum. After 24 hrs. incubation period, an inoculum from each bottle was streaked on tryptone soya agar (TSA) and incubated aerobically at 37°C for 24 hrs.

The lowest concentration of tea extract that showed bactericidal effect was selected as the MBC. Agar well diffusion technique was then applied to assess the antibacterial effect of the MBC tea extract concentrations compared with 2% NaF and 0.2% CHX (Corsodyl™, GlaxoSmithKline PLC, UK). Wells of equal size and depth were prepared on MSB, Rogosa and Mueller Hinton Agar (Oxoid) plates, and each filled with 40µl of one of the study agents. The plates were then inoculated with MS or LB and incubated at 37°C for 24 hrs. The antibacterial effect of each agent was estimated by measuring the inhibition zone around the relevant well.

Time-kill study

The time-kill method was used to compare the in-vitro activity of 35% BT and 30% GT on MS and LB. A suspension was prepared by mixing the bacterial culture with the aqueous extract of BT or GT. Dilutions were prepared at 1, 3, 5, and 30 min. From dilutions 10⁻² and 10⁻³, 0.1 ml was spread in duplicates on TSA and incubated aerobically at 37°C for 48 hrs to assess bacterial growth as follows:

- +++ : Heavy growth of bacteria (>100 colonies/plate).
- ++ : Moderate growth of bacteria (50-100 colonies/plate).
- +: Mild growth of bacteria (10-50 colonies/plates).
- : No growth (<10 colonies /plate)

In-vivo study

The effect of tea aqueous extract on salivary counts of MS and LB

Thirty healthy dental students (17 female and 13 male, age 21-23 years) were recruited according to the exclusion and inclusion criteria. Participants were randomised into three groups of ten subjects each; BT, GT and distilled water (DW).

Inclusion criteria

- No relevant medical history
- Had not been on any antibacterial agents for at least two weeks prior to the study
- Dentate with no prosthesis or orthodontic appliances
- Participants with at least one dental caries

Exclusion criteria

- Pregnant and/or lactating females.
- No active primary dental caries
- Chronic/aggressive periodontal disease.
- Purulent exudates, tooth mobility, and/or extensive bone loss risking the prognosis of the test tooth
- Any other significant disease or disorder which, in the opinion of the investigator, may either put the participants at risk because of participation in the trial, or may influence the results, or the participant's ability to participate
- Participants who were prescribed long-term systematic antibiotics
- Participation in another dental study testing different dental products during the previous three months
- The use of topical agents that may affect the results such as mouthwash, chewing gum and lozenge with active ingredients or antibacterial agents

Participants were asked to suspend all oral hygiene practices on the day of the study starting at 8 am, and to refrain from eating, drinking and smoking until the tests completed. Stimulated saliva was collected, by asking participants to chew a piece of sugar-free gum for five min at 9:00 am. Then each volunteer was asked to rinse with 10 ml of 50% aqueous extract of BT, GT, or DW for 3 min and to expectorate the solution. Stimulated saliva was re-collected at 1, 15, 30 and 60 min from the time of expectoration. Saliva samples were immediately dispersed for two min by vortex mixer and tenfold dilutions were prepared. From dilutions 10⁻² and 10⁻³, 0.1 ml was spread in duplicates on MSB and Rogosa agar plates and incubated as described above. MS and LB viable counts were measured as colony-forming unit per millilitre of saliva

(CFU/ml).

Statistical analysis

Calculation of the statistical parameters; mean, standard deviation (SD), analyses of variance (ANOVA), Student's paired t-test were employed using SPSS statistical package (IBM, NY, USA), and adjusted at 95% ($p < 0.05$) confidence interval. Power calculation was performed using StatMate statistical pack (GraphPad, USA) revealing a 95% power to detect a difference between means of 4.37 with a significance level (alpha) of 0.05 (two-tailed) for a sample size equal to 20 in each group.

Results

In-vitro study

The effect of the tea aqueous extract on viable counts

The MS total viable count tested with GT was significantly less than that tested with BT in all concentrations, and the most significant difference was found at 30%. Similar pattern was seen for LB; the counts with GT were less than with BT. The most statistically significant difference in the viable counts was found with 30% dilution of GT and BT (Table 1).

Table 1: Effect of BT and GT aqueous extract on MS and LB total viable count (in vitro).

| Bacteria | Percent concentration | Aqueous extract | Mean count $\times 10^3$ | SD | t-value | p-value | Description |
|----------|-----------------------|-----------------|--------------------------|--------------|---------|---------|-------------|
| MS | 1% | BT GT | 161.6 147.7 | 61.6 62.5 | 0.50 | 0.62 | NS |
| MS | 5% | BT GT | 132.1 79.8 | 51.0 33.5 | 2.71 | 0.016 | S |
| MS | 10% | BT GT | 82.8 51.6 | 30.1 25.1 | 2.52 | 0.022 | S |
| MS | 20% | BT GT | 39.3 25.8 | 14.2 10.3 | 2.44 | 0.027 | S |
| MS | 30% | BT GT | 22.8 7.2 | 7.57 3.46 | 5.93 | 0.000 | HS |
| LB | 1% | BT GT | 179.4 172.0 | 64.4 68.1 | 0.25 | 0.81 | NS |
| LB | 5% | BT GT | 144.6 95.2 | 54.1 32.3 | 2.48 | 0.027 | S |
| LB | 10% | BT GT | 87.3 52.3 | 33.6 19.1 | 2.86 | 0.013 | S |
| LB | 20% | BT GT | 47.3 27.2 | 21.3 10.7 | 2.66 | 0.020 | S |
| LB | 30% | BT GT | 24.2 8.50 | 10.4 4.35 | 4.41 | 0.000 | HS |

NS: Not Significant difference at level $P > 0.05$.
 S: Significant difference at level $P < 0.05$.
 HS: Highly significant difference at level $P < 0.01$.
 MS: Mutans streptococci; LB: Lactobacillus, BT: black tea; GT: green tea.

The inhibitory effect of different concentrations of the tea aqueous extract on MS and LB

The MBC of BT and GT aqueous and alcoholic extract was 35% and 30% respectively. For the aqueous extract, there was a direct positive relationship between the diameter of the inhibition zone and the concentration of the extract. The mean diameter of the inhibition zones of 50% BT and GT extract was significantly more than other concentrations. A significant difference between 10% and 20% concentrations was observed for BT and GT. However, there was no significant difference between 30% and 50% concentrations (Table 2).

Figure 1 displays inhibition zones of tea aqueous extract in comparison with CHX and

NaF. Similar pattern was observed for the LB, where the mean diameter of inhibition zones of 10% BT concentration was less than that of 50% BT. There was also significant difference among all GT concentrations against LB, and that the 50% concentration achieved larger inhibition zones than the other concentrations (Table 2).

Table 2: Comparison between BT and GT aqueous extract and alcoholic extract of each tested concentration against the sensitivity of MS and LB.

| Bacteria | Percent Concentrate | Aqueous extract | Mean (mm) | SD | t-value | p-value | Description |
|----------|-----------------------|-------------------|-----------|------|---------|---------|-------------|
| MS | 10% | BT | 8.00 | 1.08 | 6.74 | 0.000 | HS |
| | | GT | 11.82 | 1.43 | | | |
| MS | 20% | BT | 15.21 | 2.15 | 3.13 | 0.006 | HS |
| | | GT | 18.81 | 2.94 | | | |
| MS | 30% | BT | 19.84 | 1.45 | 1.05 | 0.31 | NS |
| | | GT | 20.87 | 2.75 | | | |
| MS | 50% | GT | 22.39 | 2.23 | 1.50 | 0.15 | NS |
| | | BT | 24.01 | 2.60 | | | |
| LB | 10% | BT | 8.14 | 0.76 | 8.10 | 0.000 | HS |
| | | GT | 15.90 | 2.93 | | | |
| LB | 20% | BT | 10.90 | 1.05 | 12.55 | 0.000 | HS |
| | | GT | 19.90 | 2.01 | | | |
| LB | 30% | BT | 14.40 | 0.74 | 12.61 | 0.000 | HS |
| | | GT | 23.50 | 2.16 | | | |
| LB | 50% | BT | 17.35 | 1.42 | 5.91 | 0.000 | HS |
| | | GT | 24.08 | 3.31 | | | |
| Bacteria | Percent concentration | Alcoholic extract | Mean (mm) | SD | t-value | p-value | Description |
| MS | 10% | BT | 6.57 | 0.42 | 2.43 | 0.029 | S |
| | | GT | 7.22 | 0.73 | | | |
| MS | 20% | BT | 10.79 | 0.50 | 11.08 | 0.000 | HS |
| | | GT | 18.08 | 2.02 | | | |
| MS | 30% | BT | 13.12 | 0.93 | 9.72 | 0.000 | HS |
| | | GT | 19.27 | 1.78 | | | |
| MS | 50% | BT | 14.72 | 1.00 | 6.61 | 0.000 | HS |
| | | GT | 19.95 | 2.29 | | | |
| LB | 10% | BT | 6.59 | 0.56 | 2.45 | 0.028 | S |
| | | GT | 7.43 | 0.93 | | | |
| LB | 20% | BT | 12.10 | 2.18 | 4.01 | 0.000 | HS |
| | | GT | 16.28 | 2.47 | | | |
| LB | 30% | BT | 15.19 | 2.29 | 1.96 | 0.070 | NS |
| | | GT | 17.90 | 3.73 | | | |
| LB | 50% | BT | 16.60 | 2.80 | 2.19 | 0.049 | S |
| | | GT | 18.70 | 1.16 | | | |

NS: Not Significant difference at level $P > 0.05$.
 S: Significant difference at level $P < 0.05$.
 HS: Highly significant difference at level $P < 0.01$.
 MS: Mutans streptococci; LB: Lactobacillus, BT: black tea; GT: green tea.

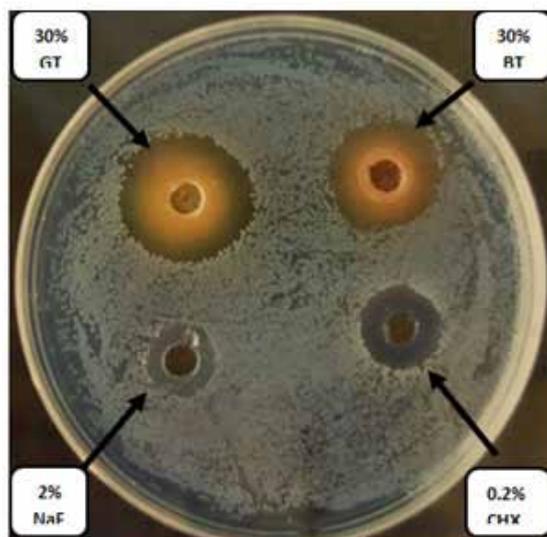


Figure 1: Sensitivity of MS on MSB agar to the aqueous extract of BT and GT, NaF and CHX by agar diffusion technique. GT has better results

The inhibitory effect of different concentrations of the tea alcoholic extract on MS and LB.

For MS, a significant difference was found between BT and GT alcoholic extract with larger inhibition zones given by GT at 10% concentration and a mean diameter of 6.57 ± 0.42 and 7.22 ± 0.73 for BT and GT respectively. Significant difference was recorded between BT and GT alcoholic extract with the larger inhibition zones given to GT extract (Table 2).

The pattern against LB was slightly different with significant difference observed between 10% and 50% concentrations of BT and GT extract with larger inhibition zones for GT, and significant difference at concentration 20%. At 30% concentration, not significant difference was recorded between both tea extracts (Table 2).

The inhibitory effect of the tea aqueous extract on total count of oral microflora.

No statistical significant difference between the GT aqueous extract and the BT in the mean dimension of inhibition zones of 10% concentration (7.30 ± 0.82 ; 6.99 ± 0.43 respectively). While the antibacterial effect of the 50% concentration of GT was superior to the BT (Mean diameter, SD= 21.42 ± 0.69 ; 16.65 ± 0.00 respectively). High significant difference was recorded among 20%, 30% and 50% concentrations of both types of tea against oral microflora. Figure 2 demonstrates the activity of the aqueous extract of both types of tea in comparison with CHX and NaF against oral microflora.

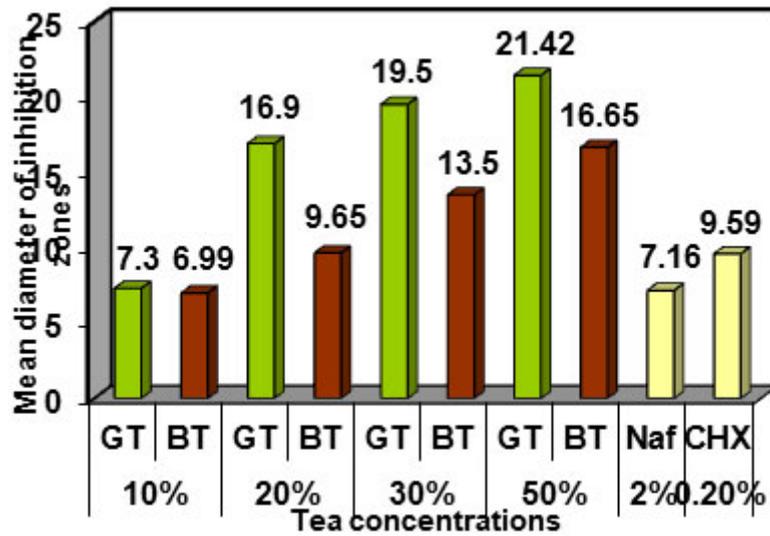


Figure 2: Comparison between the mean diameter of inhibition zones of BT and GT aqueous extracts, CHX and NaF against the sensitivity of oral microflora.

Time-kill study

The growth of MS and LB colonies were significantly reduced in the presence of BT and GT aqueous extract separately. However, findings were time dependent with an ascending decrease of bacterial count when time increased (Figure 3).

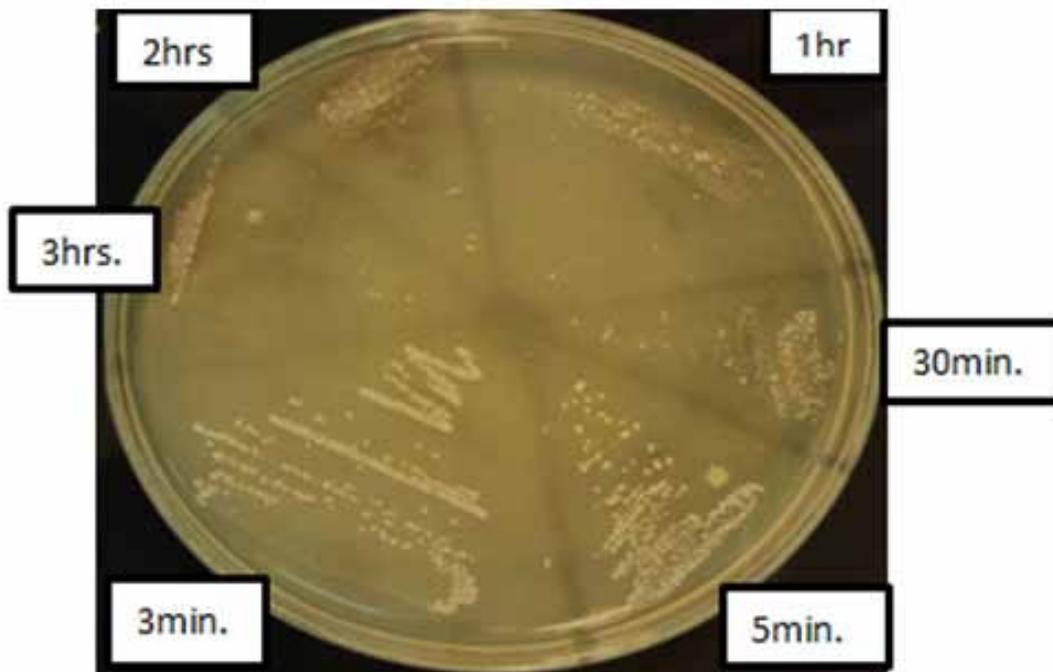


Figure 3: Effect of increase time of mixing 30% of GT aqueous extract on the growth of MS, with the best clearance of bacteria at 3hrs.

In-vivo study

The effect of the tea aqueous extract on salivary counts of MS and LB

All recruited subjects completed the study (n=30). Viable bacterial count was evaluated for the three study groups after single rinse with the selected study agents; BT, GT or DW. The maximum reduction of MS viable count from baseline count was significantly seen after rinsing with 50% GT at 30 min time point. Slightly different pattern was shown with LB, where the maximum significant reduction of the total count was at 15 min (Figure 4 and Table 3).

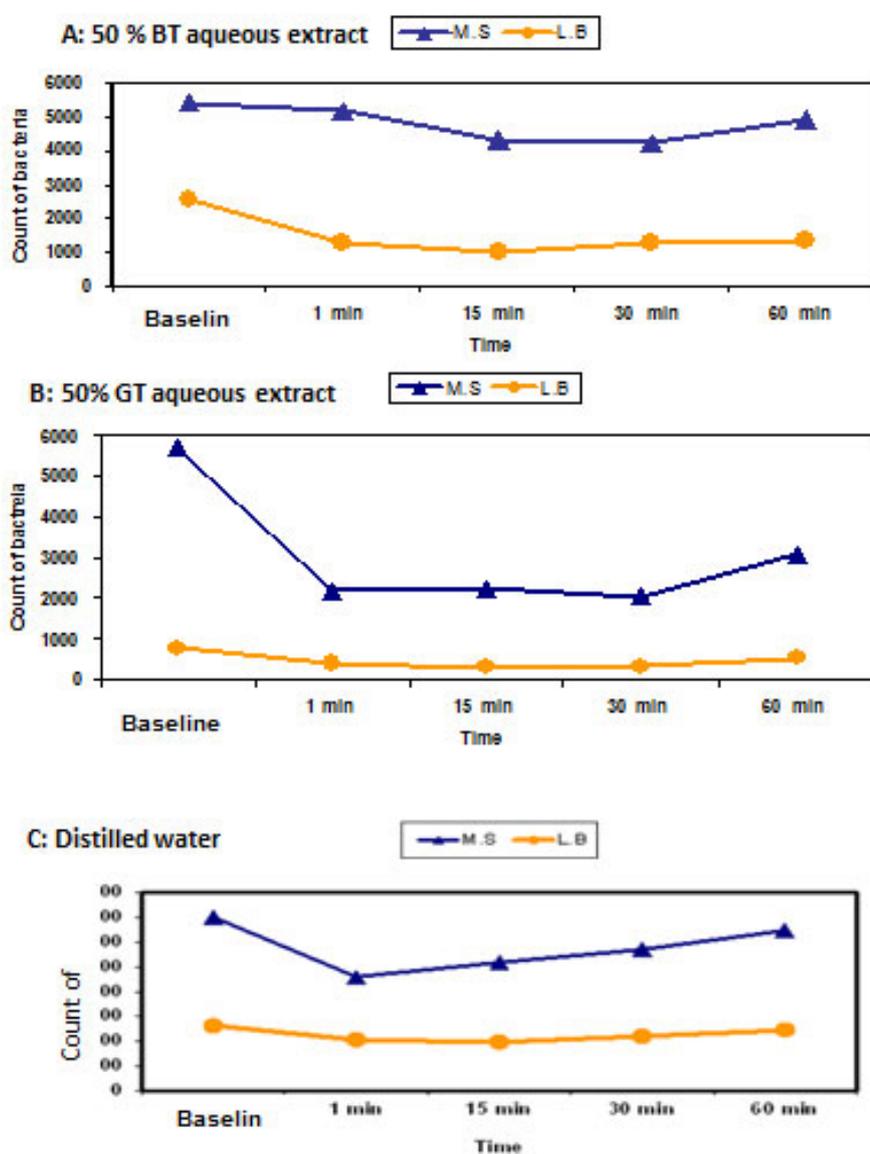


Figure 4: A. Effect of rinsing with 50 % BT aqueous extract on the viable count of MS and LB, B. Effect of rinsing with 50% GT aqueous extract on the viable count of MS and LB at each time point, C. Effect of rinsing with DW on the viable count of MS and LB.

Table 3: Comparison of the effect of rinsing with 50% BT aqueous extract on the viable count of MS and LB at each time point.

| Antibacterial agent | Time points | Between | Mean Count | SD | t-value | p-value | Description |
|--|-------------|----------|--------------|--------------|---------|---------|-------------|
| 50% BT aqueous extract | 1 min. | MS LB | 5195 1237 | 2251 1351 | 4.77 | 0.000 | HS |
| | 15 min. | MS LB | 4335 1010 | 2154 997 | 4.43 | 0.000 | HS |
| | 30 min. | MS LB | 4209 1233 | 1875 1414 | 4.01 | 0.001 | HS |
| | 60 min. | MS LB | 4920 1326 | 1876 1761 | 4.42 | 0.000 | HS |
| 50% GT aqueous extract | 1 min. | MS LB | 2182 403 | 1151 262 | 4.77 | 0.001 | HS |
| | 15min. | MS LB | 2250 286 | 1279 241 | 4.77 | 0.001 | HS |
| | 30 min. | MS LB | 2048 332 | 1225 228 | 4.35 | 0.001 | HS |
| | 60 min. | MS LB | 3075 533 | 2131 413 | 3.70 | 0.004 | HS |
| HS: highly significant difference at level $P < 0.01$. Count of MS $\times 10^3$ Count of LB $\times 10^2$ MS: Mutans streptococci; LB: Lactobacillus, BT: black tea; GT: green tea. | | | | | | | |

Discussion

This preliminary study was conducted to screen the antimicrobial properties of two types of tea extract against two cariogenic bacteria. BT and GT have similar amounts of flavonoids which known as potent antibacterial substances. These flavonoids have the ability to bind and precipitate macromolecules such as bacterial enzymes that affects the metabolic activity of bacteria. GT tea leaves reserve more catechins (simple flavonoids) than BT. While most of BT components are oxidised during the process of fermentation to become a complex mixture of polymerised catechins (theaflavins and thearubigens) which have less antimicrobial activity (Lee et al., 2002, Frei and Higdon, 2003, Cabrera et al., 2006). Other active constituents of tea extract are alkaloids, and tannins (van het Hof et al., 1999). It was reported that the alkaloids interfere with microbial cell division (Olaleye, 2007). Flavonoids have anti-glucosyl-transferase activity that prevent microbial adherence, i.e., anticaries effect (Yousef Yari Kamrani 2007, Li et al., 2004). Tannins also have an antibacterial growth capacity with their iron-binding power; to inhibit bacterial adherence and glucocyltransferase activity (Akiyama et al., 2001).

To date, a limited number of studies assessed these complex procedures to obtain a final purified organic extract (Hirasawa et al., 2006, Araghizadeh et al., 2013, Anita et al., 2014, Jazaeri et al., 2015, Naderi et al., 2011). In this study, two commonly consumed types of tea were extracted using the various laboratory procedures and comparing their antibacterial activity against MS and LB.

In our study, the crude extracts (aqueous and alcoholic) of both types of tea, recorded inhibitory effects to the growth of MS and LB, these findings agree with others (Touyz and Amsel, 2001, Sasaki et al., 2004). However, GT crude extract showed better inhibitory effect on LB than BT. This can be attributed to the presence of higher amounts of alkaloids and phytochemicals in GT as compared to BT. These findings are consistent with our earlier findings which reported that LB are sensitive to the alkaloids extracted from the seeds of the *Peganum harmala*, which is a popular plant in the Middle East to be used for folk medicine and spiritual practices (Al-Izzy, 2010). Our findings disagree with others, which reported that BT had equal inhibitory effect with GT extract (Spratt et al., 2012). Zones of inhibition on the aqueous extract plates were higher than those seen on plates of alcoholic extract. This indicates that the amount of antibacterial substances obtained from the aqueous extract is more than that obtained from alcoholic extract. Additionally, water-soluble substances of tea leaves are more potent than alcoholic soluble substances in their antibacterial activity. Sensitivities of LB to CHX and NaF were tested, and NaF was a weak antibacterial agent in comparison to CHX. The antibacterial effect of 2% NaF was enhanced by increasing its concentration. Fluoride in tea was thought to contribute to the inhibition of dental caries (Moezizadeh, 2013). However, the low levels of fluoride in BT (99.74 mg/kg) and GT (63.04 mg/kg) were reported, suggesting the presence of active substances other than fluoride that may eventually inhibit dental caries (Peng et al., 2016). It should be noted that tea aqueous extract at 50% concentration had significantly better results on the tested bacteria compared to 0.2% CHX and 2% NaF. Our results are consistent with previous studies on the effectiveness of GT on cariogenic bacteria (Tehrani et al., 2011, Anita et al., 2014).

BT and GT aqueous extract reduced the total viable count of MS and LB colonies in vitro. GT aqueous extract had significant results than BT extract in reducing LB viable count. These findings are in agreement with others who suggested that the fermentation process of BT leaves, changes its antibacterial activity (Rasheed and Haider, 1998). The opposite pattern was seen for MS (Naderi et al., 2011, Matsumoto et al., 1999).

LB isolates were sensitive to BT aqueous and alcoholic extract at 35% concentration. While at 30% concentration, GT aqueous and alcoholic extract was able to inhibit the

tested bacteria. The slightly better action of GT, denotes the greater antimicrobial constituents occur in the crude GT extract. This finding is in agreement with other published papers (Anita et al., 2014). It was noticed that a concentration of 10% of alcoholic extract had better results than higher concentrations. This can be referred to reaching the optimum saturation levels at higher concentrations. At high concentrations, the solutions became sticky and thicker that could not be diffused through the agar molecules. The 5% concentration of alcohol that was used in the preparation of alcoholic extract has no additional antimicrobial effect in our study. The role of this proportion of alcohol is only to dissolve the organic ingredients of the leaves. Tea aqueous extract has a time-dependant antibacterial agent. This can be attributed to the time required for tea molecules to bind to the microorganisms. These findings were in agreement with previously published study where significant reduction in the bacterial count from baseline was found with increasing incubation time with GT extract. However, there is no data available on the activity of BT extract in a time kill study (Farooqui et al., 2015). This study is the first to highlight the relation of bacterial count with period of incubation with BT aqueous extract.

In in-vivo study, the aqueous extract of tea was chosen over alcoholic extract, since the former showed larger inhibition zones in the agar well technique in the current study. Furthermore, alcoholic-containing mouthwash may pose ethical and cultural issues for participants. A concentration of 50% for both types of tea aqueous extract was used, as inhibition zones were maximised by 50% on oral microflora, MS and LB. Both types of tea extracts had potent antibacterial activity against MS and LB, however MS being more sensitive than LB. The viable bacterial count at baseline of each participant was significantly minimised after rinsing with both tea extracts. Furthermore, it was shown that the adherence ability of MS to the tooth surfaces was inhibited by the presence of tea extract, despite the lack of adsorbing properties of tea on teeth (data not presented). Our results are in line with previous literature and highlight the importance of tea as a useful agent in oral hygiene maintenance (Esimone et al., 2001). To the best of our knowledge, our study is the first to examine BT aqueous extract as a mouthwash agent against pure isolates of MS microorganisms, and to compare it with that of GT. Studies with experiments of longer periods monitoring for the sustainability of the tea extract are needed. The results of the current study were derived from concentrated extracts of BT and GT. Therefore, the outcome may not be applied on the common practice of drinking black or green tea. In fact, it can be speculated that adding sugar to tea may counteract its antibacterial effect.

The slight difference in the antimicrobial potency of the two types of tea aqueous extract could be due to the simple catechins (Polyphenols of low molecular weight) that

are profusely present in GT leaves in comparison to BT leaves. These catechins are oxidised and condensed to larger, dark coloured molecules during the fermentation process of BT leaves, which led to the reduced quantity of simple catechins in BT. The current study demonstrated a reduction of bacterial count in saliva for up to 60 min, after a single rinse with tea aqueous extract. The maximum decline of MS was recorded after 30 min and for LB after 15 min after rinsing with GT aqueous extract. All participants commented on the bitter taste, astringent sensation and tooth discolouration related to the high concentration of tea. The staining of the teeth after the long term use of mouthwash with high concentration of tea extracts is usually expected (Bergesch et al., 2017).

While the present study has provided useful information using natural products for dental care, study limitations must be acknowledged. The study was carried out in accordance to the local national standard operative procedures (SOP) for microbiology in Iraq, which could be different from the SOP in other countries. Moreover, the tested bacteria were not identified to the species level. Furthermore, the alcoholic extract of tea has not been tested in the in vivo study due to cultural norms. Additionally, the crude extract was used instead of extracting active substances (polyphenols) of the tealeaves. Employing the latter, could have avoided the reported side effects of teeth discolouration and bitter taste. In the in-vivo study, participants were assessed in one occasion. Finally, tea extract was prepared in high concentrations (30% and 50%) that may not be possible to be consumed on daily basis.

Conclusion

The crude extracts (aqueous and alcoholic) of BT and GT at high concentrations had inhibitory effect on oral bacteria. The tea extract at 50% concentration was more effective than 0.2% CHX and 2% NaF in reducing bacterial counts. The extract can be incorporated into dental products such as mouthwash, gel, irrigation solution for root canal treatment in clinical practice. Therefore, controlled randomised double blind clinical trials were required to provide robust evidence for the effectiveness of using tea extracts as an antibacterial agent and for caries prevention.

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Conflict of interest Authors of this work declare no conflict of interest.

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