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Hagar Abdel Tawab

Dentist at Ministry of Health, Egypt., hagar_dent@yahoo.com

Maha Niazy

Professor of operative dentistry Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt, drniazy_maha@hotmail.com

Doaa El Elsharkawy

Assistant professor of Operative Dentistry Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt, doshar@yahoo.com

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Antibacterial Effect of Ginger, Green Tea and Pomegranate versus Chlorhexidine using Stevia and Sucrose Sugar

Hagar A. Abdel Tawab^{1*}, Maha A. Niazy², Doaa A. El Elsharkawy³

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azhardentj@azhar.edu.eg

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ABSTRACT

Purpose: To evaluate the antibacterial activity of three natural plant extracts (Ginger, Green tea and Pomegranate peel) versus Chlorhexidine using Sucrose and Stevia Sugar. **Materials and Methods:** extracts of the three natural materials were prepared where an aqueous extract of green tea was prepared by boiling, and pomegranate peel and ginger were extracted using ethyl alcohol. Carious dentin samples were obtained from carious permanent molars. *Streptococcus mutans* were isolated and identified. The extracts were compared to chlorhexidine using either no sugar, sucrose or stevia sugar. Antibacterial activity was assessed through the inhibition zone, bacterial count and metabolic activity. Ginger, pomegranate peel, green tea extracts, and chlorhexidine were individually tested against the *S. mutans*. Inhibition zone test was done by the agar well diffusion method. Then, Colony forming units were counted. MIC of tested extracts was determined by a MTT micro-well dilution method. The results were statistically analyzed and the significance level was set at $p \leq 0.05$. **Results:** All groups showed a significant antibacterial effect. Chlorhexidine had the highest value followed by Pomegranate peel then Ginger while Green Tea had the lowest value. Medium with Stevia Sugar had the highest antibacterial effect followed by medium without sugar while medium with Sucrose sugar had the least effect. **Conclusion:** Pomegranate peel, ginger, and green tea could be considered as antibacterial agents against *Streptococcus mutans*. Also, Stevia sugar can be considered as a non-cariogenic agent.

INTRODUCTION

Dental caries is one of the most widely recognized diseases influencing individuals. If significant oral environmental changes have occurred, such as sugar availability increased, the acidogenic and aciduric bacteria become

KEYWORDS

Antibacterial activity,
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1. Dentist at Ministry of Health, Egypt.

2. Professor of operative dentistry Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

3. Assistant professor of Operative Dentistry Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

* Corresponding author email: hagar_dent@yahoo.com

the most prominent members of dental biofilm. One of the cariogenic bacteria is *Streptococcus mutans*⁽¹⁾. It can survive in a low pH and produce huge amounts of acid by using carbohydrates, which help the demineralization of enamel. Sucrose also can be utilized by *Streptococcus mutans* to produce extracellular polysaccharides by glucosyltransferases⁽²⁾.

Sugar intake such as sucrose is a responsible factor in caries development. Sucrose is considered the highest cariogenic factor in the caries progression^(3,4). Sucrose is fermented by oral bacteria and also enhances the growth and colonization of *Streptococcus mutans*⁽⁵⁾. Additionally, sucrose acts as a substrate for the production of EPSs in dental biofilms⁽⁶⁾. Extracellular polysaccharides improve the stability of biofilm matrixes and physical integrity of biofilm matrixes, which indicate that EPSs is a virulence factor related to cariogenic biofilm formation⁽⁷⁾.

Given the incidence of oral disease, several chemical agents are available, these chemicals can change the oral environment and have side effects such as tooth staining, vomiting, and diarrhea. So, there is an increased demand for substitute treatment options which are safe and effective. Thus, the search for natural products extracted from plants used as popular medicines come as great alternatives⁽⁸⁾.

It is demonstrated that natural plants possess antibacterial activity against most bacteria including cariogenic bacteria. Phytochemicals for the treatment and prevention of periodontal diseases are identified, such as tannins, terpenoids, flavonoids, alkaloids, etc. Antimicrobial action of these have been found to be useful for dental caries prevention⁽⁹⁾.

Therefore, the evaluation of three natural plants was done to evaluate the antibacterial activity of them against *streptococcus mutans*, and also to examine the role of different sugars in fighting cariogenic bacteria. The null hypothesis is that the pomegranate peel, ginger and green tea individually and with different types of sugars have no effect on the oral cariogenic bacteria.

MATERIALS AND METHODS

Collection of carious dentin samples

Egyptian adult patients were included in this study. Ethical approval was obtained from the Research and Ethics committee of the Faculty of Dental Medicine of Al-Azhar University (Girls Branch), Cairo - Egypt. The study was explained to the patients and they signed an informed consent denoting their consent to take part in the study. Also, verbal consent from the patients was obtained.

Different dentin samples were obtained from carious permanent molars. An occlusal carious cavity was accessed and caries was removed till leathery dentin was reached. Carious dentin samples were obtained from the center of the floor of the carious cavity using sterile sharp spoon excavator, with care not to touch the adjacent enamel to prevent contamination.

The dentin quantity removed was precisely sufficient to cover the surface of the excavator. Carious dentin samples were immediately placed into sterile eppendrofs then filled with isotonic saline to reach 0.5ml for further microbiological analysis maximum time within 2 hours.⁽¹⁰⁾

Preparation of dentin samples:

All collected dentin samples were used for isolation of *streptococcus mutans*. The carious dentin samples were homogenized by vortex mixer (2000 rpm) for 30 seconds to disperse the bacteria from dentin samples to saline. One microliter of the suspension was diluted in 99 micro L. distilled water to give a dilution of 1/100 of the original suspension using automatic micropipette⁽¹¹⁾. All microbiological processing was done by one operator to ensure standardization. The dentin samples were cultured on Mitis salivarius bacitracin agar for identification and enumeration of *S. mutans* colonies.

Isolation and identification of *Streptococcus mutans*:

10 µL of the diluted samples were uniformly spread on the MSBA plate's surface. The plates were sealed and incubated anaerobically and supplied in an anaerobic jar, for 48 hours at 37°C followed by aerobic incubation for 1 day at 37°C. Transferring of single colony from SM to 10 ml sterile Brain heart infusion broth was done and activation of inoculums were done by incubation aerobically which took 24 hours at 37°C. Colonies of *S. mutans* were identified based on its unique morphology on MSBA. Raised, convex, opaque colonies of dark blue color with rough margins and granular frosted glass appearance were identified as *S. mutans*. Under microscopic examination. *S. mutans* appeared as Gram positive cocci arranged in chains.

Extraction of natural agents:***Extraction of Ginger:***

Ginger was purchased from local market of medical herbs. The plants were washed, peeled, cut and dried at room temperature for 48 h, then stored in a plastic zip bag in 4°C until use ⁽¹²⁾. The small sections of a ginger plant were ground to coarse powder by using an electric blender. To obtain a ginger extract, 10 gm. were weighed by a sensory balance and were used for extraction by adding it to 100ml of each of methanol, ethanol and ethyl acetate at room temperature. The extracts were passed through a sterile filter paper ⁽¹³⁾. The filtrates were exposed to 40 °C in a hot air oven for evaporation of water ⁽¹⁴⁾. After that, the filtrates were concentrated with a rotary evaporator that is providing reduced pressure. Finally, the extracts were kept at 4°C until needed for use.

Extraction of pomegranate peel:

Fresh pomegranate fruits, that had been previously purchased, were cleaned, dried, after opening the fruit, the arils were separated from the peels. The collected peels were then rinsed with tap water, cut into small sections and left in an oven to dry out

at 40°C for 24h. The peels were ground into powder using an electric blender ⁽¹⁵⁾. The fine powder was then sieved through 24-mesh and maintained in an air-tight plastic bag in room temperature. To obtain pomegranate peel extract, 10 gm. of fine powder were weighted and were prepared for extract by added it to 100ml of each of methanol, ethanol and ethyl acetate at 25°C for 24 h in a shaking water bath. The extract was passed over a Millipore filter with a 0.45µm nylon membrane ⁽¹⁶⁾. The extracts were condensed by rotary evaporator. Then keeping at 4°C until use.

Extraction of Green Tea:

Five green tea packets were opened and emptied to supply 2gm/packet to give 10 gm. of green tea leaves. 10 g of green tea was boiled in 100 mL of distilled water in Reflux condenser at 100 °C for 60 minutes. The green tea extract was allowed to cool at room temperature. Then, it was filtrated by Whatman No. 1 filter paper ⁽¹⁷⁾. Evaporation was done to the filtered solution for dryness and concentrated by rotary evaporator to produce a fine powder of green tea extract through the process of evaporation and condensation ⁽¹⁸⁾. Green tea extract was saved in room temperature until used.

Sample grouping:

The groups were divided into five main groups (A0, A1, A2, A3, A4) according to the antibacterial material used, either control group (A0) (no material), Ginger (A1), pomegranate (A2), green tea (A3) and Chlorhexidine (A4). These groups were further subdivided into three subgroups according to Sugar used in media: B0: NO sugar in media. B1: Sucrose sugar in media. B2: Stevia sugar in media.

Antibacterial assays:***Inhibition Zone assay:***

Ginger, pomegranate peel, green tea extracts, and chlorhexidine were individually tested against *Streptococcus mutans*. Three types of media

were prepared for each sugar (No sugar in media, Sucrose sugar and stevia sugar). Antibacterial tests were performed using a method called the agar well diffusion method. The media had cooled and solidified, then wells (6 mm in diameter) were made in the solidified agar after that bacterial inoculum was spread uniformly using a sterile cotton swab on a sterile Petri dish containing Tryptic Soy agar (TSA). The preparation of 100 microliters of extracts were done by dissolving 10 mg of the extracts in 1 ml dimethyl sulfoxide (DMSO). Then, the inoculated plates were incubated for 1 day at 37 C. After incubation, antibacterial activity was assessed by measuring the inhibition zone in millimeters (mm)⁽¹⁹⁾. The measure was performed in triplicate for each sugar.

Statistical Analysis:

Numerical data were explored for normality by checking the data distribution, calculating the mean and median values and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Bacterial count data was positively skewed so log transformation was made. Data showed parametric distribution so; it was represented by mean and standard deviation (SD)

values. Two-way ANOVA was used to see the effect of different variables and their interaction. One-way ANOVA followed by Tukey's post hoc test was used to study simple and main effects.

The Statistics were done for Inhibition zone assay and Count, while MIC results were descriptive statistics.

RESULTS

Bacterial inhibition zone:

Results for comparison of the effect of the antibacterial agents on the inhibition zones are shown in table (1) and figure (1). There was a significant difference between different materials. Chlorhexidine (A4) had the highest (mean±SD) value of bacterial inhibition zone followed by Pomegranate (A2) then Ginger (A1) while Green Tea (A3) had the lowest value.

For all materials, there was a significant difference between different sugars. Medium with Stevia Sugar (B2) had the highest (mean±SD) value of bacterial inhibition zone followed by medium without sugar (B0) while medium with Sucrose sugar (B1) had the lowest value as shown in table (2) and figure (2).

Table (1): Mean \pm standard deviation (SD) of bacterial inhibition zones (mm) for different materials within each sugar.

Medium	Inhibition zone in mm				P-value
	Ginger (A1)	Pomegranate (A2)	Green Tea (A3)	Chlorhexidine (A4)	
No sugar (B0)	15.66±1.52 ^{bc}	19.33±1.52 ^{ab}	14.66±1.52 ^c	23.00±2.00 ^a	0.001*
Sucrose sugar (B1)	11.33±0.57 ^{bc}	14.33±1.52 ^{ab}	9.66±0.57 ^c	16.66±1.52 ^a	<0.001*
Stevia Sugar (B2)	21.33±1.52 ^{bc}	23.33±1.52 ^{ab}	18.366±1.15 ^c	26.66±1.15 ^a	0.001*

Different superscript letters indicate a statistically significant difference within the same row; significant ($p \leq 0.05$).*

Table (2): Mean \pm standard deviation (SD) of bacterial inhibition zones (mm) for different sugars within each material.

Material	No sugar (B0)	Sucrose sugar (B1)	Stevia Sugar (B2)	P-value
Ginger (A1)	15.66 \pm 1.52 ^B	11.33 \pm 0.57 ^C	21.33 \pm 1.52 ^A	<0.001*
Pomegranate (A2)	19.33 \pm 1.52 ^B	14.33 \pm 1.52 ^C	23.33 \pm 1.52 ^A	0.001*
Green Tea (A3)	14.66 \pm 1.52 ^B	9.66 \pm 0.57 ^C	18.366 \pm 1.15 ^A	<0.001*
Chlorhexidine (A4)	23.00 \pm 2.00 ^A	16.66 \pm 1.52 ^B	26.66 \pm 1.15 ^A	0.001*

Different superscript letters indicate a statistically significant difference within the same row*; significant ($p \leq 0.05$).

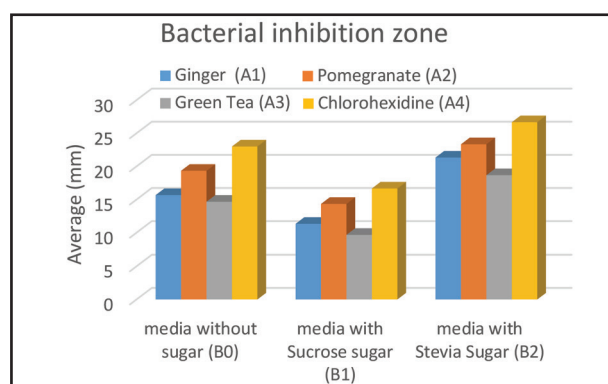


Figure (1) Bar chart showing average bacterial inhibition zones (mm) for different materials within each sugar.

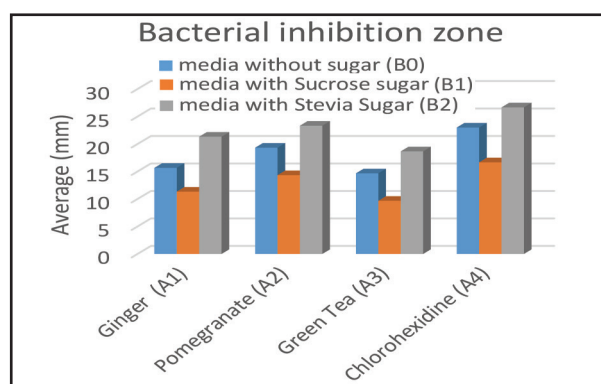


Figure (2) Bar chart showing average bacterial inhibition zones (mm) for different sugars within each material.

DISCUSSION

Dental caries is one of the most common and infectious disease in the world. Biofilms attached to tooth surfaces are basic factors related to this disease. Bacteria such as *streptococcus mutans* unite with the enamel proteins, and then convert sucrose into extracellular polysaccharides, fighting the bacteria through antibacterial agents has been advocated for disease prevention⁽²¹⁾.

In this study, the chlorhexidine mouth wash was chosen as a positive control antibacterial agent because chlorhexidine has been used in more than sixty pharmaceuticals and medical devices. Also, it has proven its broad spectrum potency and safety. Chlorhexidine has become the standard in patient care for the prevention of diseases⁽²²⁾. However a

lot of new researches demonstrated that antibacterial agents used in the treatment of oral diseases are reported to cause staining of teeth, toxicity and in the case of ethanol have been linked to cancer in oral tissue⁽²³⁾.

The increasing demand for alternative products that prevent and treat oral diseases which are effective and safe as natural products which contain biological components^(24, 25). This study examined three natural materials which are: materials; ginger, pomegranate peel and green tea for their antibacterial effects and compared then to chlorhexidine.

On comparing the antibacterial agents, results of this study showed that chlorhexidine agent was the best antibacterial agent in comparison to the other materials using all sugar medium. Its action

includes inhibition of adenosine triphosphatase activity. CHX trigger the precipitation of nucleic acids and proteins in the cytoplasm. Also, it stops the activity of the sugar transport system (phosphoenolpyruvate – phosphotransferase) and inhibits production of acids in *Streptococcus mutans* ^(27, 28).

The results revealed that pomegranate peels Possessed higher antibacterial activity than other natural extracts (ginger and Green tea) and gave best results in Zone of inhibition and with all different sugars. From pomegranate peel polyphenols, the main ingredients in the peel extract are tannins which have antibacterial potential. The tannins are able to cross the cell wall which contains proteins and polysaccharides and bind to its surface. Polyphenols inhibit enzymes by oxidizing agents, they interact with proteins, affect the bacterial cell wall, and disturb coaggregation of bacteria ⁽²⁸⁾.

These results are supported by a study ^(29, 30) which showed that pomegranate peel extracts showed antioxidant activity and antibacterial activity against oral bacteria and it was highly correlated with the total phenolics.

Pomegranate peel was followed by Ginger extract. Ginger's antibacterial activity is mainly due to the presence of gingerols which act as the main active components. In dried ginger powder, a dehydrated product of gingerol which is called shagaol is an active constituent ^(31, 32). Ginger extract has been found to affect the growth of *S. mutans* and *S. sanguinis* in previous studies. ^(33, 34)

Results have shown that green tea had the least antibacterial effect in all tests. Even though green tea had the least effect among the tested materials, it did have some antibacterial effect against the *S. mutans*. Green tea has been shown to have a bactericidal effect on *S. mutans* via one of its components, catechins ⁽³⁵⁾. These flavonoids have the ability to bind and precipitate macromolecules such as bacterial enzymes that affects the metabolic activity of bacteria. Other active constituents of tea extract are alkaloids and tannins ⁽³⁶⁾. One study showed the re-

duction of *S. mutans* counts in plaque with the use of a green tea mouthwash ⁽³⁷⁾.

When comparing the effect of sugars, it is noted the adding Stevia sugar to the tested natural extracts, enhance the antibacterial action more than each natural product alone. On the other hand, adding Sucrose sugar decreased the effect of natural agents and offer less antibacterial results than the natural agents with stevia and also on media with no sugar. The best results of stevia are related to major secondary metabolites. Steviol, stevioside and isosteviol are non-cariogenic, and they can also act as an anticariogenic product ⁽³⁸⁾. The secondary metabolites inhibit glucan induced aggregation of a cariogenic organism. The results are supported by previous research which demonstrated that dental caries was increased in rat pups in presence of sucrose solution while it was not with stevioside ⁽³⁹⁾.

The results of this study proved that pomegranate peel, ginger, and green tea had antibacterial activity against a *Streptococcus mutans* when examined individually and when added to sucrose and stevia sugar and thus the null hypothesis is rejected.

CONCLUSION

Chlorhexidine remains the gold standard against which other antibacterial agents are measured, but natural materials especially pomegranate peel have potential antibacterial activity, and may be considered as a natural alternative to synthetic antibacterial agents. Stevia sugar showed very promising results in fighting caries if used as a sugar substitute to sucrose.

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