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### Comparing The Antibacterial Effect of Psidium guajava Extract, Camellia sinensis Extract and Chlorhexidine Gluconate as Root Canal Irrigants in Primary Teeth: In-Vitro Study

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#### Abstract

##### Aim

The aim of the present study was to compare the in-vitro antimicrobial effect of: 20% Psidium guajava leaves extract, 20% Camellia sinensis leaves extract, versus 2% Chlorhexidine gluconate when used as root canal irrigants in primary teeth roots contaminated with Enterococcus faecalis.

##### Methods

In the study, 65 primary teeth roots were randomly distributed among three experimental groups, one positive control group and one negative control group as follows: Group I: consisted of 15 roots that were contaminated with Enterococcus faecalis and irrigated with 2% Chlorhexidine gluconate, Group II: consisted of 15 roots that were contaminated with Enterococcus faecalis and irrigated with Guava leaves extract, Group III: consisted of 15 roots that were contaminated with Enterococcus faecalis and irrigated with Green tea leaves extract. Group IV (positive control): consisted of 15 roots that were contaminated with Enterococcus faecalis and irrigated with sterile saline. Group V (negative control): consisted of 5 roots that were not contaminated nor irrigated.

Two sterile absorbent paper points were used to absorb the irrigation fluid from each root and transferred to a test tube containing 1 ml of saline for incubation. Colony forming units of Enterococcus faecalis per 1 ml were counted.

##### Results

There was a significant difference among the four tested groups. Positive control group had the highest (Mean±SD) value followed by Guava leaves extract group then Green tea leaves extract group while Chlorhexidine had the lowest (Mean±SD).

##### Conclusions

The three of 2% Chlorhexidine di-gluconate, 20% guava leaves extract as well as 20% green tea leaves extract irrigation solutions have an antibacterial effect against Enterococcus faecalis. 2% Chlorhexidine di-gluconate showed superior efficacy to the other two irrigation solutions against Enterococcus faecalis. Thus, it is preferred in infected canals as an irrigant. Green tea leaves and guava leaves extracts may be used as a natural alternative to Chlorhexidine irrigation in primary teeth.

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## Introduction

The early loss of primary teeth can compromise the development of permanent dentition and lead to psychological problems. The main objective of pulp therapy in primary teeth is to facilitate the proper eruption of permanent successors. (1)

Successful root canal therapy depends on the combination of proper mechanical instrumentation, disinfection and obturation. Of these three essential steps, irrigation of the root canal is the most important in the process of healing. Irrigation results in flushing out debris, dissolving tissue, and disinfecting the root canal system. (2)

Chlorhexidine gluconate is a biocompatible antimicrobial irrigant. However, discoloration of teeth, loss of taste, dryness of the oral cavity and irritation of the oral mucosa limit its use. To counter the ineffectiveness, potential side effects and safety concerns of synthetic irrigants, herbal alternatives have been introduced. Herbal irrigants are easily available and cost-effective. They have long shelf life and low toxicity and lack microbial resistance. (3)

Extracts from *Psidium guajava* species showed significant inhibitory effects against *Enterococcus faecalis*; one of the most resistant microorganisms in the peri-radicular lesions of the oral cavity. (4)

Guava has been used as a source of food and raw materials for pharmaceuticals. Its antioxidant, antibacterial and anti-inflammatory properties prioritize its use as an intra-canal irrigation. It is biocompatible and lacks the serious risks associated with the use of other irrigants. (5,6)

Green tea is produced from the leaves and buds of *Camellia sinensis*. It is a non-fermented tea and contains more catechins than black tea. Catechins have antioxidant and anti-inflammatory effects. In addition, the content of certain minerals, vitamins and fluoride in green tea potentiates its antibacterial potential. Green tea exhibits antibacterial activity on *Enterococcus faecalis* planktonic cells. (7)

The aim of the study was to compare the in-vitro antimicrobial effect of: 20% *Psidium guajava* leaves extract, 20% *Camellia sinensis* leaves extract, versus 2% Chlorhexidine gluconate when used as root canal irrigants in primary teeth roots contaminated with *Enterococcus faecalis*.

## Materials and Methods

A minimal sample size of 15 in each of the three experimental groups and the positive control group, 5 in the negative control group with a total sample size of 65, that was sufficient to detect a large effect size (1.07), a power of 80% and alpha = 0.05 according to

EpiCalc 2000 version 1.02 program.  
(Brixton Health, USA)

65 primary roots were selected for this study. Those were collected from the outpatient clinic of the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University due to:

- Caries sequela including periapical abscess and failed pulpotomy leading to failure of restoration of the crown of the tooth.
- Orthodontic tooth removal to prevent or correct malocclusion.
- Acute trauma.
- Mobility.
- Over-retention. (8,9)

Showing the following criteria:

- Primary teeth with complete root.
- Absence of internal root resorption.
- Absence of external root resorption.
- Absence of Internal root calcification or any other inadequate local anatomical factors such as dilaceration. (10)

### Sample grouping:

In the study, 65 primary teeth roots were randomly distributed among three experimental groups, one positive control group and one negative control group.

### Results

There was a significant difference among the four tested groups. Positive control group had the highest (Mean±SD) value followed by Guava leaves extract group then Green tea leaves extract group while Chlorhexidine had the lowest (Mean±SD).

Pairwise comparisons showed that after using the irrigation materials:

There was a significant difference between the means of CHX and the positive control groups.

There was no significant difference between the means of CHX and Guava leaves extract groups.

There was no significant difference between the means of CHX and Green tea leaves extract groups.

There was a significant difference between the means of Guava leaves extract and the positive control groups.

There was a significant difference between the means of Green tea leaves extract and the positive control groups.

There was no significant difference between the means of Guava leaves extract and Green tea leaves extract groups.

### Discussion

2% Chlorhexidine was chosen as a gold standard root canal irrigant for the comparison in the present study because it has not shown any long-term damage to the host tissues. (11,12)

In the current study, inoculation with a facultative anaerobe: *Enterococcus faecalis*, was implemented because root canal infections are multi-bacterial with facultative anaerobes being the most prevalent of the bacteria isolated. (13) *Enterococcus faecalis* is a predominant and frequently isolated bacteria from necrotic pulps of primary teeth. (14)

The microbial suspensions used in the present study were adjusted to match the turbidity of No. 1 MacFarland scale to standardize the microbial suspensions used through the testing procedures, as reported. (15)

The presence of *Enterococcus faecalis* in root canals of both primary and permanent necrotic teeth can be detected either by culture or by molecular techniques like polymerase chain reaction according to Cogulu et al. (16)

Molecular techniques can detect uncultivable, difficult-to-grow bacteria as well as vulnerable species. Culture methods have some limitations in the detection of obligate anaerobic bacteria. Since *Enterococcus faecalis* is facultative anaerobic bacteria, both culture and PCR methods showed similar results. Culture method was chosen for the present study as a more economical method of to identify and quantify predominant species as the PCR technique only detects the target species

but did not enumerate the total number of bacteria present in the samples. (17)

The study was carried out in-vitro as the collection of pure *Enterococcus faecalis* isolates from the root bacterial ecology is a sophisticated process which may lead to unreliable data and results. (18)

Sample size was statistically calculated to ensure the reliability of the data based on the study conducted by Sahebi et al.(10) Accordingly, 65 roots were used in the current study and were divided into three experimental groups (15 roots), one positive control group (15 roots) and one negative control group (5 roots).

Flaring of the canals with #40 K-file helped the irrigating solutions to penetrate better into the apical third of the canals causing better antimicrobial activity (19).

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, ultrasound extraction and phytonic extraction. (20)

Maceration extraction is crude extraction; solvents diffuse into solid plant material and solubilize compounds with similar polarity. Maceration was the method of choice in the present study to avoid the effect of heat, enzymes, or

radiation on the content of the guava leaves and this process has a high absorption effectiveness of the active substances contained in the leaves. (20)

Ethanol extraction of guava leaves was the method of choice in the present study, it showed higher zones of inhibition against other Gram-positive organism (*Lactobacillus acidophilus*) than those showed by water extracts. (21) Guava leaves extract was found to be more active against another Gram-positive organism: *Staphylococcus aureus* by using ethanol and water extract than with just aqueous extract. (22,23)

The efficacy of 5% and 20% ethanolic extract was found to be better than 5% and 20% water extract. Ethanolic extract yields more flavonoid content than aqueous extracts. Flavonoids are known to have antibacterial activity. (24) This difference in composition of ethanolic and water extracts can be attributed to the difference in solubility of various components of guava leaves in water and organic solvents. (25)

Extraction yield of catechins from green tea is best by using water rather than other organic solvents. Korean tea was extracted using water and ethanol as solvents and it was reported that extraction with water at 80 °C for 40 min gave maximum yield of catechins. Also, Treating the tea leaves at 80 °C for 30 min gives a maximum yield without degradation. (26)

In theory, high extraction temperatures can increase the yield of tea catechins because the cell walls of the green tea leaves become more permeable to the solvent and to the constituents and, thus, the solubility and diffusion coefficients of the tea catechins are increased. However, the catechins can also be subject to degradation when the extraction is conducted at too high temperatures due mainly to the epimerization of their structure. That is why boiling point of water was never reached during the extraction of green tea in the present study. (27)

According to the results of the present study, all of Chlorhexidine, guava leaves extract as well as green tea leaves extract irrigations showed antibacterial effect when compared with the positive control that was irrigated with saline. There was a significantly higher antibacterial effect of Chlorhexidine irrigation than that of the other two irrigants.

This agrees with Jose et al., (6) in which both 2% Chlorhexidine and a lower concentration of guava leaves extract than the one used in the present study showed significant inhibitory effects against *Enterococcus faecalis*. 2% Chlorhexidine showed superior antibacterial efficacy compared to guava leaves extract.

Results also agreed with Najafi et al. (28) in which green tea showed antibacterial activity against *Enterococcus faecalis*.

However, results were not in line with the study carried out by Thomas et al. (29) in which the antimicrobial efficacy of a lower concentration of green tea in a mouth rinse compared to that of 0.2% Chlorhexidine was significantly more effective against bacteria.

Other properties beyond antimicrobial activity must also be investigated before the final choice of an irrigant solution for clinical use, such as minimum inhibitory concentration, tissue dissolution capacity, detoxification of endotoxin and acceptable biologic compatibility.

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