



Original Research

**Comparison of Syzygium Aromaticum (clove) and Punica Granatum (Pomegranate) extracts with 2% chlorhexidine in dentinal tubule disinfection against faecalis by using real-time PCR: an in-vitro study.**

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**ABSTRACT**

**Background:** A vital and significant goal in treating a tooth with an apical infection is getting rid of the germs in the pulp space. Recurrent root canal infections are most frequently linked to Enterococcus faecalis and can happen even after endodontic therapy. E. faecalis usually resist routine endodontic disinfectants and can also survive the nutrient-deprived conditions in the root filled tooth. So, an attempt has been made to eliminate the microorganisms using the herbal extracts which have antimicrobial properties and comparing it with the well accepted and excellent antimicrobial agent 2% chlorhexidine.

**Aim:** To compare Syzygium aromaticum (Clove) and Punica granatum (Pomegranate) extracts with 2% chlorhexidine in dentinal tubule disinfection with Real-time polymerase chain reaction which was used to detect E. faecalis.

**Methods:** Thirty-six extracted premolar teeth were selected for the study; access cavity was prepared and cleaning and shaping was done. With the help of a rotating diamond disc bur, the middle part of the root was sliced. E. faecalis was applied to the tooth specimens and left on them for 21 days. Group 1 specimens had pomegranate extract, Group 2 contained clove extract, and Group 3 contained 2% CHX. After being watered by the corresponding groups, the specimens were incubated for 5 days. A Gates-Glidden drill was used to collect the dentinal shavings, which were then subjected to DNA isolation before being subjected to real-time PCR analysis.

**Statistical analysis used:** The results were statistically analyzed using one way ANOVA and Post hoc Tukey's analysis

**Results:** Threshold cycle (Ct) values showed greater inhibition of bacterial load with pomegranate extracts followed by 2% chlorhexidine. Lesser reduction of bacterial load was found with clove extract.

**Conclusion:** Pomegranate extract, an herbal extract with therapeutic potential which can be utilised as an efficient substitute for 2% CHX for treating E. faecalis.

**Keywords:** Clove; Pomegranate; E. Faecalis; Real-time PCR.

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## INTRODUCTION

The oral cavity is a unique eco-system. The presence of micro-organisms in the oral cavity in certain specific sites on teeth leads to demineralization, cavitation because of caries and periodontitis in later stages.<sup>1</sup> The microorganisms are nothing but opportunistic pathogens, which invade the root canals containing the necrotic tissue causing infections. This is one of the primary causes of endodontic infections. Endodontic infections are polymicrobial in nature, with anaerobic microbes predominating.<sup>1</sup> One of the common therapeutic procedures followed to remove these bacteria is the root canal procedure. The main idea of doing three root canal treatment is to disinfect the root canal systems of the teeth completely. Irrigants helps to lubricate the root canals, dissolve the organic compounds and flush out the debris from the canal. Most of the bacteria in an endodontic infection are “strict anaerobes”.<sup>2</sup> Most commonly found species in the canals of the teeth is *Enterococcus faecalis*. Gram-positive cocci known as enterococci can appear individually, in pairs, or in short chains. The capacity to grow either with or without oxygen makes them facultative anaerobes. They are responsible for as much as 77% of treatment failures.<sup>3</sup> An effective root canal irrigants should have strong antibacterial effectiveness, be suitable for periapical tissues, be capable of dissolving necrotic tissue, lubricate the canal, aid in the removal of the smear layer, and most significantly, it should purify the dentin of all microorganisms.<sup>4,5</sup>

Recently, according to the World Health Organization, between 75% and 95% of the inhabitants of the world's developing nations rely on traditional therapies especially on plant extracts or their active ingredients, and this is true in case of traditional medicines. component.<sup>6</sup> The herbal alternatives for endodontic use could be useful given the inefficacy, potential side effects, and safety issues with synthetic medications. Pomegranate is one of the natural products which was used in the study, which has been quoted as an effective herbal extract in Iranian medicine as it is a potent antioxidant with anti-carcinogenic and anti-inflammatory properties. However, several in vitro assays demonstrate its bactericidal activity against several pathogenic and antibiotic-resistant organisms. In ayurvedic medicine, the pomegranate is considered “a pharmacy unto itself” and as remedy for diabetes in the Unani system of medicine practiced in the Middle East and India. According to Lansky, the pomegranate is a unique fruit with a complete medicinal potency contained in its juice, peel, and seeds, despite being a strange fruit.<sup>7</sup> Clove (*Syzygium aromaticum*), a plant that is widely cultivated in the Spice Islands, Indonesia, Pemba, and Zanzibar, is the other natural extract that is employed. Earlier production of the plant took place in China.<sup>8</sup> Its essential oil extracts' ability to kill numerous Gram positive and Gram-negative species, including certain fungus, demonstrated its antibacterial properties. It is also a well-known anticarcinogenic agent and is used in Asian countries as a traditional treatment for headaches, sore throats, dental problems, and respiratory problems. Additionally, it has been found that *S. aromaticum* has antibacterial activity against oral microorganisms linked to periodontal disease and dental caries.<sup>9</sup> There are many laboratory procedures which have been followed and changed accordingly with new technology in decades. The most accurate one which is popularly followed in the studies today is Polymerase chain reaction. Dr. Kary Mullis created the PCR technique, as we know it today, to amplify target DNA in 1983.<sup>10</sup> PCR is a biochemical procedure that may quickly multiply a single DNA molecule into millions of copies. Every single cell's DNA can be amplified via PCR in order to provide enough material for cloning or sequencing. The best PCR could do, though, is only semi-quantitative. Real-time PCR tests are now possible due to recent technological advancements that utilise fluorescent probes. Real-time PCR is currently replacing many traditional PCR techniques because it enables quicker detection and quantification of the PCR result.<sup>11</sup> Hence the present study was conducted to compare and evaluate *Syzygium aromaticum* (Clove) and *Punica granatum* (Pomegranate) extracts with 2% chlorhexidine as root canal irrigants, in dentinal tubule disinfection against *Enterococcus faecalis* by using real-time polymerase chain reaction.

## MATERIALS & METHODS

***Punica granatum* (Pomegranate extract) preparation:** In Davangere city from the nearby store, India, fresh, ripe pomegranate fruits were purchased. The Bapuji Pharmacy College in Davangere, India, prepared the extracts. The peel (pericardium) was taken off after washing. 100gm of thoroughly cleaned pomegranate pulp and 2.5 ml of chloroform in 1000 ml of filtered water (Indian Pharmacopoeia) were combined and then mashed in a juicer. The supernatant was centrifuged at 8,000 rpm for 40 minutes. Then, filtering of the mixture was done through two layers of filter paper.<sup>12</sup>

***Syzygium aromaticum* (Clove extract) preparation:** Clove was bought at a local Davangere market. The Bapuji Pharmacy College created the clove aqueous extract. 25g freshly chopped cloves were combined with 150 ml of distilled water and the mixture was then stirred for 8 hours at 300 rpm and 39°C in an incubator. The extract, which has been

obtained after additional filtering and processing, was black in colour, and it was kept in storage at 40°C.<sup>9</sup>

**Agar diffusion test:** The procedure was carried out using Brain heart infusion agar (BHI). After calibrating the inoculum to a McFarland 0.5 turbidity standard, the agar plate was infected within 15 minutes. To remove extra inoculum, a sterile cotton swab was dipped into the inoculum and rubbed against the tube wall above the liquid. Three times, the entire surface was streaked onto an agar plate, with the plates being rotated by around 60 degrees in between streaks to achieve even dispersion. A minimum of three minutes was given for the inoculated plate to stand. A 5mm diameter hollow tube was heated, pushed onto an agar plate with an inoculum above it, and then immediately removed by drilling a well in the plate. On each plate, five wells were also constructed. The dilutions of the samples were made at 50mg/ml, 100mg/ml and 200mg/ml and 50µl of each of samples were added into their respective wells on each plate. Plates were inverted and stacked. Incubation was carried out for 18-24 hrs at 37 °C in the incubator. Zones of inhibition, or zones without bacterial contamination, were inspected all around each well after 24 hours of incubation at 37 °C. They were visible as a crystal-clear, elliptical halo surrounding the wells.<sup>13</sup>

**Preparation of dentine specimens:** For this investigation, 36 extracted premolar teeth were chosen. Cleaning, contouring, and preparing the access cavity were completed. To get 6 mm of the middle third of the root, the teeth below the cemento - enamel junction and the apical portion of the root were decoronated using a rotating diamond disc. On the surface of the root, cement was removed. To eliminate the organic and inorganic detritus from the smear layer, an ultrasonic bath containing 17% ethylene diamine tetraacetic acid (EDTA) for 5 minutes was followed by 3% NaOCl for the same amount of time. To get rid of any traces of chemicals, the teeth were placed in an ultrasonic bath of distilled water for ten minutes. The samples were autoclaved at 121°C for 20 minutes.<sup>13</sup>

**Contamination of species:** *E. faecalis* (ATCC No. 35550) which were grown in tryptone soya agar for 24 h was used. Each dentine block was placed in a pre-sterilized microcentrifuge tube containing 1 mL of the TSB. A total of 50µL of the inoculums containing the *E. faecalis* was transferred into each of the microcentrifuge tubes. At the end of 24 hrs, the dentine specimens were transferred into the fresh broth containing *E. faecalis*. Contamination of the dentine specimens using *E. faecalis* was carried out for a period of 21 days at 37°C.<sup>13</sup>

**Antimicrobial assessment:** The inoculated broth was removed from the blocks after 21 days by irrigating them with 5 mL of sterile water. Three groups of blocks were assigned: Group 1 received pomegranate extract, Group 2 received clove extract, and Group 3 received 2% CHX. These irrigants were applied to the canal in order to test their antibacterial effectiveness. The canals were anaerobically incubated for five days at 37°C after being sealed at both ends with paraffin wax. After five days, dentine was collected at a depth of 400 metres using a clean Gates-Glidden drill numbered 5.<sup>13</sup>

**Irrigation procedure and collection of dentin shavings:** All of the irrigants used to have a 20-minute contact time. To irrigate, a 25-G needle was employed. Approximately 6ml of the proper irrigants were used in the irrigation technique, which was done at a rate of 3ml every 15 seconds. The paper points were then used to dry the canals. Dentine samples were obtained using Glades Glidden drills no. 4 and no. 5 at thicknesses of 200 m and 400 m. The obtained dentin shavings then were put into 1 ml of TSB broth and let to incubate for 24 hours under anaerobic conditions at 37 °C.<sup>13</sup>

**DNA isolation:** DNA isolation was done using the modified proteinase-K method. Samples were transferred to the tube containing T.E.buffer (1M Tris Buffer: 0.5ml, 0.5M EDTA: 100 µl, distilled water). It was centrifuged at 5,000 rpm for 5 min. The supernatant was discarded and 500 microliters of fresh T.E.buffer was added and centrifuged for 3-4 minutes. The procedure was repeated for 3-4 times with fresh T.E. buffer. Further, 50 microlitre of lysis buffer II and 10 micro liter of proteinase-K (100ug/ml) were added and vortexed vigorously. It was kept in a water bath for 2 hours, then it was kept in a boiling water bath for 10 minutes. DNA was stored at -20<sup>0</sup> C.<sup>14</sup>

**Real-time polymerase chain reaction (PCR):** PCR primers (stock: 10 pmole concentration), readymade master mix (2x concentrated), template (DNA extracts) and approximately 100mg concentration of molecular grade water were the reagents used in the study. Primers used were; Forward primer: CCG AGT GCT TGC ACT CAA TTG G, Reverse primer: CTC TTA TGC CAT GCG GCA TAA AC. A readymade master mix was used to prepare a total reaction volume of 20µl per sample. Fast Start Universal SYBR Green Master, 2x concentrated master mix (Roche, Switzerland) that contains 2.5 mg MgCl<sub>2</sub>, Fast Start Taq DNA Polymerase, Reaction Buffer, Nucleotides (dATP, dCTP, dGTP, dUTP), and SYBR Green I was used. SYBR Green I is a DNA double-strand-specific dye used. The SYBR Green I dye, which was included

in the reaction mix, binds to the amplified PCR products during each stage of DNA synthesis, and the amplicon was identified by its fluorescence. The PCR conditions were 95 0 C for 7 minutes of initial denaturation, then 40 cycles of denaturation (94 0 C for 30 seconds), annealing (60 0 C for 30 seconds), and extension (72 0 C, 30 seconds). The study was performed use of the common strain of *E. faecalis*, ATCC No. 35550. By utilising a 0.5 McFarland turbidity standard, the concentration of the standard strain was adjusted to 10<sup>7</sup> CFU/ml. This sample was serially diluted to get the lowest concentration of 10<sup>2</sup> CFU/ml (10<sup>7</sup> to 10<sup>2</sup> CFU/ml). From each of these samples, DNA was extracted and used as standards for real-time PCR. Double-stranded DNAs that were selectively amplified by *E. faecalis* specific primers bind with SYBR Green I dye. The fluorescence that the dye produces takes the shape of a graph. The amount of fluorescence was plotted versus the number of cycles on the graph. For all of the samples, the cycle threshold (Ct), or value cycle number, at which fluorescence has begun, was determined from the graph. Plotting a graph of Ct values versus CFU/ml values led to the creation of a standard curve. Each test sample's CT value was plotted on the standard curve, and the corresponding CFU/ml value was calculated.<sup>14</sup>

#### STATISTICAL ANALYSIS:

Data collected was subjected to statistical analysis using one way ANOVA for comparison of bacterial load in between study groups and to compare these two groups' bacterial inhibition levels, the Tukey Post Hoc test for pair - wise comparisons was used.

#### RESULTS:

Amplification plot is a graph of fluorescence against thermal cycle numbers. Each line represents each sample as PCR progresses. Peaks seen earlier indicated a greater number of the organism was present. Peak seen at later stage indicated a smaller number of the organism was present. The cycle number at which peak was generated is called Ct value. The Ct values which showed the inhibition of bacterial load for each sample were tabulated in Table 1. It shows the average mean and its standard deviation (SD) of inhibition of bacterial load for each group against *E. faecalis*. Group I (samples treated with the pomegranate extracts), group II (samples treated with clove extracts) and group III (samples treated with 2% Chlorhexidine) all showed a significant reduction in the bacterial load against *Faecalis*. (P value-0.001). The comparison between the groups for the efficacy of extracts against *Faecalis* were tabulated in Table 2. It shows a pairwise comparison between the groups. When the group I were compared with group II, there was a significant difference in inhibition of bacterial load against *E. faecalis* with the P value (0.009). When the group I were compared with group III, there was a significant difference in inhibition of bacterial load against *E. faecalis* with the p-value (0.001). When group II was compared with group III, there was no significant difference in inhibition of bacterial load against *E. faecalis* with the P value (0.77).

Study groups	Number	Mean	SD	ANOVA
				P – value
Pomegranate	12	6.45	1.33	0.001
Clove	12	5.36.	0.22	
2% chlorhexidine	12	5.13	0.56	

**Table 1: Comparison of inhibition of bacterial load of *E. faecalis* between the study groups.**

Study groups	Comparison groups	p- value	95% confidence interval	
			Lower bound	Upper bound
Pomegranate	Clove	0.009	0.24	1.93
	2% chlorhexidine	0.001	0.48	2.16
Clove	2% chlorhexidine	0.77 (NS)	-0.61	1.08

**Table 2: Pairwise comparison of group 1 (pomegranate extract), group 2 (clove extract), group 3 (2% chlorhexidine) against *E. faecalis***

**DISCUSSION:**

Modern endodontic procedures are successful, durable, and dependable because in order to clean, shape, and disinfect root canals, the effectiveness of endodontic files, rotary tools, irrigating solutions, and chelating agents plays a very important role. The long-term success of endodontic therapy is dependent on full debridement and effective disinfection of the canal space. The fundamental microbes' significance in the emergence of preapical inflammatory has been clearly demonstrated in recent years. Necrotic pulp becomes infected with microorganisms then it causes apical periodontitis.<sup>1</sup>

Most of the commercial irrigants such as sodium hypo chlorite and hydrogen peroxide are reported to cause cytotoxic reactions, unfavorable side effects, safety concerns and ineffectiveness in dental tubule disinfection of conventional commercial formulations, recent medicine has turned its attention towards biological medicinal preparations from natural plants over the last few decades.

According to WHO, herbal medicine is any substance or preparation that is produced from plants and comprises raw or processed components from one or more plants that have therapeutic benefits. The excellent antibacterial activity, biocompatibility, anti-inflammatory, and antioxidant characteristics of herbal medications have proven them more popular in modern dentistry than they have been in dental and medicinal procedures for countless years. Modern endodontic procedures are successful, durable, and dependable because of the efficiency of endodontic files, rotary tools, irrigating solutions, and chelating agents for cleaning, shaping, and disinfecting root canals.<sup>4,5</sup>

In the present study, Agar well diffusion method is used, as it is widely accepted to evaluate the anti-microbial activity of plant extracts. The anti-microbial efficacy of the extracts is known well when the antimicrobial agents diffuse in the Agar and inhibits the growth of the microbial strain tested.<sup>[8]</sup>

PCR is a highly effective practical research instrument with its own benefits. The PCR has been used to determine the sequencing of numerous diseases' unidentified etiological agents. The method can assist in determining the relationships between previously unknown viruses and those that are currently known, which will help us better understand disease itself. Sequencing, cloning, and analysis are aided by it, as was previously said regarding the major benefits. Real time PCR has the same benefits of PCR, plus the ability to quantify the produced product. The prior knowledge of the target sequences required to generate the primers that will allow for the selective amplification of PCR is one of its major drawbacks. Like all enzymes, DNA polymerases are prone to making mistakes, which leads to mutations in the PCR fragments produced. Another disadvantage of PCR is that even minute amounts of contaminated DNA can be amplified, producing results that are incorrect or unclear.<sup>11, 14</sup>

A common laboratory method for producing numerous copies (millions or billions) of a specific DNA region is the polymerase chain reaction. The aim of this technique is to typically to produce enough of the target DNA area for analysis or other uses. In many sectors of biology and medicine, including medical diagnostics, molecular biology research, and even some aspects of ecology, PCR is used. Using two oligonucleotide primers that hybridize the opposing strands and surround the region of interest in the target DNA, The PCR technique uses specific DNA sequences to be synthesized enzymatically in vitro.<sup>10</sup> The 3' ends of the DNA, which are the growing ends of newly produced strands, face each other as these oligonucleotides anneal to these separated templates DNA strands. When one primer's new strand stretches across the other primer side as a result of DNA polymerase, the new strand turns into a new template. The number of duplicates of the target DNA typically doubles per cycle. due to the fact that the primer extension products created in one cycle can act as a template in the following one. As a result, the particular fragments whose termini are determined by a sequence of cycles that repeatedly involve template denaturation, primer annealing, and the extension of annealed primers by the DNA. These cycles are represented by the 5' ends of primers. Twenty rounds of PCR, assuming 100% efficiency, produce approximately 220 million-fold amplifications. Each cycle consists of a number of processes, including a heating step to separate the two DNA strands and steps to anneal and extend primers at the DNA polymerase's preferred temperature. PCR-based detection techniques are extremely sensitive and specific allow for the quick identification of microbial species that can be grown and those that cannot. At best, PCR is only semi-quantitative. Real-time PCR was created as a solution to this problem. By using fluorescence detection of tagged PCR products, a new improvement to the polymerase chain reaction (PCR) makes it possible to precisely identify particular nucleic acids in a complicated mixture. Both non-specific and specific fluorescence probes can be used to detect dictation. This eliminates the need for gel electrophoresis to detect amplification products and merges the DNA amplification and detection process into a single homogenous

experiment. For Amplicon identification, appropriate data processing and the application of appropriate chemistry also render southern blotting or DNA sequencing unnecessary. Real-time PCR is the gold standard for the detection of DNA because of its ease of use, sensitivity, potential for higher results, and the development of novel chemicals, dependable instruments, and improved protocols. Real-time PCR is frequently employed to measure gene expression levels. Real-time tools ascertain the time during cycling when amplification of a PCR product is first observed, as compared to the need to take into account how much DNA target has accumulated after a certain number of cycles. This is calculated as the cycle number at which the reporter die emission strength can be detected over background noise. The term used to refer to that cycle number is the threshold cycle, or CT. The CT is calculated during the PCR reaction's exponential phase and is inversely proportional to the target's copy number. Therefore, a noticeable increase in fluorescence is seen and the value decreases the greater the nucleic acid target's start copy number. However, over a huge range, the fluorescence output is linear to sample concentration. The amount of template present at the start of the reaction can be determined using the linear connection between TCR products and fluorescence intensity.<sup>10</sup>

In the present study, pomegranate extracts (Group I) showed the best efficacy against *E. faecalis*. Pomegranate polyphenolic chemicals may serve as an effective adjuvant for the prevention and treatment of dental caries, according to a study by Gianmaria et al. that evaluated the antibacterial activity of hydroalcoholic extracts of pomegranate peel and juice against *S. mutans*.<sup>15</sup> Pagliarulo et al, in his study has proved that the antimicrobial properties of *punica granatum* against isolated organisms like *S. aureus* and *E. coli*, *Clostridium difficile*, *B. megaterium* and *B. subtilis*<sup>16</sup>. Arvind et al, in his study showed that *Punica granatum* was effective against the test organisms but when compared to standard antibiotics the extracts were found to be less effective against the test organisms which is contradictory to the results of this study.<sup>17</sup> The 2<sup>nd</sup> best efficacy against *E. faecalis* was shown by 2% CHX (Group II). The findings were consistent across research conducted by several authors; Krithikadatta et al., the effectiveness of calcium hydroxide as an intracanal medication was compared to the disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, and bioactive glass; Gomes et al, where 2 % chlorhexidine gel and calcium hydroxide were tested against *faecalis* in root dentine<sup>18</sup>; Bhardwaj et al compared the antibacterial activity of *Morinda citrifolia*, papain, and aloe vera, 2% chlorhexidine gel and calcium hydroxide, against *Enterococcus faecalis*.<sup>19</sup> In our study, Clove (Group III), samples treated with clove extracts showed significant inhibition in bacterial load, but the results were not significant statistically which is in accordance with studies by Shah et al, where clove oil was tested for its antibacterial effect as intracanal medicaments against *E. fecalis*.<sup>20</sup> Mistry et al, in his study showed that clove had an antibacterial effect against *faecalis* when the clove is used at 5 %, 10% and 50% concentrations.<sup>21</sup>

#### **LIMITATIONS:**

Limitations of this study includes the need to prepare fresh solutions each time, the unacceptable odour and taste requiring flavouring agents to make it palatable and short lifespan.

#### **CONCLUSION:**

Irrigants that are biocompatible in nature, active in action, less cytotoxic and effective against polymicrobial community should be chosen. In this regard use of herbal agents like pomegranate would be appropriate. However, their clinical implementation requires more scientific evidence and in vivo trials.

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#### **REFERENCES**

1. Eswar K, Venkateshbabu N, Rajeswari K, Kandaswamy D. Dentinal tubule disinfection with 2% chlorhexidine, garlic extract, and calcium hydroxide against *Enterococcus faecalis* by using real-time polymerase chain reaction: In vitro study. *J Conserv Dent* 2013; 16:194-8.
2. Elakanti S, Cherukuri G, Rao VG, Chandrasekhar V, Rao AS, Tummala M. Comparative evaluation of antimicrobial efficacy of QMix™ 2 in 1, sodium hypochlorite, and chlorhexidine against *Enterococcus faecalis* and *Candida albicans*. *J Conserv Dent* 2015 Mar; 18(2):128.

3. Gajan EB, Aghazadeh M, Abashov R, Milani AS, Moosavi Z. Microbial flora of root canals of pulpally-infected teeth: *Enterococcus faecalis* a prevalent species. *J Dent Res Dent Clin Dent Prospects*. 2009; 3(1):24.
4. Oliveira DP, Barbizam JV, Trope M, Teixeira FB. In vitro antibacterial efficacy of endodontic irrigants against *Enterococcus faecalis*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007; 103:702–6.
5. Retamozo B, Shabahang S, Johnson N, Aprecio RM, Torabinejad M. Minimum contact time and concentration of sodium hypochlorite required to eliminate *Enterococcus faecalis*. *J Endod*. 2010; 36:520–6.
6. Pujar M, Makandar S. Herbal usage in endodontics- a review. *Int J Contemp Dent*. 2011; 2:34-7.
7. Lansky EP. Beware of pomegranates bearing 40% ellagic acid. *J Med Food Mar* 2006; 9(1):119-22.
8. Deans SG, Ritchie GA. Antimicrobial properties of plant essential oils. *Int J Food Microbiol*. 1987; 5:165–180.
9. Lee S, Najiah M, Wendy W, Nadirah M. Chemical composition and antimicrobial activity of the essential oil of *Syzygium aromaticum* flower bud (Clove) against fish systemic bacteria isolated from aquaculture sites. *Front Agric China*. 2009 Sep 3(3):332-6.
10. Nelson K, Brannan J, Kretz K. The fidelity of TaqPlus DNA polymerase in PCR. *Strategies Mol Biol*. 1995; 8:24-5.
11. Briozzo J, Nunez L, Chirife J, Herszage L, D'Aquino M. Antimicrobial Activity of Clove Oil Dispersed in a Concentrated Sugar Solution. *J Appl Bact*. 1989; 66:69–75.
12. Mehta VV, Rajesh G, Rao A, Shenoy R, BH MP. Antimicrobial Efficacy of *Punica granatum* mesocarp, *Nelumbo nucifera* Leaf, *Psidium guajava* Leaf and *Coffea Canephora* Extract on Common Oral Pathogens: An In-vitro Study. *J Clin Diagn Res*. 2014 Jul; 8(7):65-8.
13. Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide. *Int Endod J*. 2010; 43:419–23.
14. Vanpelt E, van Belkum A, Hays JP. The PCR in practice. Principles and Technical Aspects of PCR Amplification. 2008:17-23.
15. Ferrazzano GF, Scioscia E, Sateriale D, Pastore G, Colicchio R, Pagliuca C, Cantile T, Alcidi B, Coda M, Ingenito A, Scaglione E. In vitro antibacterial activity of Pomegranate juice and peel extracts on cariogenic bacteria. *Int J Biomater*. 2017.
16. Pagliarulo C, De Vito V, Picariello G, Colicchio R, Pastore G. Inhibitory effect of pomegranate polyphenol extracts on the bacterial growth and survival of clinical isolates of pathogenic *Staphylococcus aureus* and *Escherichia coli*. *Food chem*. 2016 Jan 1; 190:824-31.
17. Aravindraj S, Preethi M, Sivapathasundharam B. Antimicrobial Effects of *Punica granatum* extracts on *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis* and *Candida albicans*. *Int. J. Curr. Microbiol. App. Sci*. 2017; 6(9):2762-74.
18. Gomes B. Valdrighi and Souza-Filho. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine in vitro. *Int End J*. 2003:267.
19. Bhardwaj A, Ballal S, Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of *Morinda citrifolia*, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against *Enterococcus faecalis*: An in vitro study. *J Conserv Dent*. 2012 Jul; 15(3):293.
20. Shah A, Jani M, Shah H, Chaudhary N, Shah A. Antimicrobial effect of Clove oil (Laung) extract on *Enterococcus faecalis*. *J Adv Oral Res*. 2014 Sep 1; 5(3).
21. Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of *Azadirachta indica*, *Mimusops elengi*, *Tinospora cardifolia*, *Ocimum sanctum* and 2% chlorhexidine gluconate on common endodontic pathogens. An in vitro study. *Eur J Dent*. 2014 Apr; 8(2):172.



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