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RESEARCH ARTICLE

Antimicrobial Action of Novel *Panax ginseng* Paste, Calcium Hydroxide and Bio-C Temp as an Intracanal Medicament Against *Enterococcus faecalis* and *Streptococcus mutans*

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Abstract

Background: Intracanal medicaments are substances placed inside the root canal system during endodontic treatment to help achieve optimal healing and infection control. Their use is critical for managing infections, reducing inflammation, and promoting tissue regeneration between appointments, especially when dealing with persistent microbial infections. **Aim:** To evaluate the antimicrobial action of novel *Panax ginseng* paste, Calcium hydroxide and Bio-C temp as an Intracanal Medicament (ICM) against *Enterococcus faecalis* and *Streptococcus mutans* by Modified Direct Contact Test (DCT). **Methods:** Two bacterial strains were grouped into (A) *Enterococcus faecalis* and (B) *Streptococcus mutans* and subgrouped (n=7) as follows: A1 and B1- Control, A2 and B2- Calcium hydroxide, A3 and B3- Bio-C Temp, A4 and B4- *Panax ginseng.* The ICM were freshly prepared and coated on a fixed area of 96-well microtiter plates followed by the addition of the bacterial suspension. Antimicrobial action was evaluated by applying the Modified DCT. **Results:** In both groups, the order of bacterial load reduction was- *Panax ginseng* > Calcium hydroxide > Bio-C Temp > Control. A significant difference was observed in control groups compared with the three ICMs ($P<0.05$). Bio-C Temp exhibited a significantly lower reduction as compared to Calcium hydroxide and *Panax ginseng* in both groups ($P<0.05$). *Panax ginseng* exhibited better antimicrobial action than $Ca(OH)_2$ in both the groups although it was not statistically significant. Intergroup comparison showed that all three ICMs led to more Mean Reduction (MR) in *S. mutans* than *E. faecalis* which was statistically significant in Calcium hydroxide and *Panax ginseng* groups. **Conclusion:** All three ICMs led to the reduction of both *E. faecalis* and *S. mutans. Panax ginseng* exhibited the best antimicrobial activity, and further research can be conducted on its biocompatibility on oral tissues for its application as a potential intracanal medicament.

Keywords: Bio-C Temp, Calcium Hydroxide, *Enterococcus faecalis, Panax ginseng, Streptococcus mutans*

1. Introduction

Bacteria significantly contribute to the emergence of pulpal pathology. Hence its elimination remains one of the primary objectives of endodontic treatment[1].

Enterococcus faecalis, a major prevalent microorganism has been isolated from root canals of chronic apical periodontitis cases. It is notoriously implicated as the major organism associated with failed endodontic treatment[2]. *Streptococcus mutans* has been isolated from both asymptomatic and symptomatic endodontic infections at high prevalence. The interdependency of *E. faecalis* and *S. mutans* can be substantiated by the impact of *S. mutans* on the biofilm formation of *E. faecalis* through coaggregation[3].

The invasion of microbes in the lateral canals remains to be a challenge for mechanical preparation alone. Additional steps, such as placement of intracanal medicament have been recommended in extensively

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infected root canals and cases of long interappointment intervals[4].

Calcium hydroxide as an intracanal medicament was suggested in 1920 by Hermann, and since then it has been the most used intracanal medicament[4]. It has been observed from clinical studies that although it reduces the microbial load from root canals, complete elimination has not been achieved[5].

Bio-C Temp, a bioceramic tricalcium silicate paste is applicable in conventional root canal treatment, retreatments, perforations, external and internal resorptions, etc. Guerreiro JCM *et al.,* found that it had similar cytocompatibility with calcium hydroxide but inferior antimicrobial activity[6].

The benefits of using traditional medicines derived from medicinal plants and their potential as sources of new drugs have been recognized by the World Health Organization. *Panax ginseng* has been a fundamental element of traditional Chinese herbal medicine for centuries. The presence of ginsenosides and polysaccharides in *P. ginseng* has been attributed as being responsible for its medicinal properties[7].

A modified DCT is a method that offers quantifiable and consistent results that can be employed in standardized settings and circumvents the limitations of the Agar Diffusion Test (ADT)[8].

Although numerous studies in the literature have reported the medicinal benefits and antimicrobial action of *P. ginseng,* its antimicrobial action against endodontic pathogens and its potential use as an ICM have not been evaluated. Therefore the purpose of this *in vitro* study was to evaluate and compare the antimicrobial action of *Panax ginseng* with $Ca(OH)_2$ and Bio-C Temp against *E. faecalis* and *S. mutans* by Modified DCT. The null hypothesis tested is that there will be no difference in the antimicrobial action of the three intracanal medicaments against both *E. faecalis* and *S. mutans.*

2. Materials and Methods

2.1 Study Protocol and Registration

This study was approved by the Ethical Committee of the I.T.S. Centre for Dental Studies and Research with the ethical code of ITSCDSR/IIEC/RP/2023/004.

2.2 Bacterial Strains and Culture Conditions

Enterococcus faecalis ATCC 29212 (Himedia Laboratories Pvt. Ltd., India) and *S. mutans* ATCC 25175 (Himedia Laboratories Pvt. Ltd., India) were procured in the lyophilized form. The hydrating medium at the upper opposite end was pinched to mix with the lyophilized pellet following the manufacturer's instructions. The hydrated strains were plated on Brain Heart Infusion (BHI) agar plates and incubated for 48 hours at 37°C anaerobically.

2.3 Preparation of Bacterial Suspension

From the subculture of the two bacterial species grown in BHI agar plates, a 0.5 McFarland standard suspension was prepared in BHI broth and then diluted to obtain an initial bacterial suspension of approximately 3×10^6 colony forming unit per millilitre (CFU/ml).

2.4 Preparation of the Intracanal Medicaments

1000 mg of *Panax ginseng* extract powder (Greendorse Health Products Private Limited., India) measured from an Electronic compact scale and 2 ml of propylene glycol measured from a micropipette (DLAB Scientific Co., Ltd., China) were dispensed onto a glass slab. The powder and vehicle were mixed with a Cement spatula (GDC Fine Crafted Dental Pvt. Ltd., India) until a paste-like consistency was achieved. $Ca(OH)_2$ Powder (Samit, India) with propylene glycol was prepared similarly. Bio-C Temp (Angelus, Londrina, PR, Brazil) was directly dispensed from the tube following the manufacturer's instructions. Table 1 shows the

Table 1. Intracanal Medicaments (ICM), manufacturers and compositions

ICM	MANUFACTURER	COMPOSITION
Calcium hydroxide	Samit, India	Calcium hydroxide
Bio-C Temp	Angelus, Londrina, PR, Brazil	Calcium silicates, calcium aluminate, calcium oxide, calcium tungstate, and titanium oxide.
Panax ginseng	Greendorse health products private limited., India	*Panax ginseng* extract (20% Ginsenosides)

Intracanal Medicaments (ICM) tested in the study along with manufacturers and composition.

2.5 Serial Dilution

100 µl of either bacterial suspension was added to 1ml of Phosphate-Buffered Saline (PBS) and vortexed in a Vortex mixer (Scientech Instruments, India) to ensure thorough mixing. Starting with the first test tube, a 100 µl portion of the solution was transferred to the second test tube. This process was repeated successively up to the sixth test tube, The resulting dilutions ranged from 10^{-1} to 10^{-6}. Subsequently, 100 µl of the suspensions from this dilution were carefully transferred to sterile BHI plates using a micropipette (DLAB Scientific Co., Ltd., China).

2.6 Modified Direct Contact Test

A 96-well microtiter plate (Himedia Laboratories Pvt. Ltd., India) was positioned upright, and a consistent area on both the inner side wall and bottom of each well was covered with an equal quantity of each medication using a sterile cotton swab. A volume of 10 µl from the bacterial suspension, which contained $3x10^6$ bacteria, was meticulously applied to the surface of each medicament. Bacterial suspensions were also applied to the walls of wells that had not been coated with any medicament, serving as a control group. The microtiter plates containing the medicaments were put in an incubator (Jindal Medical and Scientific Instruments Pvt., Ltd.) at 100% humidity at 37°C for 24 hours. After incubation, 240 **µl** of BHI Broth (Himedia Laboratories Pvt. Ltd., Mumbai, India) was deposited into each well. Following a mild agitation using a Micropipette (DLAB Scientific Co., Ltd., China) for 1 minute, the contents from each well were then transferred and diluted in a stepwise manner in PBS. An aliquot of 100 **µl** was plated onto the BHI plate after 6-fold serial dilutions. The BHI Plates underwent incubation for 24 hrs at 37°C. The survival of bacteria was calculated by adding up the colonies on each plate with a Digital colony counter (Simtronics analytical and laboratory instruments). The CFU/ml was then calculated after a contact period of 24 hours, at a one-point time interval.

2.7 Statistical Analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 22.0 (IBM Corp 2013, New York, USA). For comparison of the reduction in colony-forming units of both *E. faecalis* and *S. mutans*, one-way ANOVA was applied and Tukey's test for pairwise comparison of means in different groups.

An Independent t-test was applied for intergroup comparison. The level of significance and the confidence interval were 5% and 95% respectively. P ≤0.05 was considered statistically significant.

3. Results

Comparing the reduction of *E. faecalis* count (cfu/ml) in the ICM**,** the highest reduction was shown by A4 (*Panax ginseng*) followed by A2 (Calcium hydroxide), A3 (Bio-C Temp), and A1 (Control). A significant difference was obtained in *E. faecalis* count reduction in each of the three ICMs compared with the control group (Table 2).

On a Pairwise comparison of reduction in *E. faecalis* count (cfu/ml) in different ICM, A1 (Control group) showed a significantly lower reduction of *E. faecalis* count when compared to the other three groups. A3 (Bio-C Temp) showed a significantly lower reduction as compared to the A2 (Calcium hydroxide) and A4 (*Panax ginseng*). No significant difference was observed between A2 (calcium hydroxide) and A4 (*P. ginseng*) (Table 3).

On Comparison of reduction in *S. mutans* count (cfu/ml) in different ICM, the highest reduction was shown by A4 (*P. ginseng*) followed by A2 (Calcium hydroxide), A3 (Bio-C Temp) and A1 (Control). A significant difference was made in the reduction of *S. mutans* count in each of the three ICMs compared with the control group (Table 4).

On Pairwise, of reduction in *S. mutans* count (cfu/ml) in different ICM (Table 5), B1(Control group) showed

Table 2. Comparison of reduction in *E. faecalis count* (cfu/ml) in different intracanal medicaments

Pair	p-value
A1 (Control) vs A2 (Calcium hydroxide)	<0.001*
A1 (Control) vs A3 (Bio-C Temp)	<0.001*
A1 (Control) vs A4 (*P. ginseng*)	<0.001*
A2 (Calcium hydroxide) vs A3 (Bio-C Temp)	<0.001*
A2 (Calcium hydroxide) vs A4 (*P. ginseng)*	0.240
A3 (Bio-C Temp) vs A4 (*P. ginseng*)	<0.001*

One-way ANOVA test; * indicates significant difference at p≤0.05

Table 3. Pairwise comparison of reduction in *E. faecalis* count (cfu/ml) in different intracanal medicaments

Group	N	Mean	SD	p-value
A1 (Control)	7	0.8194	0.0517	<0.001*
A2 (Calcium hydroxide)	7	21.0950	0.8402	
A3 (Bio-C Temp)	7	18.5736	1.2493	
A4 (*Panax ginseng*)	7	21.9746	0.7889	

Post hoc Tukey test; * indicates significant difference at p≤0.05

Table 4. Comparison of reduction in *S. mutans count* (cfu/ml) in different intracanal medicaments

Group	N	Mean	SD	p-value
B1 (Control)	7	0.8079	0.0497	<0.001*
B2 (Calcium hydroxide)	7	22.9657	1.0501	
B3 (Bio-C Temp)	7	18.7193	1.0727	
B4 (*Panax ginseng*)	7	23.0954	0.8989	

One-way ANOVA test; * indicates significant difference at p≤0.05

Table 5. Pairwise comparison of reduction in *S. mutans* count (cfu/ml) in different intracanal medicaments

Pair	p-value
B1 (Control) vs B2 (Calcium hydroxide)	<0.001*
B1 (Control) vs B3 (Bio-C Temp)	<0.001*
B1 (Control) vs B4 *(Panax ginseng)*	<0.001*
B2 (Calcium hydroxide) vs B3 (Bio-C Temp)	<0.001*
B1 (Control) vs B4 *(Panax ginseng)*	0.992
B3 (Bio-C Temp) vs B4 *(Panax ginseng)*	<0.001*

Post hoc tukey test; * indicates significant difference at p≤0.05

a significantly lower reduction of *S. mutans* count as compared to the other three groups. B3 (Bio-C Temp) exhibited a significantly lower reduction as compared to B2(calcium hydroxide) and B4 (*P. ginseng)*. No significant difference was observed between B2(calcium hydroxide) and B4 (*P. ginseng*).

On intergroup comparison of reduction of *E. faecalis* vs *S. mutans* (Table 6), In the control groups (A1 and B1), the MR in *E. faecalis* count was 0.8194 units whereas the MR in *S. mutans* was 0.8079 units but no significant difference was observed (p>0.05).

In the Calcium hydroxide groups (A2 and B2), the MR in *E. faecalis* count was 21.0950 units whereas the MR in *S. mutans* was 22.9657 units but no significant difference was observed (p<0.05).

Table 6. Intergroup comparison of reduction of *E. faecalis* vs *S. mutans* of each group

Pair	Group	N	Mean	Std. Deviation	Difference	p-value
A1 vs B1 CONTROL	**A1**	7	0.8194	0.0517	-0.0115	0.677
	B1	7	0.8079	0.0497		
A2 vs B2 Ca(OH)2	**A2**	7	21.0950	0.8402	1.8707	0.003*
	B2	7	22.9657	1.0501		
A3 vs B3 BIO-C TEMP	**A3**	7	18.5736	1.2493	0.1457	0.819
	B3	7	18.7193	1.0727		
A4 vs B4 PANAX GINSENG	**A4**	7	21.9746	0.7889	1.1208	0.029*
	B4	7	23.0954	0.8989		

Independent t test; * indicates significant difference at p≤0.05

In the Bio-C Temp groups (A3 and B3), the MR in *E. faecalis* count was 18.5736 units whereas the MR in *S. mutans* was 18.7193 units but no significant difference was observed (p>0.05).

In the Panax ginseng groups (A4 and B4), the MR in *E. faecalis* count was 21.9746 units whereas the MR in *S. mutans* was 23.0954 units. A significant reduction in the bacterial load was observed (p<0.05).

3. Discussion

Microorganisms and their by-products have a crucial influence on the genesis, progression, and establishment of pulpal and periradicular disorders[9]. Root canal infections have a polymicrobial nature, consequently; anaerobic and facultative anaerobic bacteria are commonly detected together in endodontic infections, including *Enterococcus faecalis* and *Streptococcus mutans*[10].

Enterococcus faecalis was selected for the study due to its frequent isolation in teeth with persistent endodontic infections[2]. Notably, it is one of the most prevalent species in teeth that have undergone RCT, with prevalence rates reaching as high as 90%[10]. *E. faecalis* was selected due to its easy availability and established use in previous studies, forming colonies within 24-48 hours[11,12].

Streptococcus mutans has been found in association with both symptomatic and asymptomatic endodontic infections. Its presence in pulp increases the likelihood

of bacteremia and extra-oral pathologies linked with the extrusion of debris during RCT[13]. It has been discovered in 47% of endodontically treated teeth and is strongly associated with a microorganism contributing to biofilm formation[3]. *Streptococcus mutans* ATCC 25175 was selected due to its easy availability and ease of culturing the colonies[14].

The bacterial load of both *E. faecalis* and *S. mutans* were standardized to 3 $\times 10^6$ colony forming units (CFU/ml). This is following studies by Zhang H *et al.,*[8] and Miranda RG *et al*[15]. The assessment of antimicrobial action of the ICM with modified DCT was assessed after incubating the culture plate for 24 hours[8].

In the treatment of root canal infections, it is essential to go beyond chemo-mechanical preparation and employ ICM. Its purposes vary from eliminating residual microorganisms after biomechanical preparation, reducing pulp remnants and periapical tissue inflammation, rendering the contents of root canals inert, functioning as a barrier of seepage from the temporary filling, and aiding in drying persistently wet canals[16].

Calcium hydroxide (Samit, India) was chosen due to its established effectiveness against *E. faecalis* and *S. mutans* when employed as an intracanal medicament[15].

Guerreiro JCM *et al.,*[6] demonstrated Bio-C Temp's antimicrobial effectiveness against *E. faecalis* but there have been no prior studies assessing its antimicrobial activity against *S. mutans.*

Over the years, the use of herbal extracts as antimicrobial agents has been explored tremendously[17]. Ginseng is one among various herbal extracts with a known potent antimicrobial action. A study by Wu H *et al.,*[18] demonstrated the ability of ginseng extract to disrupt mature biofilm. This implies the capacity of *P. ginseng* to eliminate biofilm-associated chronic infections, including endodontic infections. Although several studies have proved the antimicrobial action of *Panax ginseng* on different organisms, its action on *Enterococcus faecalis* and *Streptococcus mutans* has not been studied to date. Additionally, the use of *Panax ginseng* in the field of endodontics or as an ICM has not been tested. *Panax ginseng* extract powder (Greendorse Health Products Private Limited., India) was selected as it is a pure extract without any added constituents and contains 20% ginsenosides.

Propylene glycol was chosen as the vehicle for both $Ca(OH)_2$ and *Panax ginseng* powder. 1000 mg of each powder was mixed with 2 ml of propylene glycol to achieve a paste-like consistency as previously described by Srinivas S *et al*[19]. Propylene glycol has been documented to be less cytotoxic and more biocompatible than other regularly applied carriers for intracanal medications, according to Thomas *et al*[20].

ADT has various limitations including limited diffusion and penetration, lack of standardization, the potential for false negatives or positives, and lack of clinical relevance[21]. Weiss *et al.,* originally introduced the Direct Contact Test (DCT) as an improvement over the ADT. The test can be used in controlled environments and provides a quantitative and reproducible approach for mimicking the interaction between the test microorganism and the material being tested. In this particular study, DCT was adapted to include a process where plating was carried out immediately after each contact, guaranteeing the assessment of the bactericidal impact of the materials, as opposed to their bacteriostatic effects. Additionally, this adjustment allows for the precise determination of the number of viable bacteria following each contact duration. This modified DCT followed the methodology outlined by Hui Zang *et al*[8].

Panax ginseng exhibited the most antimicrobial action against both bacteria. This finding can be ascribed to its content of ginsenosides and polysaccharides and its various mechanisms including biofilm disruption leading to an increase in motility and shifting of bacterial cells to a predominantly planktonic form, thereby enhancing their vulnerability to *P. ginseng*, reduction of quorum sensing signalling molecules leading to decrease in virulence, reduction in bacterial adherence, modulation of microbial gene expression and by impeding and inhibiting the growth of bacterial cells. The antimicrobial properties of *P. ginseng* are analogous to that of *Aloe vera* due to the content of polysaccharides. A study by Bhardwaj A *et al.,*[22] showed that *E. faecalis* was better inhibited by *Aloe vera* gel (78.9%) than by $Ca(OH)_2$ (64.3%). This justifies our finding that *Panax ginseng* has a better bactericidal effect than $Ca(OH)_2$ against both *Enterococcus faecalis* and *Streptococcus mutans* although not statistically significant($p>0.05$). When *P. ginseng* was compared

with Bio-C Temp, it exhibited better antimicrobial action against both *E. faecalis* and *S. mutans* which was statistically significant($p<0.05$).

$Ca(OH)_2$ exhibited an SR ($p<0.05$) in bacterial count in comparison with Bio-C Temp against both *E. faecalis* and *S. mutans*. Its antimicrobial effect occurs through the dissociation of calcium and hydroxyl ions when it encounters aqueous fluids. The hydroxyl ions have a PH ranging from 12.5 - 12.8 and thus create a highly alkaline environment and eliminate microorganisms when they come in direct contact with them. Additionally, hydroxyl ions can diffuse into the dentinal tubules and penetrate the biofilm to kill the viable bacteria. This finding is in line with a study done by Guerreiro JCM *et al.*,[6] in which Bio-C Temp showed similar cytocompatibility with $Ca(OH)_2$ but significantly less antibacterial and antibiofilm activity against *E. faecalis*.

Bio-C Temp exhibited the most inferior antimicrobial action among the three ICMs. It exerts its action through its alkalinity, calcium ion release, reactive oxygen species generation, and ion exchange. Its antimicrobial action can be attributed to the hydroxyl (OH-) ions release which is continuous and gradual. Its limited solubility enables it to persist in contact with the canal walls for a prolonged duration, and this property can be advantageous to $Ca(OH)_2$. However, its inferior property in the present study can be explained due to fewer CH molecules formed during the hydration reaction. This finding is per the previously mentioned study done by Guerreiro JCM *et al*[6].

Intergroup comparison showed that all ICM led to more reduction in *S. mutans* than *E. faecalis*. *Panax ginseng* also showed a significant reduction($p<0.05$). Haripriya S. and Ajitha P. (2017)[23] tested silver nanoparticles of *Aloe vera* and also confirmed the better antimicrobial action of a herbal agent against *S. mutans* than *E. faecalis*. These findings can be attributed to the resistance mechanism of *E. faecalis*. It has a variety of strategies for surviving in unfavourable environments, such as the ability to thrive in environments with high pH levels, low oxygen levels and a broad temperature range between 10° to 60°C, high salinity, or environments with inadequate nutrients. It can survive in the presence of ICM by utilizing the periodontal ligament fluid as a source of nutrients thereby forming biofilms.

The null hypothesis was thus rejected as there was a difference in the antimicrobial action of the three ICMs tested.

A strength of the present study was that it is the first study to test the antimicrobial action of *P. ginseng on E. faecalis* and *S. mutans* and its potential application in dentistry including the field of endodontics. Additionally, the Modified DCT was used that overcome the limitations of the agar diffusion test.

A limitation of the present study was that it is an *ex vivo* study hence it could not simulate ideal clinical conditions and the physiological actions of various biological entities on the intracanal medicaments within and or surrounding the root canal could not be taken into account. Additionally, the antimicrobial action of the medicaments at various time intervals was not evaluated.

The future scope of this study includes different concentrations of *P. ginseng* that exert optimal antimicrobial action and can be evaluated. Furthermore, its use as an intracanal medicament *in vivo* can be tested.

4. Conclusion

Within the limitations of the study, it can be concluded that Calcium hydroxide, Bio-C Temp, and *Panax ginseng* showed antimicrobial action against both *Enterococcus faecalis* and *Streptococcus mutans*. *Panax ginseng* exhibited the most antimicrobial activity and further research can be conducted on its biocompatibility on oral tissues for its application as a potential intracanal medicament.

5. Authors contribution

Conceptualization, methodology, review- Zadeno Kithan, Sonali Taneja; Software, validation- Zadeno Kithan, Abhik Mukherjee; Investigation, Data curation- Zadeno Kithan, Sonali Taneja, Abhik Mukherjee; Writing—original draft preparation- Zadeno Kithan; Supervision- Sonali Taneja, Abhik Mukherjee; All authors have read and agreed to the published version of the manuscript.

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