

Cavity disinfection in minimally invasive dentistry - comparative evaluation of *Aloe vera* and propolis: A randomized clinical trial

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Abstract

Context: The survival of atraumatic restorative treatment (ART) restorations would probably increase if near total elimination of cariogenic microorganisms could be done in the process of cavity cleaning before going ahead with the restoration. Thus, use of naturally occurring disinfecting agents for achieving this goal could herald a new beginning in the field of contemporary minimum intervention dentistry. **Aims:** To evaluate the efficacy of hand instruments in excavating dental caries and comparatively evaluate the roles of *Aloe vera* and propolis as potential cavity disinfecting agents after minimally invasive hand excavation of dental caries. **Settings and Designs:** Experimental, *in vivo* intergroup split mouth, randomized clinical trial. **Subjects and Methods:** The study included Group I (Control), Group II (*A. vera*) and Group III (propolis). Ten patients with three teeth each have occlusal/occlusoproximal lesions suitable for ART were selected. Dentinal samples were collected three times from each tooth viz., preexcavation, postexcavation and postdisinfection of the cavities. These dentinal samples were subjected to microbiological analyses for total viable count. **Statistical Analysis Used:** Repeated measures of analysis of variance (ANOVA) with Bonferroni *post-hoc* test and one-way ANOVA with Tukey *post-hoc* test. **Results:** In all the three groups, significant amount of bacteria were left behind after hand excavation. Group II and Group III, in which cavities were treated with *A. vera* and propolis extracts respectively, showed a significant reduction in the bacterial counts when compared to control the group. **Conclusions:** Hand excavation alone does not completely eliminate bacteria, which may predispose treated teeth to secondary caries. Both propolis and *A. vera* extracts can be used as potential natural disinfecting agents, thereby embracing the concept of phytotherapy in minimum intervention dentistry.

Keywords: *Aloe vera*, atraumatic restorative treatment, cavity disinfection, minimum intervention dentistry, phytotherapy, propolis, total viable count

Introduction

Minimum intervention, the key phrase in contemporary dentistry focuses on the least invasive treatment option available to minimize tissue loss and patient discomfort.^[1] Atraumatic restorative treatment (ART) is one such minimally invasive modality, which uses hand instruments to remove soft, demineralized dental tissues followed by the placement of an adhesive restoration.^[2]

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Though this technique is most suited for the developing countries with sparse resources, it is gaining wide acceptance in the developed countries as well. ART is the currently the intervention of choice for (i) Treatment of early childhood caries,^[2,3] (ii) very young children who are being introduced to oral care, (iii) patients who experience extreme fear or anxiety about dental procedures, (iv) mentally and/or physically handicapped patients, and (v) home-bound elderly patients and residents of nursing homes.^[4]

The essence behind atraumatic restorative treatment

The goal of every stepwise intervention/excavation in both primary and permanent dentitions is to induce “self-repair”, to arrest the carious process and thus maintain pulp vitality.^[5] Such an intervention bears fruit if we manage to perform the same in sterile conditions and then back it up with an adhesive restoration thereafter.

However, the available evidence pertaining to efficacy of hand instruments in elimination of cariogenic bacteria suggests that neither color nor dentin consistency is effective in differentiating the level of infection. Bacteria remain in the cavity even when excavation is carried to firm tissue and hence quite a few ART restorations fail due to secondary caries development subsequently.^[2] Thus, the survival of ART restorations would probably increase if near total elimination of cariogenic microorganisms could be done in the process

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of cavity cleaning before going ahead with the restoration.^[6] The use of disinfecting agents for achieving this goal could herald a new beginning for the field of contemporary, minimal intervention dentistry.^[7]

Recently there has been a growing trend to seek natural remedies as part of medical and dental therapeutics, which has been termed as “phytotherapeutics” or “ethnopharmacology”.^[8] Naturally occurring *A. vera* and propolis are emerging as very good antibacterial agents in various domains of dentistry.

Aloe barbadensis Mill (*A. vera*), is a short succulent herb resembling a cactus, with green dagger-shaped fleshy, spiny and marginated leaves, filled with a clear viscous gel which has potent antibacterial, antifungal, and antiviral properties.^[9,10] Propolis is a resinous substance collected by honeybees (*Apis mellifera*), from different plant buds. Presence of flavonoids accounts for most of the biological activities of propolis, such as its’ antimicrobial, antioxidant, and antitumor activities.^[11]

Though *A. vera* and propolis extracts have been used in dentistry for various purposes, no study till date has been conducted using them as cavity disinfectants. Thus, the present study was carried out to evaluate and compare the roles of *A. vera* and propolis as potential cavity disinfecting agents after minimally invasive hand excavation of dental caries.

Subjects and Methods

Children aged 5–12 years suitable for ART with at least three cavitated dentinal lesions in primary molars clinically involving occlusal and occlusoproximal surfaces, were selected irrespective of sex, race and socioeconomic status.

Study setting

Outpatient clinics of Department of Pedodontics and Preventive Dentistry at Bapuji Dental College and Hospital, Davanagere.

Study design

Experimental, *in vivo*, split mouth, randomized clinical trial.

Study groups

The study consisted of following groups [Table 1].

Inclusion/exclusion criteria and ethical clearance

Teeth with deep dentinal lesions with pulpal involvement, abscess, pain or swelling, developmental disorders and adjacent soft tissue lesions and children with systemic illness were excluded. Informed consent explaining the rationale of the study was read and signed by the parents of the children selected for the study. The study was conducted in strict adherence to the guidelines by Helsinki Declaration of 1975, (revised in 2000). Ethical clearance was obtained by institutional review board.

Study groups

Preparation of cavity disinfectants

Propolis extract

Hand collected propolis samples produced by honeybees (*A. mellifera*) were kept desiccated in the dark before processing. The samples were ground mechanically and bottled in 10 g portions. The portions of 10 g were put into flasks, and 100 ml of 70% ethanol was added. Propolis was subjected to 14 days of extraction in order to obtain ethanolic extract of propolis. The flask was placed in laboratory shaker for 2 weeks at room temperature. After that the extract was cooled at 4°C for 24 h in order to precipitate all insoluble substances. Rough particles were removed from the propolis extract by filtering through filter paper (Whatman no. 1). The filtrate obtained was evaporated, using rotary vacuum evaporator at 40°C. This way, a viscous substance having brown color was obtained.^[12]

Aloe vera extract

The leaves of the plant were washed with distilled water, cut opened, and fresh pulp was collected. The gel was dried in an oven at 80°C for 48 h and then powdered. An ethanolic extract was obtained by dissolving 20 g of the powder in 200 ml of ethanol. The contents were then filtered using Whatman filter paper no. 1, and the filtrate was evaporated for dryness.^[13]

Procedure

Dentin sampling procedure

In each patient, three cavitated lesions were selected. A baseline sample of the carious lesion was obtained using a sterile spoon excavator from the center of each lesion, after isolation with a rubber dam prior to excavation of caries.

After the removal of caries, a second dentin sample for microbial evaluation was collected using another sterile spoon excavator from the hard dentin. Cavities of Groups I, II and III was disinfected with distilled water, propolis extract and *A. vera* extract respectively for 60 s. Then these cavities were air dried. New dentin samples were collected for microbial analysis using a spoon excavator from the same place. After collection of the third sample, the teeth were restored with glass ionomer cement (Fuji 9).^[7]

Thus, dentinal samples were collected three times from each carious tooth, viz., baseline-before excavation of caries, after hand excavation of caries and after disinfection of the cavity. These samples were subjected to microbiological evaluation for total viable count (TVC).

Table 1: Study consisted of following groups

Group 1	Distilled water
Group 2	Propolis extract
Group 3	<i>Aloe vera</i> extract

Microbiological procedures

The samples collected were immediately transferred to brain heart infusion broth, then subjected to microbiological processing and were cultivated so that the total number of viable bacteria could be detected. The samples were homogenized in a tube shaker for 3 min, and 25 μ l aliquots of this solution would be placed onto the plate surfaces containing blood agar, with a micropipette. Subsequently, the cultures were incubated after which it was possible to make a visual assessment of the total number of viable bacteria unit-forming colonies.^[5]

Results

Table 2 shows a comparison of the viable bacterial colony count in each study group using repeated measures analysis of variance (ANOVA). A statistically significant reduction in the bacterial count was observed in all the three groups postexcavation (compared to preexcavation) and postcavity disinfection (compared to postexcavation) ($P < 0.001$).

Pairwise comparison of viable bacterial colony counts at different phases viz preexcavation, postexcavation and postcavity disinfection was done in each of the study groups using Bonferroni *post-hoc* test. It showed statistically significant difference in the number of bacterial colonies between each phase in all the three study groups ($P < 0.001$) [Table 3].

When inter group comparisons were made using one-way ANOVA [Table 4 and Illustration 1], and pair wise comparisons were done using *post-hoc* Tukey's test [Table 5], there was no statistically significant difference among the three study groups in the bacterial colony count at two phases of experiment viz preexcavation and also postexcavation. But when bacterial counts were compared postcavity disinfection there were statistically significant differences among the groups ($P < 0.01$). The reduction in the number of bacterial colonies after cavity disinfection was statistically significant in both Group II and Group III, when compared to Group I ($P < 0.001$). However, the difference observed between Group II and Group III in terms of TVC after cavity disinfection was not statistically significant ($P = 0.99$) [Figures 1-9].



Figure 1: Total viable count preexcavation in Group I



Figure 2: Total viable count postexcavation in Group I

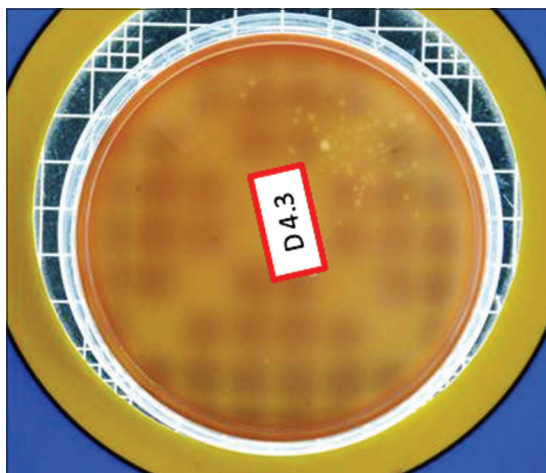


Figure 3: Total viable count postdisinfection in Group I

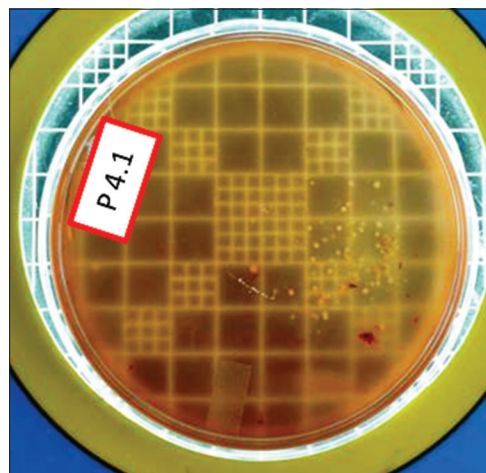


Figure 4: Total viable count preexcavation in Group II

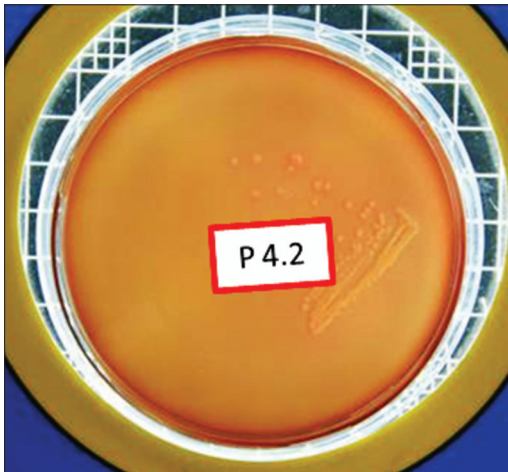


Figure 5: Total viable count postexcavation in Group II

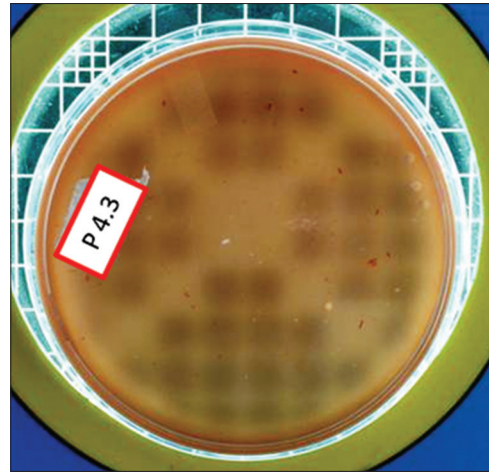


Figure 6: Total viable count postdisinfection in Group II



Figure 7: Total viable count preexcavation in Group III

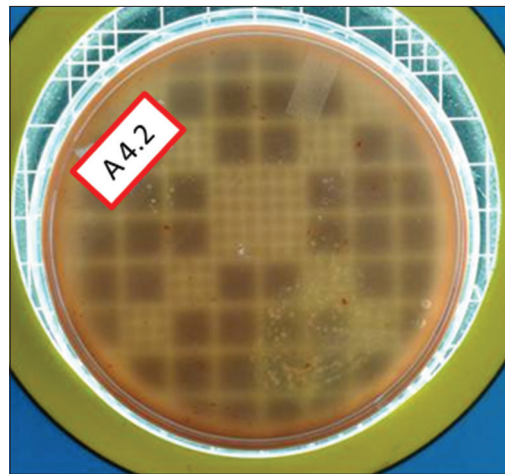


Figure 8: Total viable count postexcavation in Group III

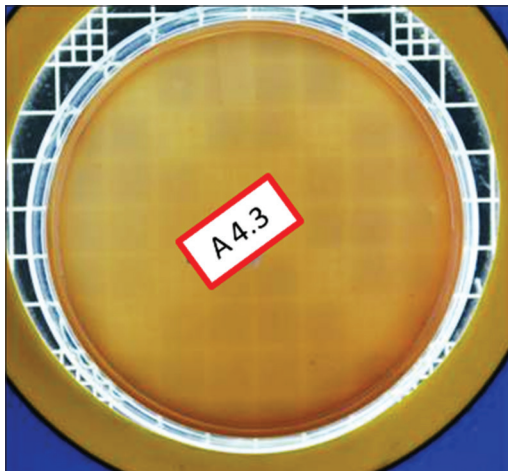


Figure 9: Total viable count postdisinfection in Group III

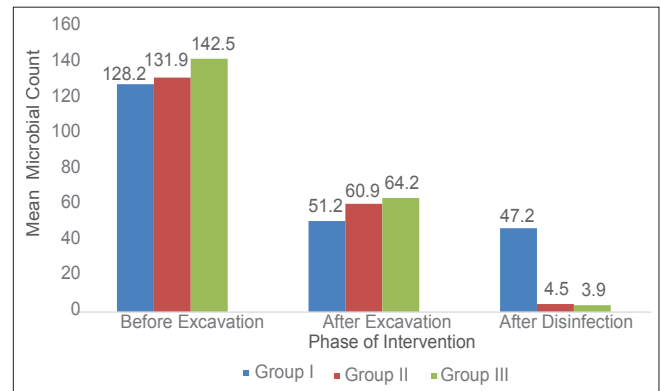


Illustration 1: Comparison of the mean bacterial count between the study groups before excavation, after excavation and after cavity disinfection

Discussion

Restorative dentistry was traditionally based on the premise that bacterial infection of demineralized dentine should and

must prompt operative intervention. However, the more recent concepts encompassing the MID philosophy emphasize the need to create a favorable environment for arresting the progress of caries with bare minimum operative intervention.^[14] While using

minimally invasive techniques such as ART, the limitations in terms of accessibility and fatigue of hand and wrists, increase the risk of incomplete excavation,^[14,15] thereby allowing cariogenic bacteria to survive incarceration under adhesive restorations.^[16] Even in conventional restorative techniques, residual or secondary caries is one of the most common reasons for the ultimate failure of the restoration.^[14,17,18]

Several concepts using antimicrobial agents have been tried to eliminate bacteria underneath such restorations, the predominant one being the use of cavity disinfectants. In this regard, chlorhexidine has been studied extensively^[7,19] and it was found to reduce residual bacteria after cavity excavation, but it has shown adverse effects on the microtensile^[20] and shear bond strengths^[21] as well as on microleakage.^[20,22]

Thus, there is a global need for an alternate cavity disinfectant, which is safe, effective and economical. With the growing popularity of phytotherapy, natural products are increasingly being considered as better alternatives to synthetic chemicals.^[23] Thus, in the present study, we used ethanolic extracts of naturally available *A. vera* and propolis for disinfecting the cavities before restorative procedure.

Table 2: Comparison of the bacterial colony count in each of the study groups using repeated measures ANOVA

Group	Procedure	Mean	SD	F statistic	P
I	Postexcavation	128.20	47.35	61.64 (1.01, 9.13)**	<0.001
	Postexcavation	51.20	27.07		
	Postdisinfection	47.20	26.61		
II	Preexcavation	131.90	51.73	59.90 (1.13, 10.18)**	<0.001
	Postexcavation	60.90	28.77		
	Postdisinfection	4.50	5.23		
III	Preexcavation	142.50	82.23	28.80 (1.17, 10.61)**	<0.001
	Postexcavation	64.20	47.35		
	Postdisinfection	3.90	5.60		

**Corrected using Greenhouse-Geisser estimate of sphericity.
SD: Standard deviation; ANOVA: Analysis of variance

The results of our study showed that there were no statistically significant difference between the number of bacterial colonies among the three different study groups at various phases like preexcavation and postexcavation, thereby reiterating the importance of split mouth technique as well the use of a standardized method of caries excavation adapted in this study. When the TVC between pre and postexcavation of caries were compared, there was a significant reduction in the counts. However, hand excavation alone was not efficient enough to completely eliminate cariogenic bacteria. This is in agreement with previous studies, which have been critical about the ART technique based on the argument that hand excavation can never come close to its rotary counterpart as far as the efficiency of eliminating the carious tissues is concerned.^[14,24]

An important and clinically relevant finding of the present study is that there was a significant reduction in the bacterial counts after cavity disinfection with propolis and *A. vera* when compared to the control group (Distilled water). These findings can be supported by the findings of previous investigators such as Mohammad Zadeh *et al.* (2007) who postulated that propolis had no significant clinical toxicity and was capable of preventing the growth of all the tested microorganisms including bacteria and fungi.^[25] Similarly, Fani and Kohanteb (2012) had suggested that *A. vera* gel at optimum concentration could be used as an antiseptic for preventing dental caries and periodontal diseases.^[26]

Mechanism of action of propolis

Flavonoids and Cinnamic acid derivatives have been widely cited as the main biologically active compounds in propolis.^[27,28] Though, antimicrobial activities of propolis have usually been attributed to the aforementioned entities, other constituents of propolis too have been found to be contributing to the same cause. For instance, Ikeno *et al.* reported that cinnamic acid identified from Chinese and Japanese propolis demonstrated antimicrobial activity against *Streptococcus mutan*.^[29] Similarly, Aga *et al.* stated that some hydroxycinnamic acid derivatives isolated in Brazilian propolis showed significant antimicrobial

Table 3: Pairwise comparison of different phases in each of the study groups using Bonferroni post-hoc test

Group	(I) Factor	(J) Factor	Mean difference	Percentage reduction	95% CI	P
I	Preexcavation	Postexcavation	77.00	60.06	47.48-106.51	<0.01*
	Preexcavation	Postdisinfection	81.00	63.18	51.48-110.51	<0.01*
	Postexcavation	Postdisinfection	4.00	7.81	1.03-6.96	<0.01*
II	Preexcavation	Postexcavation	71.00	53.82	44.59-97.40	<0.01*
	Preexcavation	Postdisinfection	127.40	96.58	80.54-174.26	<0.01*
	Postexcavation	Postdisinfection	56.40	92.61	31.51-81.28	<0.01*
III	Preexcavation	Postexcavation	78.30	54.94	36.28-120.31	<0.01*
	Preexcavation	Postdisinfection	138.60	97.26	65.86-211.33	<0.01*
	Postexcavation	Postdisinfection	60.30	93.92	20.29-100.30	<0.01*

*P<0.01 statistically significant. CI: Confidence interval

activity.^[30] Further, Iio *et al.* demonstrated that Chrysin (one of the key components in propolis) inhibited glucosyltransferase (GTF) activity as well as glucan formation.^[31] Results of several studies confirmed that other propolis constituents such as Pinocembrin and Naringenin possess antibacterial activity as well.^[32,33] In addition, Duarte *et al.* found that fatty acids were the putative active compounds in Brazilian propolis and influenced some of the critical virulence factors associated with the pathogenesis of dental caries, including acid production, F-adenosinetriphosphatase Pase, and GTF activities.^[34] Takaisi-Kikuni and Schilcher reported that propolis prevented bacterial cell division and also broke down bacterial walls and cytoplasm similar to the action of some antibiotics.^[35] Thus, the antibacterial characteristic of propolis have been explained based on the synergy between these compounds along with unique properties of each constituent.^[36-38]

Mechanism of action of *Aloe vera*

Aloe vera has been used therapeutically, since Roman times and perhaps long before.^[39,40] The antimicrobial activity

of *A. vera* is attributed to a number of pharmacologically active compounds including anthraquinones, aloin, aloe-emodin, aloetic acid, anthracene, aloe mannan, aloeride, antranol, chrysophanic acid, resistanol, and saponin.^[41] Aloin, a bitter-tasting yellow compound, is the C-glycoside derivative of an anthraquinone.^[42] Aloin and aloe-emodin possess strong antibacterial and antiviral activities as well as laxative, hepatoprotective, and antineoplastic characteristics.^[43] Aloin and aloe emodin are the major anthrquinones in aloe plants, and these can inhibit protein synthesis from bacterial cells, thus explaining their antimicrobial activity.^[44] It is noteworthy that some compounds like anthraquinones and saponin present in *A. vera* gel have direct antibacterial activities while some other components, such as acemannan, have been considered to exert indirect bactericidal activity through stimulation of phagocytosis.^[45] The advantage of alcohol extract of *A. vera* is that the active ingredients of *A. vera* are highly soluble in this liquid extract media, thereby enhancing the antimicrobial activity.^[46] Hence, we used an ethanolic extract of *A. vera* in the present study.

In the present study, though *A. vera* was found to be marginally more efficacious when compared to propolis, there was no statistically significant difference between their disinfecting abilities. Thus, it would be fair to suggest ethanolic extracts of both *A. vera* and propolis as potential cavity disinfecting agents although further long-term assessments are recommended.

Conclusion and Points of Clinical Relevance

- Excavation alone cannot completely eliminate cariogenic bacteria
- Ethanolic extracts of *A. vera* or propolis can be used as effective cavity disinfectants, which in turn may help to reduce secondary caries thus contributing to long-term restorative success.

Table 4: Comparison of the bacterial colony counts between the study groups before excavation, after excavation and after disinfection using one-way ANOVA

Phase	Groups	Mean (SD)	F-statistic	P
Before excavation	I	128.20 (47.35)	0.14	0.86
	II	131.90 (51.73)		
	III	142.50 (82.23)		
After excavation	I	51.20 (27.07)	0.36	0.70
	II	60.90 (28.77)		
	III	64.20 (47.35)		
After disinfection	I	47.20 (26.61)	24.10	<0.01*
	II	4.50 (5.23)		
	III	3.90 (5.60)		

*P<0.01 statistically significant. SD: Standard deviation; ANOVA: Analysis of variance

Table 5: Pairwise comparison of the number of bacterial colonies between the study groups before excavation, after excavation and after disinfection using Tukey post-hoc test

	(I) Group	(J) Group	Mean difference (I-J)	95% CI		P
				Lower bound	Upper bound	
Before excavation	III	I	14.30	-54.89	83.49	0.86
	III	II	10.60	-58.59	79.79	0.92
	II	I	3.70	-65.49	72.89	0.99
After excavation	III	I	13.00	-26.48	52.48	0.69
	III	II	3.30	-36.18	42.78	0.97
	II	I	9.70	-29.78	49.18	0.81
After disinfection	I	II	42.70	24.97	60.43	<0.01*
	I	III	43.30	25.57	61.03	<0.01*
	II	III	0.60	-17.13	18.33	0.99

*P<0.01 statistically significant. CI: Confidence interval

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