

Antimicrobial efficacy of apple cider vinegar against *Enterococcus faecalis* and *Candida albicans*: An *in vitro* study

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ABSTRACT

The aim of this *in vitro* study was to compare the antimicrobial efficacy of apple cider vinegar against *Enterococcus faecalis* and *Candida albicans* with 5% sodium hypochlorite. Fresh strains of *E. faecalis* and *C. albicans* were cultured. The antimicrobial efficacy of apple cider vinegar and 5% sodium hypochlorite against both the strains were assessed individually using antimicrobial susceptibility testing - microtiter plate method. In Group A (*E. faecalis* + test group), both the test groups showed similar results, that is, 20% of sodium hypochlorite and 20% of apple cider vinegar showed turbidity and the subculture also was positive for the same. In Group B (*C. albicans* + test group), only 20% of apple cider vinegar showed turbidity; subculture was positive for the same. Apple cider vinegar is as effective as 5% sodium hypochlorite in its antimicrobial efficacy against *E. faecalis* and *C. albicans*.

Keywords: Antimicrobial, apple cider vinegar, root canal irrigant, sodium hypochlorite

Introduction

Adequate root canal irrigation is extremely important for the success of root canal treatment.^[1,2] Root canal failures are very often encountered by endodontic practitioners. Majority of the failures are caused by persistent microorganisms in the root canal even after proper treatment.^[3-5] Studies have shown that 45.8% of the failures are caused by *Enterococcus faecalis*, followed by Peptostreptococci, *Candida albicans*, and *Actinomyces* among the others. Sodium hypochlorite has been commonly used as an endodontic irrigant for the decades. However, along with its various advantages such as tissue dissolving, bactericidal, and virucidal properties, it has major disadvantages principally being its toxicity, risk of emphysema, disagreeable smell, and taste.^[6-8] Hence, many studies are being carried out to find an efficient organic alternative for it. Apple cider vinegar is a type of vinegar made from cider or apple must that has a pale-to-medium amber color. It is claimed to possess anti-inflammatory and antibacterial properties and is being currently used widely recommended for diabetes, weight loss, and healthy nails and skin.

Apple cider vinegar has been tested by researchers in the field of dentistry as a chelating agent.^[9] However, there are no studies to the best of our knowledge that talks about the antimicrobial efficacy of apple cider vinegar against *E. faecalis* and *C. albicans*. This study, thus, aims to assess the antimicrobial efficacy of apple cider vinegar against two of the predominant persistent microbes that is *E. faecalis* and *C. albicans* and compare it with 5% sodium hypochlorite.

Materials and Methods

This *in vitro* study was carried out in the Department of Microbiology in Saveetha Dental College, Saveetha University, and Chennai. This is an *in vitro* experimental study done on microtiter plates. Commercially available apple cider vinegar was autoclaved and used as the test group. 5% sodium hypochlorite was used as the control (Figure 1). Standard strains of *E. faecalis* (Group A) and *C. albicans* (Group B) were used for the study purpose as shown in Figure 2. A micropipette was used to dispense the liquids into the microplates as shown in Figure 3.

Standard strains of microbial suspensions were used. These suspensions were compared with 0.5 McFarland standard. 20 µL containing the bacterial suspension was mixed with 200 µL of the test groups in each of the wells (A1-A5 and B1-B5). The positive and negative control groups were placed in C1-C2 and E1-E4, respectively, as shown in the flowchart in Figure 4.

The microtiter plates, after being filled according to their assigned wells, were placed into the incubator at 37°C for 4 h (Figure 5a and b). After this, 10 ml of the solution from each well was transferred to

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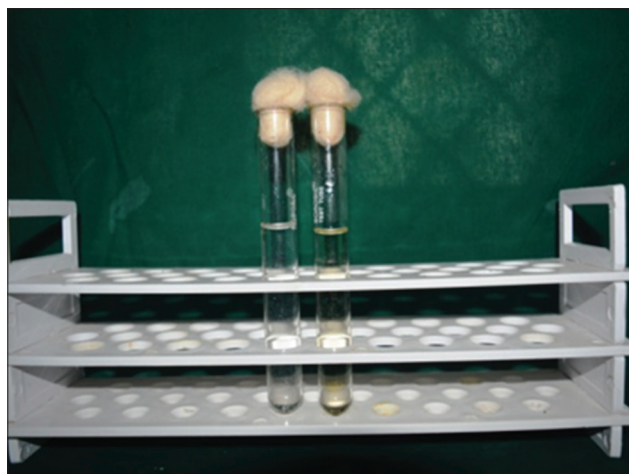


Figure 1: Test Group 1: Apple cider vinegar (autoclaved before use) Test Group 2: 5% sodium hypochlorite

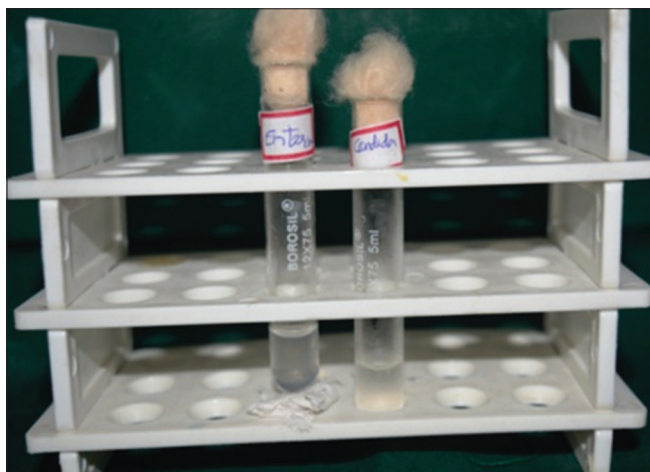


Figure 2: Group A: *Enterococcus Faecalis*; Group B: *Candida albicans*



Figure 3: Microtiter plates and micropipette

brain heart infusion agar. Brain heart infusion agar was prepared for subculture to check the viability of the microbial species. The agar plates were divided accordingly to the assigned groups in the microtiter plates. Soon after, the subculture was done. The plates were incubated aerobically at 37°C for 24 h (Figures 6-8).

Results

In Group A (*E. faecalis* + test group), both the test groups showed similar results, that is, 20% of sodium hypochlorite and 20% of apple cider vinegar showed turbidity, and the subculture also was positive for the same (Figures 9 and 10). In Group B (*C. albicans* + test group), only 20% of apple cider vinegar showed turbidity; subculture was positive for the same (Figures 11 and 12). The efficacy of sodium hypochlorite (5%) was similar to apple cider vinegar against *E. faecalis*, but apple cider vinegar was not as effective and sodium hypochlorite (5%) against *C. albicans*.

Discussion

The use of irrigating solutions in combination with canal instrumentation, loosen debris, pulp tissue, and microorganisms from the irregular dentin walls so that they can be removed from the canal. The main objectives of any irrigating solution are mechanical and chemical flushing, lubrication, dissolution antimicrobial, and smear layer removal. Apart from these, it should be non-irritating to the periapical tissues and not cause any potential harm.

The most popular root canal irrigant currently used is sodium hypochlorite. Its popularity is due to its tissue dissolving property along with being antimicrobial and potent lubricant.^[6,10-13] The high pH of sodium hypochlorite interferes in the cytoplasmic integrity with an irreversible enzymatic inhibition, biosynthesis alteration in cellular metabolism, and phospholipids degradation.^[14-16] However, along with its many advantages, it has many disadvantages including toxic and bad odor. Many cases of sodium hypochlorite accidents have been reported.^[17,18] Thus, many alternative irrigants have been researched over the years that will overcome sodium hypochlorite various drawbacks.

Apple cider vinegar is used in a wide number of health-related issues such as in cancer, cardiovascular diseases, body and joint pains, diabetes, and weight loss. Its antimicrobial action is mainly due to the presence of acetic acid in it, that is, it causes loss of cell integrity. This also can be used in dentistry as a potent root canal irrigant. Very few studies have been carried out using apple cider vinegar as a potential root canal irrigant. In a study by Dormelles *et al.*, in 2011, the combination of 2.5% sodium hypochlorite and apple cider vinegar proved to be less potent than plain 2.5% sodium hypochlorite or 2% chlorhexidine.^[19]

Yet, another study used various vinegar solutions concluding that 5% apple cider vinegar eradicated streptococcal biofilm successfully.^[20]

A study by Mota *et al.* in 2014 concluded that apple cider vinegar showed antifungal properties against *C. albicans* and can be used as a therapeutic alternative for patients with denture stomatitis.^[21] In our present study, both 5% sodium hypochlorite and apple cider vinegar showed similar antimicrobial effect in this *in vitro* study against *E. faecalis* (Table 1). However, against *C. albicans*, 20% of apple cider vinegar showed turbidity; subculture was positive for the same

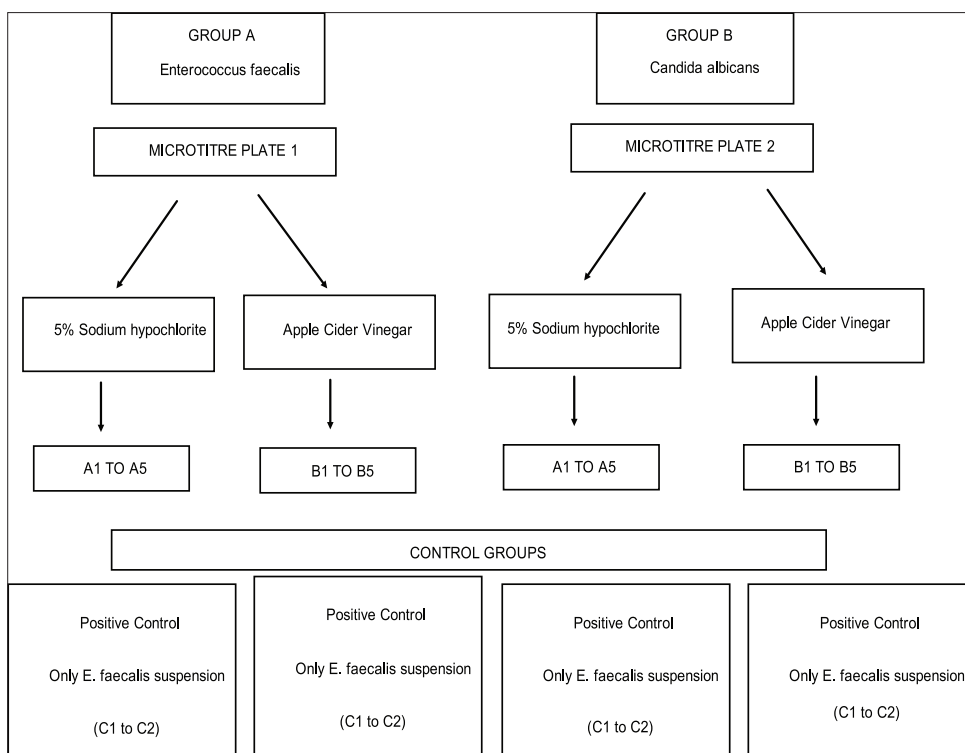


Figure 4: Flowchart depicting the cells and their corresponding numbers on the microtiter plate which the test group and control group were placed using a micropipette

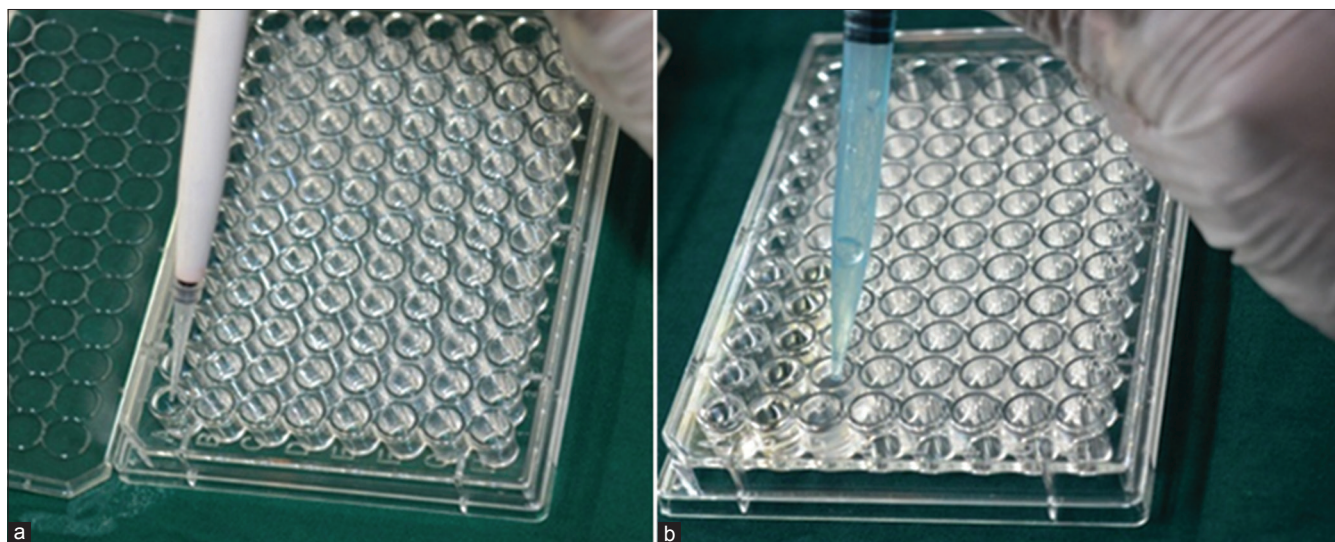


Figure 5: (a) – Dispensing the solutions into the assigned microtiter wells microtiter Plate 1: *Enterococcus faecalis*, (b) - Dispensing the solutions into the assigned microtiter wells microtiter Plate 2: *Candida albicans*

Table 1: The antimicrobial activity of 5% sodium hypochlorite and apple cider vinegar against *Enterococcus faecalis*

Group A: *Enterococcus faecalis* + Test Group

Test Group 1: Sodium hypochlorite (5%)			Test Group 2: Apple cider vinegar		
S. No.	Well No.	Result	S. No.	Well No.	Result
1	A1	Negative	1	B1	Negative
2	A2	Negative	2	B2	Negative
3	A3	Positive	3	B3	Positive
4	A4	Negative	4	B3	Negative
5	A5	Negative	5	B5	Negative

Table 2: Antimicrobial activity of 5% sodium hypochlorite and apple cider vinegar against *Candida albicans*

Group B: *Candida albicans* + Test Group

Test Group 1: Sodium hypochlorite (5%)			Test Group 2: Apple cider vinegar		
S. No.	Well No.	Result	S. No.	Well No.	Result
1	A1	Negative	1	B1	Positive
2	A2	Negative	2	B2	Negative
3	A3	Negative	3	B3	Negative
4	A4	Negative	4	B3	Negative
5	A5	Negative	5	B5	Negative



Figure 6: Incubation done: 4 h at 37°C



Figure 9: Test Group 1: *Enterococcus faecalis* + 5% sodium hypochlorite

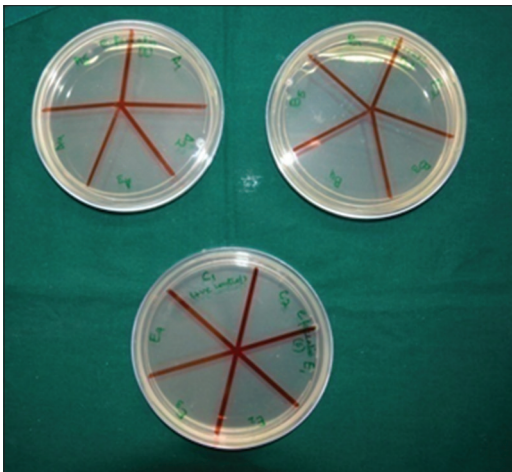


Figure 7: Division of plates according to the assigned groups in the microtiter plates

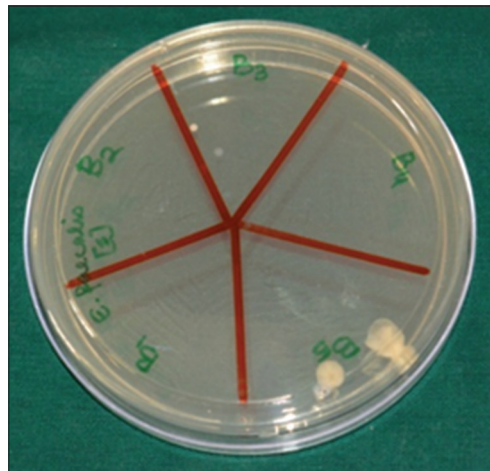


Figure 10: Test Group 2: *Enterococcus faecalis* + apple cider vinegar

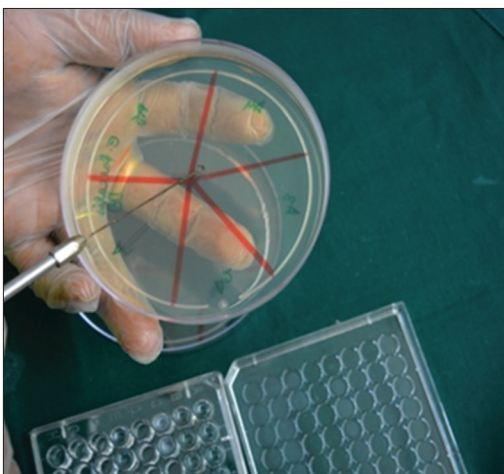


Figure 8: Subculture



Figure 11: Test Group 1: *Candida albicans* + 5% sodium hypochlorite

(Table 2). Since the study is a qualitative analysis, further testing needs to be done to find quantitative analysis of the antimicrobial activity of apple cider vinegar so that it can be used as an intracanal

medicament in root canal treatment. Further studies need to be done on the bioavailability of apple cider vinegar on prolonged exposure in root canal.

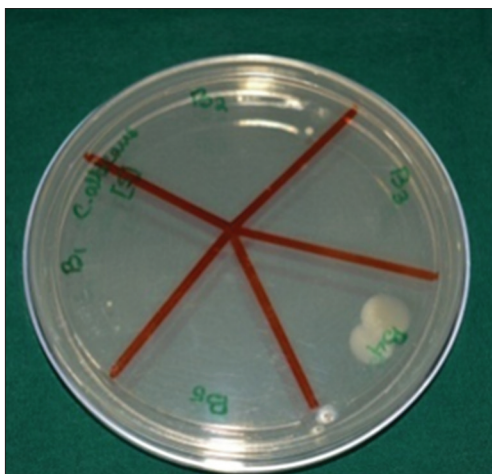


Figure 12: Test Group 2: *Candida albicans* + apple cider vinegar

Conclusion

The antimicrobial activity of apple cider vinegar is almost similar to 5% sodium hypochlorite against *E. faecalis*. However, it is not as effective as 5% sodium hypochlorite against *C. albicans*.

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