Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 19s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Evaluating The Antibacterial Efficacy of Avocado Seeds Extract Against Enterococcus Faecalis: An In Vitro Study for Root Canal Irrigation

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Cite this paper as: Dr. Om Prasad Pal, Dr. Swetha H B, Dr. Thokala Dhamodaran, Dr. Kavana M.G, Dr. Aathees A S, Dr. Sandhra Johnson, (2025) Evaluating The Antibacterial Efficacy of Avocado Seeds Extract Against Enterococcus Faecalis: An In Vitro Study for Root Canal Irrigation. *Journal of Neonatal Surgery*, 14 (19s), 32-37.

ABSTRACT
Root canal infections often involve Enterococcus faecalis, a resilient bacterium that can hide within tooth tubules. Traditional root canal irrigants have limitations, prompting research into alternative agents. One such candidate is an ethanol extract from avocado seeds, known for their bioactive compounds. If effective, avocado seed extract could offer a natural solution for combating Enterococcus faecalis during endodontic treatments, benefiting both patients and clinicians seeking improved strategies for root canal therapy..

Keywords: *Avocado seeds, Ethanol, Enterococcus faecalis, Herbal irrigant.*

1. INTRODUCTION

Pulp and periapical disease occurs because of an opportunistic infection by pathogenic bacteria that invade the pulp and periapical tissues[1]. Therefore, the succesful of endodontic treatment depends on a decrease in the number of microorganisms in the root canal. Some studies indicate that in infected root canal is more than 90% dominated by obligate anaerob bacteria [3]. To achieve the goal of endodontic treatment, an ideal irrigation solution is needed. And other counter artificial chemical irrigant under study are unable to show good biocompatibilities and need for more favourable and naturaly available form of irrigant is required. One such approach is use of herbal irrigants, as a means to reduce microbial load and improved biocompatibility of tissues at the same time.

Enterococcus faecalis is an anaerobic gram-positive coccus that normally commences in the human oral cavity with rich nutrient and low oxygen levels and complex ecology[4]. E. faecalis is an extensively evaluated biological indicator. E. faecalis can survive in very harsh environments, with poor nutrient supply and high alkaline pH reaching up to 11.5.

Avocado seed (Persea americana Mill.) is one of the natural products that are currently widely studied for its antibacterial properties. Persea americana Mill. Is a plant that originated in Central America which is also found in many tropical regions like Indonesia.Phytochemical analysis results show that the avocado seed has secondary metabolites such as flavonoids, saponins, tannins, alkaloids, steroids and terpenoids. The active component act as antibacterial of the seed extract of avocado[2].

The antibacterial efficacy of ethanol extract from avocado seeds, as avocados are known for their diverse bioactive compounds.If proven effective, avocado seed extract could serve as a promising alternative for root canal irrigation, offering a natural and potentially sustainable solution for combating Enterococcus faecalis in endodontic treatments.

2. MATERIALS AND METHODS:

A total of 30 non-carious Single rooted premolar teeth extracted for periodontal or orthodontic purpose were collected for study. Teeth with carious lesion, severe attrition, fractures, cracks & developmental defects and with multiple roots were excluded.

SAMPLE PREPARATION AND METHODOLOGY:

This study used 30 freshly extracted human teeth with a confirmed single canal. Root canals were prepared using Pro taper rotary files up to #30. The sterility of each sample was verified by immersing them in sterile brain heart infusion broth and incubating at 37°C for 48 hours. E. faecalis in freeze-dried form was inoculated in tryptone soy broth and confirmed using bile esculin azide agar. Each sample was immersed in a test tube with sterile BHI broth and contaminated with E. faecalis colonies picked from agar plates. Incubation was done at 37°C for at least seven days. Infected samples were removed from the turbid broth, rinsed with distilled water, and dipped into fresh sterile BHI broth. Turbidity indicated E. faecalis infection.

Avocado Seed Extraction:

Preparation:

Avocado seeds (2 kg) washed and dried.Dried seeds blended and shifted to obtain 300 grams of simplicia powder.

Extraction:

Simplicia powder soaked in 50% ethanol for 15 minutes.Simplicia powder soaked in Saline for non-ethanol extract.

GROUP 1: Sodium hypochlorite irrigation. [CONTROL GROUP]

GROUP 2: Avocado seed extract irrigation with ethanol extract.

GROUP 3: Avocado seed extract irrigation without ethanol extract.

3. METHODOLOGY

IN **GROUP 1** : 2.5% sodium hypochlorite used to irrigate the canal with 10ml solution for 10mins, Then the sample were dabbed in dry sterile gauze, and the canals were dried with the sterile paper points which were then immersed in sterile brain heart infusion broth and incubation was done for 72hours at 37°C. Same methodology were followed for **Group 2** and **Group 3** as well.

Antimicrobial Assessment After Irrigation:

Each group were tested separately using a sufficient 10 number of samples. The bacterial growth after treatment was measured using a colony-forming unit (CFU) count, and the data was statistically analyzed to compare the antimicrobial efficacy of the different treatments against Enterococcus faecalis. All the samples whether it showed turbidity or not were serially diluted five times to obtain the final suspension. 1 mL of suspension from the last dilution was inoculated on bile esculin azide agar with a micropipette and incubated at 37°C for 24 hours. The plates were then observed for growth or no growth. Colony-forming units were obtained from the plates in which growth was observed by dilution plate method.

STATISTICAL ANALYSIS:

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Descriptive Statistics:

Descriptive analysis includes expression of CFUs in terms of mean and standard deviation for each group.

Inferential Statistics:

One-way ANOVA test followed by Tukey's post hoc analysis / Kruskal Wallis Test followed by Dunn's post hoc test [based on data distribution] will be used to compare the mean CFUs between 3 groups.

The level of significance [P-Value] will be set at $P<0.05$.

And any other relevant test, if found appropriate during the time of data analysis were dealt accordingly.

4. RESULTS :

Comparison of mean CFUs / ml (x 10^3) between different groups using Kruskal Wallis Test						
Groups	N	Mean	SD	Min	Max	p-value
Group 1	10	3.33	0.68	3.8	6.4	
Group 2	10	4.38	0.49	4.6	6.6	<0.001*
Group 3	10	6.60	0.59	5.2	7.4	

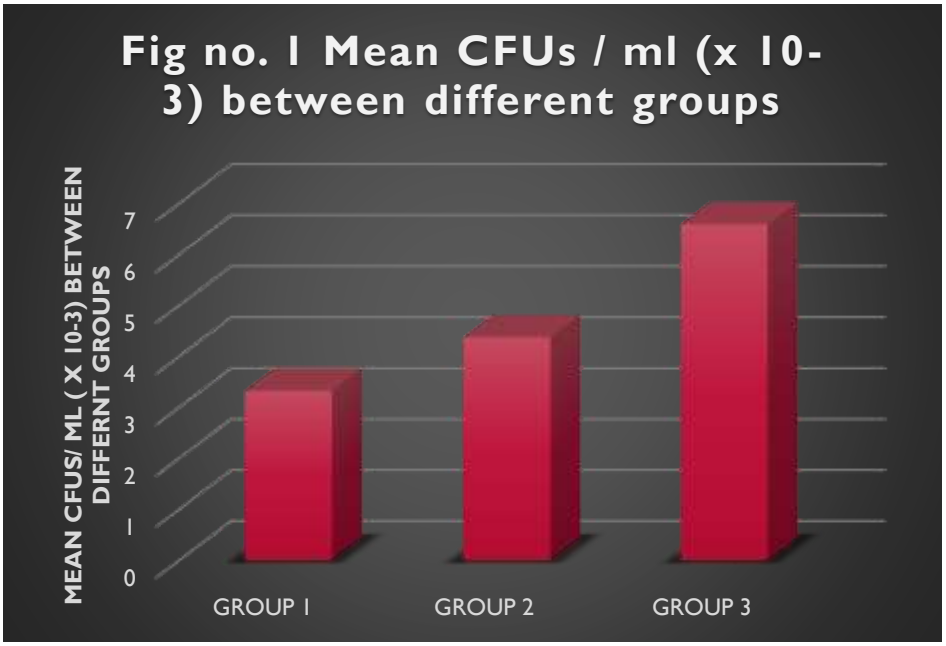
* - Statistically Significant

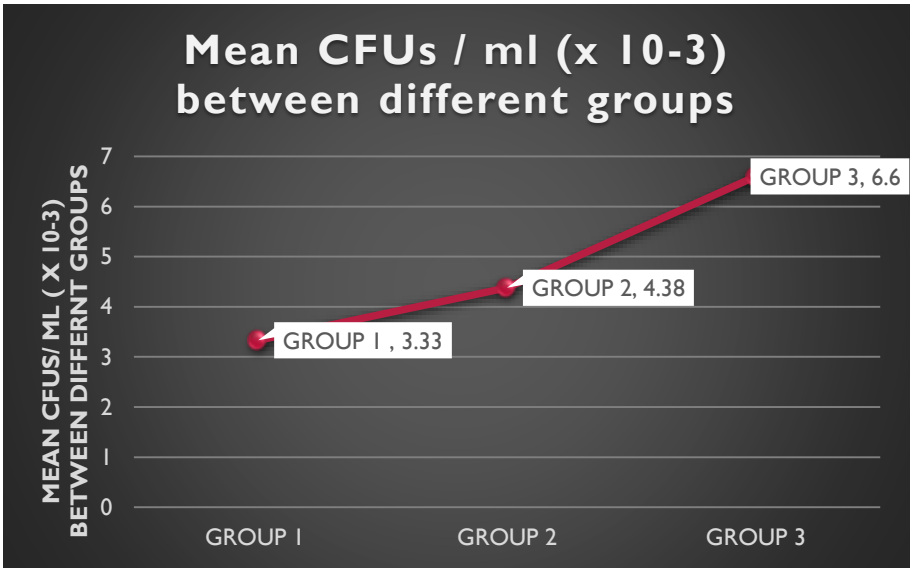
Comparing to control group it is statistically significant.

Note: Group 1 - Sodium Hypochlorite 2.5% group, Group 2 – Avocado seed extract with Ethanol & Group 3 - Avocado seed extract without Ethanol.

The test result showed the mean CFUs count for Group 1 as 3.33 ± 0.68, Group 2 as 4.38 ± 0.49, Group 3 as 6.60 ± 0.59.

The difference in the mean CFUs count between 3 groups was statistically significant at p< 0.001 . [Refer Fig no.1]





5. DISCUSSION:

The study, the avocado seed extraction is done with ethanol. Ethanol used in this study is 50%, which is able to attract the active substances in the seeds of avocado. Ethanol is safer than methanol and effective for extracting antibacterial compounds.The test of antibacterial activity against Enterococcus faecalis done the bacterial growth after treatment was measured using a colony-forming unit (CFU) count.The plates were then observed for growth or no growth. Colony-forming units were obtained from the plates in which growth was observed by dilution plate method.Potentially different outcomes may occur in root canals, where bacterial biofilms and a microbial environment are present. Differences in antibacterial activity in studies are attributed to factors like extract quality, solvent type, and bacterial strain differences.

Various active coumpounds found in Avocado seed extract with ethanol :

Flavonoids, Inhibit nucleic acid synthesis, disrupt cell membranes, and interfere with energy metabolism. Saponins, Act like detergents, reduce cell wall surface tension, and disrupt membrane stability, leading to cell lysis. Tannins, Precipitate proteins, react with cell membranes, and inhibit enzymes, affecting cell adhesion and transport. Steroids, Alter membrane permeability and reduce membrane integrity, leading to cell fragility and lysis.

In Group 1, which is control group showed the least CFU's value among the three groups containing Sodium hypochlorite 2.5% as the irrigant. Goel P, Galhotra VA (2016)in various studies, have already proven sodium hypochlorite as go to irrigant for the successful root canal treatment. But it has got various disadvantages such as Tissue Toxicity, Hypochlorite Accident, Unpleasant Odor and Taste, Dentine Weakening, Allergic Reactions.. Mohammadi Z, Shalavi S (2017). To overcome the following adverse effects , more biocompatible root canal irrigants is to be invastigated and researched .

In Group 2, Avocado seed powder extracted with 50% ethanol was used which showed significant difference in CFU's value when compared to group 1. Nurliza et al (2017) studies have proven that MIC of 50% ethanol enhances the extracting of

active compounds which are present in avocado seeds. Following which the use of 50% Ethanol was necessary to do the same. The adverse effects of using sodium hypochlorite 2.5% such as Tissue Toxicity, Hypochlorite Accident, Unpleasant Odor and Taste, avocado seed extraction with 50% ethanol was used as it is less toxic and more bio compatible.

In Group 3, Avocado seed powder extract without ethanol was used as the irrigant which showed Highest CFU's value comparing the all three groups. Nurliza et al (2017) Various studies have proven that MIC of 50% ethanol enhances the extracting of active compounds which are present in avocado seeds. Without the use of ethanol the extraction of active compounds were inhibited due to which following results were seen.

6. CONCLUSION :

Within the limitations of the study it concludes that the ethanol extract of avocado seed has an in vitro antibacterial effect against E. Faecalis, with MIC at 50% comparing with non ethanolic extract. In Group 1, which is control group showed the least CFU's value among the three groups containing Sodium hypochlorite 2.5% as the irrigant. Group 2, Avocado seed powder extracted with 50% ethanol was used which showed significant difference in CFU's value when compared to group 1. Group 3, Avocado seed powder extract without ethanol was used as the irrigant which showed Highest CFU's value comparing the all three groups. Sodium hypochlorite 2.5% < Avocado seed extract with 50% ethanol < Without ethanol group. Potentially different outcomes may occur in root canals, where bacterial biofilms and a polymicrobial environment are present. Still various studies to be conducted to use the irrigant as useful in clinically

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