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AN INVITRO CROSS-CORRELATION STUDY OF APPLE CIDER VINEGAR AND MORINDA CITRIFOLIA AS EFFECTIVE ALTERNATIVE ANTIMICROBIAL HERBAL IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Aim: The aim of this in vitro study was to compare the antimicrobial efficacy of commercially available herbal agents -Apple cider vinegar, Morinda citrifolia with the gold standard sodium hypochlorite against Enterococcus faecalis when used as root canal irrigants for disinfection of root canal system. **Methodology:** Fresh strains of E. faecalis were cultured. The antimicrobial efficacy of apple cider vinegar, Morinda citrifolia and Sodium hypochlorite against the strains of E. faecalis were assessed individually using antimicrobial susceptibility testing. Group A control group (E. faecalis + saline), group B (E. faecalis + NaOCl), group C (E. faecalis + Morinda citrifolia), and group D (E. faecalis + apple cider vinegar). Brain heart infusion agar was prepared for a subculture to check the viability of the microbial species. **Results:** The efficacy of apple cider vinegar against E. faecalis was comparable to that of sodium hypochlorite (5.25%). Morinda citrifolia showed inhibitory action against E. faecalis, but it has a lower inhibitory effect than NaOCl and apple cider vinegar, the least efficacy was seen in the normal saline group. **Clinical Relevance:** "Nature itself is the best Physician" - In order to achieve proper microbial control of infected root canal flora significant research on the properties and mechanism of action of various antimicrobial solutions have been extensively researched. Though the adequacy & potency of chemical disinfectants have been proven, there is an earnest search among researches for more safer, less harsh, non-chemical natural herbal alternatives. This invitro

cross-correlation analysis highlights the potency of two popular herbal disinfecting solutions namely apple cider vinegar and Morinda citrifolia in eliminating E. faecalis strains from radicular dentin, thereby substituting natural phytates over harmful chemical regimens for disinfection of the root canal system.

KEYWORDS: E. faecalis, 5.25% NaOCl, Morinda citrifolia, Apple cider vinegar.

ABBREVIATIONS: Sodium hypochlorite (NaOCl), chlorhexidine (CHX), Apple Cider vinegar (ACV), Morinda citrifolia Juice (MCJ), ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide (H_2O_2).

INTRODUCTION

The primary cause of apical periodontitis is infection of the root canal system. Although chemical and physical factors can induce inflammation in the periradicular tissues, a large body of scientific evidence indicates that microorganisms play an important role in the progression and persistence of apical periodontitis.[1]

Root canal infections have a polymicrobial etiology caused by a diverse consortium of more than 600 species of bacteria arranged in biofilms. One of the most common microbes in infected root canals and retreatment cases of apical periodontitis has been identified as E.faecalis. The prevalence of C.albicans in association with E.faecalis increases most commonly in cases of secondary persistent infections in retreatment cases. Both these microorganisms have notorious reputation of being most persistent and resistant, especially in secondary infections, owing to multiple virulent factors.[2]

E.faecalis is a gram-positive facultative anaerobe that can invade dentin tubules and is resistant to a variety of irrigating solutions and intra-canal medications. In primary endodontic infections, it has a prevalence of 4-40%, while in persistent endodontic infections, its prevalence varies between 24 - 77 %.[3]

Chemo mechanical preparation plays a significant role in achieving successful endodontic therapy. The use of an irrigant with good penetrability and bactericidal activity is required to inhibit microorganisms present in biofilms, dissolve necrotic pulp remnants, inactivate endotoxins, and remove the tenacious smear layer due to the limitations of mechanical instruments to reach far and beyond in narrow isthmuses, accessory canals, and dentinal tubules. Till date, many irrigants have been used such as 0.5%–6% sodium hypochlorite

(NaOCl), 0.2%–2% chlorhexidine (CHX), 3%–30% hydrogen peroxide (H2O2), 2%–5% iodine potassium iodide, 0.5% dequalinium acetate, 10%–17% ethylenediaminetetraacetic acid (EDTA), 10%–50% citric acid, proteolytic enzymes, sodium hydroxide, urea, potassium hydroxide, local anesthetic solutions, and normal saline. Various more recent irrigants, including tetraclean, electrochemically activated solutions, ozonated water, and photon-activated disinfection, have also been tested in varying clinical situations. However, NaOCl has remained the gold standard against which efficacy of other irrigants is adjudged,[2] due to its easy availability and broad spectrum of activity and tissue dissolving ability. However, it has a number of drawbacks, including tissue toxicity, emphysema, and a burning sensation when contacting oral tissues. As a result, alternate irrigating solutions other that successfully eradicate E. faecalis from radicular dentin are required.[4]

Research has been exploring the benefits of introducing phytates into the clinical realm of allopathic treatments due to its myriad benefits. Herbal agents are increasingly being researched in root canal disinfection since they are less expensive and have less side effects than standard NaOCl. Noni is the fruit of the Morinda citrifolia tree, which has a wide range of antibacterial characteristics. Murrey et al. (2008) demonstrated that Morinda citrifolia has efficacy against E. faecalis that is comparable to NaOCl and with no tissue toxicity or adverse effects.[5]

MCJ though nascent to endodontics, it has been suggested as an alternative to NaOCl because of its chelating ability to remove smear layer which helps to improve the penetration of the disinfectants deeper into the tissue and enhance their antimicrobial properties, especially against anaerobic bacteria such as *E. faecalis* and *C. albicans*.[2]

Due to its abundance of bioactive components, apple vinegar is a natural product that is frequently used in food and traditional medicine. Apple Cider vinegar (ACV) is an alcoholic or an acetic fermentation of apple fruits[6], produced either by the traditional method, called the Orleans method, or by the rapid (or submerged) method used for industrial purposes with variant types of apples. Vinegar is considered as a safe and natural food without any additions, as described by GRAS (Generally Recognized As Safe).[7] It is well-known for its various biological activities, such as antioxidant, antibacterial, and antifungal activity. ACV is found to have potent antimicrobial activity due to its phytochemical compounds, organic acids, phenolic compounds, tannins, flavonoids, and carotenoids, all of which contribute to its antioxidant and antibacterial action against many pathogenic microbes.[8]

Although it is effective at removing smear layers, there are limited studies in literature that compare the efficacy of ACV against E. faecalis.

The objective of this research is to evaluate and compare the antimicrobial efficacy of Morinda citrifolia, and ACV with NaOCl, against E. faecalis.

MATERIALS AND METHODOLOGY

This in vitro study was conducted at the Panineeya Mahavidhyalaya Institute of Dental Sciences and Research Centre in Hyderabad, in the Department of Oral Microbiology.

For the investigation, standard strains of E. faecalis microbiological suspensions were used, as shown in (fig1). The 0.5 McFarland standard was used to compare these suspensions. A micropipette was used to distribute the liquids into the Eppendorf tubes.

SAMPLE SIZE

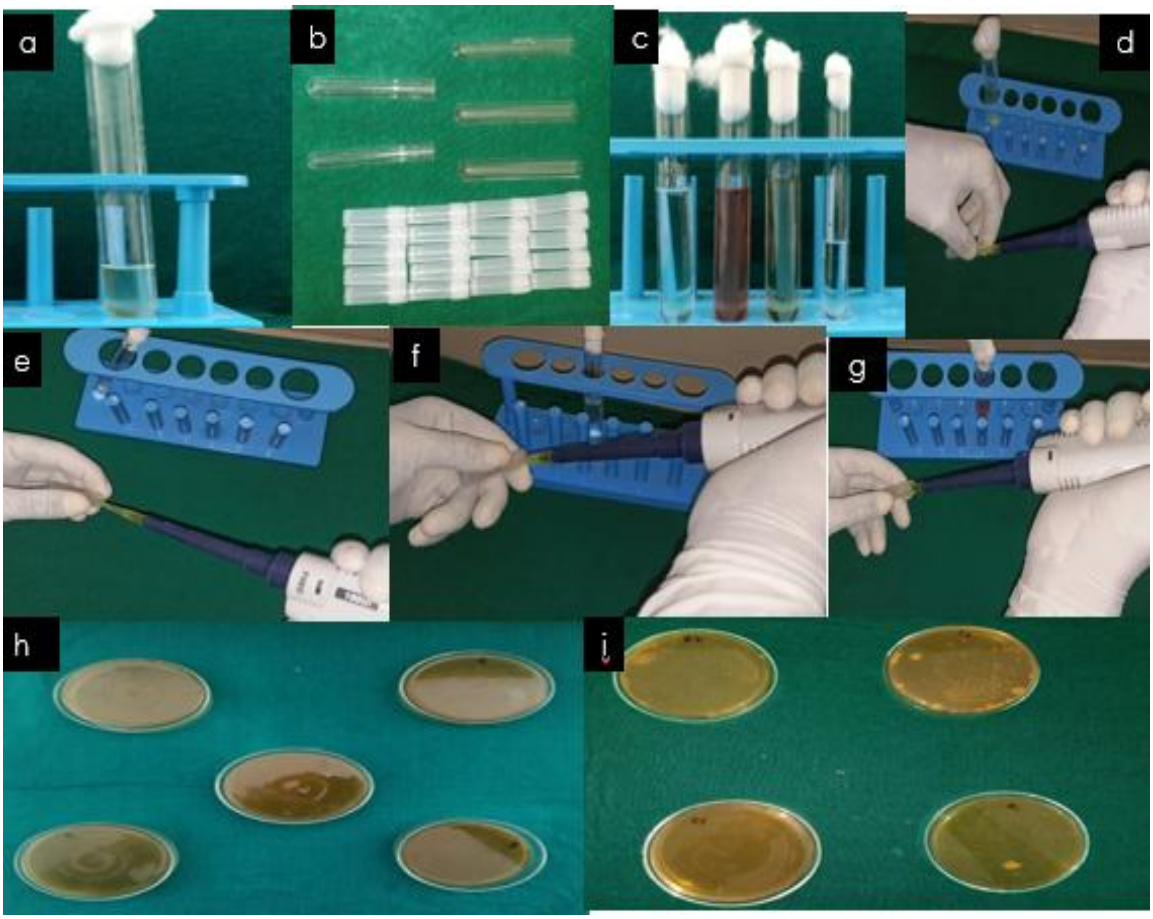
40 Eppendorf tubes (fig 2) were taken and divided into test and control groups (fig3). each group contains 10 samples. The control group contains saline, while the test group contains commercially available apple cider vinegar (PATANJALI" AYURVED PVT LTD, Haridwar, India), 6% Morinda citrifolia (SPECTRUM NONI FRUIT JUICE DHANIKUL FOOD AND BEVERAGES PVT LTD, Mumbai, Maharashtra, India 6 percent dilution) and 5.25 percent sodium hypochlorite(PRIME DENTAL PRODUCTS PVT LTD, Bhiwandi, Maharashtra, India) (fig4).

1) GROUP A – Normal Saline + E.fecalis (A1-A10)(fig5),
2) GROUP B – NaOCl 5.25 % + E.fecalis (B1-B10)(fig6),
3) GROUP C – Morinda citrifolia 6% + E.fecalis (C1-C10)(fig7), and
4) GROUP D – Apple cider vinegar + E.fecalis (D1-D10)(fig8)

MICROBIAL INOCULATION

In each of the Eppendorf tubes, 20 µL of bacterial suspension [fig 1(a)] were mixed with 200 µL of the test groups (A1-A10, B1-B10, C1-C10, D1-D10) [fig1(c-g)]. The eppendorf tubes were placed in the incubator at 37°C for 4 hours after being filled according to their assigned eppendorf tubes. Following that, 10 mL of each Eppendorf tube's solution was transferred to brain heart infusion agar [fig 1(h)]. To test the viability of the microbial species, brain heart infusion agar was produced for subculture. The agar plates were separated into test and control groups. After 24 hours of aerobic incubation at 37°C, colony forming units were

counted in a digital colony counting machine [fig 1(i)]. The data was tallied and analyzed using One-way ANOVA.



[Fig 1]: a) E.faecalis bacterial suspension b) Eppendorf tubes c) Preparation of test irrigants

d) E.faecalis+ Normal saline e) E.faecalis+ NaOCl f) E.faecalis + Apple cider vinegar

g) E.faecalis+ Morinda citrifolia h) Brain heart infusion agar subculture

i) Microbial colonies after 24 hrs inoculation

RESULTS

Table 1: Comparison of overall effect of each test irrigant against E.faecalis.

	Endodontic Irrigant	CFU	CFU	CFU	CFU	N
1	Saline	120.7000	2.243200	116.1506	125.2494	10
2	NaOCl	1.5000	2.243200	-3.0494	6.0494	10
3	Morinda	32.1000	2.243200	27.5506	36.6494	10
4	Apple cider vinegar	2.0000	2.243200	-2.5494	6.5494	10

Table 2: Summarizes the mean CFU/mL of *E. faecalis* after use of different test root canal irrigants.

	Group	Average colonies	- 95 % CI	+ 95 % CI	Sample size
GROUP A	Saline	120.7000	116.1506	125.2494	10
GROUP B	NaOCl	1.5000	-3.0494	6.0494	10
GROUP C	Morinda	32.1000	27.5506	36.6494	10
GROUP D	Apple cider vinegar	2.0000	-2.5494	6.5494	10

	SS	Degr. of	MS	F	p
Intercept	61074.23	1	61074.23	1213.730	<0.0001
Group	94977.28	3	31659.09	629.162	<0.0001
Error	1811.50	36	50.32		

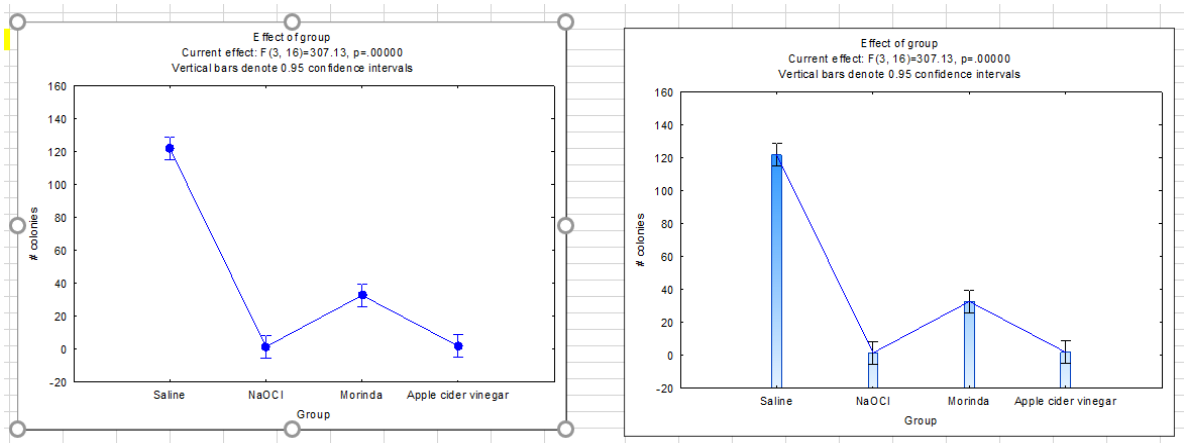


Figure 2: Compares the effect of different root canal irrigants on mean microbial counts (cfus/ml) of e. Faecalis after a period 0f 24hours.

There was a decrease in microbial counts of *E. faecalis* in all control groups. NaOCl group shows lowest amount of colony forming units which is comparable to apple cider vinegar. Morinda citrifolia showed inhibitory action against E. faecalis, but it has a lower inhibitory effect than NaOCl and apple cider vinegar, the least efficacy was seen in the normal saline group. (fig2).

The decrease in microbial counts was found to be statistically significant ($P < 0.001$) on intergroup comparisons by using one-way ANOVA test.

DISCUSSION

The chemo mechanical phase of endodontic therapy is crucial to the success of endodontic treatment. Thorough cleaning removes microorganisms, permits a better adaptation of filling

materials, and enhances the action of intracanal medicaments. The role of various irrigating solutions when used in combination with root canal instrumentation procedures is to help loosen and remove organic debris, pulp tissue remnants and microbiota embedded within the irregular dentin walls from the canal system. Irrigating solutions should possess main properties like mechanical and chemical flushing, lubrication, dissolution of canal contents, antimicrobial, and smear layer removal. The chemical disinfecting solutions should also be non-toxic, inert and not cause any injury to the tissues of the periapical region.

The most often used standard solution in root canal disinfection is sodium hypochlorite (NaOCl), which is a low-cost approach with excellent antibacterial action against the microbiota of infected root canals.[9]

Sodium hypochlorite (NaOCl) was first recognized as an antibacterial agent in 1843 when surgeons performed hand washing with hypochlorite solution between patients and found unusually low rates of infection transmission between patients. This began its inception as a disinfectant solution worldwide with its use, first recorded as endodontic irrigant in 1920.

Its ability to oxidise and hydrolyse cell proteins, as well as its tissue solvent capacity, add to its utility as a potent irrigating solution. Also, this chemical agent reaches new locations within root canals and cleans them, dissolving necrotic-purulent tissues. Its cytotoxic effects, on the other hand, are proportional to the NaOCl concentration utilized.[8,10] In the literature, serious tissue reactions have been reported as a result of the unintentional extrusion of NaOCl into periapical tissues.[10]

Reeh and Messer (1989) reported a case of injection of sodium hypochlorite (1%) through a midroot perforation of a maxillary central incisor with the typical symptoms of immediate severe pain and swelling, followed by fistulation and erythema which extended upto the infraorbital area. Paraesthesia of the floor and ala of the patient's nose persisted for more than 15 months.[11]

In a case report presented by Sabala and Powell (1989) 5.25% sodium hypochlorite was injected into the periapical tissues of a left maxillary second premolar. The patient developed symptoms of sudden, severe pain and a swelling which rapidly developed, followed by ecchymosis of the skin with completion of root canal treatment at the same visit. Antibiotics

were prescribed and a surgical drainage was also performed to prevent secondary infections. The symptoms had resolved only after nine days.[12]

Ingram (1990) recorded a case of accidental spillage of 5.25% sodium hypochlorite into a patient's eye during endodontic therapy.[13] Allergy to hypochlorite was first reported in 1940 by Sulzberger[14] and subsequently by Cohen and Burns (1984).[15]

Gatot et al. (1991) reported that the patient immediately experienced severe pain and marked oedema developed extending from the lip to the right eye, following injection of 5.25% NaOCl during endodontic treatment of a right maxillary central incisor.[16]

Ehrich et al. (1993) reported that following inadvertent injection of 5.25% NaOCl via the palatal root canal of a maxillary first molar into the maxillary sinus, patient only complained of a taste of sodium hypochlorite but no further symptoms developed.[17]

Caliskan et al. (1994) presented a case where a 32-year-old female developed rapid onset pain, swelling, difficulty in breathing and subsequently hypotension following application of 0.5 ml of 1% NaOCl.[18]

Kavanagh and Taylor (1998) reported that during endodontic treatment of a right second maxillary premolar; NaOCl of unknown concentration was injected beyond the apex. The patient experienced acute severe facial pain and swelling.[19]

Witton et al (2005) reported a case causing paraesthesia and anaesthesia affecting the mental, inferior dental and infraorbital branches of the trigeminal nerve following inadvertent extrusion of sodium hypochlorite beyond the root canals. Witton et al. in 2005 was the 1st person who described reversible facial nerve damage.[20]

Paola Campos et al (2017) reported a case of a 45-year-old woman with moderate pain and crown fracture of first upper right premolar. The tooth was non-responsive to pulp testing. The diagnosis of pulp necrosis was confirmed and RCT was initiated. Bruises and severe inflammation of the right side of the face appeared immediately after irrigation and extrusion of NaOCl 5.25%.[21]

Anubha Sejra et al (2019) reported a case of a 72-year-old female patient complaining of pain in upper right back tooth region in the last 5 days. Intraoral examination revealed maxillary

second premolar with attrition, and it was tender on percussion. During a final rinse of the canal with Naocl, the patient developed sudden distress and intense episode of pain. Immediately, she had a tense, shiny, and warm swelling extending from right lower eyelid to corner of mouth and from ala of nose to angle of mandible.[22]

Mariana et al (2021) presented a case of a 7-year-old female child who was referred to the emergency department for left facial oedema and haematoma after an endodontic treatment. During root canal treatment of a primary first left upper molar the patient felt serious pain during irrigation of NaOCl into the tooth.[23]

Cytotoxicity and adverse reactions of NaOCl is well documented in evidence-based literature reviews. Most often the reason for complications after the use of Naocl irrigation occurs due to extrusion of the solution beyond the apex of the root due to forceful or active/positive pressure application at the time of needle insertion into the canal system. This could lead to tissue reactions at the periapical region and in certain cases allergic responses to Naocl extrusion may also occur. All these adverse effects of hypochlorite led to the search for other tissue compatible irrigants with similar efficacy. In this study we compared the commercially available herbal irrigants like ACV and MC.[24]

The scientific name for the commercially available Noni is Morinda citrifolia. The botanical name Morinda citrifolia is derived from the two Latin words "morus" meaning mulberry and "indicus" meaning Indian. It belongs to the Rubiaceae family (Nelson, 2006) and is used as a popular folk medicine.[25] Its juice possesses antibacterial, antifungal, antiviral, anticancer, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune-stimulating properties.

It is the earliest herbal alternative to be used for disinfecting the root canal system. Studies have shown that oral streptococci which causes dental caries are inhibited by it. Morinda citrifolia juice (MCJ) is a biocompatible irrigant that aids in the attachment of dental pulp stem cells to root canal dentin, which is necessary for regenerative endodontic treatment, its prime components are L-asperuloside and alizarin which are secondary metabolic phenolic compounds.[26,27]

In a study conducted by Prabhakar AR et al (2013), Morinda citrifolia was compared to Chlorhexidine as an anti-microbial endodontic irrigant. The presence of alizarin, scopoletin,

aucubin, and asperuloside in Morinda citrifolia was reported to have considerable antibacterial action. However, it was less than 0.2% Chlorhexidine.[28]

Murray et al. (2008) examined the efficacy of MCJ, sodium hypochlorite, and chlorhexidine gluconate in removing the smear layer from the walls of instrumented root canals, and found that 6% MCJ could be utilised as an endodontic irrigant.[5]

Because it is a biocompatible antioxidant, the use of MCJ as an endodontic irrigant may be beneficial because it is less likely to cause severe injuries to patients that might occur through NaOCl accidents. Before MCJ can be definitively suggested as an intracanal irrigating solution, preclinical and clinical research are required to assess its biocompatibility and safety. However, Yoshida etal (1995) in their in vitro observations suggest that the effectiveness of MCJ when combined with an EDTA rinse is promising.[29]

Apple cider vinegar, also known as cider vinegar or ACV, is pale medium-colored vinegar prepared from cider or apple must. ACV is obtained from the made from the crushed pulp of fermented apples. It contains pectin, vitamin b1, vitamin b2, and vitamin b6, as well as biotin, folic acid, niacin, pantothenic acid, and vitamin c.[30]

It is used to treat cancer, cardiovascular disease, body and joint discomfort, diabetes, and weight reduction, among other ailments. This can also be used as a potential root canal irrigant in dentistry. However there have been limited studies on ACV as a root canal irrigant.

Its antibacterial activity is mostly owing to the presence of acetic acid in it. Vinegar's organic acids, particularly acetic acid, penetrate through microorganism cell membranes, causing bacterial cell death. The antibacterial action of organic acids is influenced by bacterial strains, temperature, pH, acid concentration, and ionic strength.

According to Zhang et al (2001), organic acids have a residual effect in preventing the growth of harmful microorganisms. Organic acids have the ability to release protons H+ into cells, which lowers intracellular pH, causing microbial membrane cells to be destroyed. High amounts of hydrogen ions, on the other hand, cause the protonation of cell macromolecules, which causes the bacteria to become destabilized and induces their death.[31]

According to Dormelles et al., in 2011 the combination of 2.5 percent sodium hypochlorite and ACV was less effective than 2.5% NaOCl or 2% chlorhexidine.[32]

Estrela et al (2005), tested the antimicrobial capacity of four vinegars (apple, rice, red, and white wine) in a mixed suspension of microorganisms (S. aureus + E. faecalis + P. aeruginus + B. subtilis + C. albicans) and a pure suspension of E. faecalis for 24, 48, 72, and 7 days, although all of the solutions were efficient against E. faecalis, ACV showed the best results against the mixed suspension.[33]

Ismael NF et al. (2013) used various vinegar solutions and found that 5% ACV successfully eliminated streptococcal biofilm.[34]

Soorya M.S (2018) evaluated the anti-microbial and antifungal efficacy of ginger extract, commercially available ACV and fruit vinegar and compared it with that of 5.25%NaOCl and concluded that commercially available ACV and FV were effective against E. faecalis and C.albicans, however the ginger extract was not effective against both the tested organisms.[35]

In the present study antimicrobial efficacy of ACV, Morinda citrifolia and Sodium hypochlorite against the strains of E. faecalis were assessed individually using antimicrobial susceptibility testing. The results showed that both 5.25% sodium hypochlorite and ACV demonstrated equal antibacterial effects with statistically comparable results(table-2). When compared to the control group, M.citrifolia had less colony forming units(table-1), but the mean colony forming units(table-2, fig-1) were larger when compared to 5.25 % NaOCl and ACV. However, in a clinical scenario the root canal biofilm has multispecies microorganisms which may alter the efficacy of these irrigating solutions. Thus, further invitro and exvivo studies with large sample size are required to evaluate the role of test irrigants on all possible microbes which are present in root canal biofilm.

CONCLUSION

From the observations made from this research design the antimicrobial activity of ACV is almost similar to 5.25% NaOCl against E. faecalis, although none of the two test irrigants were effective in completely disinfecting the root canal system. Morinda citrifolia showed inhibitory action against E. faecalis, but it has a lower inhibitory effect than NaOCl and ACV with the least efficacy seen in the normal saline group. The overall antibacterial effect of different irrigants against E.faecalis was maximum with similar comparable efficacy in the NaOCl and ACV groups. Even though the natural herbal irrigants showed promising results, further research on their strength, long-term efficacy and interaction with intra canal medicaments /sealers should be made to extend their benefits as endodontic irrigants. This

would help open avenues to safer, kinder and inert herbal disinfecting solutions as opposed to harsher chemical regimens for cleaning of the radicular system.

CONFLICTS OF INTEREST: The authors declared no conflicts of interest.

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