

Comparative Antimicrobial Efficacy of *Chrysopogon Zizanioides* and *Matricaria Chamomilla* along with 5.25% Sodium Hypochlorite and 2% Chlorhexidine against *Enterococcus Faecalis*: An In-Vitro Study

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Abstract: Aim and Objective: The Aim of The Study Was To Compare the Antimicrobial Efficacy of *Chrysopogon Zizanioides* and *Matricaria Chamomilla* Along With 5.25 % Sodium Hypochlorite and 2 % Chlorhexidine against *Enterococcus Faecalis*. Material and Method: In This Study, Essential Oils (100 % Pure Extract Free From Chemicals) Which Are Commercially Available Have Been Used. For media preparation, 15 g of agar powder were weighed and dissolved in 1000 mL by stirring in distilled water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min. Total 16 agar plates were prepared by using nutrient agar. *E. faecalis* strain was inoculated and then streaked on nutrient agar plate to get single isolated colony. *E. faecalis* were grown overnight at 37°C in nutrient agar. The antibacterial activity of: Vetiver essential oil [Group A], Chamomile essential oil [Group B], 5.25 % Sodium Hypochlorite [Group C] and, 2 % Chlorhexidine [Group D] were determined. Microbial zones of inhibition were measured in the number of colony forming units. Results: There was a significant reduction in the mean colony forming units in group C, which showed fluctuation from 41.75 % to only 6.75 % after the use of irrigant. The maximum percentage reduction was seen in group C being 83.83 % with minimum reduction in group A being only 22.67 %. Group B showed 58.95 % reduction which was comparable with Group D results showing 37.93 % reduction in the number of colonies Conclusion: The present study proves the efficacy of *Chrysopogon Zizanioides* and *Matricaria Chamomilla* against *Enterococcus Faecalis* which is comparable to 5.25 % Sodium Hypochlorite [I] and 2 % Chlorhexidine [III]. The Vetiver Essential oil [IV] proven to be the weaker irrigant than Chamomile Essential Oil [II] and 5.25 % sodium hypochlorite being most efficient amongst all as mentioned above.

Keywords: Antimicrobial, Endodontics, Essential Oils, Root Canal Treatment, Irrigating solutions

1. Introduction

Microorganisms play a fundamental role in the etiology of pulp and periapical diseases. Their control and elimination are important during endodontic treatment⁽⁹⁾

The complex morphology of dentine and the presence of bacteria, especially in the apical part of root canal, even after the disinfection protocols, make this task very tedious, especially *Enterococcus faecalis* infection, which is the most common pathogen responsible for the failure of root canal therapy.⁽¹⁾

The most widely used irrigant for root canal treatment is sodium hypochlorite (NaOCl) at concentrations of 0.5 to 5.25 %. The tissue dissolving capacity and microicidal activity of NaOCl make it an excellent irrigating solution (Pe´rezHeredia et al. 2006).⁽⁵⁾

Chlorhexidine has also been shown to be a potent broad-spectrum antimicrobial that has the advantage of substantivity. CHX has been shown to be more effective against gram-positive organisms than gram-negative organisms.⁽⁸⁾

The field of dentistry is constantly evolving and adopting the best suitable and available antimicrobial agent for the treatment. Since long time, many agents have been used to eradicate or reduce endodontic microflora; however, most of them are found ineffective to combat complete infection and led to the failure of root canal treatment. Therefore, the

search for the novel materials and new techniques which can take dentistry to new heights and make the patient more comfortable is incessant.⁽¹⁾

Matricaria Chamomilla known as (Chamomile) is an annual plant of the composite family Asteraceae. *M. chamomilla* is the most popular source of the herbal product.⁽⁵⁾ Chamomile was also found to be effective when used as a mouthwash to treat irritations and minor infections of the mouth and gingiva (Fidler et al. 1996). It is also used in some tooth pastes (Ruszynska et al. 1986).⁽⁵⁾

Chrysopogon zizanioides, commonly known as **vetiver** is a perennial bunchgrass of the family Poaceae, native to India. Vetiver Essential Oil works as a natural anti-oxidant that promotes relief from various types of inflammation. Its anti-septic properties eliminate and prevent the growth of harmful bacteria.⁽⁷⁾

Considering all the above factors, we planned our study to evaluate the antimicrobial potential of two plant-derived essential oils, namely, chamomile and vetiver, along with traditional irrigants 5.25% Sodium Hypochlorite and 2% chlorhexidine against *E. faecalis*.⁽⁶⁾

2. Materials and Methods

Preparation of Plant Extract

The plant materials (roots) of *Chrysopogon zizanioides* (L.) Roberty (Poaceae) and flowers of *Matricaria chamomilla* L. (Asteraceae) were subjected to steam distillation for 4–6 hrs

using a Clevenger type apparatus. The essential oils were collected after decantation.

Media Preparation

15 g of agar powder were weighed and dissolved in 1000 mL by stirring in distilled water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min. Total 16 agar plates were prepared by using nutrient agar. Agar was mixed according to the manufacturer’s directions, and then stored at room temperature for 2 days before use to verify that they had remained sterile. The growth inhibition and minimum inhibitory concentration of all tested materials were determined *in vitro* following nutrient agar diffusion.

Maintenance of Bacterial Strain

E faecalis was obtained from Government Dental College, Ahmedabad. *E. faecalis* strain was inoculated and then streaked on nutrient agar plate to get single-isolated colony. The Cultures of *E faecalis* were grown overnight at 37°C in nutrient agar in the Department of Pathology and Microbiology at Goenka Research Institute of dental sciences, Gandhinagar.

Making Of Petri Dishes for the Experiment

Sixteen agar plates were prepared. Agar was mixed according to the manufacturer’s directions, and enough agar was poured to cover the surface of a 125-mm diameter Petri dish. The plating was done, and the plates were incubated at 37°C overnight in an incubator at the institution.

The plates were randomly divided into four groups. The Petri dishes were marked on the bottom of the plate to identify the medicament. Each Group [A, B, C, D] (n=4) was incubated aerobically at 37°C for 48 hours.



Figure 1: Marking of Petri-dish at the Bottom to Identify the Medicament

Four disks in each group were saturated with one of the four test solutions, with pipette with Doses of

- 1) Group A : (2.5 ml) of essential oils of Chrysopogon Zizanioides,
- 2) Group B : (2.5 ml) of essential oils of Matricaria Chamomilla,
- 3) Group C : (2.5 ml) of 5.25% Sodium Hypochlorite (by V-Dent Care) and
- 4) Group D: (2.5 ml) of 2% Chlorhexidine (V-Dent Care).

Microbial zones of inhibition were measured in the number of colony forming units. Results were recorded by group and were statistically analyzed with repeated measures analysis of variance (ANOVA)

3. Results

There was a significant reduction in the mean colony forming units in group C, which showed fluctuation from 41.75% to only 6.75% after the use of irrigant. The maximum percentage reduction was seen in group C being 83.83% with minimum reduction in group A being only 22.67%. Group B showed 58.95% reduction which was comparable with Group D results showing 37.93% reduction in the number of colonies

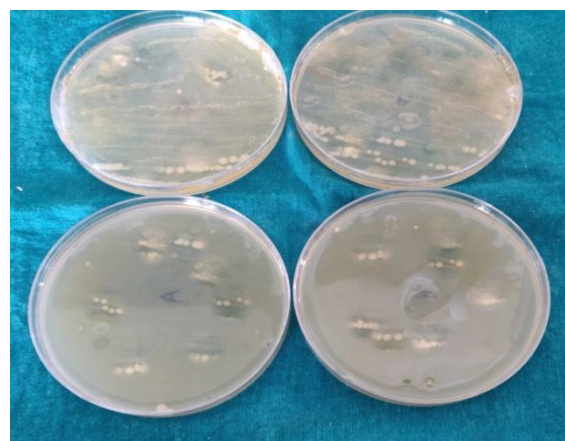


Figure 2: Remaining Colonies after the Use Of Vetiver Essential Oil

Table 1: Analyzing the Antimicrobial Potential of Vetiver Essential Oil

Group A	No. of Colonies	No. of Remaining Colonies	Percentage Reduction
Plate I	32	25	21.87%
Plate II	28	21	25%
Plate III	41	33	19.51%
Plate IV	36	27	25%

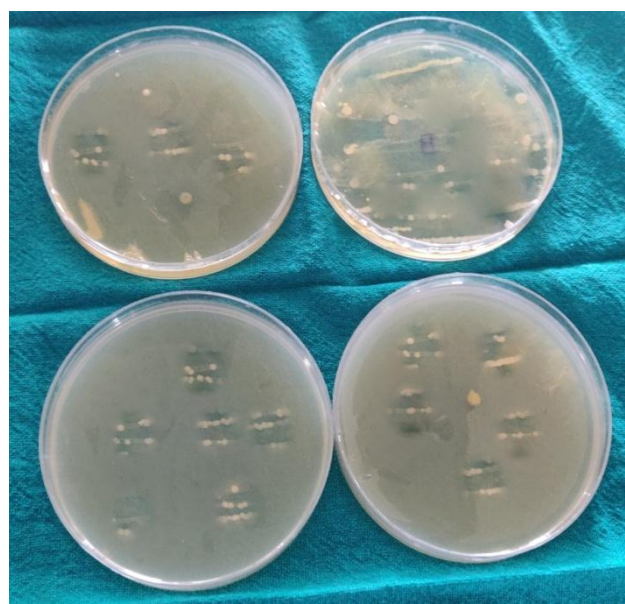


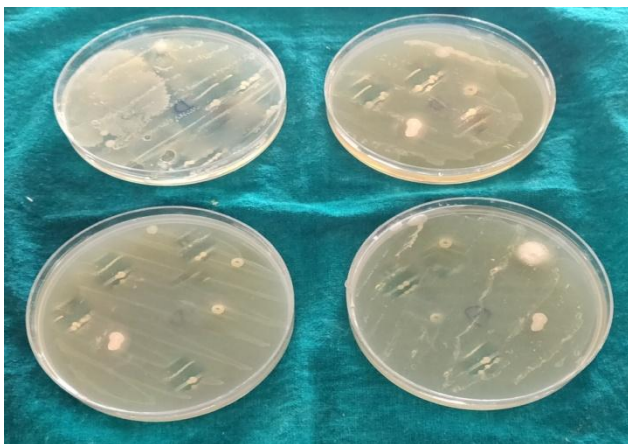
Figure 3: Remaining Colonies after the Use of Chamomile Essential Oil

Table 2: Analyzing the Antimicrobial Potential of Chamomile Essential Oil

Group B	No. of Colonies	No. of Remaining Colonies	Percentage Reduction
Plate I	54	20	62.96%
Plate II	47	23	51.06%
Plate III	33	13	60.60%
Plate IV	39	15	61.53%

**Figure 4:** Remaining Colonies after the Use Of 5.25% Sodium Hypochlorite**Table 3:** Analyzing the Antimicrobial Potential Of 5.25% Sodium Hypochlorite

Group C	No. of Colonies	No. of Remaining Colonies	Percentage Reduction
Plate I	50	9	82%
Plate II	43	6	86.04%
Plate III	35	5	85.71%
Plate IV	39	7	82.05%

**Figure 5:** Remaining Colonies after the Use of 2% Chlorhexidine**Table 4:** Analyzing the Antimicrobial Potential of 2% Chlorhexidine

Group D	No of Colonies	No of Remaining Colonies	Percentage Reduction
Plate I	40	22	45%
Plate II	26	15	42.30%
Plate III	37	25	32.56%
Plate IV	42	28	33.33%

4. Discussion

This *in vitro* study investigated the antibacterial potential of two essential oils, that is, *vetiver* and *chamomile*, and it was observed that activity was comparable with that of

standards, namely, 5.25% Sodium Hypochlorite And 2% Chlorhexidine against *E. faecalis* on nutrient agar plate. This study was planned to provide an alternative for quick, preliminary assessment of antibacterial potential of essential oils of *vetiver* and *chamomile*, and its role as an intracanal medicament. *E. faecalis* infection is commonly associated with failed root canal cases (77%), and approximately 50% in chronic apical periodontitis. These are some of the known reasons which prompt us to choose this microbe for our study.

Sodium hypochlorite has long been the irrigant of choice for nonsurgical endodontic procedures. Sodium hypochlorite dissolves necrotic and vital tissue, has antimicrobial activity, and aids as a lubricant in the canal. It also has minimal "clinical toxicity" when kept within the confines of the canals.

Chlorhexidine has also been shown to be a potent broad-spectrum antimicrobial that has the advantage of substantivity. CHX has been shown to be more effective against gram-positive organisms than gram-negative organisms. Chlorhexidine is positively charged particle reacts with negatively charged molecule present on the bacterial cell wall and destroyed cell hemostasis. It has been known for its activity against *E. faecalis* ⁽¹⁾

Matricaria chamomilla commonly known as **chamomile** is an annual plant of the composite family Asteraceae. *M. chamomilla* is the most popular source of the herbal product. *Matricaria chamomilla* has been found to contain fairly strong anti-spasmodic and anti-inflammatory constituents and is particularly effective in treating stomach and intestinal cramps. The chamomile plant is known to have antibacterial, anti-inflammatory, antiviral, and antioxidant. Its antibacterial properties are mainly due to the presence of α -bisabolol, luteolin, quercetin, and apigenin against many bacteria including *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus salivarius*, and fungi like *Candida albicans*. The effectiveness and clinical efficacy of chamomile have also been reported selectively in removing smear layer, oral mucositis, plaque, scurvy, gingivitis, and patient undergoing orthodontic treatment. ⁽¹⁾ The chamomile oil has a high level of safety, acute oral LD₅₀, and dermal LD₅₀ was found >5 g/kg body weight, and Food and Drug Administration classified it as generally regarded as safe. ⁽¹⁰⁾

Chrysopogon zizanioides, commonly known as **vetiver**, originates in India; *C. zizanioides* is widely cultivated in tropical regions. The major *vetiver* producers include Haiti, India, Indonesia, and Réunion. Its anti-septic properties eliminate and prevent the growth of harmful bacteria. Its tonic properties are reputed to show regenerative effects as they enhance and maintain immunity.

Previously published reports advocate the antimicrobial action of *vetiver* and *chamomile* oils against *E. faecalis*. Other natural products, that is, propolis produced by honey bee tested against *E. faecalis* was found to be highly effective when compared with calcium hydroxide and triple antibiotic mixture. Another study compared *Morinda citrifolia*, papain, and aloe vera. They concluded that *M.*

citrifolia having good antibacterial activity against *E. faecalis*; however, it was not as effective as chlorhexidine. ⁽¹⁾

The Results of the Present Investigations showed that although 2% CHX had less antimicrobial effect on *E. faecalis* than Chamomile Oil, both the medicaments still had an observable effectiveness against this bacterium. The Maximum reduction in Colony forming units was shown by 5.25% Sodium Hypochlorite (83.38%), followed by Chamomile Essential Oil (58.95%). The Minimum reduction in Colony Forming Units was shown by Vertiver Essential Oil (22.67%). However, 2% Chlorhexidine (showing 37.93% reduction) was more effective than Vertiver Essential Oil but Less Effective than Chamomile Essential Oil against *E. Faecalis*. The results of this study support the findings of another study in which they proved the antibacterial role of chamomile essential oil (Vijay kumar shakya et al; 2019; J Conserv Dent). It can be an herbal alternative intracanal medicament to disinfect the root canal.

Table 5: Analysis of Colony Forming Units

Groups	Mean Number of Colonies Present	Mean Number of Colonies Remaining	Percentage Reduction
Group A [Vertiver Oil]	34.25	26.5	22.67%
Group B [Chamomile Oil]	43.25	17.75	58.95%
Group C [5.25% Sodium Hypochlorite]	41.75	6.75	83.83%
Group D [2% Chlorhexidine]	36.25	22.5	37.93%

Since this was an in vitro study, further ex vivo studies are required regarding the same to corroborate with the results of this study before this can be used for the clinical purpose.

5. Conclusion

The present study proves the efficacy of **Chrysopogon Zizanioides** and **Matricaria Chamomilla** against *Enterococcus Faecalis* which is comparable to 5.25% Sodium Hypochlorite [I] and 2% Chlorhexidine [III]. The Vertiver Essential oil [IV] proven to be the weaker irrigant than Chamomile Essential Oil [II] and 5.25% sodium hypochlorite being most efficient amongst all as mentioned above.

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