

Innovations

Antimicrobial Efficacy of Essential Oils against the Growth of Oral Pathogenic Flora in Root Canal using Agar Diffusion Test (ADT): An *in-Vitro* Study

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Abstract

Background: Plant essential oils are well-known for their antimicrobial properties, effective against both gram-positive and gram-negative bacteria, as well as pathogenic fungi. Easily absorbed into the bloodstream, these oils work effectively in the body. For example, clove oil is used for dental pain, while neem oil has antibacterial and antifungal properties. Turmeric oil and thymol are used in dental products like mouth rinses and fillings. Rich in bioactive compounds, these oils show promise for developing new antimicrobial drugs targeting root canal bacteria.

Methodology: The essential oils in this study were divided into five groups: Group 1 (Eugenol), Group 2 (Thyme Oil), Group 3 (Turmeric Oil), Group 4 (Neem Oil), and Group 5 (Cinnamon Oil), all at 5% concentration. Selected based on traditional medicinal use, their antimicrobial properties were compared to chlorhexidine. The oils were tested against five bacterial strains (*S. mutans*, *Lactobacilli*, *E. coli*, *Pseudomonas*, *E. faecalis*) and one fungal strain (*C. albicans*) by measuring inhibition zones using the agar diffusion test at 24, 36, 48, and 72 hours. **Results:** *S. mutans* growth was inhibited at 72 hr by eugenol which showed no activity in 24hr, 36hr and 48hr. Cinnamon oil inhibited *S. mutans* growth at all time intervals. *Lactobacilli* growth was inhibited at all time intervals by thyme oil and neem oil, with neem oil showing better efficacy than thyme oil. For *E. coli*, statistically significant inhibition zones were observed at all time intervals for Groups 1, 2, 3,

and 4, with the effectiveness ranking as Group 4 > Group 2 > Group 1 > Group 3. *Pseudomonas* growth was inhibited at all time intervals by neem oil only. *E. faecalis* growth was inhibited at all time intervals by Groups 2, 3, and 4, with Group 2 > Group 4 > Group 3. *C. albicans* growth was significantly inhibited at all time intervals by thyme oil only. **Conclusion:** Traditionally used medicaments have been shown to have efficacy against the oral pathogenic microflora. Neem oil showed anti-microbial effect in the following order against, *E. coli* > *Lactobacilli* > *E. faecalis* > *Pseudomonas*. Thyme oil showed anti-microbial effect in the following order *E. faecalis* > *E. coli* > *Lactobacilli* > *C. albicans*. Cinnamon oil and eugenol showed anti-microbial effect against *S. mutans*. Eugenol and thymol have been already incorporated into several dental products and they are known to be efficacious in reducing the microbial load. Neem oil can be developed as a paste or an intracanal medicament.

Keywords: Antimicrobial, Essential oils, Pathogenic Flora, Root Canal

Introduction

Dental caries is a preventable multifactorial oral disease caused primarily by an imbalance of the oral flora (biofilm). At the tooth level, dental caries activity is characterized by localized demineralization and loss of tooth structure. Microorganisms and their products are considered to be the primary etiological agents in endodontic diseases.¹ Root canal dentinal tubules harbour microorganisms. Also, bacterial biofilm may be present at the apical portion of root canal and extra-radicular regions.² Microbiological analyses have shown that Gram-positive (*Streptococcus mutans*), (*Lactobacilli*) and Gram-negative bacteria (*Escherichia coli*), (*Enterococcus faecalis*), (*Pseudomonas*) as well as fungi (*Candida albicans*) are present in infected root canals.¹

Endodontic treatment aims to completely eliminate microbes from the pulp space through thorough chemo-mechanical preparation and three-dimensional obturation of the root canal system.³ Despite effective mechanical instrumentation, some bacteria may remain in canal complexities, leading to periradicular inflammation. Thus, mechanical preparation is supplemented with irrigation solutions, with sodium hypochlorite and 2% chlorhexidine being preferred.⁴ Natural products, including various plant parts like roots, bark, seeds, and oils, have been used for centuries for their antimicrobial properties, primarily due to their terpenoid and phenol compounds.⁵ Following table shows examples of few essential oils and their bioactive compounds with their function on micro-organisms (Table 1):^{6, 7, 8, 9, 10}

These herbs have been traditionally used in medicine, but their application in dentistry has not been extensively explored. In the search for plant essential oils with antimicrobial properties, this study aims to evaluate the *in vitro* antimicrobial effects of eugenol, thyme oil, neem oil, turmeric oil, and cinnamon oil against six root canal pathogens. Chlorhexidine, widely recognized as the gold standard in

antimicrobial testing in dentistry due to its broad-spectrum activity and long-lasting effects, was used as a control. To date, no study has assessed the efficacy of five different essential oils against six root canal pathogens. Therefore, this study seeks to determine *in vitro* antimicrobial efficacy of five essential oils against six root canal pathogens by measuring the zone of inhibition.

Materials and Methods

Study Groups

The essential oils were divided into five groups, i.e. group 1, group 2 group 3, group 4, group 5 along with a control group, to assess their efficacy against five bacterial strains (both Gram-positive and Gram-negative) and one fungal strain commonly found in necrotic pulps and endodontic lesions. The antimicrobial activity was evaluated using the agar diffusion test (ADT) at different time intervals. The results were compared with the control group, which was tested against the same microorganisms (Figure 1). Essential oils were sourced from Sugandhco, Lucknow, and eugenol and chlorhexidine from a dental shop, all of analytical grade. The oils were prepared at a 5% concentration, using stock cultures of bacteria and fungus.

Growth Conditions and Culture Media

The culture media used in this study were sourced from HI-Media Laboratories Pvt. Ltd., Mumbai, India. Muller Hinton Agar (MHA) was utilized for the enrichment of *Pseudomonas*, *E. coli*, *E. faecalis*, and *S. mutans*, while MRS media was used for enriching *Lactobacilli*. For the enrichment of *C. albicans*, Sabouraud Dextrose Agar (SDA) was employed. MHA, MRS, and SDA media were prepared according to the standard compositions provided by HI-Media and sterilized in an autoclave (Gentek India Pvt. Ltd.) at 121°C and 15 psi for 15 minutes. After sterilization, the media were poured into sterile glass petri dishes inside a laminar airflow cabinet (Toshiba, India) using aseptic techniques. Each plate was filled with 20 ml of the culture media.

Preparation of Inoculum

After the plates solidified with the nutrient medium, they were inoculated with the respective bacterial and fungal isolates. The inoculum was spread evenly across the plates using the spread plate technique with a sterile glass rod. Ten minutes after spreading, 6mm filter paper discs loaded with the respective essential oils were placed onto the media plates using sterile forceps. (Figure 2, 3, 4, 5, 6) Additionally, two control discs were included on each plate: a positive control disc containing 20 µl of chlorhexidine and a negative control disc loaded with distilled water.

Incubation

The samples were allowed to diffuse from the discs into the media, after which the plates were sealed with paraffin and incubated at 34°C in a digital incubator—a

laboratory growth chamber. Bacterial isolates were incubated for 24 hours, and fungal isolates for 48 hours. Following incubation, the plates were examined for clear zones around the discs, known as zones of inhibition. The diameters of these zones were measured in millimetres using an endodontic millimeter ruler and recorded. For aerobic bacteria (*Pseudomonas*) and facultative anaerobes (*E. coli*, *S. mutans*, *E. faecalis* and *Lactobacilli*), the zones of inhibition were measured at 24, 36, 48, and 72 hours. For *C.albicans*, measurements were taken after 5, 7, 10, and 15 days. The entire experiment was repeated nine times for each isolate, and the mean zone of inhibition was calculated.

Recording the Zone of Inhibition

The growth inhibitory zones around each essential oil were identified by the absence of bacterial colonization (clearing of the agar) near each oil. The most uniform diameter of the inhibition zone was measured using an endodontic millimeter ruler. Larger zones of inhibition were interpreted as an indication of stronger antimicrobial activity of the respective essential oil.

Results

Intragroup comparison of ZoI against *S mutans* at 24 hours, 36 hours, 48 hours and 72 hours, time interval respectively showed only Group 1(Eugenol), Group 5 (Cinnamon oil) showed a statistically significant difference ($p\text{-value}<0.05$) in the ZoI (Table 2).

Intergroup comparison of ZoI against *S mutans* showed that after 24 hours, 36 hours & 48 hours, the mean ZoI, among Group 5 (Cinnamon oil) was significantly more when compared to other groups. At 72 hours, Group 5 (Cinnamon oil) showed the maximum mean ZoI, followed by Group 1 (Eugenol). No other groups exhibited anti-microbial activity against *S mutans* even after 72 hours (Table 2).

Intragroup comparison of ZoI against *Lactobacilli* at 24 hours, 36 hours, 48 hours and 72 hours' time interval showed only Group 2 (thyme oil), Group 4 (neem oil) showed a statistically significant difference in the ZoI ($p\text{-value}<0.05$) against *Lactobacilli*(Table 3).

Intergroup comparison of ZoI against *Lactobacilli* at 24 hours, 36 hours, 48 hours,&72 hours group 4 (Neem oil) showed the maximum antimicrobial efficacy than group 2 (thyme oil) (Table 3).

Intragroup comparison of ZoI against *E coli* after 24 hours, 36 hours, 48 hours and 72 hours, time interval showed that all groups except group 5 (cinnamon oil) showed a statistically significant difference. In group 1 (Eugenol) & group 3 (Turmeric oil), the mean ZoI decreased significantly from 24 hours to 36 hours and then increased from 36 hours to 48 hours following which it decreased significantly from 48 hours to 72 hours (Table 4).In group 2 (thymol oil) & group 4 (neem oil), the mean ZoI increased significantly from 24 hours to 36 hours and then further decreased from 36 hours to 48 hours and 48hours to 72 hours respectively.

Intergroup comparison of ZoI against *E coli* at 24 hours, 36 hours, 48 hours & 72 hours showed that the mean ZoI of group 4 (Neem oil), was greatest followed decreasing order by group 2 (thymol oil), group 1 (Eugenol), and group 3 (turmeric oil) respectively (Table 4).

Intragroup comparison of ZoI against *Pseudomonas* after 24 hours, 36 hours, 48 hours and 72 hours' time interval showed that group 4 (neem oil) showed a statistically significant difference in the ZoI (Table 5).

Intergroup comparison of ZoI at 24 hours, 36 hours, 48 hours & 72 hours' time interval showed that the mean ZoI of group 4 (Neem oil) was significant and increased from 24 hours to 36 hours following which it decreased from 36 hours to 72 hours respectively (Table 5).

Intragroup comparison showed that only group 2 (thymol oil), group 3 (turmeric oil), group 4 (neem oil) showed a statistically significant difference. Among group 2 (thymol oil), group 3 (turmeric oil), group 4 (neem oil) the mean ZoI reduced significantly from 24 to 72 hours (Table 6).

At 24 hours, 36 hours, 48 hours & 72 hours' time interval intergroup comparison showed that ZoI of group 2 (thymol oil) was significantly greater than group 4 (neem oil) followed in descending order by group 3 (turmeric oil) (Table 6).

Intragroup comparison of ZoI against *C albicans* at 5th day, 7th day, 10th day, and 15th day time interval showed that only group 2 (Thymol oil) showed a statistically significant difference which increased from 5th day to 10th day and then decreased from 10th day to 15th day respectively (Table 7).

Intergroup comparison at 5th day, 7th day, 10th day, and 15th day time intervals showed that mean ZoI was significantly more in group 2 (thyme oil). The rest groups did not show any ZoI (Table 7).

Discussion

Root canal treatment aims to eliminate bacteria by cleaning, shaping, and obturating the canal system.¹¹ To achieve this, effective antimicrobial agents with minimal toxicity to periapical tissues is crucial. With growing bacterial resistance, medicinal plants and essential oils are gaining attention for their antimicrobial potential. Essential oils, composed of numerous chemical constituents, owe their biological properties to key bioactive compounds.¹² For example, cinnamaldehyde and eugenol in cinnamon oil, and nimbodin in neem oil, exhibit antimicrobial effects. These oils are incorporated into endodontic sealers like Maisto paste (thymol oil) and Walkhoff paste (camphor) for use in dentistry.

In the current study we explored five essential oils called Eugenol, Cinnamon oil, Thyme oil, Neem oil, Turmeric oil with 2% chlorhexidine as the positive control group against endodontic pathogens - Gram-positive (*Streptococcus mutans*), (*Lactobacilli*) and Gram-negative bacteria (*Escherichia coli*), (*Enterococcus faecalis*), (*Pseudomonas*) as well as fungi (*Candidaalbicans*). Chlorhexidine was chosen as it is considered the gold standard for antimicrobial tests in dentistry

against which other antimicrobial agents are compared due to its wide range of activity and persistent effect.¹

S. mutans is believed to be the most common bacteria associated with dental caries. It causes demineralization of tooth structure. *S. mutans* along with *S. aureus* and is frequently isolated from primary endodontic infection, as well as in root filled teeth. They are more strongly associated with pre-operative symptoms and presence of swelling.³

E. faecalis remains to be the most frequently identified species in canals of root filled teeth as established by different molecular methods. This may be due to its ability to survive the effects of a wide range of antimicrobial solutions and intracanal medicaments used during endodontic treatment procedures. *E. faecalis* survives on serum components from the dentinal fluid. Therefore, even in a well debrided and coronally well-sealed root canal, remaining or surviving cells of *E. faecalis* may still grow and utilize local sources of energy and nutrients.³

P. aeruginosa has been recovered from primary and persistent endodontic infections. Leonardo *et al.* (2000) reported that growth of *P. aeruginosa* was not inhibited by several of the commonly used root canal sealers and pastes.¹³ Hence, it was chosen as a test microorganism.

Gram-negative rods such as *Escherichia coli* was selected because Gomes *et al.* (2004) considered *E. coli* and *Staphylococcus aureus* to be the most resistant species in the oral cavity and the causative organisms for the failure of root canal treatment. Earlier studies reported 80.64% of *E. coli* infecting Indian patients were Extended Spectrum beta-Lactamase (ESBL) producers. Due to ESBL the emergence of resistance occurs and therefore, it is critical to develop alternative approaches to overcome this problem.¹⁴

Lactobacilli are a diverse group of strictly fermentative, non-sporing Gram-positive bacilli and facultative anaerobes. *Lactobacilli* invading the oral cavity are believed to be opportunistic invaders of existing carious lesions taking advantage of the retentive niche created by the early colonizers such as *Streptococcus mutans*.³

Candidal organisms are commensals of the oral cavity and their opportunistic infection depends on the interplay of local and systemic factors. Endodontic treatment failure has been associated with the persistence of microbial flora including fungi like *Candida*, which are resistant to conventional root canal irrigant. Caries has been considered as the portal of entry for fungi to enter the root canal system. In a study done by Kumar *et al.* (2015) cases with endodontic failure were included shows more fungal growth compared to saliva suggesting that the retained candidal organisms from caries or by salivary contamination during initial treatment may be the cause of failure of the endodontic treatment.³

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Due to presence of these micro-organisms in the smear layer before and after endodontic treatment it was necessary to find alternative treatment options to the conventional used intracanal medicaments. Hence, the purpose of the study was

to evaluate the antimicrobial efficacy of essential oils against these microorganisms which can cause endodontic failures.

Group 1 (Eugenol) showed zone of inhibition against *E. coli* and *S. mutans*. Group 1 showed the presence of zone of inhibition after 72 hours against *S. mutans* and it was lower than group 5 at 72 hours and the result was significant (p -value < 0.05). The result states that eugenol can have long term effects on the bacteria when used as intracanal medicaments. The results were in accordance to Yanakiev S(2020) who concluded that eugenol in different concentration acts against *S. mutans*.¹⁶ Saha *et al.* (2010) compared eugenol and non-eugenol-based sealers and found that eugenol-based sealers showed largest inhibitory zones which continued up to period of 7 days showing its long-term effects.¹¹ Against *E. coli* the zone of inhibition decreased from 5.00 ± 1.22 to 3.78 ± 1.48 which was statistically significant (p -value < 0.05). The results were in contrast to Dhara and Tripathi (2013) who stated that cinnamon oil has a better efficacy against *E. coli* than eugenol.¹⁴ In our study we found no zone of inhibition in group 5.

Group 2 (thyme oil) showed efficacy against *Lactobacilli*, *E. coli*, *E. faecalis* and *C. albicans* in all the time intervals. The efficacy against these bacteria and fungi follows the order *E. faecalis* > *E. coli* > *Lactobacilli* > *C. albicans*. Our result was in accordance to Thosar *et al.* (2018), Thosar *et al.* (2013), Shu *et al.* (2016).^{17, 12, 18} Against *E. faecalis* and *Lactobacilli* a component of thyme, known as thymol, appears to inhibit growth of these oral pathogens in the mouth. The mode of action of the major components, carvacrol and thymol, as explained by Burt, consists of the disintegration of the outer cell membranes of bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP.⁹ Thosar *et al.* (2013) in their study had shown the antimicrobial susceptibility for thyme oil for *E. coli* which was with minimum inhibitory concentration.¹² Against *C. albicans* thymol may act, at least in part, through the function of the p38 MAPK signalling pathway to protect against *C. albicans* infection and maintain the host innate immune response to *C. albicans*.¹⁸

Group 3 (turmeric oil) showed efficacy against *E. coli* and *E. faecalis*. The efficacy against these bacteria follows the order *E. faecalis* > *E. coli*. Our result was in accordance with Chamele and Bhat (2014), Neelakantan *et al.* (2013) who concluded that there was 50% reduction in colony count of *E. faecalis*.¹⁹ Haukyiket *et al.* (2009) studied the phototoxic effects of curcumin against gram positive bacteria like *E. faecalis*, *Streptococcus intermedius* and gram-negative bacteria *E. coli* were investigated in aqueous preparations (Kaushik *et al.*, 2013).^{21, 22}

Group 4 (Neem oil) showed efficacy against *Lactobacilli*, *E. coli*, *Pseudomonas*, *E. faecalis* in all the time interval. The efficacy against these bacteria follows the order *E. coli* > *Lactobacilli* > *E. faecalis* > *Pseudomonas*. The results were statistically significant (p -value < 0.05). Our result was in accordance to Wylie and Merelle (2022), Tasanarong T *et al.* (2021), Saleem *et al.* (2018), Theilmann *et al.* (2019) and Singh *et al.* (2017).^{23, 24, 25, 26, 27} In contrast to our results Dhara and Tripathi (2013)

stated that cinnamon oil has a better efficacy against *E. coli* than neem oil.¹⁴ Against *E. coli* Theilmann *et al.* (2019) stated that neem-tree essential oil, mainly consists of the compounds Azadirachtin and Nimbin. The compounds are known to possess antimicrobial activity, but are predominately used as spermicides.²⁷ Azadirachta indica is found to be beneficial in reducing the plaque index and salivary bacterial count (*Lactobacilli*) comparatively better than chlorhexidine gluconate mouthwash Tasanaronget *al.* (2021) and Srivastavet *al.* (2020).^{24, 28} Against *Pseudomonas* the mechanism of action of the neem extracts is mainly attributed to the inhibition of cell-membrane synthesis in the bacteria. Numerous biological and pharmacological activities of neem leaf have been reported including antibacterial, antifungal, and anti-inflammatory activities. Phytol is a diterpenes, can decrease the level of bacterial counts *in vivo*. Dodecanoic acid or lauric acid, a type of medium-chain fatty acids, was also obtained but in a small amount. This constituent reduces biofilm formation *in vitro* and restrains oral bacterial growth.³

Group 5 (cinnamon oil) showed maximum efficacy against *S. mutans*, after 24 hours which decreased from 7.11 ± 1.76 to 4.89 ± 2.67 after 72 hours and the result was significant (p -value < 0.05). This result was similar to the studies done by Shivkumaret *al.* (2023), Marcoux *et al.* (2020), Yanakiev S (2020), Dhara and Tripathi (2013).^{29, 30, 16, 14} Yanakiev S (2020) stated that the cinnamon tree has cinnamon oil, eugenol in different concentration when obtained from different parts of a plant, hence cinnamon shows dual effects of cinnamaldehyde and eugenol.¹⁶ Didryet *al.* (1994) stated that cinnamon oil contains a compound that help in the antimicrobial efficacy against *S. mutans*.³¹ Later on, Dhara and Tripathi (2013) named the bioactive compound as cinnamaldehyde which is a potent antimicrobial agent present.¹⁴

Chlorhexidine is widely regarded as the gold standard for antimicrobial tests in dentistry due to its broad-spectrum activity, persistence, and low toxicity. It effectively targets Gram-positive and Gram-negative bacteria, yeasts, dermatophytes, and certain lipophilic viruses, with a strong affinity for skin and mucous membranes. In our study, we compared five essential oils with chlorhexidine as the positive control against common root canal pathogens. Both neem oil and thyme oil demonstrated antimicrobial efficacy comparable to chlorhexidine, effectively targeting a wide range of microorganisms. As non-toxic and commonly used household products, they could serve as suitable alternatives to chlorhexidine in dental applications. Additionally, the other essential oils in the study showed some antimicrobial activity and may be used in combination to achieve a synergistic effect when blended in the right proportions.

Conclusion

Plant extracts can be used as an alternate agent to synthetically produced antimicrobial agents. Neem oil and thyme oil have been used earlier and, in our study, it showed antimicrobial activity its effect on oral pathogenic flora.

Based on the following results following conclusions can be drawn:

- Neem oil showed efficacy against *Lactobacilli* and *E. coli* more than thyme oil.
- Against *Pseudomonas* only neem oil was effective and results were comparable to chlorhexidine.
- Thyme oil exhibited inhibitory effect against *E. faecalis* more than neem oil.
- Against *C. albicans* only thyme oil was effective.
- Eugenol and cinnamon oil exhibited inhibitory effect against *S. mutans*.

References

1. Salma RS, Matar MA, Darwish SS, Elseoudy N, Kandil M, Mehelba M et al. The antimicrobial effect of eugenol on lactobacilli isolated from children's saliva compared to chlorhexidine (in-vitro study). *Egypt Dent J.* 2022; 68(2): 1141-1148.
2. Saha S, Dhinsa G, Ghoshal U, Hussain AN, Nag S, Garg A. Influence of plant extracts mixed with endodontic sealers on the growth of oral pathogens in root canal: An in vitro study. *J Indian Soc Pedod Prev Dent* 2019; 37:39-45.
3. Devi MT, Saha S, Tripathi AM, Dhinsa K, Kalra KS, Ghoshal U. Evaluation of the Antimicrobial Efficacy of Herbal Extracts Added to Root Canal Sealers of Different Bases: An In Vitro Study. *Int J Clin Pediatr Dent.* 2019;12(5):398-404.
4. Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of *Azadirachta indica*, *Mimusops elengi*, *Tinospora cardifolia*, *Ocimum sanctum* and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. *Eur J Dent.* 2014;8(2):172-177.
5. Farjana A, Zerín N, Kabir S. Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. *Asian Pac J Trop Dis.* 2014;4(2): S920-S923.
6. Mohammad MA. Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Evid Based Complement Alternat Med.* 2016; 6:1-11.
7. Elbestawy MKM, El-Sherbiny GM, Moughannem SA. Antibacterial, Antibiofilm and Anti-Inflammatory Activities of Eugenol Clove Essential Oil against Resistant *Helicobacter pylori*. *Molecules.* 2023; 28(6):2448.
8. El Atki Y, Aouam I, El Kamari F, Taroq A, Nayme K, Timinouni M et al. Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. *J Adv Pharm Technol Res.* 2019;10(2):63-67.

9. Nzeako BC, Al-Kharousi ZS, Al-Mahrooqui Z. Antimicrobial activities of clove and thyme extracts. *Sultan Qaboos Univ Med J.* 2006;6(1):33-9.
10. Odo EO, Ikwuegbu JA, Obeagu EI, Chibueze SA, Ochiaka RE. Analysis of the antibacterial effects of turmeric on particular bacteria. *Medicine (Baltimore).* 2023;102(48): e36492.
11. Saha S, Samadi F, Jaiswal JN, Ghoshal U. Antimicrobial activity of different endodontic sealers: an in vitro evaluation. *J Indian Soc Pedod Prev Dent.* 2010;28(4):251-7.
12. Thosar N, Basak S, Bahadure RN, Rajurkar M. Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study. *Eur J Dent.* 2013;7(Suppl 1): S071-S077.
13. Zichichi L, Asta G, Noto G. *Pseudomonas aeruginosa* folliculitis after shower/bath exposure. *Int J Dermatol.* 2000;39(4):270–273.
14. Dhara L and Tripathi A. Antimicrobial activity of eugenol and cinnamaldehyde against extended spectrum beta lactamase producing *Enterobacteriaceae* by in vitro and molecular docking analysis. *Eur J Integr Med.* 2013; 5:527-536.
15. Kumar J, Sharma R, Sharma M, Prabhavathi V, Paul J, Chowdary CD. Presence of *Candida albicans* in Root Canals of Teeth with Apical Periodontitis and Evaluation of their Possible Role in Failure of Endodontic Treatment. *J Int Oral Health.* 2015;7(2):42-5.
16. Yanakiev S. Effects of Cinnamon (*Cinnamomum* spp.) in Dentistry: A Review. *Molecules.* 2020;25(18):4184.
17. Thosar NR, Chandak M, Bhat M, Basak S. Evaluation of Antimicrobial Activity of Two Endodontic Sealers: Zinc Oxide with Thyme Oil and Zinc Oxide Eugenol against Root Canal Microorganisms- An in vitro Study. *Int J Clin Pediatr Dent.* 2018;11(2):79-82.
18. Shu C, Sun L, Zhang W. Thymol has antifungal activity against *Candida albicans* during infection and maintains the innate immune response required for function of the p38 MAPK signaling pathway in *Caenorhabditis elegans*. *Immunol Res.* 2016;64(4):1013-24.
19. Chamele J and Bhat C. Efficacy of turmeric extract as an intracanal medicament in deciduous teeth against *Enterococcus faecalis*: An in vitro study. *Int J Curr Microbiol App Sci.* 2014;3(9):17-25.
20. Neelakantan P, Subbarao C, Sharma S, Subbarao CV, Garcia-Godoy F, Gutmann JL. Effectiveness of curcumin against *Enterococcus faecalis* biofilm. *Acta Odontol Scand.* 2013;71(6):1453-7.
21. Haukvik T, Bruzell E, Kristensen S, Tonnesen HH. Photokilling of bacteria by curcumin in different aqueous preparations. *Studies on curcumin and curcuminoids XXXVII. Pharmazie.* 2009;64(10): 666673.
22. Kaushik N, Rehani U, Agarwal A, Kaushik M, Adlakha V. Antimicrobial Efficacy of Endodontic Irrigants against *Enterococcus Faecalis* and *Escherichia Coli*: An in vitro study. *Int J Clin Pediatr Dent.* 2013;6(3):178-82.

23. Wylie MR and Merrell DS. *The Antimicrobial Potential of the Neem Tree Azadirachta indica*. *Front Pharmacol*. 2022; 13:891535.
24. Tasanarong T, Patntirapong S, Aupaphong V. *The inhibitory effect of a novel neem pastes against cariogenic bacteria*. *J Clin Exp Dent*. 2021;13(11): e1083-e1088.
25. Saleem S, Muhammad G, Hussain MA, Bukhari SNA. *A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica*. *Phytother Res*. 2018;32(7):1241–1272.
26. Thielmann J, Muranyi P, Kazman P. *Screening essential oils for their antimicrobial activities against the foodborne pathogenic bacteria Escherichia coli and Staphylococcus aureus*. *Heliyon*. 2019;5(6): e01860
27. Singh H, Kaur M, Dhillon J, Batra M, Khurana J. *Neem: a magical herb in endodontics*. *Stomatological Dis Sci*. 2017;1(2):50-54.
28. Srivastava KS, Agrawal B, Kumar A, Pandey A. *Phytochemicals of Azadirachta Indica Source of Active Medicinal Constituent Used for Cure of Various Diseases: A Review*. *J Sci Res*. 2020;64(1):385-390
29. Shivakumar B, SS, BRC, Sundaravadivelu M. *Antimicrobial efficacy of essential oils of clove, neem and cinnamon on Streptococcus mutants and lactobacillus acidophilus in comparison with 0.2% chlorhexidine – An in vitro study*. *J Dent Med Sci*. 2023;22(4):28-33.
30. Marcoux E, Lagha BA, Gauthier P, Grenier D. *Antimicrobial activities of natural plant compounds against endodontic pathogens and biocompatibility with human gingival fibroblasts*. *Arch Oral Biol*. 2020;116: 104734.
31. Didry N, Dubreuil L, Pinkas M. *Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria*. *Pharm Acta Helv*. 1994;69(1):25-28.

Table 1: Essential Oil with their Bio-Active Compound and Susceptible Micro-organism

Essential Oils	Bio-Active Compound	Function	Susceptible Micro-organisms
Neem ⁶ (<i>Azadirachta indica</i>)	<ul style="list-style-type: none"> • Oleic acid • Linoleic acid • Hexadecanoic acid • Alkaloids like Nimbin, Tannins and Nimbidin 	The disruption and disintegration of the bacterial cell wall, along with significant alterations in its morphological structure, lead	Gram-positive (<i>Lactobacilli</i>) Gram-negative bacteria (<i>E. coli</i>), (<i>E. faecalis</i>), (<i>Pseudomonas</i>) and <i>Candida</i> species.

		to bacterial cell death.	
Eugenol ⁷ (<i>Syzygium aromaticum</i>)	<ul style="list-style-type: none"> • Flavonoids • Kaempferol • Rhamnetin • β-caryophyllene 	Eugenol destroys the cell wall and membrane, causing the loss of vital intracellular materials and leading to bacterial death.	<i>S. aureus</i> , <i>E. coli</i> , <i>H. pylori</i> and <i>L. monocytogenes</i>
Cinnamon Oil ⁸ (<i>Cinnamomum verum</i>)	<ul style="list-style-type: none"> • Cinnamaldehyde • Eugenol 	It damages the cell membrane, alters lipid profiles, and inhibits ATPases, cell division, membrane porins, motility, and biofilm formation through anti-quorum sensing effects.	<i>E. coli</i> , <i>S. aureus</i> , and <i>P. aeruginosa</i>
Thyme oil ⁹ (<i>Thymus vulgaris</i>)	<ul style="list-style-type: none"> • Thymol • Carvacrol • B-cymene • Pinene • Triterpenic acid 	Damage to the cell membrane, leading to the leakage of cytoplasmic content and changes in cell morphology.	<i>P. aeruginosa</i> and <i>C. albicans</i> .
Turmeric ¹⁰ (<i>Curcuma longa</i>)	<ul style="list-style-type: none"> • Curcuminoids • Sesquiterpenes 	Phototoxic effect against gram positive	<i>B. subtilis</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>B. coagulans</i> , <i>E.</i>

		and gram-negative bacteria Anti-oxidant effect	<i>coli</i> and <i>P. aeruginosa</i>
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Table 2: Intragroup and Intergroup comparison of zone of inhibition (ZoI) against *S. mutans* after 24-hour, 36 hour and 72-hour time interval *mutans* after 24-hour, 36 hour and 72-hour

	At 24 hr		At 36 hr		At 48 hr		At 72 hr		P value of Intragroup comparison
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Group 1 (Eugenol)	.00	.00	.00	.00	.00	.00	3.56	1.59	<0.001, S
Group 2 (Thymol oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 3 (Turmeric oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 4 (Neem oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 5 (Cinnamon oil)	7.11	1.76	6.56	1.88	6.89	1.97	4.89	2.67	<0.001, S
(Chlorhexidine) Positive control for Group 1, Group 2, Group 3	10.22	1.20	10.22	1.20	9.44	1.13	8.78	1.30	<0.001, S
(Chlorhexidine) Positive control for Group 4, Group 5	9.67	.50	9.44	.53	8.44	.53	8.33	.50	<0.001, S
P value of Intergroup		<0.001, S		<0.001, S		<0.001, S		<0.001, S	

comparison									
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Table 3: Intragroup and Intergroup comparison of Zone of Inhibition (ZOI) against *Lactobacilli* after 24 hour, 36 hour, 72 hour time interval

ZOI against <i>Lactobacilli</i>									
	At 24 hr		At 36 hr		At 48 hr		At 72 hr		P value of Intragroup comparison
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Group 1 (Eugenol)	.00	.000	.00	.000	.00	.000	.00	.000	-
Group 2 (Thymol oil)	6.11	1.54	5.67	1.12	5.89	1.27	4.67	1.41	<0.001, S
Group 3 (Turmeric oil)	.00	.000	.00	.000	.00	.000	.00	.000	-
Group 4 (Neem oil)	7.78	.97	7.56	1.01	6.44	1.24	5.11	1.05	<0.001, S
Group 5 (Cinnamon oil)	.00	.000	.00	.000	.00	.000	.00	.000	-
(Chlorhexidine) Positive control for Group 1, Group 2, Group 3	8.67	1.22	8.56	1.13	7.33	.87	6.78	1.30	<0.001, S
(Chlorhexidine) Positive control for Group 4, Group 5	12.00	.71	11.89	.93	14.33	2.35	10.33	.50	<0.001, S
P value of Intergroup comparison	<0.001, S		<0.001, S		<0.001, S		<0.001, S		

Table 4: Intragroup and Intergroup comparison of Zone of Inhibition (ZOI) against *E. coli* after 24 hour, 36 hour, 72 hour time interval

ZOI against <i>E. coli</i>									
	At 24 hr		At 36 hr		At 48 hr		At 72 hr		P value of Intragroup comparison
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Group 1 (Eugenol)	5.00	1.22	3.89	1.76	4.56	1.33	3.78	1.48	<0.001, S
Group 2 (Thymol oil)	6.56	1.13	6.89	1.05	5.56	1.24	4.56	.88	<0.001, S
Group 3 (Turmeric oil)	2.89	3.44	1.67	2.65	2.89	3.44	2.56	2.74	<0.001, S
Group 4 (Neem oil)	8.22	.67	8.44	.53	7.67	.87	7.22	.67	<0.001, S
Group 5 (Cinnamon oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
(Chlorhexidine) Positive control for Group 1, Group 2, Group 3	10.67	1.22	10.67	1.22	9.56	1.13	9.11	1.05	<0.001, S
(Chlorhexidine) Positive control for Group 4, Group 5	9.56	.53	9.44	.73	8.56	.53	8.33	.50	<0.001, S
P value of Intergroup comparison	<0.001, S		<0.001, S		<0.001, S		<0.001, S		

Table 5: Intragroup and Intergroup comparison of Zone of Inhibition (ZOI) against *Pseudomonas* after 24 hour, 36 hour, 72 hour time interval

ZOI against <i>Pseudomonas</i>									
	At 24 hr		At 36 hr		At 48 hr		At 72 hr		P value of Intragroup comparison
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Group 1 (Eugenol)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 2 (Thymol oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 3 (Turmeric oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 4 (Neem oil)	3.00	2.88	3.67	2.78	2.44	2.35	1.56	1.88	<0.001, S
Group 5 (Cinnamon oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 6 (Chlorhexidine) Positive control for Group 1, Group 2, Group 3	9.00	1.12	8.67	1.22	6.67	1.00	6.56	1.13	<0.001, S
Group 7 (Chlorhexidine) Positive control for Group 4, Group 5	10.33	1.12	9.22	1.20	9.44	1.24	8.67	1.41	<0.001, S
P value of Intergroup comparison	<0.001, S		<0.001, S		<0.001, S		<0.001, S		

Table 6: Intragroup and Intergroup comparison of Zone of Inhibition (ZoI) against *E. faecalis* after 24 hour, 36 hour, 72 hour time interval

ZoI against <i>E faecalis</i>									
	At 24 hr		At 36 hr		At 48 hr		At 72 hr		P value of Intragroup comparison
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Group 1 (Eugenol)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 2 (Thymol oil)	8.33	.70	8.00	.87	7.67	.87	7.11	.60	<0.001, S
Group 3 (Turmeric oil)	4.67	1.32	4.33	1.22	3.67	1.00	3.67	1.00	<0.001, S
Group 4 (Neem oil)	6.00	1.50	5.44	1.13	5.00	1.58	4.11	1.54	<0.001, S
Group 5 (Cinnamon oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
(Chlorhexidine) Positive control for Group 1, Group 2, Group 3	10.56	1.13	9.44	1.24	9.44	1.42	8.78	1.48	<0.001, S
(Chlorhexidine) Positive control for Group 4, Group 5	9.33	1.22	9.00	1.00	9.11	1.27	8.56	1.13	<0.001, S
P value of Intergroup comparison	<0.001, S		<0.001, S		<0.001, S		<0.001, S		

Table 7: Intragroup and Intergroup comparison of Zone of Inhibition (ZoI) against *C. albicans* after 24 hour, 36 hour, 72 hour time interval

ZoI against <i>C. albicans</i>									
	At 5 th day		At 7 th day		At 10 th day		At 15 th day		P value of Intragroup comparison
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Group 1 (Eugenol)	.00	.000	.00	.00	.00	.00	.00	.00	-
Group 2 (Thymol oil)	4.33	.71	4.33	.86	4.56	.53	3.33	.71	<0.001, S
Group 3 (Turmeric oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 4 (Neem oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
Group 5 (Cinnamon oil)	.00	.00	.00	.00	.00	.00	.00	.00	-
(Chlorhexidine) Positive control for Group 1, Group 2, Group 3	10.11	1.05	10.11	1.27	9.33	1.22	7.67	.87	<0.001, S
(Chlorhexidine) Positive control for Group 4, Group 5	7.44	1.33	7.11	1.54	7.00	1.41	5.89	1.54	<0.001, S
P value of Intergroup comparison	<0.001, S		<0.001, S		<0.001, S		<0.001, S		

Figure1: Flowchart showing Study Groups and the test Micro-Organisms

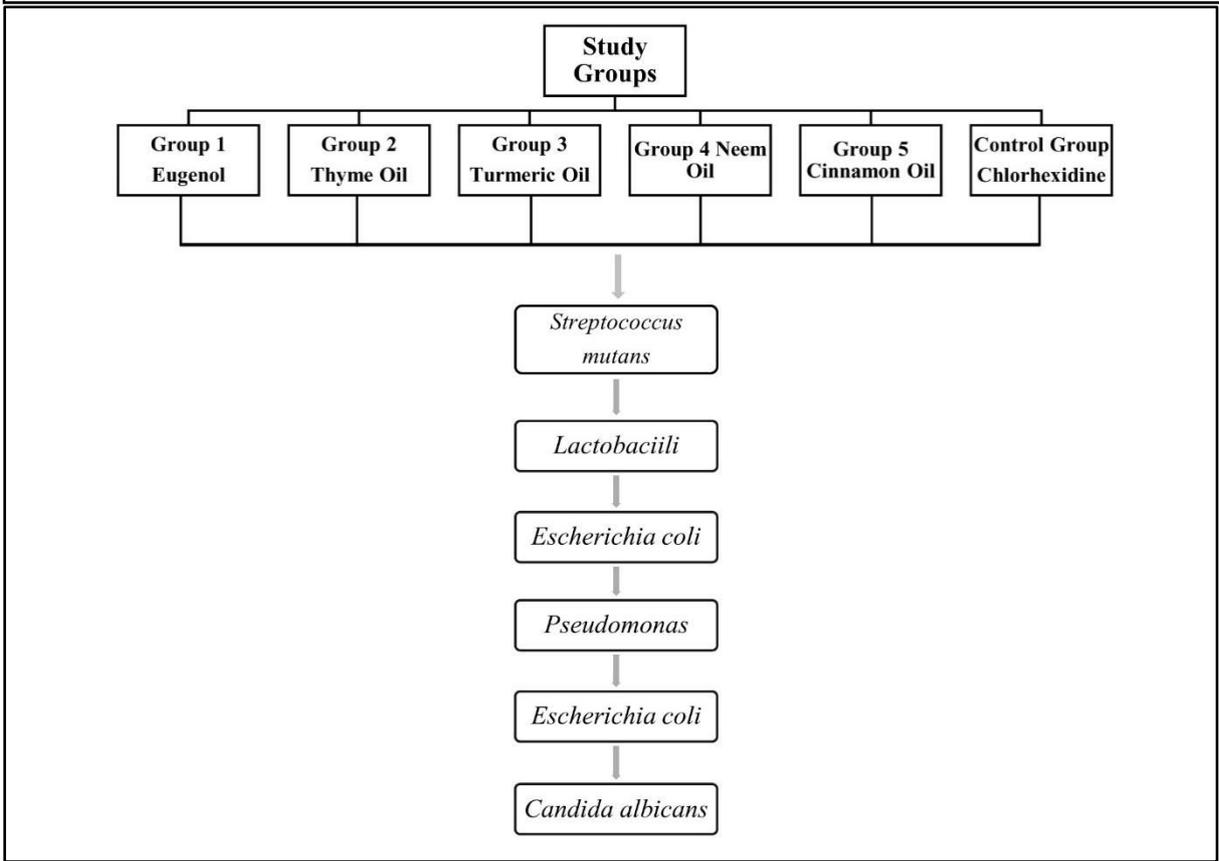


Figure2: Zone of Inhibition for *E. coli*

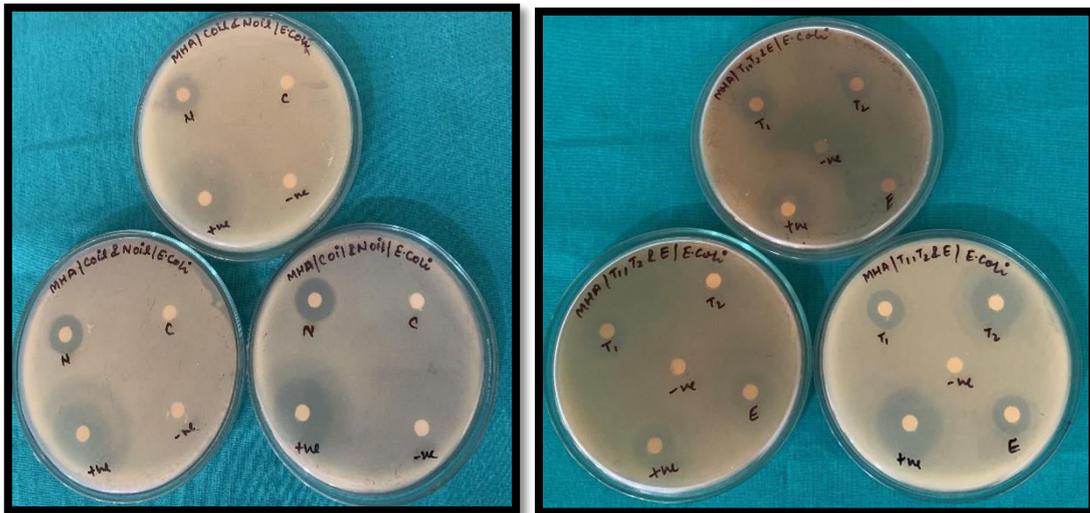


Figure3: Zone of Inhibition for *Lactobacilli*

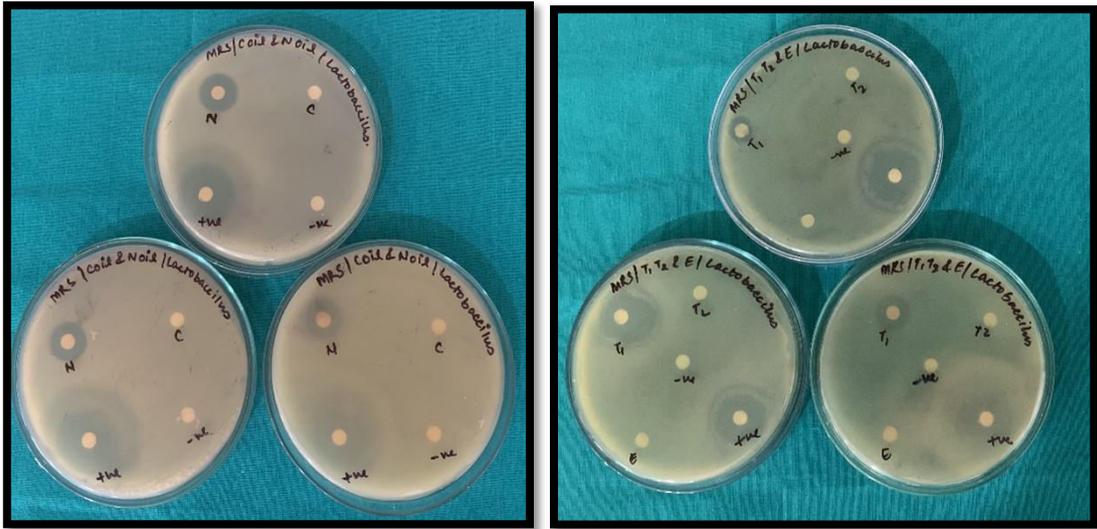


Figure4: Zone of Inhibition for *Pseudomonas*

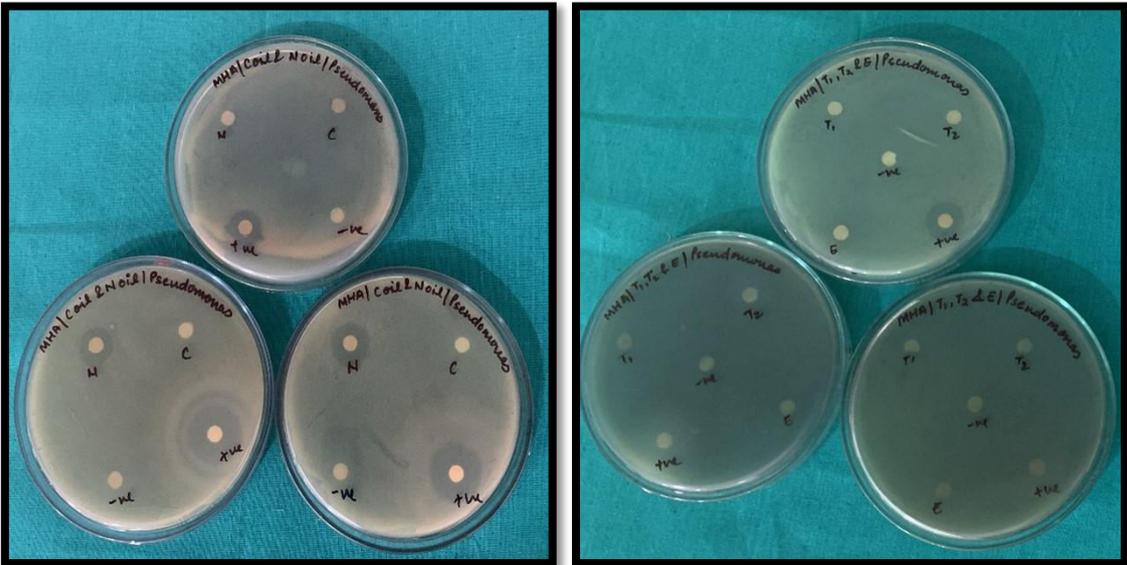


Figure5: Zone of Inhibition for *S. mutans*

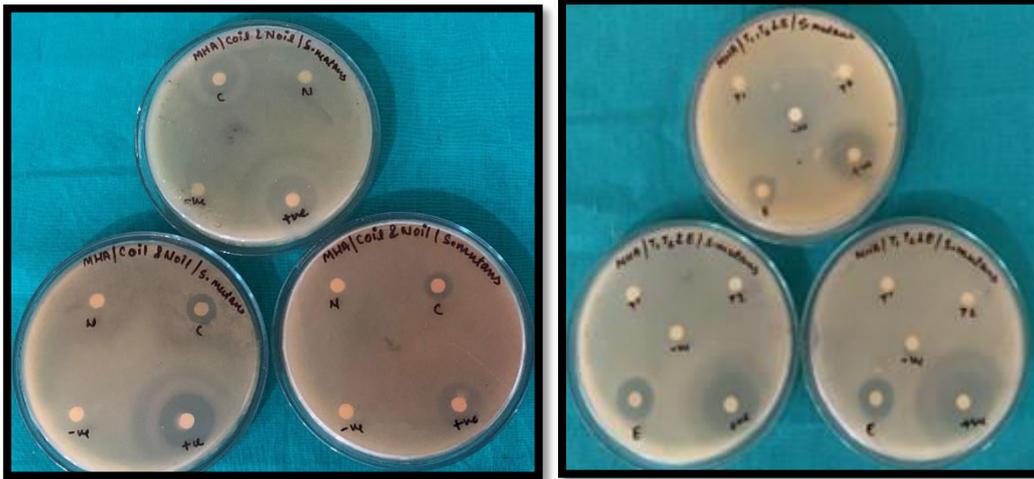


Figure6: Zone of Inhibition for *C. albicans*

