Original Article

Microbiologic Evaluation of Matricaria and Chlorhexidine against *E. faecalis* and *C. albicans*

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Abstract

**Objective:** To evaluate the antimicrobial activity of different concentrations of *Matricaria chamomilla* and Chlorhexidine gel against *Candida albicans* and *Enterococcus faecalis*. **Materials and Methods:** The agar diffusion test was used to evaluate the antimicrobial activity of 15%, 25% *Matricaria chamomilla* in aq. base and 2% Chlorhexidine gel against *C. albicans* (ATCC 24433) and *E. faecalis* (ATCC 24212) strains. Vancomycin was used as the positive control for *E. faecalis* and fluconazole for *C. albicans*. The agar plates were incubated at 37°C for 48 h after which the zone of inhibition were measured separately for each material. Data thus obtained were statistically analyzed using the Wilcoxon rank–order test. **Results:** 2% chlorhexidine showed maximum inhibitory zone for *C. albicans* (33.26 mm) and *E. faecalis* (24.54 mm). 25% Matricaria showed zones of 24.16 mm and 20.62 mm for *C. albicans* and *E. faecalis*, respectively. 15% Matricaria did not show any antimicrobial activity (0 mm). **Conclusion:** The results of the current *in vitro* study suggest that 25% Matricaria can be used as an antimicrobial agent, but it is less effective than 2% chlorhexidine gluconate gel against *C. albicans* and *E. faecalis*. Matricaria at a lesser concentration of 15% aq. base is ineffective against both the microorganisms.

Key words: Agar diffusion test, *Candida albicans*, chlorhexidine, *Enterococcus faecalis*, *Matricaria chamomilla*

INTRODUCTION

Debridement of root canal space is essential for the success of endodontic treatment. The microbes most commonly found in failed endodontic treatment cases are *Enterococcus faecalis* and *Candida albicans*. *E. faecalis* has demonstrated a high resistance and ability to inactivate antimicrobial agents, survival capacity in harsh environments, with scarce nutrient supply and extreme alkaline pH, and the capacity for growth as a biofilm on root canal walls.

There are evidences that indicate the presence of fungi in the root canal system. Fungi, especially *C. albicans*, have been demonstrated in the pulp space and periapical area through light electron microscopy and culture techniques, where they have been associated with persistent infections. *C. albicans* releases collagenolytic enzymes that make it possible to use dentin as a nutrient source.

According to various studies, both *C. albicans* and *E. faecalis* are resistant to the antimicrobial action of calcium hydroxide, a commonly used intracanal medicament, but are sensitive to the antimicrobial action of chlorhexidine gluconate (CHX). To achieve long-term substantive antimicrobial effects, the infected root dentin must be exposed to CHX for a longer time than that afforded by irrigation.

Antibiotics are most commonly used for eradicating infectious disease. But, nowadays, some microbial infectious agents have become resistant against relevant antibiotics as a result of irregular use of drugs by people, which results in failure of antibiotic therapy. However, man has found that the therapeutic effects of herbal extracts, unlike many chemical drugs, have no side-effects. Some commonly used herbs in modern medicine are
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Glycyrrhiza glabra, Commiphora mukul, Plantago ovata, Aloe barbadensis and Azadirachta indica. The plants Glycyrrhiza glabra, Piper longum, Adhatoda vasica, Withania somnifera, Cyperus rotundus, Tinospora cordifolia, Berberis aristata, Tribulus terrestris, Holarhena antidysenterica and Boerhavia diffusa have been used in 52–141 herbal formulations and triphala (Terminalia chebula, Terminalia belerica and Embelia officinalis) alone has been used in 219 formulations. Among these plants, Matricaria chamomilla can be mentioned for its remedial features.

Chamomile is a widely recognized herb in Western culture. It is a common ingredient today in herbal teas because of its calming, carminative and spasmytic properties. It is also a popular ingredient in topical health and beauty products for its soothing and anti-inflammatory effects on skin. It also has a role in the treatment of recurrent aphthous stomatitis, mucositis and oral ulcers. The aim of this in vitro study is to comparatively evaluate the antibacterial and antifungal activities of 15%, 25% Matricaria chamomilla in aq. base and 2% chlorhexidine gel against C. albicans and E. faecalis.

**MATERIALS AND METHODS**

The materials evaluated for antimicrobial activity were 15%, 25% Matricaria chamomilla in aq. base and 2% CHX gel (Endogel; Itapetininga, SP, Brazil) against C. albicans (ATCC 24433) and E. faecalis (ATCC 24212) strains. Vancomycin (antibacterial) was used as a positive control for E. faecalis and fluconazole (antifungal) was used for C. albicans. Plant extracts of chamomile were obtained from the National Botanical and Research Institute, Lucknow.

**Aqueous extraction (cold water)**
The method of Al-Magboul et al., as modified by Okigbo and Omodamiro, was used. The extract was filtered with sterile filter paper (labline filter paper) inserted in a funnel and the filtrate was evaporated in a water bath at 100°C to dryness. The standard extracts obtained were stored in a refrigerator at 4°C until required for use.

**Aqueous extraction (hot water)**
Fifteen and 25 g of the weighed plant material were soaked in 100 mL of hot water boiled for 30 min in a conical flask for 24 h. The solution was filtered using filter paper and evaporated.

**Plant extract disc preparation**
The plant extract discs were prepared from labline filter paper by punching with a cork borer of 6 mm diameter. Discs with concentration of 1.5 mg (15%), 2.5 mg (25%) were prepared for M. chamomilla. The discs were autoclaved at 121°C for 15 min. The plant extract discs were dried in an oven and stored in a refrigerator until required for use.

**Agar diffusion test**
C. albicans (ATCC 24433) and E. faecalis (ATCC 24212) strains were cultured on Sabouraud’s dextrose agar (Difco; BD Diagnostic Systems, Denmark, US) and blood agar, respectively. The organisms were incubated under aerobic condition. The agar plates were prepared in sterile glass Petri dishes and kept overnight for sterility at 37°C. After ensuring sterility, inoculae of the strains were prepared with sterile saline and the turbidity was compared using McFarland’s turbidity standard tube No. 0.5. This results in 1–2 x 10^6 CFU/mL of E. faecalis and 1–5 x 10^6 CFU/mL of C. albicans. These inoculae were used to make the lawn culture of the organism using sterile cotton swabs on Sabouraud’s agar and blood agar. Wells of 4 mm deep and 6 mm wide in diameter were then punched in the agar plates with a sterile punch for 2% chlorhexidine. The wells in the plates were filled with 2% CHX gel. The plant extract discs were placed in the cultured plates using a sterile forceps. The discs were placed far from each other to avoid overlap of zone of inhibition.

Sensitivity to all these drugs was seen on the Muller–Hilton Agar (MHA) (Oxoid, Cambridge, UK) plate for E. faecalis and CHX gel with 2% glucose and 0.5 µg methylene blue for C. albicans. Media were lawn cultured with the respective organism. The agar plates were then incubated at 37°C for 24 h, after which time the zone of inhibition was measured using a plastic ruler and was recorded for each material. The results thus obtained were statistically analyzed using the Wilcoxon rank–order test.

**RESULTS**
The mean values of microbial growth inhibition produced by M. chamomilla and CHX gel are shown in Tables 1 and 2. All the test groups were statistically different (P < 0.005) for both organisms tested.

2% CHX gel showed the strongest antimicrobial action, producing the largest zones of inhibition, followed by 25% Matricaria, and 15% Matricaria did not show any antimicrobial activity [Figure 1].

**DISCUSSION**
The tested microorganisms were selected because they represent bacteria and fungus commonly
isolated from necrotic canals. *E. faecalis* is associated with persistent apical inflammation and, in clinical situations, it is difficult to eliminate from the root canal system, and therefore selected as a test organism in this study.\(^{[23,24]}\) *C. albicans* is more often isolated from infected root canals and is one of the common microorganisms that survive chemical–mechanical procedures and the application of root canal medicaments.\(^{[10,11]}\) The agar diffusion method was used to test antimicrobial activity because of its simplicity and rapidity. The advantage of this method is that it allows direct comparison of materials against the organism, indicating the potential of the test material to eliminate bacteria in the local microenvironment of the root canal system. However, the disadvantage of this method is that the result does not only depend on the toxicity of material for a particular organism but is also highly influenced by the ability of the material to diffuse across the medium.\(^{[25,26]}\)

Chlorhexidine in gel formulation was chosen for this study because of its low toxicity on the periapical tissues, solubility in water as well as viscosity that keeps the active agent in contact with the root canal walls and dentinal tubules.\(^{[27,28]}\) The antimicrobial property of CHX is due to its permeability to the cell wall or outer membrane of bacteria and ability to attack the bacterial cytoplasmic membrane and inner plasma membrane of the yeast. In high concentrations, CHX causes coagulation of intracellular components.\(^{[28]}\) This could be the reason for its strong antimicrobial action against microbes.

German chamomile is one of the oldest favorites among garden herbs, and its reputation as a medicinal plant shows little signs of abatement. It is especially suitable for children with teething problems and in those who have been in a highly emotional state over a long period of time.\(^{[30]}\) The herb kills certain bacteria and can be used as a mouth wash for dental abscesses and tonsillitis;\(^{[31]}\) it is excellent in treating any type of inflammation, whether internal or external.\(^{[32]}\)

While for German Chamomile extract, the antimicrobial effects are primarily the result of the active components α-bisabolol and azulenes, which have an anti-inflammatory activity.\(^{[33]}\) This activity has been demonstrated not only by long empirical use but also by a number of different laboratory models.\(^{[34]}\) More than 120 chemical constituents have been identified in the chamomile flower as secondary metabolites,\(^{[35,36]}\) including 28 terpenoids, 36 flavonoids\(^{[37-39]}\) and 52 additional compounds with potential pharmacological activity.\(^{[40]}\) Components such as α-bisabolol and cyclic ethers are antimicrobial,\(^{[41,42]}\) umbelliferone is fungistatic, whereas chamazulene and α-bisabolol are antiseptic.\(^{[43]}\) The chamomile was found to have the most effective antileishmanial activity.\(^{[42]}\) But, the total anti-inflammatory effect of whole chamomile depends on the presence of flavonoids such as apigine and luteoline.\(^{[44,45]}\)

Although chamomile oil, at a concentration of 25 mg/mL, demonstrated antibacterial activity against Gram positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans* and *Streptococcus salivarius* in previous studies performed by Berry *et al.*, 1995 and Cinco *et al.*, 1972.\(^{[16,46,47]}\) It showed no microbial action against *E. faecalis* and *C. albicans* at the concentration of 150 mg/mL in the present study.\(^{[48]}\) However, at a higher concentration of 250 mg/mL, it is effective against both microorganisms, but the efficacy is not more than 2% CHX. These concentrations were used to determine the effect of the commercial preparations available in the market for dental uses.

### Table 1: Antifungal activities of materials used against *C. albicans*

| Samples    | n  | Mean | SD   | P value (compared with the positive control) |
|------------|----|------|------|---------------------------------------------|
| 15% Matricaria | 5  | 0    | 0    | 0.025                                       |
| 25% Matricaria | 5  | 24.16| 0.50299 | 0.042                                      |
| 2% Chlorhexidine | 5  | 33.26| 0.79874 | 0.043                                      |
| Fluconazole  | 5  | 44   |      |                                              |

**SD:** Standard deviation

### Table 2: Antibacterial activities of materials used against *E. faecalis*

| Samples    | n  | Mean | SD   | P value (compared with the positive control) |
|------------|----|------|------|---------------------------------------------|
| 15% Matricaria | 5  | 0    | 0    | 0.025                                       |
| 25% Matricaria | 5  | 20.62| 0.61806 | 0.043                                      |
| 2% Chlorhexidine | 5  | 24.54| 0.45607 | 0.042                                      |
| Vancomycin  | 5  | 22   |      |                                              |

**SD:** Standard deviation
A natural substance MC was compared with a synthetic substance (CHX) because of the known side-effects of CHX like brown discolorations of teeth, some restorative materials and the dorsum of tongue, taste alterations, oral mucosa erosion, unilateral or bilateral parotid swelling (extremely rare occurrence), enhanced supragingival calculus and bitter taste,[43] whereas herbal medicines are free from side-effects, easy to obtain, considered healthy and easily accepted by the host and have a lot of useful pharmacological actions comparable to synthetic drugs.[44]

A herbal approach MC can be used as an alternate to drugs available as antimicrobial agents for dental uses if used in proper concentrations, although CHX still represents a satisfactory result against microbes used in the study even at lower concentrations. However, further studies using the same medicament in failed root canal cases in vivo have to be conducted.

CONCLUSION

The results of the current in vitro study suggest that 25% Matricaria is less effective than 2% CHX gel against C. albicans and E. faecalis. Matricaria at a lower concentration of 15% aq. base is not effective. Therefore, it can be concluded that Matricaria can be used as an antimicrobial agent in various fields of dentistry also because of its advantage of being a natural substance, free from side-effects, easy to obtain, considered healthy and acceptable host response.

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How to cite this article: Rahman H, Chandra A. Microbiologic Evaluation of Matricaria and Chlorhexidine against E. faecalis and C. albicans. Indian J Dent 2015;6:60-4.

Source of Support: Nil. Conflict of Interest: None declared.