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Evaluation of the antimicrobial activity of different concentrations of chamomile extract and chlorhexidine gel against *Candida albicans* and *Enterococcus faecalis*

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Abstract
Background: Important goals of endodontic treatment are elimination of microorganisms and prevention of reinfection within the root canal system. Microbial agents most commonly found in failed endodontic treatment procedures are *Enterococcus faecalis* and *Candida albicans*.
Aim: To compare the antimicrobial activity of different concentrations of Chamomile Extract and Chlorhexidine gel against *Candida albicans* and *Enterococcus faecalis*.
Methods: We evaluated the antimicrobial activity of 15%, 25% *Matricaria chamomilla* in aq. base and 2% chlorhexidine gel against *C. albicans* and *E. faecalis* strains by agar diffusion test. 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.
Results: The mean values of microbial growth inhibition against *C. albicans* produced by 25% *M. chamomilla* was 25.25±0.478, by 2% Chlorhexidine was 34.82±0.825. The mean values of microbial growth inhibition against *E. faecalis* produced by 25% *M. chamomilla* was 21.27±0.652, by 2% Chlorhexidine was 25.84±0.494. 2% Chlorhexidine gel showed the strongest antimicrobial action, producing the largest zones of inhibition, followed by 25% Matricaria, whereas 15% Matricaria did not show any antimicrobial activity.
Conclusion: It is clear that *Matricaria chamomilla* in concentrations more than 15% aq. base is effective and provides a good alternative as an antimicrobial agent in dental practice.

Keywords: *Matricaria chamomilla*, antimicrobial activity, chlorhexidine

Introduction
Important goals of endodontic treatment are elimination of microorganisms and prevention of reinfection within the root canal system. Complex anatomy of root canal system helps resistant microorganisms to remain there after mechanical instrumentation and irrigation procedures [1]. Microbial persistence and growth in dentinal tubules, lateral canals and apical ramifications have also been shown. Various authors have demonstrated the effectiveness of intra-canal medications against antimicrobial resistant microorganisms [2, 3].
Microbial agents most commonly found in failed endodontic treatment procedures are *Enterococcus faecalis* and *Candida albicans* [4, 5]. As per various reports, 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 [6].
Matricaria recutita (Chamomile) is a popular herb present in herbal tea due to its calming, carminative and spasmolytic properties. It has soothing and anti-inflammatory effects on skin [7]. It also has a role in the treatment of recurrent apthous stomatitis, mucositis and oral ulcers [8]. At the same time, antimicrobial chlorhexidine efficacy toward endodontic infections is not very clear. Therefore, we planned this study to compare the antimicrobial activity of different Concentrations of Chamomile Extract and Chlorhexidine gel against *Candida albicans* and *Enterococcus faecalis*.

Methods
This investigation was conducted at a tertiary care dental teaching hospital located in the region of Jammu and Kashmir. Convenient sampling technique was adopted.

Substances assessed for antimicrobial activity were 15%, 25% *Matricaria chamomilla* in aq. base and 2% CHX gel against *C. albicans* and *E. faecalis* 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 institute.

Modified Okıgbo and Omodamıro method was used for aqueous extraction (cold water) [9]. 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 100C to dryness. The standard extracts obtained were stored in a refrigerator at 4 °C until required for use. For aqueous extraction (hot water), 15 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.

For plant extract disc preparation, 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.

Strains of *C. albicans* and *E. faecalis* were cultured on Sabouraud's dextrose agar 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. This results in 1–2 x 108 CFU/mL of *E. faecalis* and 1–5 x 106 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% Chlorhexidine 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 plate for *E. faecalis* and MHA 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.

Written and informed consent was obtained from study subjects. Permission of ethical committee was obtained from the Institutional Ethics Committee. All the questionnaires were manually checked and edited for completeness and consistency and were then coded for computer entry. After compilation of collected data, analysis was done using Statistical Package for Social Sciences (SPSS), version 21 (IBM, Chicago, USA). The results were expressed using appropriate statistical variables.

Results

The mean values of microbial growth inhibition against *C. albicans* produced by 25% *M. chamomilla* was 25.25±0.478, by 2% Chlorhexidine was 34.82±0.825. These values tested statistically significant. (Table 1)

Table 1: Antifungal activities of different concentrations of Chamomile Extract and Chlorhexidine gel used against *C. albicans*

Variable	Mean	Standard deviation	P value
15% *matricaria*	0	0	<0.05 (Significant)
25% *matricaria*	25.28	0.478	<0.05 (Significant)
2% Chlorhexidine	34.82	0.825	<0.05 (Significant)
Fluconazole	45	Positive control	

The mean values of microbial growth inhibition against *E. faecalis* produced by 25% *M. chamomilla* was 21.27±0.652, by 2% Chlorhexidine was 25.84±0.494. These values tested statistically significant. (Table 2)

Table 2: Antifungal activities of different concentrations of Chamomile Extract and Chlorhexidine gel used against *E. faecalis*

Variable	Mean	Standard deviation	P value
15% Matricaria	0	0	<0.05 (Significant)
25% Matricaria	21.27	0.652	<0.05 (Significant)
2% Chlorhexidine	25.84	0.494	<0.05 (Significant)
Vancomycin	21	Positive control	

2% Chlorhexidine gel showed the strongest antimicrobial action, producing the largest zones of inhibition, followed by 25% Matricaria, whereas 15% Matricaria did not show any antimicrobial activity.

Discussion

C. albicans is a member of the normal human microbiota and colonizes the oral cavity, the gastrointestinal tract and the genitourinary tract of 70%, or more, of the population, as a commensal agent, but, inducing infection. In the mouth, it occurs when there is an alteration in such environment or immunological dysfunction conditions. Proteinases and phospholipases located on the surface of the yeast and at the end of the germ tube, acts on the phospholipids hydrolysis and causes damage to the epithelial cell [10].

E. faecalis is found in 4 to 40% of primary endodontic infections. *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 [11]. *E. faecalis* is able to form a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing organisms.

In the present work, the mean values of microbial growth inhibition against *C. albicans* produced by 25% *M. chamomilla* was 25.25±0.478, by 2% Chlorhexidine was 34.82±0.825. These values tested statistically significant. The mean values of microbial growth inhibition against *E. faecalis* produced by 25% *M. chamomilla* was 21.27±0.652, by 2%

Chlorhexidine was 25.84±0.494. These values tested statistically significant. Our findings are comparable with results of Rahman H, *et al* [12]. Authors observed that 2% chlorhexidine showed maximum inhibitory zone for *C. albicans* (33.26mm) and *E. faecalis* (24.54mm). 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).

Chlorhexidine, in a 2% gel or liquid concentration, is effective at reducing or completely eliminating *E. faecalis* from the root canal space and dentinal tubules [13]. Two percent chlorhexidine gel is effective at completely eliminating *E. faecalis* from dentinal tubules for up to 15 days. This may be in part attributed to its substantive antimicrobial activity [14]. It is questionable as to whether 0.12% chlorhexidine is more effective than calcium hydroxide. Some studies suggest it is more effective, yet neither will completely eradicate *E. faecalis*. Another study suggests 10% calcium hydroxide alone is more effective. When heated to 46 °C, both 0.12% chlorhexidine and 10% calcium hydroxide have greater antimicrobial effects against *E. faecalis* than at normal body temperature [15].

In our study, 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 [16, 17]. It showed no microbial action against *E. faecalis* and *C. albicans* at the concentration of 150 mg/mL in the present study [18]. However, at a higher concentration of 250 mg/mL, it is effective against both microorganisms, but the efficacy is not more than 2% Chlorhexidine. These concentrations were used to determine the effect of the commercial preparations available in the market for dental uses.

Conclusion

This study observed that 2% Chlorhexidine gel showed the strongest antimicrobial action, producing the largest zones of inhibition, followed by 25% Matricaria, whereas 15% Matricaria did not show any antimicrobial activity. It is clear that *Matricaria chamomilla* in concentrations more than 15% aq. base is effective and provides a good alternative as an antimicrobial agent in dental practice.

References

1. Oliver JD. The viable but non culturable state in bacteria. J Microbiol. 2005; 43:93-100.
2. Turk BT, Sen BH, Ozturk T. *In vitro* antimicrobial activity of calcium hydroxide mixed with different vehicles against *Enterococcus faecalis* and *Candida albicans*. Oral Surg. Oral Med Oral Pathol Oral Radiol Endod. 2009; 108(2):297-301.
3. Valera MC, da Rosa JA, Maekawa LE *et al*. Action of propolis and medications against *Escherichia coli* and endotoxin in root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 110(4):70-74.
4. Baumgartner JC, Watts CM, Xia T. Occurance of *Candida albicans* in infections of endodontic origin. J Endod. 2000; 26:695-8.
5. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006; 32:93-8.
6. White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod. 1997; 23:29-31.
7. Carmen C, Reyes A, Rafael G. Beneficial effects of green tea-a review. J AM Coll Nutr. 2006; 25:79-99.
8. Sahba S, Alipour M. Evaluation of the Effects of Chamomill Mouthrinse on Recurrent Aphthous Stomatitis. J Dent Tehran Univ Med Sci. 2005; 2:147-51.
9. Okigbo RN, Omodamiro OD. Antimicrobial Effects of Leaf Extracts of *Piegon pea Cajanus cajan* L. Millsp. On Some Human Pathogens. J Herbs Spices Med Plants. 2006; 12:117-27.
10. Nobile CJ, Johnson AD. *Candida albicans biofilms* and human disease. Annu Rev Microbiol. 2015; 69:71-92.
11. Portenier I, Haapasalo H, Orstavik D, Yamauchi M, Haapasalo M. Inactivation of the antibacterial activity of iodine, potassium iodide and chlorhexidine digluconate against *Enterococcus faecalis* by dentin, dentin matrix, type-I collagen, and heat-killed microbial whole cells. J Endod. 2002; 28:634-7.
12. Rahman H, Chandra A. Microbiologic Evaluation of Matricaria and Chlorhexidine against *E. faecalis* and *C. albicans*. Indian J Dent. 2015; 6:60-4.
13. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules *in vitro*. Endod Dent Traumatol. 1993; 9:243-8.
14. White R, Hays G, Janer L. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod. 1997; 23:229-31.
15. Evanov C, Liewehr F, Buxton TB, Joyce AP. Antibacterial efficacy of calcium hydroxide and chlorhexidine gluconate irrigants at 37 degrees C and 46 degrees. CJ Endod. 2004; 30:653-7.
16. Kunde R, Isaac O. On the flavones of chamomile *Matricaria chamomilla* L. and a new acetylated apigenin-7-glucoside. Planta Med. 1980; 37:124-30.
17. Budzinski JW, Foster BC, Vandenhoek S, Amason JT. An *in vitro* evaluation of human cytochrome P4503A4 inhibition by selected commercial herbal extracts and tinctures. Phytomed. 2000; 7:273-82.
18. Bayoub K, Baibai T, Mountassif D, Retmane A, Soukri A. Antibacterial activities of the crude ethanol extracts of medicinal plants against *Listeria monocytogenes* and some other pathogenic strains. Af J Biotechnol. 2010; 9:4251-8.