

Research Article



Evaluation of antimicrobial activity and efficacy of herbal oils and extracts in disinfection of gutta percha cones before obturation

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Received: Jul 22, 2016

Accepted: Jul 2, 2017

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Conflict of Interest

No potential conflict of interest relevant to this
article was reported.

Author Contributions

Conceptualization: Makade CS, Shenoi
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Funding acquisition: Makade CS, Shenoi PR;
Investigation: Makade CS; Methodology:
Makade CS, Shenoi PR, Morey E; Project
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ABSTRACT

Objectives: Literature has shown that micro-organisms contaminate gutta percha (GP) during storage and manipulation. Till date herbal extracts are not explored as an alternative medicament for pre-operative chairside disinfection of GP cones. The purpose of our study was to evaluate the antimicrobial activity and efficacy of lemon grass oil (LG), basil oil (BO), and obicure tea extract (OT) in disinfecting GP cones before obturation.

Materials and Methods: Agar diffusion method was used to evaluate the antimicrobial efficacy of LG, BO, OT, and sodium hypochlorite (control) against common contaminants, namely, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*. One hundred and twenty GP cones were contaminated and cut into 2. First half was placed in the broth and incubated; whereas the second was treated with herbal extracts for 1 minute and then incubated for 24 hours in the broth. Any inhibition in bacterial growth was noted with presence/absence of turbidity. Two-way analysis of variance and χ^2 test were used to assess the effectiveness of herbal extracts to decontaminate GP.

Results: LG showed the highest inhibition zones (29.9 ± 6.9 mm) for all tested organisms, followed by OT extract (16.3 ± 1.8 mm), sodium hypochlorite (16.0 ± 1.6 mm), and BO (14.5 ± 5.3 mm). Statistically significant difference was observed between LG and other herbal extracts ($p < 0.05$).

Conclusions: All extracts proved to be potential rapid chairside disinfectants of GP cones with LG showing the highest antimicrobial activity.




Keywords: Basil; Disinfection; Gutta percha; Lemon grass; Obicure tea extract

INTRODUCTION

The success of endodontic therapy principally depends on removal of micro-organisms from the canal and prevention of reinfection in root canal [1]. Gutta percha (GP) is a proven obturating material as it is dimensionally stable, biocompatible, radiopaque, thermoplastic with antimicrobial properties. It is suggested that disinfection of GP cones before obturation is not mandatory owing to presence of zinc [2]. Literature reveals that most clinicians use

Makade CS; Software: Makade CS, Paralikar AV; Supervision: Shenoi PR; Validation: Makade CS, Shenoi PR; Visualization: Makade CS, Shenoi PR; Writing - original draft: Makade CS; Writing - review & editing: Makade CS, Shenoi PR, Morey E, Paralikar AV.

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GP cones directly from their packages, further imposing the risk of contamination by glove, handling, and/or advertent storage [3,4]. Panugant *et al.* [5] recently reported that almost 75% endodontic post-graduates did not practice GP disinfection despite its established role in success of root canal therapy. Hence, to maintain aseptic chain during root canal treatment, rapid chair side disinfection of GP cones is of utmost importance along with careful handling of disinfected GP cones with sterile tweezers. Various chemicals like ethyl alcohol, paraformaldehyde, formocresol, glutaraldehyde, polyvinylpyrrolidone iodine, quaternary ammonium compounds, and hydrogen peroxide are being commonly used as disinfectants. Recent studies have advocated the use of 1% peracetic acid and BioPure MTAD antibacterial root canal cleanser (DENTSPLY Tulsa Dental Specialties, DENTSPLY International, Inc., Johnson City, TN, USA) for disinfection of GP [6-8].

Sodium hypochlorite (5.25%) is the coveted choice for effective disinfection of GP in 1 minute. Milton's solution (1%) and Dakin liquid (0.5%) were also used with time varying from 3–25 minutes for disinfection [9]. However, in all the concentrations, crystal deposition is reported to hamper the bond between the sealers and canal walls, leading to microleakage [10]. Pallotta *et al.* [11] advocated ciprofloxacin (CFC), metronidazole (Flagyl, G.D. Searle LCC, Division of Pfizer Inc., New York City, NY, USA) and calcium hydroxide to kill *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa*, *Enterococcus faecalis* (*E. faecalis*), and *Bacteroides fragilis*. But it was concluded that CFC required a minimum of 5 minutes exposure for GP disinfection [8]. Chlorhexidine gluconate (0.2%, 1%, and 2%) both in liquid and gel forms were ineffective against *Bacteroides subtilis* (*B. subtilis*) spores even after 72 hours. Chlorhexidine gel (0.2%) took 2 hours to kill *E. faecalis* and the vegetative form of *B. subtilis*, making its use practically difficult.

Later, it was found that immersion of GP cones in 2% chlorhexidine gluconate for 1 minute was an effective method for GP disinfection [12,13]. All 3 chemical disinfectants (sodium hypochlorite, chlorhexidine, and ChlorPrep [solution of 75% isopropyl alcohol and 2% chlorhexidine in 1:1 vol.; Medi-flex, Leawood, KS, USA]) were effective in the rapid disinfection of GP cones against *Staphylococcus* species (spp), and 1 minute immersion of GP cone was adequate. All disinfectants significantly increased the elongation rate of the GP cones compared with fresh GP cones ($p < 0.05$), especially in the ChlorPrep group [14].

Scientific literature suggests that herbs possess antimicrobial, antiseptic, antiviral, antifungal, and immunomodulatory properties. Hence, they are safely used in food and pharmaceutical industries with little or no side effects [15,16]. Herbal agents are eco-friendly but have not been explored as an alternative pre-operative disinfective medicament for GP cones. In recent studies, Shenoi *et al.* [17] and Athiban *et al.* [18], recommended the use of neem bark and aloe vera extract respectively for disinfection of GP. Lemon grass (*Cymbopogon citrates*), basil (*Ocimum basilicum* L.), green tea (*Camellia sinensis*) extract are popular culinary herbs in Asian subcontinent and are used in many dental and oral products.

Therefore, in this study, an attempt was made to find herbal substitutes for disinfection of GP cones. The null hypothesis tested was that there is no difference in the antimicrobial efficacy of lemon grass oil (LG), basil oil (BO), and obicure tea extract (OT). The aims were to evaluate the antimicrobial efficacy of OT, LG, and BO, to evaluate the efficacy of these herbal extracts in disinfecting GP cones, and to find the best herbal extract to disinfect the GP cones in 1 minute.

MATERIALS AND METHODS

This was an experimental *in vitro* study using a 2-factorial design: micro-organisms (*E. faecalis* [American Type Culture Collection {ATCC}-29212], *S. aureus* [ATCC-25923], *Candida albicans* [*C. albicans*, ATCC-90028]; ATCC, Belgaum, KA, India) and medicaments.

Preparation of extracts

Fresh lemon grass and basil leaves were collected, washed and oil was extracted using distillation procedure (Sanghavi Labs Private Ltd., Anjangaonsurji, MH, India), and stored in sterile bottles till use [19]. A commercially proprietary food product 'Obicure' tea extract (Uth Healthcare Ltd., Pune, MH, India) and sterile water were mixed in a ratio of 1 g/5 mL to obtain an extract 0.005 µg/mL.

Evaluation of antimicrobial efficacy of herbal extracts

The inoculums of *S. aureus* and *E. faecalis* were incubated overnight in nutrient broth (Hi-media Laboratories, Mumbai, MH, India) to collect sufficient number of microbial colonies. Each species was then lawn cultured on 10 petri-dishes (Hi-media Laboratories, Mumbai, MH, India). The strain of *C. albicans* species was cultured on Sabouraud dextrose agar medium (Hi-media Laboratories).

Each petri-dish was punched with sterile template on its surface to make 4 wells of 5 × 5 mm. Each well was marked according to the medicament dispensed as group A (control group; 5.25% sodium hypochlorite, Vishal Dentocare Private Ltd., Ahmedabad, GJ, India), group B (OT), group C (LG), and group D (BO). About 0.5 mL of the medicament to be tested was dispensed into the wells using a micropipette.

All strains were tested using agar well diffusion technique (Kirby-Bauer method) [20], incubated at 37°C for 24 hours, and observed for the development of clear zones around the extracts. The antibacterial activity was assessed by measuring the diameter of the clear zone of inhibition in millimeters (mm) against light.

Verification of contamination of GP cones

A total of 120 GP cones (0.02 taper and size 40; Dentsply Maillefer, Ballaigues, Switzerland) were selected from freshly opened manufacturers' pack. These cones were put in a sterile container and sent to the department of microbiology. They were divided into 4 groups ($n = 30$). Each cone was cut into 2 halves with a sterile GP cutter. The first half of the cone was placed in individual test tubes containing brain heart infusion (BHI) broth (Hi-media Laboratories) [18], while the remaining cones were assigned to treatment by respective medicament for 1 minute. The treated cones were placed on absorbent paper to remove excess medicament before immersing in BHI broth. All the test tubes were incubated for 24 hours and recorded for the formation of turbidity. Growth noted at 24 hours was considered positive; however, it was observed for 72 hours and results were confirmed by sub-culturing the bacterial colony.

Statistical analysis

Descriptive statistics including mean, standard deviation (SD), and range were used to summarize antimicrobial properties of herbal extracts. Statistical analysis was performed using STATA (version 10.1, Stata Corp. LLC, College Station, TX, USA). Two-way analysis of

variance (ANOVA) was used to assess effects of herbal extracts on tested micro-organisms by groups and their interaction (group×micro-organisms). One-way ANOVA with *post hoc* Bonferroni's test was used to compare mean scores of inhibition zones. The contamination of GP cones and effectiveness of herbal extracts to decontaminate GP cones was evaluated using χ^2 test. *p* value less than 0.05 was considered statistically significant.

RESULTS

For all 3 tested micro-organisms, group C (LG) showed largest zones of inhibition (mean \pm SD), 26.1 \pm 1.0 mm, 39.3 \pm 1.2 mm, and 24.3 \pm 1.0 mm against *E. faecalis*, *S. aureus*, and *C. albicans*, respectively (Table 1 and Figure 1). Two-way ANOVA (Table 2) revealed that efficacy of herbal extracts differed significantly across the 4 compared groups ($p = 0.0001$). Significant differences in efficacy of herbal extracts for 3 tested microorganisms were also observed ($p = 0.0001$). There was significant interaction between the medicaments and the organisms ($p = 0.0001$). One-way ANOVA for tested micro-organisms revealed statistically significant difference in all possible pair wise comparisons for medicament groups, except group A (control) vs. group B (OT). Microbial assays showed maximum efficacy with group C (LG; 100%) for decontamination of GP cones in 1 minute, followed by group D (BO; 93.3%), group A (control; 86.7%), and group B (OT; 83.3%) (Table 3). However, the differences between the groups were not statistically significant ($p = 0.110$).

DISCUSSION

Maintaining the aseptic chain during cleaning, shaping, and obturation is the most important step during the endodontic treatment. However, it is quite difficult to completely eradicate microorganisms from root canal system. Hence, it becomes imperative to disinfect GP cones to minimize the chances of reinfection and to enhance peri-radicular healing [1].

Table 1. Descriptive statistics for tested organisms by 4 comparison groups ($n = 10$)

Organism	Group A (control)	Group B (OT)	Group C (LG)	Group D (BO)
<i>E. faecalis</i>	15.30 \pm 0.78	17.70 \pm 0.90	26.10 \pm 0.94	10.40 \pm 1.02
<i>S. aureus</i>	17.70 \pm 1.10	17.20 \pm 0.75	39.30 \pm 1.19	21.70 \pm 0.90
<i>C. albicans</i>	14.90 \pm 0.94	14.00 \pm 0.63	24.30 \pm 0.90	11.40 \pm 0.80

All the data are presented as means and standard deviations; Unit, mm.

Group A (control), 5.25% sodium hypochlorite; group B (OT), obicure tea extract; group C (LG), lemon grass oil; group D (BO), basil oil.

Table 2. Two-way analysis of variance (ANOVA) for tested organisms and medicaments

Source	Partial SS	df	MS	F	<i>p</i> value
Model	6,913.87	11	628.53	673.43	0.0001
Group	4,663.2	3	1,554.4	1,665.43	0.0001
Org.	1,417.22	2	708.61	759.22	0.0001
Interaction (group×org.)	833.45	6	138.91	148.83	0.0001
Residual	100.8	108	0.93	-	-
Total	7,014.67	119	58.95	-	-

SS, sum of squares; df, degrees of freedom; MS, mean sum of squares; org., organism.

Table 3. Efficacy of herbal extracts in decontamination of gutta percha (GP) cones ($n = 30$)

Turbidity absent	Group A (control)	Group B (OT)	Group C (LG)	Group D (BO)	χ^2 test	<i>p</i> value
No. (%)	26 (86.7)	25 (83.3)	30 (100.0)	28 (93.3)	5.90	0.110 (NS)

Group A (control), sodium hypochlorite; group B (OT), obicure tea extract; group C (LG), lemon grass oil; Group D (BO), basil oil; NS, not significant.

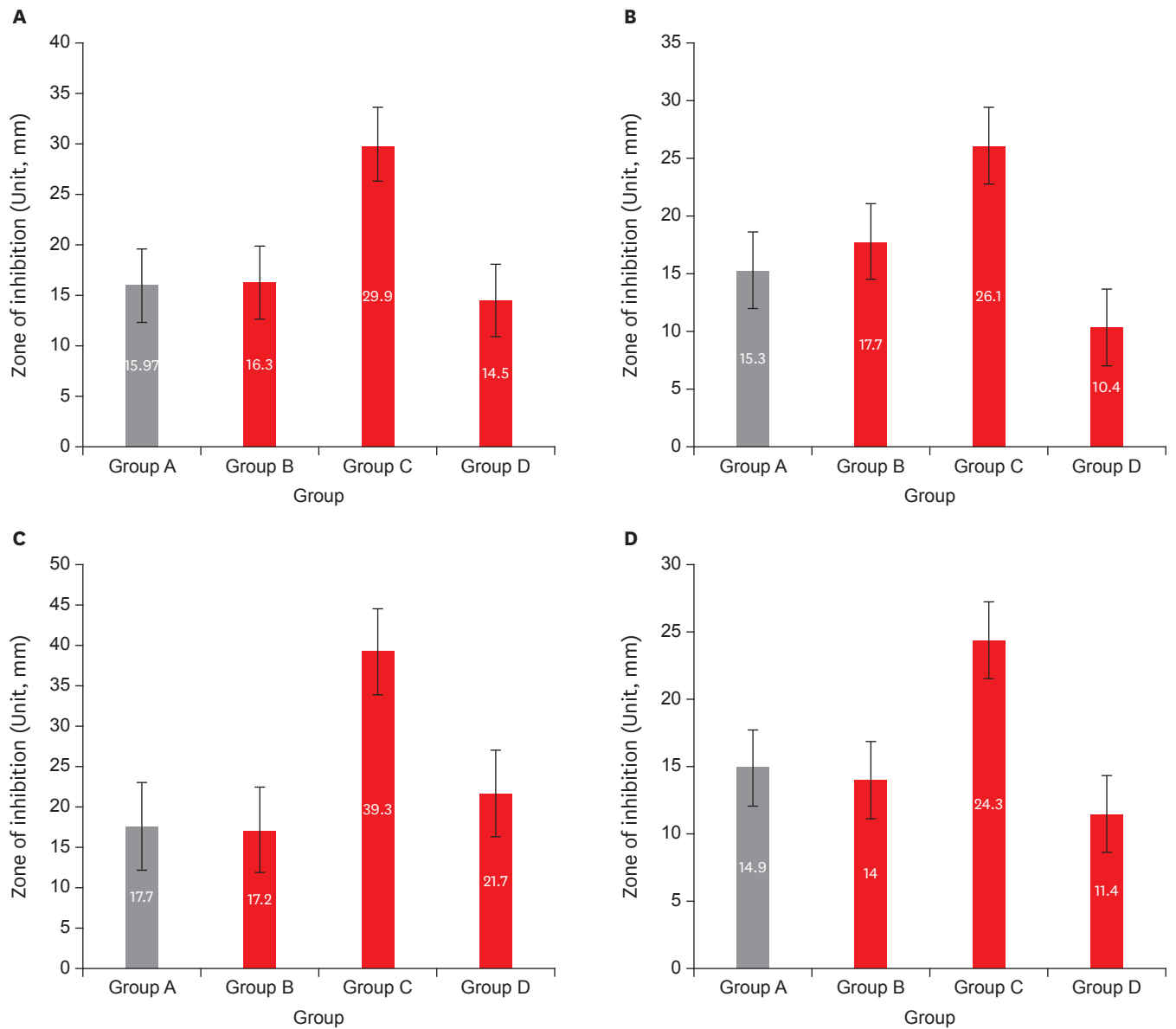


Figure 1. Comparison of inhibition zones of control group A vs. Experimental groups B, C, and D. (A) For all tested organisms; (B) for *E. faecalis*; (C) for *S. aureus*; (D) for *C. albicans*.

Group A (control), sodium hypochlorite; group B (OT), obscure tea extract; group C (LG), lemon grass oil; group D (BO), basil oil.

The foremost cause for failure of root canal treatment is the presence of facultative and resistant microbial species like *E. faecalis*, *C. albicans*, and *S. aureus* [21]. *E. faecalis* is a gram positive facultative anaerobe and an outstanding survivor in the root canal system. It causes failure of endodontically treated teeth (5% in untreated teeth whereas 29%–77% in root filled teeth) [22]. *E. faecalis* and *C. albicans*, have been repeatedly identified as the species most commonly recovered from root canals that undergo retreatment, and in cases with persistent infections [23]. Similarly, Waltimo *et al.* [24] concluded in their study that *C. albicans*, was also associated with failed endodontic therapy. According to Baumgartner *et al.* [25], extraradicular microbial biofilm of *S. aureus* on tissue or biomaterial surface is related to refractory periapical disease. Therefore, these pathogens were selected for this study.

Several herbal extracts are commonly known for their medicinal properties, *viz.* antimicrobials, anti-cancer, anti-diabetic, immune-modulatory, gastro-protective, as well as protective against many systemic diseases, liver disorders and also as cosmetics agents [26,27]. Furthermore, they are comparatively economical and eco-friendly; although their use in endodontic practice for sterilization of GP cones is scarcely reported [17,18]. Therefore, this study was undertaken to analyse the anti-microbial property of herbal extracts and to check their ability to disinfect GP cones in 1 minute. This may provide a practical solution for rapid chairside disinfection of GP cones and help increase success rate of root canal treatment.

LG, BO, and OT were used in the study for their higher occurrence in tropical countries. *Cymbopogon citratus* is largely cultivated in tropical and subtropical countries. Saddiq *et al.* [28] and Falcao *et al.* [19] reported its antibacterial activity against *S. aureus* because of high activity of citral epoxide. De Silva *et al.* [29] proved 100% toxicity (American Association of Microbiology) of LG against fungal growth as citral formed a large transfer complex with an electron donor of fungal cells resulting in fungal death. Moreover, Tyagi *et al.* [30] found the oil form (vapour phase) to be highly effective against *C. albicans* causing deleterious morphological changes in cellular structure. In addition, study by Naik *et al.* [31] proved it to be more effective against gram positive than gram negative bacteria at low concentrations. Lemon grass essential oil consists mainly of citral, which is a racemic mixture of 2 monoterpene aldehydes; the geranial (*cis*-citral) and the neral (*trans*-citral). It is anti-inflammatory, and antioxidant; hence recommended for use in mouth rinses, tooth pastes, and in diseases of oral cavity [16]. However, this is being used for the first time in the field of endodontics.

Basil (*Ocimum basilicum L.*) is a popular culinary herb [32] and also used for many dental and oral products owing to its antimicrobial activity (*Bacillus cereus*, *B. subtilis*, *Bacillus megaterium*, *S. aureus*, *Listeria monocytogenes*, *Escherichia coli* [*E. coli*], *E. faecalis*) [33], and antifungal activity against *C. albicans* [34,35]. These herbal extracts were used in the form of essential oils as they are a complex mixture of natural substances composed of terpenoids and phenylpropanoid molecules possessing many biological qualities. It directly acts on the cell by reducing metabolites and ions from the cytoplasm due to the partition of membranes and lipids, coagulation of cytoplasm, and disruption of the cell wall [36]. Essential oils used in the study were extracted using distillation procedure to prevent degradation of thermolabile compounds for operational ease and low operating cost [19].

We used 'Obicure', a proprietary herbal product constituting Green tea extract, 400 g; *Garcinia cambogia* extract, 150 mg; Ginger extract, 20 mg; Pepirene extract, 5 mg, which is commonly used for treating obesity. Green tea (*Camellia sinensis*) extract, a known chelating agent & antioxidant, is also known for its antibacterial activity against *E. faecalis* and planktonic cells [37]. Piperene extract (*piper nigrum*) is a potent antibacterial (both gram positive and gram negative), anti-inflammatory, antioxidant, with analgesic effect [38]. Ginger extract (*Zingiber officinale*) possesses antimicrobial activity against many gram negative and gram positive bacteria and was found to be effective against *E. faecalis* and also against *C. albicans* [39]. *Garcinia cambogia* extract contains hydroxy citric acid (HCA) and is considered safe. In our study, antimicrobial efficacy was assessed using agar diffusion technique as it does not alter properties of medicaments and is an easy and less technique sensitive method. The antimicrobial efficacy of herbal agents was assessed by comparing the diameters of inhibition zone against *E. faecalis*, *S. aureus*, and *C. albicans*. All the tested groups (control and experimental) showed significant zones of inhibition suggesting potent antimicrobial activity. However, inhibition zones (mean \pm SD) for LG (29.9 \pm 6.9 mm) was reported largest,

followed by OT extract (16.3 ± 1.8 mm), sodium hypochlorite (16.0 ± 1.6 mm) and BO (14.5 ± 5.3 mm) when compared with all 3 microbes in the study (**Figure 1**). One-way ANOVA test performed for each organism suggested significant differences existed across all the groups. Further, all pair-wise Bonferroni comparisons also suggested significant differences except between control and OT. Thus, it can be concluded that LG and BO have superior antimicrobial activity than control and OT.

In the second part of study, LG showed 100% decontamination followed by BO (93.3%), sodium hypochlorite (86.7%), and OT extract (83.3%) in 1 minute. Chi-square test showed no significant difference in the formation of turbidity between the control and experimental groups. Sheno *et al.* [17] evaluated neem, aloe vera, and turmeric gels against *E. coli*, *E. faecalis*, and *S. aureus*. Of all the herbal extracts used in GP cone decontamination for 3 minutes, neem gel showed decontamination equivalent to sodium hypochlorite. Athiban *et al.* [18] recommended aloe vera for decontaminating GP cones.

GP cones are usually sterile during storage but can be easily contaminated if incorrectly handled. LG is a potent antimicrobial agent for rapid disinfection of GP cones and can be an alternative to sodium hypochlorite. So, the null hypothesis tested was rejected as there was a significant difference in the antimicrobial efficacy of OT, LG, and BO.

CONCLUSIONS

The study found LG to be the most effective herbal substitute followed by BO, mixture of OT, and sodium hypochlorite. Herbal extracts are eco-friendly and cost effective, and may serve as a promising tool for rapid chair-side disinfection of GP cones before obturation.

ACKNOWLEDGEMENT

The authors acknowledge the support and efforts of Department of Microbiology, NKPSIMS, Nagpur & Dr. Kishore G. Bhat, Prof. & Head, Department of Microbiology; and Director, Department of Molecular Biology and Immunology, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum, Karnataka for their help during this study.

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