Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2019;10:1s:263-271.

Comparative Evaluation of Antimicrobial Efficacy of Phytomedicinal Extracts and Chemical Irrigant against Enterococcus faecalis and Candida albicans: In vitro study

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ABSTRACT

Background: To compare and evaluate the antimicrobial activity of phytomedicinal extracts and chemical irrigant against E.faecalis and C. albicans using disc diffusion method.

Materials and Methods: For the study, microbial strain of Enterococcus faecalis and Candida albicans were used. Four experimental groups were divided. These were as follow- Group I- Garlic Extracts, Group II- Ginger extracts, Group III- 5.25% Sodium Hypochlorite and Group IV- 2 % Chlorohexidine Gluconate. For the agar disc diffusion test, Petri plates of Muller Hinton agar were inoculated with 0.1 ml of the microbial suspensions. Subsequently, the paper discs of four groups were placed on the Muller Hinton agar plate. After 24 hours, the diameter of inhibition zone was measured around the papers discs containing the substances.

Results: Statistically significant difference ($p<0.05$) was found between the groups against E. faecalis and C. albicans. Order of antimicrobial activity is Garlic >2 % Chlorohexidine gluconate > 5.25% Sodium Hypochlorite> Ginger.

Conclusions: Garlic extract is advantageous in cases of secondary endodontic infections which are dominated by organisms like E. faecalis and C. albicans.

Keywords: Enterococcus faecalis, Candida albicans, disc diffusion, ginger extract, garlic extract.

INTRODUCTION

One of the key reasons behind the success of root canal treatment is the chemo -mechanical preparation which aids in removal of remaining tissue, various microbes and debris from the complex root canal areas and allow the canals to be obturated to achieve a fluid tight seal which will prevent future ingress of microbes into the canals(1). Although a plethora of microbes have been found to reside in oral cavity, the lower oxygen potential inside root canals and nutrient availability leads to colony formation of facultative anaerobes predominantly.

Received: Nov. 15, 2019**: Accepted**: Dec. 17, 2019
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pISSN 2278-0076
eISSN 2321-9270

Enterococcus faecalis, a facultative anaerobe found in 4 to 40% of primary endodontic infections, survives unaided inside canals and periapical area and multiply by genetic polymorphism, causes triggering infection and local bone resorption[2]. Because of its virulence factors like aggregation substance, cytolysin and lytic enzymes can alter host responses and hence it is able to strive better than other microbes and is the strain isolated after unsuccessful endodontic treatment[3]. Moreover, they invade dentinal tubules and adhere to collagen due to their ability to form biofilm and therefore it may act as a nidus for recurrence of infection in failed root canal treated teeth[4].

Recent studies have shown that fungi also cause root canal infections. Fungi have been detected in root canals with primary and secondary endodontic infections[4] abscesses or cellulitis of endodontic origin, and Periapical lesions in asymptomatic teeth[5].

Candida albicans is the most commonly isolated, potentially pathogenic yeast species from infected root canals[5]. Candida is a versatile micro-organism, each form of growth depends on environmental conditions such as the pH level, temperature, and nutritional source[6]. Furthermore, it also exhibits a variety of virulence factors such as adherence, thigmotropism and phenotypic switching and secretes a degenerative enzyme ''aspartyl protease'' that degrades the dentinal collagen[7].

Because of these unique qualities, able to survive in the harsh ecologic environment of the root canal, E. faecalis and C. albicans choose as test micro-organism.

Hence, to attain lasting successful root canal treatment, it necessitates a complete disinfection of root canal and limiting the recurrence of infection. Use of irrigants to thoroughly clean root canal systems during instrumentation is central to successful endodontic treatment. Irrigation is complementary to instrumentation and facilitates the removal of pulp tissues and/or microorganisms. In addition to providing mechanical flushing action, an irrigant should exert microbicidal effects without damaging periradicular tissues[8].

Sodium hypochlorite (NaOCl) is a potent irrigant with a broad-spectrum antimicrobial activity that causes the breakdown of proteins and amino acids through its "free chlorine release." It has the potential to dissolve the necrotic pulp tissue and organic debris[9].

Chlorohexidine gluconate (CHX) is also a broad-spectrum antimicrobial agent with a wide range of action. CHX has a unique feature in that dentin medicated with it acquires antimicrobial substantivity. The positively charged ions released by CHX can adsorb into dentin and prevent microbial colonization for some time beyond the time of the medicament[10]. Side effects of synthetic drugs have prompted researchers to search for herbal alternatives. Pharmacological studies have acknowledged the value of medicinal plants as potential sources of bioactive compounds[11]. Some natural plant extracts have antimicrobial and therapeutic properties, suggesting their potential use as endodontic irrigants or intracanal medications[12]. In addition to antimicrobial effects, herbal irrigants are safe and nontoxic to host tissues.

The aim of the present study was to evaluate the antimicrobial efficacy of phytomedicinal extracts of garlic and ginger with chemical irrigant of 5.25% sodium hypochlorite (NaOCl) and 2% chlorhexidine gluconate (CHX) against E.faecalis and C. albicans.

Aim: To evaluate the antimicrobial efficacy of phytomedicinal extracts of Garlic and Ginger as endodontic irrigants with chemical irrigants of 5.25% NaOCl and 2% Chlorohexidine gluconate against Enterococcus faecalis and Candida albicans.

MATERIALS AND METHODS

The following materials were used in the study:

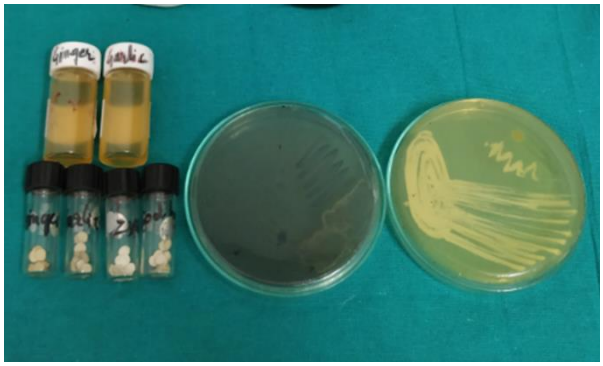
Extract of garlic, ginger, 2% Chlorohexidine Gluconate and 5.25% Sodium hypochlorite, Candida albicans strain, E. faecalis strain, Chromagar, Mitis Salivarius Agar, Mueller Hinton Agar, sterile test-tubes and sterile droppers, Whatman filter paper no.1.

Preparation of extracts

Preparation of garlic and ginger extract

Fresh natural garlic and ginger were purchased from local market. The cloves were separated from

Fig 1: Materials used in study.



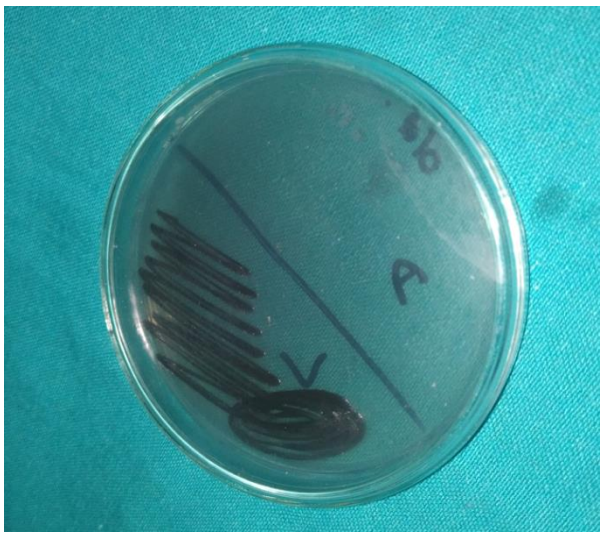


Fig 2: Growth of E. faecalis on Mitis Salivarius Agar.

Fig 3: Growth of C. albicans on Chromagar.

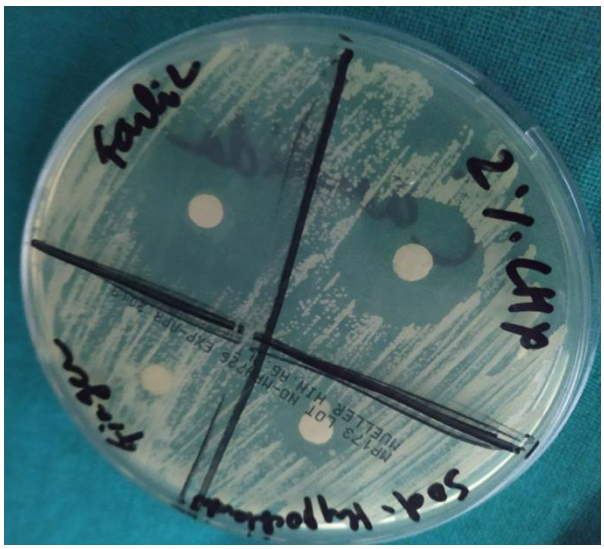
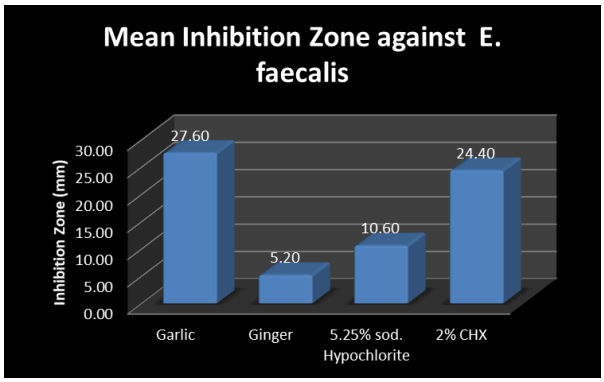


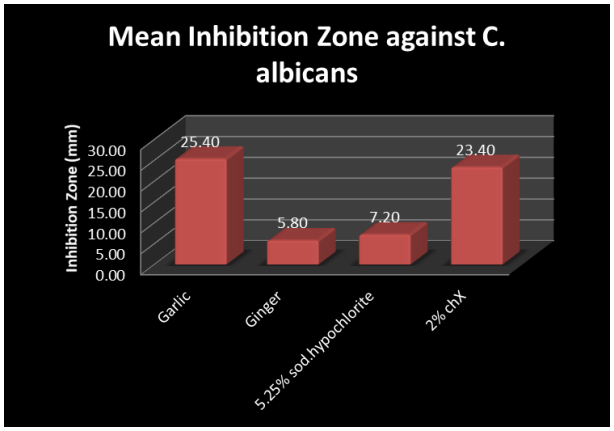
Fig 4: Inhibition Zone of study group against E. faecalis.

Fig 5: Inhibition Zone of study group against C. albicans.

Graph 1: Mean zone of inhibition of all group against E. faecalis.



Graph 2: Mean zone of inhibition of all group against C. albicans.



garlic and peeled to obtain the edible portion. Fifty gram of the edible portion was chopped and homogenized in 100 ml of autoclaved water in a blender. The homogenized was then filtered by passing through a 25µm pore size filter to give a crude aqueous extract of 500mg of extracts/ml. This was collected in a sterile screw cork bottle and stored at 4^0C until used.

Microbial strains

Two strains including Candida albicans and Enterococci faecalis were obtained from the Microbial Culture Collection Bank, Department of Pathology and Microbiology. The Candida strains were maintained on Sabouraud's dextrose agar slants and E. faecalis were maintained on Brain Heart Infusion. The culture was kept under refrigerated conditions and was sub cultured every 15 days. After that, growth of pure colonies of E. faecalis on Mitis Salivarius agar with 1% potassium tellurite and C. albicans on Chromagar were maintained.

Preparation of antimicrobial disc

Whatman filter paper no.1 was used for preparation of antimicrobial disc. The paper disc were prepared and kept in hot air oven at 160^0C for 1 hr for sterilization. The antimicrobials agents that are to be used are impregnated on sterile filter paper disc. Prepared antimicrobial disc divided into four group.

Group I- Garlic Extracts
Group II- Ginger Extracts
Group III- 5.25% Sodium Hypochlorite
Group IV – 2% Chlorohexidine Gluconate

These disc were kept for drying in oven. Prepared antimicrobial discs were stored in refrigerators.

Preparation of microbial inoculum

The density of selected organisms was adjusted equal to that of the 0.5 McFarland standards by adding them to BHI broth for E. faecalis and SDA for C. albicans. A 24 h old culture was used for the preparation of bacterial suspension. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms would be within a given range.

Antimicrobial susceptibility test

Disc diffusion method was used to conduct the antimicrobial susceptibility test on Muller Hinton agar by Kirby Bauer Method. The agar media plates were inoculated with the organisms by even streaking of the swab over the entire surface of the Muller Hinton agar plate 3 times, rotating the plate approximately 60° after each application to ensure an even distribution of the inoculums. Prepared antimicrobial bio disc were placed equidistance on agar plate. The plates were maintained for 1 h at room temperature, and then the plates, thus prepared were incubated for 24 h at 37°C. The zone of inhibition was measured and expressed in millimetres. Experiments were performed 5 times and mean of the zone of inhibition was recorded in mm. The mean and standard deviation of the diameter of inhibition zones were calculated.

Table 1: Mean, Standard deviation, Standard error of inhibition zone against E. faecalis.

Group	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Garlic	27.60	1.673	.748	25.52	29.68
Ginger	5.20	1.483	.663	3.36	7.04
5.25 % Sodium Hypochlorite	10.60	1.342	.600	8.93	12.27
2% CHX	24.40	1.517	.678	22.52	26.28
Total	16.95	9.660	2.160	12.43	21.47

Table 2: Mean, Standard deviation, Standard error of inhibition zone against C. albicans.

Group	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Garlic	25.40	.894	.400	24.29	26.51
Ginger	5.80	.837	.374	4.76	6.84
5.25% Sodium Hypochlorite	7.20	2.168	.970	4.51	9.89
2% CHX	23.40	.894	.400	22.29	24.51
Total	15.45	9.305	2.081	11.10	19.80

STASTICAL ANALYSIS

All data were analysed by using 1BM SPSS-20 software. The data was interpreted at a confidence interval of 95%. Analysis of variance (ANOVA) was used to compare the antimicrobial activity of herbal extracts with chemical irrigant. Significance level was set at $P \leq 0.05$.

RESULTS

The mean values of inhibition zone were obtained in the groups by zone scale. Mean zone of inhibition were presented in Table 1and Graph 1 against E. faecalis and Table 2 and Graph 2 against C. albicans. Order of antimicrobial activity is Garlic > 2 % chlorohexidine gluconate > 5.25 % sodium hypochlorite > ginger against E.faecalis and C. albicans. The antimicrobial activity of garlic extract is more effective against E .faecalis and C.albicans than the 2 % chlorohexidine gluconate and the difference being statistically significant. The antimicrobial activity of 2% Chlorohexidine gluconate is more than sodium hypochlorite but less than garlic extract against E. faecalis and C. albicans. The antimicrobial property of 5% sodium hypochlorite and ginger were found least in both group against E.faecalis and C. albicans.

DISCUSSION

Primary root canal infections consist of different types of microbes, mostly consisting of obligate anaerobes[13]. It is believed that both mechanical enlargement and copious irrigation of root canals aids is maximum removal of microorganisms as possible[14,15]. Various studies show that the obligate anaerobes are easier to eliminate through endodontic procedures than facultative anaerobes (isolated in 19% to 22% of cases); the latter prove more resistant to chemo-mechanical procedures. Facultative anaerobic micro-organisms such as Enterococcus faecalis, Staphylococcus aureus, and Candida albicans are considered to have the highest

resistance in the oral cavity, with the potential to cause failure of root canal treatment[16]. In particular Enterococcus faecalis and C. albicans have gained attention in the endodontic literature, as associated with therapy resistant apical periodontitis. Therefore, E. faecalis and C. albicans choose for study purpose as test organism.

Irrigation is important for root canal treatment as it assists in removing bacteria and debris within the intricacies of the root canal. An irrigant should impart mechanical, microbicidal properties, be biocompatible and aid in dissolving organic tissues without damaging the periradicular tissues if extrusion of irrigant occurs into the periodontium.

Sodium hypochlorite (NaOCl) is the most popular irrigant used during instrumentation. It is a nonspecific proteolytic agent with wide-ranging activities against endodontic microorganisms. Higher the concentration of NaOCl, better are the antibacterial and tissue dissolution properties, but at the same time, more are the chances of tissue reaction. Hence, 5.25% NaOCl is clinically more acceptable and has been used in this study.

Chlorohexidine gluconate (CHX) is widely used for disinfection in dentistry because of its good antimicrobial activity. CHX has been shown to have long-term antimicrobial properties because of its unique ability to bind to hydroxyapatite. it was proved that 2% CHX liquid was more potent than 1% and 0.2% liquid against selected microorganisms[16]. Hence, in this study, 2% CHX liquid was used.

A wide variety of synthetic antimicrobial agents have been used over the years as endodontic irrigants. Because of the increased antibiotic resistance to these antimicrobial agents, toxic and harmful side effects of few common antibacterial agents, there is a need for alternative agents which are affordable, nontoxic, and effective. It has been found that natural plant extracts could be used as effective endodontic irrigants[17].

Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of micro-organism[18,19].These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds[20].

Garlic has antimicrobial[21] anticancer[22] and antiplatelet activity[23] **Eswar et al.** investigated the efficacy of Garlic against E. faecalis. Garlic showed better antibacterial efficacy due to the presence of allicin, an organosulphur compound in garlic[24,25].

Ginger-It belongs to *Zingiberaceae* family and it has also been used as a medicine from Vedic days due to its antibacterial and antifungal properties. **Maekawa *et al***[26] evaluated the antimicrobial efficacy of Glycolic propolis, ginger extract, $Ca(OH)_2$, CHX and their combinations against E. faecalis, C. albicans, Escherichia coli and endotoxins in root canals. Ginger contains flavonoids, It was observed that this extract was effective in eliminating the microorganisms from the root canals[27].

In the present study, garlic extract showed maximum antimicrobial efficacy against E. faecalis and C. albicans. Garlic contains at least 33 sulphur compounds, several enzymes, amino acids, and minerals such as selenium. It contains a higher concentration of sulphur compounds than any other Allium species[28]. Allicin in garlic extracts have been found to be effective as an antifungal, antibacterial, antiviral and anti-parasitic agent. The present study was in with **Suresh Pandey et al**[29] also concluded that Garlic extract is advantageous in cases of secondary endodontic infections which are dominated by organisms like E. faecalis and C. albicans.

In our study, 2% CHX showed better antibacterial efficacy compared to sodium hypochlorite and ginger. The possible reasons might be due to bactericidal dosage of 2% CHX and increased diffusion of the medicament into the test media. Its efficacy is because of the interaction of the positive charge of the molecule with the negatively charged phosphate groups on microbial cell walls, which alters the cells osmotic equilibrium. This increases the permeability of the cell wall, allowing the CHX molecule to penetrate into the bacteria. Damage to this delicate membrane is followed by leakage of intracellular constituents, particularly phosphate entities such as adenosine triphosphate and nucleic acids. As a consequence, the cytoplasm becomes

congealed, with resultant reduction in leakage; thus, there is a biphasic effect on membrane permeability[30]. The result of this present study was similar to that of **Krithikadatta *et al*[31]** **Gomes *et al*[32]** and **Bhardwaj *et al*[33]**.

In our study sodium hypochlorite and ginger shows less inhibition zone, might be due to experimental methods, biological indicators and exposure time.

In this study, the antimicrobial activity of herbal extracts of Garlic had more inhibition zone than ginger. However, several disadvantages of herbal irrigants like fresh solutions has to be prepared each time, the unacceptable odour and taste, short shelf life have to be overcome. The smell and taste of the herbal irrigants has to be modified by adding flavouring agents to make it palatable and acceptable by the patient. More research for prolonging the shelf life of

these irrigants has to be done so that these irrigants are more widely accepted.[40]

CONCLUSION

Under the conditions of the present study and within its limitations, it can be concluded that:

1. With ever increasing resistance to synthetic drugs and typical features of resistant of antimicrobial agents of E.faecalis and C. albicans, herbal extracts can be an alternative option provided all the ideal properties of an irrigant are satisfied. Garlic extracts have antimicrobial properties than ginger.

2. 2% Chlorohexidine solution is a better endodontic irrigant with maximum antimicrobial potency compared to 5.25% sodium hypochlorite and ginger.

3. Agar diffusion test have its own drawbacks and cannot simulate the *in vivo* conditions which might vary the results obtained in the present study. Further research is needed to investigate the availability of aqueous and other properties of garlic that has more adequate properties for irrigant. Although the *in vitro* test methods are helpful in conducting and evaluating the antibacterial efficacy of various irrigants, a detailed and standardised procedure is required to enhance the reproducibility among different researchers in future.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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