Avances en Biomedicina
Publicación Oficial del Instituto de Inmunología Clínica
Mérida-Venezuela
Volumen 14(1), Enero-Junio 2025, p 60-66

Depósito Legal: PPI201102ME3935
ISSN: 2477-9369
PII: S2477-9369(25)14008-O

Comparative evaluation of antimicrobial efficacy of bitter guard (momordica charantia) & garlic (allium sativum) as endodontic irrigants against *E. faecalis*-an in vitro study

(Evaluación comparativa de la eficacia antimicrobiana de Momordica charantia y ajo (Allium sativum) como irrigantes endodónticos contra E. faecalis: un estudio in vitro)

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Received: 2nd February 2025.
Accepted: 22nd June 2025.
Online publication: 17th July 2025

[Original paper]

PII: S2477-9369(25)14008-OP

Abstract (english)

To evaluate the antimicrobial efficacy of two herbal extracts i.e, Bitter Guard (Momordica charantia) and Garlic (Allium sativum) as endodontic irrigants against Enterococcus faecalis. Single rooted human mandibular premolars extracted for orthodontic reasons were selected for the study. Teeth were decoronated to standardize the length to 12±1mm. Cleaning and shaping of root canals were done by crown down technique using protaper universal rotary files till F3. Specimens were placed in steel containers containing BHI broth and sterilized in autoclave. From a stock culture of MTCC 2527 E. faecalis strain, subculture was made onto a plate of Diagnostic Sensitivity Test Agar. Enumeration of live bacteria (CFU) was carried by serial dilution method. The root canals were inoculated with E. faecalis suspension and incubated at 37° c for 21 days. The specimens were divided into five groups, each containing ten teeth. Test irrigating solutions were used as follows. Group 1 - Normal Saline, Group 2 - 5.25% NaOCl, Group 3 - 2% CHX, Group 4 – Bitter guard, Group 5- Garlic. Dentinal shavings were collected using no 40 H file in an aseptic condition. After incubation the numbers of colonies were counted. The mean CFU from low to high with all irrigants tested was as follows Group 2 - 5. 25% NaOCl (0.00); Group 3 - 2% chlorhexidine (1.14 x 10 -3); Group 4 - Bitter Guard (1.40 x 10 -3); Group 5 - Garlic (10.30 x 10 -3) and Group 1 - Normal saline (28.60 x 10 -3) . Extracts of Bitter Guard (Momordica charantia) and Garlic (Allium sativum) are effective against E. faecalis. Bitter

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Guard (Momordica charantia) and 2% Chlorhexidine are equally effective against E. faecalis. 5.25% NaOCl showed complete inhibition of E. faecalis and proved as a gold standard endodontic irrigant..

Keywords(english)

Momordica charantiao, Allium sativum, E. faecalis.

Resumen(español)

Para evaluar la eficacia antimicrobiana de dos extractos herbales, es decir, Bitter Guard (Momordica charantia) y Ajo (Allium sativum) como irrigantes endodóncicos contra Enterococcus faecalis. Se seleccionaron para el estudio premolares mandibulares humanos unirradiculares extraídos por razones de ortodoncia. Los dientes fueron descoronadizos para estandarizar la longitud a 12 ± 1 mm. La limpieza y conformación de los conductos radiculares se realizó mediante la técnica de corona hacia abajo utilizando limas rotatorias universales protaper hasta F3. Las muestras se colocaron en recipientes de acero que contenían caldo BHI y se esterilizaron en autoclave. A partir de un cultivo madre de la cepa MTCC 2527 de E. faecalis, se realizó un subcultivo en una placa de Agar de prueba de sensibilidad diagnóstica. La enumeración de bacterias vivas (UFC) se realizó mediante el método de dilución seriada. Los conductos radiculares se inocularon con suspensión de E. faecalis y se incubaron a 37 °C durante 21 días. Las muestras se dividieron en cinco grupos, cada uno con diez dientes. Las soluciones de irrigación de prueba se utilizaron de la siguiente manera. Grupo 1: solución salina normal, Grupo 2: NaOCl al 5,25 %, Grupo 3: CHX al 2 %, Grupo 4: Bitter Guard, Grupo 5: ajo. Las virutas dentinarias se recogieron sin una lima de 40 H en condiciones asépticas. Después de la incubación, se contaron las colonias. La media de UFC de baja a alta con todos los irrigantes probados fue la siguiente: Grupo 2: NaOCl al 5,25 % (0,00); Grupo 3: clorhexidina al 2 % (1,14 x 10 -3); Grupo 4: Bitter Guard (1,40 x 10 -3); Grupo 5: ajo (10,30 x 10 -3) y Grupo 1: solución salina normal (28,60 x 10 -3). Los extractos de Bitter Guard (Momordica charantia) y Ajo (Allium sativum) son eficaces contra E. faecalis. Bitter Guard (Momordica charantia) y clorhexidina al 2% son igualmente eficaces contra E. faecalis. El NaOCl al 5,25% demostró una inhibición completa de E. faecalis y se convirtió en el irrigante endodóntico de referencia.

Palabras clave(español)

Momordica charantia, Allium sativum, E. faecalis.

Introduction

One of the most important objectives of root canal treatment is the elimination of microorganisms from the root canal system (1). During endodontic treatment; number of microorganisms within the root canals is reduced as much as possible using mechanical and chemical procedure. However, there is possibility that some of them are left in the canal (2). Persistent endodontic infections are mainly due to retention of microorganism in the dentinal tubules. Enterococcus faecalis is the primary organism detected in persistent asymptomatic infections. Enterococcus faecalis is facultative anaerobic gram positive rods which can invade the dentinal tubules endure prolonged periods of starvation and possess certain virulence factors and lytic enzymes (3).

The most effective method for eliminating E. faecalis from the root canal space and dentinal tubules is the use of Sodium hypochlorite and 2% Chlorhexidine. Due to the disadvantages of sodium hypochlorite like unpleasant taste, toxicity and potential weakening of the tooth structure by decreasing the hardness and the structural integrity of the dentine with in the root canal (4)

Another widely accepted irrigant is 2% Chlorhexidine digluconate. It has a broad spectrum antimicrobial action, low toxicity and property of substantivity, but it cannot dissolve the organic substrate and necrotic tissue from the root canal system. Allergic reactions have also been reported against 2% CHX such as contact dermatitis, desquamative gingivitis, discolouration of the teeth and tongue and dysgeusia (5). The constant increase in antimicrobial resistance and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives. In recent years there is an exponential growth in the field of herbal medicine because of their natural origin, easy availability, efficacy, safety and less side effects (6).

Studies confirm that the growth of gram +ve and gram - ve food borne bacteria, yeast and moulds can be inhibited by Garlic (Allium sativum), Bitter Guard (Momordica charantia), neem (Azadirichta Indica), Clove (Syzgium aromaticum) and other herbal extracts (7,8). This study comparatively evaluate the

antimicrobial efficacy of two herbal extracts Bitter Guard (Momordica charantia) and Garlic (Allium sativum) as endodontic irrigants in comparison with NaOCl and Chlorhexidine against *E. faecalis*.

Materials and methods

Selection of teeth and canal preparation. Fifty Single rooted human mandibular premolars extracted for orthodontic reasons were selected for the study. The teeth with extremely curved roots, fracture lines, severely calcified roots and root caries were excluded from the study. The teeth selected had single canal with straight roots measuring approximately 21mm. All the teeth were decoranated at Cemento enamel junction (CEJ) perpendicular to the long axis of the teeth with remaining roots measured 12 ±1mm. The exploration of the canal was accomplished with no 10 and no 15K file to make sure that the roots had only one canal and it was patent. Then the working length was determined one mm short of the file penetration into the canal. Cleaning and shaping of root canals were done by crown down technique using Protaper universal rotary files till F3. The canals were recapitulated and irrigated with 5.25% NaOCl, 17% EDTA and final rinse was with normal saline. Subsequent to the canal preparation the apical foramen of all the specimens were sealed with cyanoacrylate glue to prevent bacterial microleakage.

Specimens were placed in steel containers containing BHI broth and subjected to autoclave at 121ºc at 15psi for 20 minutes for sterilization. Subsequent to sterilization all the specimens were transported and manipulated under laminar flow using sterile instruments and equipments.

Preparation of E. faecalis suspension and tooth inoculation. In order to get a controlled and standard suspension of the organism the following procedure was adopted. From a stock culture of MTCC 2527 E. faecalis strain, subculture was made onto a plate of Diagnostic Sensitivity Test Agar. From this a typical colony was sub- cultured into 50 ml of Streptococcus Selection Broth contained in a 100 ml conical flask. This was incubated at 37° C for 24 hours. Enumeration of live bacteria (CFU) was carried by serial dilution method. For injecting into the tooth a suspension of bacteria containing 10µg CFU per ml was used. The root canals were inoculated with E. Faecalis suspension using sterile 1ml tuberculin syringes and specimens were separately placed in steel containers containing 2ml of broth. The steel containers containing the specimens were kept in incubators at 37° c for 21 days.

Preparation of Extract. Bitter Guard (Momordica charantia): M. charantia were washed with distilled water, and the seeds were separated. The fruits were then sliced into small pieces and dried in drying oven at 50°C. The dried plant materials were then blended into powder using an electric blender for extraction. The powdered seeds and fruits of M. charantia were separately extracted with ethanol by using Soxhlet apparatus for 24 hours. The extract was concentrated using a rotary evaporator (9)

Garlic (Allium sativum) peeled and washed with distilled water. The bulbs were squeezed and were sucked in methanol for 8hours with 10 minutes interval shaking. The extraction was filtered using muslin cloth and then Whatman no. 1 filter paper. The filtrate was evaporated at 450C to dryness and the dried substance was kept in sterile bottle under refrigerated condition until use (10).

Antimicrobial assessment. After 21 days all the specimens were retrieved and each specimen was transferred into test tubes containing 3ml of saline and was shaken three times for 30 seconds each time on a rotator to remove the excess culture medium. In addition large amount of bacteria present on the surface of the specimen were removed during rinsing and irrigation. The samples were divided into five groups, each containing ten teeth (Table 1).

Samples in each were irrigated with respective irrigating solutions and were immersed in test tubes containing 2ml of the solution for 5minutes. Subsequent to the removal of specimens from the test tubes each specimen was transferred in to test tube containing 3ml of saline and shaken in a rotator for 3 times for 30 second each.

Dentinal shavings were collected using no 40 H file in an aseptic condition. Shavings were transferred into test tubes containing 10 ml sterile normal saline. Three serial dilution as carried out. From this one ml was pipetted on to a sterile 100 mm diameter in duplicate. To each of these plates 15 ml of agar medium, melted, cooled and was added mixed well and allowed to solidify. These plates were incubated for 24hours at 37° C. After incubation the number of colonies was counted in suitable plates. The number of the colonies multiplied by the dilution factor gives the total number of CFU in the scrapings per tooth.

Results

Test herbal extracts Bitter Guard (Momordica charantia) and Garlic (Allium sativum) are effective against *E. faecalis*. 5. 25% NaOCl showed complete

Table. 1. Irrigating solutions.

Groups N=10	Test Solution
Group 1	Normal Saline
Group 2	5.25% NaOCl
Group 3	2% CHX
Group 4	Bitter guard
Group 5	Garlic

inhibition of E. faecalis. Among the two herbal extract Bitter Guard (Momordica charantia) was more effective than Garlic (Allium sativum), which is nearly equal to 2% Chlorhexidine. (Table 2).

The inter group comparison between normal saline and Garlic, Bitter guard, 5.25% NaOCl, 2% CHX there was a significance difference (p=0.000).The inter group comparison between 5.25% NaOCl and Garlic and Normal saline there was a significance difference (p=0.000) & there was no significance difference between 5.25% NaOCl and Bitter Guard (p= 0.646); 5.25% NaOCl and 2% CHX (p=0.790). Kruskall wallis ANOVA test showed statistical significance difference between the 5 groups (p< 0 .001)

Discussion

Although all the bacteria in the oral cavity can invade the root canal, only a few microbes have been identified in infected root canals. Endodontic infections with E. faecalis are probably not derived from patients own micro flora, which indicates that in these infection E. faccalis is of exogenous origin (11).

Enterococcus faecalis is the most commonly implicated microorganism in asymptomatic persistent infections. Enterococci are gram positive cocci that can occur singly, in pairs or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen (12). It can withstand harsh environmental conditions. Enterococci can grow at 10° C and 45°C, at pH 9.6 and survive at 60°C for 30 minutes. The ability of E faecalis to tolerate or adapt to harsh environmental conditions may act as an advantage over other species. It may explain its survival in root canal infections, where nutrients are scarce and there are limited means of escape from root canal medicaments (13-15).

In the present study E. faccalis was chosen as the test organism as it is the most commonly isolated intracanal bacteria from treatment failure cases, its association with persistent apical inflammation and its resistance to elimination by irrigating solutions and medicaments (16/0. *E. faecalis* can penetrate dentinal tubules to a depth of 300 - 400 µm within 3 weeks. Prolonged incubation period increased the number of infected dentinal tubules but depth of penetration of bacteria increases slowly with time (17). Another important factor for the survival of bacteria is the availability of a nutrient source (18). The teeth were immersed in the streptococcus selection broth and the broth was replaced on alternate days during the 21 day incubation period. Subsequent change of the broth allowed the microorganism to rearrange in bio- films which is a structure known to confer resistance of microbial cells to different antimicrobial agents (19)

Chlorexidine has been used as an antibacterial agent in dentistry since 1962. It is cationic bis-biguanide, which is active against gram positive and gram negative bacterial spores, lipophillic virus, yeast and dermatophytes, being bacteriostatic at low concentrations and bactericidal at high concentrations. Several advantages for the clinical use of CHX as a root canal disinfectant include, its low toxicity, substantivity, more tolerable odor than sodium hypochlorite, better taste and non bleaching effects (20). But the most important disadvantage of CHX is its inability to dissolve remnants of necrotic tissues and chemically clean the root canal system (21). When used as an intracanal medicament, Chlorhexidine is more effective than Calcium Hydroxide against *E. faecalis* infection in dentinal tubules. Chlorhexidine has also been shown to have long-term antimicrobial properties because of its unique ability to bind to hydroxyapatite. A gradual release of this bound chlorhexidine could maintain an

Table. 2. Mean CFU.

Groups N=10	Cfu X 10^{-3}
Group 1	28 .60
Group 2	0.00
Group 3	1 .14
Group 4	1 .40
Group 5	10.30

even level of the molecule sufficient to create a bacteriostatic effect in the root canal over a prolonged period of time. This is in contrast to the effect of other disinfectants, which rapidly dissipate from the pulp space and have no residual antimicrobial effects (21)

The role of natural extracts for endodontic purpose has been evaluated for plants such as Arctium lappa Morinda citrifolia Triphala. Green Tea Polyphenols and Liquorice, Allium Sativum. Garlic, Cinnamomum zeylanicum, Azadiracta indica, Ginger extract, Mishwak, Aloe vera Linne Myristica fragans, Teminalia chebula, in terms of their antimicrobial efficacy against *E. faecalis* (3, 22-,25).

Garlic (Allium Sativum) is a bulbous perennial medicinal plant which belongs to the family Liliaceae. The antmicrobial activity is attributed to thiosulphinates. Studies proved that extracts of garlic are bactericidal and are effective against E.coli, S.aureus B.cereus, Salmonella, listeria, proteus and Streptococcal species (26). Bitter Guard (Momordica charantia) a member of the Cucurbitacea family, has long been used as food and medicine. Antioxidant, anti diabetes, anti inflammatory, anti bacterial and anti cancer effectrs of M. charantia have been reported (27).

Sodium hypochlorite in present study show complete inhibition of E. faecalis with mean CFU of 0.00. NaOCl, was considered as gold standard, also showed higher zones of inhibition. In the present study 2% CHX showed mean CFU of (1.14 x 10 -3) and Bitter guard showed mean CFU of (1.40 x 10 -3). Hence bitter guard is comparable to 2% CHX.

In the present study garlic showed mean CFU of (10.30 x 10 -3) which is comparatively higher than bitter guard, 2% CH X and 5.25% NaOCl. Eswar et al showed 2% CHX showed better antimicrobial efficacy compared to garlic extract, which is concurrence with the present study, as in this study 2% CHX showed better antibacterial efficacy compared to bitter guard and garlic extracts. The possible reasons might be due to bactericidal dosage of 2% CHX and increased diffusion of the medicament into the dentinal tubules. Chlorhexidine is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysacharides on the cell membrane of bacteria and enters the cell through some type of active or passive transport mechanism. As a consequence, the cytoplasm becomes congealed, with resultant reduction in leakage; thus, there is a biphasic effect on membrane permeability (28).

Contrary to NaOCl other commonly used alternate endodontic irrigant was Chlorhexidine. The constant increase in antimicrobial resistance and side effects caused by synthetic drugs has prompted researchers for alternatives. In recent years there is an exponential growth in the field of herbal medicine because of their natural origin, easy availability, efficacy, safety and less side effects.

In the light of the problems associated in usage of high Naocl concentrations and promising results obtained in the present study with Bitter Guard (Momordica charantia) and Garlic (Allium sativum) assures a long and successful use in endodontic field. Bitter Guard is equally effective as compared to Chlorhexidine.

Further clinical and in vitro studies determining its tissue dissolving efficacy and establishing this herbal extracts usage as endodontic irrigant is the need of the hour.

In conclusion, within the limitations of the present in vitro study, based on the employed methodology and according to the results obtained, it was concluded that

5.25 % NaOCl showed complete inhibition of E. faecalis and proved as a gold standard endodontic irrigant.

Bitter Guard (Momordica charantia) and 2% Chlorhexidine are equally effective against E. faecalis.

Inhibition of E. faecalis was more effective with Bitter Guard (Momordica charantia) compared with Garlic (Allium sativum).

Herbal extracts Bitter Guard (Momordica charantia) and Garlic (Allium sativum) are effective against *E. faecalis*.

Conflicts of interest

None to declare

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How to cite this article. Yalamanchi A, Raghunathan N, Snigdha Priya C, Shobana P, Sri Satyavathi E, Sujayeendranatha Reddy E. Comparative evaluation of antimicrobial efficacy of bitter guard (momordica charantia) & garlic (allium sativum) as endodontic irrigants against E. faecalis-an in vitro study. ***Avan Biomed*** 2025; 14: 60-6

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