

The Antibacterial Effect of Green Tea on *Enterococcus faecalis*, Iraq

Ehsan Mansoor Kadhim, Bushra Karem Amin¹, Bassam Karem Amin

Conservative Department, Hawler medical University, College of Dentistry, ¹Department of Biology, College of Science, Salahaddin University-Erbil, Erbil, Iraq

Abstract

Background: For centuries, people have employed plants as a source of therapeutic substances. Herbal remedies are used as analgesics, antibacterial, and anti-inflammatory medicines in dentistry. The present study aimed to show the antimicrobial activity of green tea, against *faecalis* strains (ATCC 29212) *in vitro*, with various concentration of different extracted solvents. **Materials and Methods:** In this study, different solvents were used for the extraction active substances from dried green tea, followed by determining the antimicrobial actions of green tea against *faecalis* strains (ATCC 29212) *in vitro*. Green tea's antibacterial properties were present in every component when tested on *Enterococcus faecalis*. The least amount of hexane extract, however, significantly reduced the growth of the aforementioned bacteria. **Conclusions:** According to the findings of this study, green tea extract is a suitable replacement for sodium hypochlorite and other endodontics irrigation solutions. To identify the ideal concentration, more research is necessary.

Keywords: Bacteria, chloroform, green tea, herbal, pathogen, root canal

INTRODUCTION

A resistant bacterium called *Enterococcus faecalis* is a major factor in the development of chronic endodontic infections^[1]. The most frequently isolated or recognized species from oral infections, such as marginal periodontitis, is *Enterococcus faecalis*^[2], infected root canals^[3], and peri-radicular abscesses^[4]. This microorganism can endure in an environment with few bacterial commensals and low nutrition levels. It develops by creating a biofilm, which is an adaptive process that enables the bacterium to endure in challenging conditions like obturated root canals^[5].

There are numerous disinfection options, each with unique benefits and drawbacks^[6]. NaOCl has antibacterial activity against a wide spectrum of bacteria, including *Enterococcus faecalis*, and has an excellent tissue solubility. However, there are various drawbacks to this irrigation solution, including cytotoxicity, tissue burning, unpleasant taste and odor, and staining of the patient's clothing^[7,8]. Chlorhexidine has proven to be especially effective^[9]. Chlorhexidine, on the other hand, is a synthetic compound that has been shown to be toxic to

fibroblasts, polymorphonuclear leukocytes (PMNs), and other leukocytes, macrophages, and erythrocytes^[10]. The use of diode laser irradiation as an extra antibacterial disinfectant in the field of dentistry^[11]. However, more research is needed to validate the biological safety of diode laser use^[12]. In addition, *Enterococcus faecalis* is becoming more resistant to common antibiotics including vancomycin^[13]. New antibacterial drugs specialized for this bacteria must be studied in order to achieve endodontic procedures with a higher success rate and a better prognosis for the tooth.

Plants have long been used as a source of medicinal compounds^[14]. The antibacterial, antifungal, and anticancer effects of several plants have been the subject of numerous investigations^[15,16]. Herbal medications have long been utilized in dentistry as anti-inflammatory,

Address for correspondence: Dr. Ehsan Mansoor Kadhim, Conservative Department, Hawler Medical University, College of Dentistry, 5257 + GFV, Erbil, Iraq. E-mail: doctorihsanmansoor@gmail.com

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analgesic, and antibacterial agents^[17]. The present study aimed to show the antimicrobial activity of green tea during 24 hours of growth phase, against *faecalis* strains (ATCC 29212) *in vitro*, with various concentration of different extracted solvents.

MATERIALS AND METHODS

Enterococcus faecalis (ATCC 29,212) was selected bacterial to determine antimicrobial effect of extracted green tea with various solvents, as to show the effects of green tea in inhibiting the growth of bacteria that cause root canal infections. The strain of *Enterococcus faecalis* (ATCC 29,212) were obtained from (Medical hospital hawler Rizgari) and stored in TSB (Tryptic soy broth, Merck, Germany) at -70°C^[18,19].

The green tea was bought in Erbil, the capital of the Kurdistan region, in Iraq. The Biology Department of Salahaddin University possesses a voucher specimen of the sample that has been kept. The solvent was combined with 1000g of the green tea, which was then boiled, filtered, concentrated by evaporation, and the crude extract was combined with a 10% DMSO solution before being used. Same technique has been used with the other solvents (standard hexane, chloroform, ethyl acetate, n-butanol, ethyl alcohol, and water) in the present study the extracted solvents are expressed as fraction 1,2,3,4,5 and 6 correspondingly. In order to rule out the influence of the solvents, all of the solvents used in the extraction procedure were employed as blanks and controls in all experiments throughout the inquiry.

The minimum inhibitory concentration (MIC) was interpreted as the lowest concentration of the agents that completely inhibit the growth of target species. The antibacterial activity assay of green tea was determined by the broth dilution method^[20]. The powder of green tea dissolved in 10% DMSO solution to concentration of 100mg/ml, then ten different concentrations (5, 10, 20, 25, 30, 35, 40, 50, 60, 70 and 100mg/ml) prepared by dilution method in TSB (Tryptic soy broth, Merck, Germany). Test tubes contained equal amount of both activated culture of *Enterococcus faecalis* and pure green tea extract which were incubated at 37°C for 24hr. The MIC was determined by reading the absorbency at wave length 590 nm. All samples were tested in quadruplicate.

The usual biochemical tests recommended in recognized microbiological sources were carried out, to confirm that the bacterial isolates were *E. faecalis*^[21]. The corresponding author hereby attests that ethics were taken into consideration throughout the study process.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and

analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 538.B.44 (12/2/2021) to get this approval.

RESULTS

Enterococcus faecalis (ATCC 29,212) and pure green tea extract where incubated at 37°C for 24hr and then results obtained by reading the absorbency at wave length 590 nm. The MIC results are listed in Table 1 by (mg) of tested antibacterial agent per (ml). All the fraction of the green tea showed antibacterial activity against *Enterococcus faecalis*. However, hexane extract inhibited the growth of mentioned bacteria with the lowest concentration and green tea extract with the ethyl acetate and chloroform inhibited the growth of *Enterococcus faecalis* at the concentration of 35 and 40 mg/ml, respectively.

The results in the Figure 1 shows the absorbency reading after incubation of bacterial culture with various concentration of fraction 1, 2, and 3 of extracted green tea which stand for Hexane, chloroform and ethyl acetate. The lowest absorbency reading at various concentration were recorded for hexane green tea extract.

DISCUSSION

The most frequently isolated bacteria from the root canal systems was *Enterococcus faecalis* by (45.8%) in previously treated cases^[22]. Same results have been reported by other

Table 1: Minimum Inhibitory Concentration (MIC) for green tea extract against *Enterococcus faecalis* (ATCC 29,212) shown in mg/ml

Bacterial strain	Hexane/ green tea	Chloroform/ green tea	Ethyl acetate / green tea
<i>Enterococcus faecalis</i>	30mg/ml	40mg/ml	35mg/ml

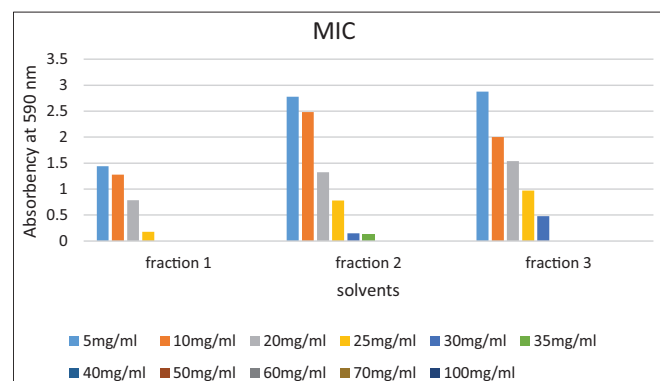


Figure 1: The results of MIC according absorbency reading for different extracted solvent

studies^[23,24]. Endodontic therapy failure has been linked to *Enterococcus faecalis* living inside different layers of the oral biofilm^[25].

The root canal system is cleaned using a variety of irrigants. In general, a drug is more suitable if it also exhibits higher antibacterial activity while having fewer negative effects^[26]. *Enterococcus faecalis* is just one of the many bacteria that sodium hypochlorite has antibacterial activity against, and it has good tissue solubility^[7], Chlorhexidine also considered as an effects agent^[9], while diode laser irradiation is an additional options for cleaning and minimizing re-infections^[11]. However, each of the above method has it is own disadvantage so new substance and techniques are required with the minimum side effects.

Around the world, medicinal plants have been utilized for centuries to cure a wide range of illnesses^[27]. Natural products have been used in cleaning and disinfecting root canals either as intra-canal medicaments or as irrigations solutions and potent antibacterial properties of different plants reported by many studies^[28-30]. Additionally, the antibacterial activity of green tea has been detected by many previous studies^[31-33]. Also antibacterial activity of green tea has been investigated with disk diffusion method in Iraq and nearby countries^[6,34]. But few studies used minimum inhibitory concentration to determine antibacterial activity of green tea against *Enterococcus faecalis*. Thus, this study used minimum inhibitory concentration for determining antimicrobial activity of green tea, against *Enterococcus faecalis* strains (ATCC 29212) *in vitro*, as plant samples need special techniques to diffuse in the used medium so standard diffusion method not working^[35,36]. In order to find the optimal extraction technique, many solvents have also been explored to extract green tea, all the fractions of the green tea showed antibacterial activity against *Enterococcus faecalis*. However, hexane extracted selected as the best extracted solvent the extract of green tea which inhibited the growth of *faecalis* strains (ATCC 29212) with the lowest concentration (30mg/ml). Previous study concluded that 10% green tea can be used as a possible irrigations solution to disinfect the root canal system especially against *Enterococcus faecalis* during endodontic treatment^[34]. Green tea can be a viable substitute for sodium hypochlorite and other root canal irrigants because of their therapeutic characteristics, which include anti-oxidant, anti-inflammatory, radical scavenging capabilities, and also contains citric acid removes layers^[37].

CONCLUSIONS

1. The root canal irrigants sodium hypochlorite and other can be successfully replaced with green tea.
2. Hexane green tea extract can reduce the growth phases of *faecalis* strains (ATCC 29212) at 30mg/ml.

3. More research is needed to identify the optimal herbal extract as a viable irrigation solution for disinfecting the root canal system during endodontic therapy, particularly against *Enterococcus faecalis*.

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Nil.

Conflict of Interest

The authors have no conflicts of interest to declare.

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