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Antibacterial Efficacy of *Pistacia atlantica* Resin on Bacteria of Root Canal

Mohammad Goodarzi [1], Ramin Abiri [2], Roohollah Sharifi [3], Hamid Reza Mozaffari [4,5], Masoud Modarresi [6] and Reza Hatam[3]*

[1] Student Research Committee, Faculty of Dentistry, Kermanshah University of Medical Sciences, Kermanshah,Iran
[2] Department of Microbiology, Kermanshah University of Medical Science, Kermanshah, Iran
[3] Endodontics Department, Faculty of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4] Department of Oral and Maxillofacial Medicine, Faculty of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
[5] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[6]Pharmaceutical Science Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

*Corresponding Author**
Reza Hatam
Endodontics Department, Faculty of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
E-mail: reza_hatam@yahoo.com

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Abstract

Introduction: The main goal of root canal treatment is removing or reducing bacteria from root canal system and to this purpose, different materials and methods have been evaluated.
Aim: The aim of this study was to evaluate the antibacterial effect of *Pistacia atlantica* resin on *Enterococcus faecalis* bacteria in vitro.
Materials and Methods: In this experimental study, collected resin of *Pistacia atlantica* was extracted with Clevenger apparatus. Then, to determine the concentration of growth decreasing and growth inhibition on *Enterococcus faecalis* bacteria, concentration of 40% of essential oil was affected in ELISA plate. After 24 hours of incubation, the opacity of the walls, which indicates the growth rate of the bacteria, was read by an ELISA reader at 640 nm.
Result: The results of this study showed that there is a significant difference in the mean of Opacity between different concentrations of *Pistacia atlantica* resin (P<0.001). So that concentrations of 40 and 20% caused complete growth inhibition and other concentrations caused a significant decrease in growth. Also, there is no difference between the inhibitory growth rate of the extract and the different concentrations of sodium hypochlorite and chlorhexidine (P=0.4).
Conclusion: From the findings of this study, it can be concluded that extract of *Pistacia atlantica* has a significant growth inhibition effect at high concentrations and decreasing growth effect in lower concentrations on *Enterococcus faecalis*.

Keywords: *Pistacia atlantica* • *Enterococcus faecalis* • Hypochlorite • Chlorhexidine

Introduction

Bacteria and their productions are important as the chief etiological agents in causing the pulpal necrosis and periapical lesions [1].
Bacteria infecting of the root canal system led to diverse disease. The foundation for effective endodontic treatment is to preserve the root canal free of infection and maximal decreasing of the bacteria infecting in the root canal system. Thus, their delestion is one of the main steps in root-canal treatment [2, 3].
It is identified that bacteria deeply penetrate into dental tubules especially in the apical portion of the root canals the complete elimination is difficult and bacterial regrowth may occur in root canals [4, 5]. Many mechanical instrumentation systems and chemical preparation have emerged to cleaning and disinfection of root canals [6, 7]. In greatest cases failure of root-canal treatment take place when treatment procedures have not met a suitable standard for control and deletion of infection.
Enterococcus faecalis, gram-positive coccus, is more resistant to instrumentation and to antiseptic agents, indicating that it can be estimated to persist more commonly in the root canal after poor root-canal preparation and obscuration [8, 9].
Enteroccocus faecalis has the ability to survive in an environment with little available nutrients and it can remain without the synergistic support of another bacterium [10]. Therefore, persisting microorganisms or their products can remain in infectious process and basis treatment failure [10, 11]. Channel detergents are constantly changing and evolving for better performance. There is currently no detergent solution that has all the desired properties. Today, the design and create of natural compounds with antibacterial properties has received attention due to the concerns of side effects of Channel detergents [12].
Pistacia atlantica is the main species of Pistachio in Iran. Essential oils and crude extracts of Pistacia species compose of antimicrobial, anti-inflammatory, insecticidal and antioxidant activities that proved in the several studies [13, 14].
Due to using of artificial antimicrobial dressing applied to the clean canal accompanied to side effects, recently it is important create of natural antibacterial compounds. Therefore, the aim of the present study was to evaluate the antibacterial effect of *Pistacia atlantica* resin on *Enterococcus faecalis* bacteria in vitro.

Material and Methods

Plant material and extraction

Oleoresin of P.atlantica was collected between May-June from Kermanshah province, west of Iran. Essential oil of P.atlantica was extracted according to method described previously [15]. Briefly, using a clevenger apparatus, oleoresin was subjected to hydrodistillation for 6h. The obtained essential oil was dehydrated using anhydrous sodium sulfate.

Microbial strain and culture condition

The *Enterococcus faecalis* strain (PTCC 1778 and ATCC 29212) was used for the antimicrobial testing and was obtained from Persian Type Culture Collection. The lyophilized ampoules of bacteria were first incubated in aseptic conditions and in the culture medium recommended by the Iranian Scientific and Industrial Research Organization for 24 hours at 37°C and then transferred from the initial culture to other cultures until activation. A fresh 24-hour culture was prepared for each test to check the antibacterial effects. Using a sterile pipette, the required amount of fresh 24-hour culture medium was transferred to sterile saline solution tubes and centrifuged at a dose of

3000 U/min. Then, the turbidity of the prepared microbial suspension was adjusted to about 108 CFU/ml using 0.5 McFarland solution. Mueller Hinton Broth (MHB) culture medium was used in ELISA plate dilution experiments.

Evaluation of the antibacterial activity

Due to the low solubility of essential oil in pure water, a solution containing 0.4% alcohol was prepared. To prepare 40% essential oil, 80 microliters of essential oil are thoroughly mixed with 20 microliters of 4% alcohol. To determine the Minimum Inhibition Concentration (MIC) by Liquid Broth Culture method, 100 microliters of Mueller Hinton Broth culture medium was added to the 10 wells of the first row of the 96-well ELISA plate that was completely sterilized. In the first row, where the concentration was 80 microliters, 50 microliters of the extract was added in the first well and mixed with good sampling. 50 microliters were taken from this well, added to the second well and sampled. Then 50 microliters were removed from the second well and added to the third well, and this dilution continued until the tenth well. 50 µL was taken from the tenth well and discarded. Then, 50 µL of McFarland quarter diluted bacteria was added to all wells. House number eleven was considered as a positive control, that is, it only contained bacteria and culture medium, and well number twelve was considered as a negative control, which included culture medium and distilled water with 0.4% alcohol. Thus, different concentrations of essential oil containing 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078% were prepared in ten wells and the plate was kept for 24 The warm hour was set. For sodium hypochlorite, as in the above method, different concentrations of (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019 and 0.009) percent and for Chlorhexidine concentrations of (0.2, 0.1, 0.05, 0.025, 0.012, 0.006, 0.003, 0.001, 0.0007 and 0.0003) percent were prepared. After 24 hours of incubation, the amount of turbidity of the wells, which indicates the growth rate of bacteria, was read by an ELISA reader (BioTek-USA) at a wavelength of 640 nm, and the results were as a positive sign, which means no growth reduction, and a negative sign means A significant decrease in growth ($p<0.05$) and a zero mark means no growth. The tests were repeated 3 times and the averages were reported in comparison with the positive and negative control groups with a confidence factor of 99%.

Statistical analysis

The data of the present study were analyzed using descriptive and inferential statistics. In the descriptive statistics, measures of centrality and dispersion were reported along with tables and graphs. In the inferential statistics, the normality of the data distribution was analyzed using Kolmogorov-Smirnov test. According to this test, the data were normally distributed, and thus the data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to pairwise comparisons. The significance level in this study was considered 0.05.

Results

The normality of the data distribution was analyzed using Kolmogorov-Smirnov test. According to this test, the data were normally distributed. (Figure 1). shows the results obtained from the effect of different concentrations of *Pistacia atlantica* resin on *Enterococcus faecalis* bacteria. The analysis showed that there is a significant difference in the mean of opacity between different concentrations of *Pistacia atlantica* resin (P<0.001). In addition, there was no significant difference between concentrations of 40% and 20% of *Pistacia atlantica* resin and negative control while there was a significant difference between all groups and positive control (Table 1).

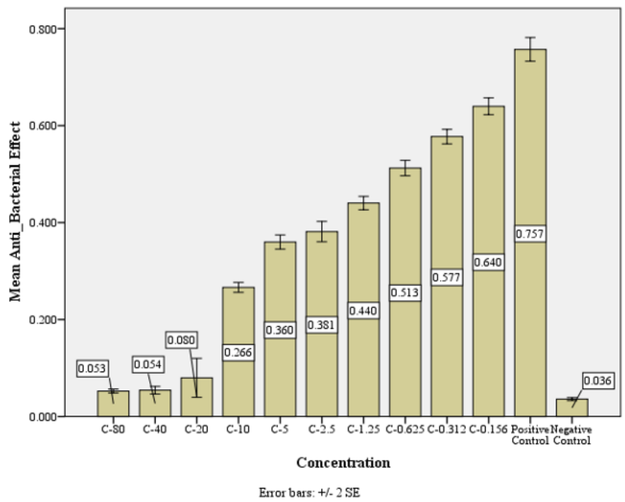

Figure 1. 4 years old child admitted for a generalized epileptic seizur.

Table 1. Effect of different concentrations of Pistacia atlantica resin on Enterococcus faecalis bacteria.

		Anti-Bacterial Effect	
		Mean	Standard Deviation
Concentration	40	.053ab	0.006
	20	.054ab	0.012
	10	.080b	0.064
	5	.266c	0.017
	2.5	.360d	0.025
	1.25	.381d	0.039
	0.625	.440e	0.025
	0.312	.513f	0.025
	0.156	.577g	0.029
	0.078	.640h	0.03
	Positive Control	.757i	0.042
	Negative Control	.036a	0.005
Means with same superscript letters are not significantly different (p > 0.05).			

The antibacterial effect of different concentrations of Cholorhexidine on *Enterococcus faecalis* bacteria is shown in (Figure 2). There was a significant difference in the mean of opacity between different concentrations of Cholorhexidin (P<0.001). There was no significant difference between concentrations of 0.1 and 0.2 of Cholorhexidine and negative control. Also, there was no significant difference between concentrations of 0.0125, 0.003 and 0.0003 of Cholorhexidine and positive control (Table 2).

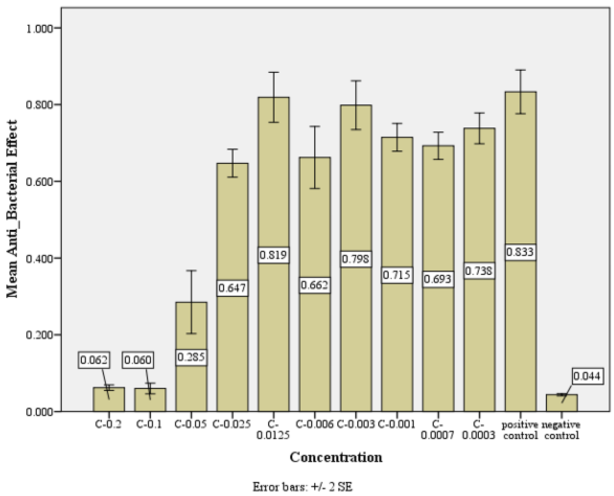

Figure 2. 4 years old child admitted for a generalized epileptic seizur.

Table 2. Antibacterial effect of different concentrations of Cholorhexidine on Enterococcus faecalis bacteria.

		Anti_Bacterial Effect	
		Mean	Standard Deviation
Concentration/	C-0.2	.062^{a}	0.01
	C-0.1	.060^{a}	0.02
	C-0.05	.285^{b}	0.082
	C-0.025	.647^{c}	0.041
	C-0.0125	.819^{ef}	0.086
	C-0.006	.662^{c}	0.081
	C-0.003	.798^{def}	0.078
	C-0.001	.715^{cde}	0.044
	C-0.0007	.693^{cd}	0.04
	C-0.0003	.738^{cdef}	0.045
	positive control	.833^{f}	0.075
	negative control	.044^{a}	0.004
Means with same superscript letters are not significantly different ($p > 0.05$).			

The results of this study showed that there is a significant difference in the mean of opacity between different concentrations of Hypochlorite sodium ($P<0.001$). There was no significant difference between concentrations of 5, 2.5, 1.25, 0.625, 0.312 and 0.156 of Hypochlorite sodium and negative control while there was a significant difference among all groups compared to positive control (Table 3) and (Figure 3).

Table 3. Effect of different concentrations of Hypochlorite sodium on *Enterococcus faecalis* bacteria.

		Anti_Bacterial Effect	
		Mean	Standard Deviation
Concentration	C-5	0.055^{a}	0.013
	C-2.5	0.057^{a}	0.01
	C-1.25	0.052^{a}	0.012
	C-0.625	0.050^{a}	0.008
	C-0.312	0.045^{a}	0.005
	C-0.156	0.081^{a}	0.056
	C-0.078	0.563^{bc}	0.112
	C-0.039	0.597^{bc}	0.103
	C-0.02	0.487^{b}	0.253
	C-0.009	0.659^{c}	0.045
	positive control	0.839^{d}	0.058
	negative control	0.042^{a}	0.003
Means with same superscript letters are not significantly different ($p > 0.05$).			

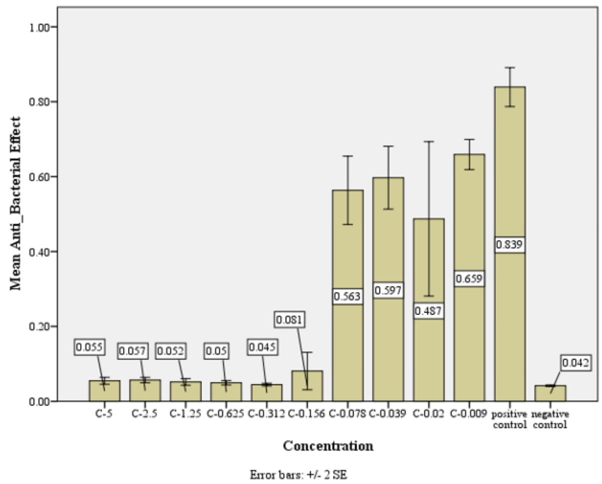

Figure 3. 4 years old child admitted for a generalized epileptic seizur.

The comparison of antibacterial effect of Pistacia atlantica, Cholorhexidine and Hypochlorite sodium is shown in (Figure 4). The analysis of data showed that there is no significant difference in the mean of opacity among different groups (Table 4).

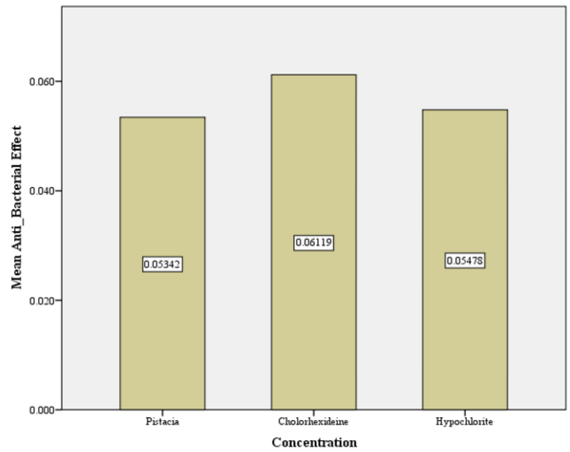

Figure 4. 4 years old child admitted for a generalized epileptic seizur.

Table 4. Comparison of antibacterial effect of Pistacia atlantica, Cholorhexidine and Hypochlorite sodium

		Anti_Bacterial Effect	
		Mean	Standard Deviation
Concentration	Pistacia	.053^{a}	0.01
	Cholorhexideine	.061^{a}	0.015
	Hypochlorite	.055^{a}	0.022
Means with same superscript letters are not significantly different ($p > 0.05$).			

Discussion

Enterococcus faecalis is one of the resistant species that is found in the oral cavity and has the ability to live in hard environmental conditions. This bacterium can be remaining in infectious process and basis treatment failure, the elimination of intracanal bacteria is very important phase because the existence of bacteria is the important cause of periodontal infection and basis treatment failure.

Therefore, in this study evaluated the antibacterial activity of *Pistacia atlantica* resin on *Enterococcus faecalis*. The results of present study exhibited that concentrations of 40% and 20% of *Pistacia atlantica* resin caused complete growth inhibition and other concentrations caused a significant decrease in growth. Also, there is no difference between the inhibitory growth rate of the extract and the different concentrations of sodium hypochlorite and chlorhexidine. Therefore, this finding propose that the resin of *Pistacia atlantica* has antimicrobial activity against *Enterococcus faecalis* bacteria in vitro In line with, in a previous study Rezaei et al. investigated the antibacterial and antioxidant properties of *Pistacia atlantica* on Staphylococcus aureus, Escherichia coli. They reported that concentrations of 6 µg/ml and 12.5 µg/ml had antibacterial effects [15]. Also, in another study, carried out by Edrah et al. reported that *Pistacia atlantica* leaf extract have antibacterial effects on gram-negative and gram-positive bacteria [16].

The results of the study of Omidi et al. also showed that the methanolic extract of *Pistacia atlantica* leaf with a concentration of 625 mg / ml has a inhibitory effect on Pseudomonas aeruginosa bacteria [17].

It is described that there is an association between the chemical constructions of the most abundant in the tested essential oil and their antimicrobial influence. Essential oils rich in phenolic compounds for example Pistacia specie are extensively described to retain high levels of antimicrobial influence [18]. According to the study that carry out by Memariani ,α-pinene, β-pinene, limonene, camphene, myrcene are the major components of *Pistacia atlantica* [19].

Tsokou et al reported that the two main volatile constituents, α-pinene and terpinolene in the Pistacia specie are compounds with interesting antibacterial activity [20]. The percentage of α-pinene in the extract of this speice is more than 93%, and this component is probably the antibacterial agent against various bacterial species [21].

Conclusion

Therefore, with regard to above, it may be concluded that the antibacterial efficacy of Pistacia atlantica resin on bacteria of root canal is at least relatively due to the presence of α-pinene.

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