

In Vitro Antimicrobial Activities of Pistachio Hydroalcoholic Extract Against Oral Facultative Anaerobes

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Information	Abstract
<p>Article Type: Original Article</p>	<p>Introduction: Research on herbal agents is justifiable since they are often safe for the host. <i>Pistacia vera</i> seed (pistachio) is edible with various therapeutic effects such as antioxidant and anti-inflammatory. This study aims to evaluate the antibacterial effects of pistachio extract on oral facultative anaerobes.</p> <p>Materials and Methods: The hydroalcoholic extract of pistachio was prepared by the maceration method. The agar diffusion method was used to determine the antibacterial effects of 5%, 10%, and 20% extracts against <i>Streptococcus mutans</i>, <i>Actinomyces viscosus</i>, and <i>Enterococcus faecalis</i>. Chlorohexidine (0.2%) and DMSO (10%) were employed as positive and negative controls, respectively. A 0.5 McFarland scale of each bacteria was incubated in 5 mL of brain heart infusion broth at 37 °C for 48 h in a rotatory shaker and swabbed on agar medium plates. Afterward, the sterile discs were impregnated with different concentrations of extracts. The discs were aseptically placed onto pathogen-seeded plates and incubated at 37 °C for 48 h. Then, The zone of inhibition was measured. All the tests were carried out in triplicate on three separate days.</p> <p>Results: The antibacterial effects against <i>Streptococcus mutans</i> and <i>Actinomyces viscosus</i> were significantly increased with higher extract concentrations.</p> <p>Conclusion: The present study showed that the hydroalcoholic extract of pistachio could be considered as a potential treatment for oral facultative anaerobes.</p>
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1. Introduction

Oral pathogens include hundreds of different bacteria, fungi, and viruses, living in various areas of the oral cavity [1]. These pathogens cause a wide range of oral diseases, such as dental caries, gingivitis, periodontitis, and endodontic infections, and lead to several systemic disorders, including stroke, diabetes, pneumonia, and cardiovascular diseases [2]. On the other hand, oral bacteria inhibit the healing of oral ulcers by infecting the wound, causing a significant challenge for clinicians [3]. Therefore, an appropriate and low-complication treatment is essential. It is well-established that *Streptococcus mutans* is the leading cause of dental caries via adhesion to the tooth surface and the conversion of sucrose and other sugars to harmful acids in the tooth enamel [4]. Moreover, *Enterococcus faecalis* is the most common species isolated from root canals after unsuccessful endodontic treatment [5]. Some studies have also reported a relationship between *Enterococcus faecalis* and the incidence of periodontal diseases [6]. *Actinomycosis viscosus* is one of the most common pathogens of the oral cavity associated with periodontitis by stimulating inflammatory reactions [7, 8].

Herbal medicines are available and inexpensive, and scientists use plants to discover new drugs. For example, the efficacy of some medicinal plants with antibacterial effects has been proven in several *in vitro*, *in vivo*, and clinical trials.

Pistacia vera (Anacardiaceae) has been known for its medicinal properties since ancient times [9, 10]. The seeds of *P. vera* (pistachio) contain phytochemicals such as oleoresins, triterpenoids, β -carotenes, phytosterols, fatty acids, α -tocopherols, and

lutein [11, 12]. Moreover, pistachio extract has many pharmacological effects such as antimicrobial [13], antioxidant [14, 15], and anti-inflammatory [16]. It is well-documented that agents with antimicrobial properties have beneficial effects on wound treatment [17, 18].

To the best of the authors' knowledge, there is no previous report in the field of dentistry about the effects of pistachio extract on oral bacteria. This study aims to investigate the effects of pistachio extract on three oral facultative anaerobes using the antibiogram test.

2. Materials and methods

Ethical considerations

The study was approved by the local ethics committee of Rafsanjan University of Medical Sciences (Ethical number: IR.RUMS.REC.1398.047).

Chemicals

Brain heart infusion (BHI) broth was purchased from Merck KGaA (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich Company (Darmstadt, Germany). Chlorohexidine was purchased from IRAN NAJO Company (Tehran, Iran).

Pistachio hydroalcoholic extract (PHE)

Dried Akbari pistachios (the plant authenticated by an expert in Pistachio Research Institute of Iran; genetic code: M30) were collected from Rafsanjan, Iran, in 2018. Pistachio nuts were pulverized (100 g) and soaked in 1 L of ethanol (80%) for 72 h to prepare the extract. The extract was then concentrated in a rotary under low pressure and stored at -20°C .

Microbial strains and disc diffusion assay

Streptococcus mutans (PTCC 1683), *Enterococcus faecalis* (PTCC 1393), and *Actinomyces viscosus* (PTCC 1202) were obtained from the Persian type culture collection (PTCC; Tehran, Iran) and utilized for antimicrobial testing.

Each bacterial inoculum was prepared in 5 mL of BHI broth, set to a 0.5 McFarland scale (1×10^6 CFU/mL), and incubated at 37 °C for 48 h in a rotatory shaker. The bacterial cultures were then swabbed on selective agar medium plates. Next, the sterile discs (6 mm) were impregnated with extracts at the concentrations of 5%, 10%, and 20% and left to soak for 10-15 min. The discs were aseptically placed onto pathogen-seeded plates and incubated at 37 °C for 48 h. The zone of inhibition (mm) generated by the extracts against the indicator pathogenic bacteria was measured after 48 h. 0.2% Chlorhexidine and vehicle of the extract (10% DMSO) were employed as positive and

negative controls, respectively. All the tests were performed in triplicate on three separate days.

Statistical analysis

Statistical analysis was conducted using the GraphPad Prism program (version 6.01, GraphPad Software, USA). The results were expressed as mean±standard deviation (SD). The normality of values was evaluated by the Shapiro-Wilk test. The differences between the groups were assessed using a one-way analysis of variance (ANOVA), followed by the Tukey post-hoc analysis. Statistical significance was defined as $p < 0.05$.

3. Results

According to the results of this study, PHE at all concentrations showed the most antibacterial effects against *Streptococcus mutans* ($p < 0.05$) and *Actinomyces viscosus* ($p < 0.05$); however, its effects were not as strong as the antimicrobial effects of chlorhexidine (Table 1).

Table 1. The antimicrobial activity of the pistachio hydroalcoholic extract

	Negative control	Positive control	PHE 5%	PHE 10%	PHE 20%
<i>Actinomyces viscosus</i>	0 ± 0	33.0 ± 0.7***	8.8 ± 0.7*** ###	11.8 ± 0.7*** ###	16.2 ± 0.5### ***
<i>Streptococcus mutans</i>	0 ± 0	25.0 ± 0.4***	6.1 ± 0.4*** ###	8.6 ± 0.2### ***	11.6 ± 0.5### ***
<i>Enterococcus faecalis</i>	0 ± 0	13.9 ± 0.5***	0 ± 0###	0 ± 0###	0 ± 0###

The numbers stand for inhibition zones. The results were expressed as mean ± SD.

*** $p < 0.001$ as compared with the negative control group.

$p < 0.001$ as compared with the positive control group.

PHE: Pistachio hydroalcoholic extract.

4. Discussion

The significant increase in the resistance of pathogenic microorganisms to antimicrobial agents is one of the most central health challenges at present [19]. Therefore, herbal products have become an important source for the production of antimicrobial agents. In the present study, the effects of pistachio extract against three bacteria (*Streptococcus mutans*, *Actinomyces viscosus*, and *Enterococcus faecalis*) that play key roles in oral infections are studied.

The study showed PHE antibacterial effects against *Streptococcus mutans* and *Actinomyces viscosus* but not against *Enterococcus faecalis*.

In line with this finding, Magi et al. found that oleoresin, which is one of the components of *Pistacia vera* L., has antimicrobial activity against *Streptococcus mutans* [20]. It has been shown that oleoresin has antibacterial properties via deleterious effects on the bacterial membrane and membrane pumps [21]. According to the study by Gutiérrez-Morales et al., the methanolic leaf extract of *P. vera* has antibacterial activity against different *staphylococcus* species [22]. In a study by Bisignano et al., the antimicrobial and antifungal activity of pistachio extract on different pathogenic microorganisms is reported [23]. Alma et al. attribute the antimicrobial activity of the essential oils from the gum of *P. vera* to its bioactive compounds, such as carvacrol, kampen, and limonene [24]. Sharifi et al. show that the mastic gum of *Pistacia lentiscose* and *Pistacia atlantica* contains a high molecular weight polymer (cis-1,4-poly- β -myrcene), which has antimicrobial properties [25]. The present study shows that pistachio extract has no antibacterial effects against *Enterococcus faecalis*. Contrary to the present work results,

Ozçelik et al. show that the lipophilic extract of pistachio has antimicrobial effects against *Enterococcus faecalis* [26]. These conflicting results can be attributed to the differences the herbal extract source and the preparation method (hydroalcoholic vs. lipophilic extract). Our results also indicate that 10% DMSO has no inhibitory effect on the growth of the studied bacterial species; it can be concluded that the observed antibacterial effect against *Streptococcus mutans* and *Actinomyces viscosus* is exclusively related to pistachio extract.

5. Conclusion

The study demonstrated PHE antibacterial effects against *Streptococcus mutans* and *Actinomyces viscosus*, which were completely dependent on the concentration of the extract. Therefore, the results of the current study open new horizons to the clinical usage of PHE for oral infections.

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Author contributions

SSS and IF conceived and designed the experiments. GM performed the experiments. SSS and MS analyzed the data. IF and SSS contributed reagents/materials/analysis tools. IF and FK wrote the paper. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest.

Abbreviations

PHE: Pistachio hydroalcoholic extract;
DMSO: dimethyl sulfoxide; PTCC: Persian

type culture collection; BHI: brain heart infusion.

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