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Research Article

A Comparison between the Antibacterial Activity of Propolis and Ginseng Extracts on *Porphyromonas gingivalis* and *Prevotella intermedia* Isolated from Patients with Chronic Periodontitis

Heba A Shawky[1] and Walid A Lotfy[2]*

[1]*Associate Professor of Periodontics, Preventive Dental Science Department, College of Dentistry, Princess Nourah Bint Abdulrahman University, Kingdom of Saudi Arabia*

[2]*Microbiology Department, Faculty of Dentistry, Pharos University in Alexandria, Alexandria, Egypt*

***Corresponding Author:** Walid A Lotfy, Microbiology Department, Faculty of Dentistry, Pharos University in Alexandria, Alexandria, Egypt.

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Abstract

The antimicrobial activity of ginseng extract was investigated *in-vitro* against 24 clinical isolates of *Porphyromonas gingivalis* and *Prevotella intermedia*. To our knowledge, there is a lack of researches on the evaluation of the antimicrobial effectiveness of ginseng extract on periodontopathic bacteria. We also compared the *in-vitro* antibacterial action of ginseng extract to that of propolis extract by agar diffusion method. The MIC values of ginseng extract against *Porphyromonas gingivalis* and *Prevotella intermedia* were 512 and 1024 µg/ml, respectively. While the MIC obtained for propolis extract were 256 and 512 µg/ml against *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively. Moreover, the statistical analysis for the effect of ginseng and propolis extracts on *Porphyromonas gingivalis* and *Prevotella intermedia* using student paired *t*-test revealed a significant difference between the two groups ($p < 0.001$). However, the anti-*Porphyromonas gingivalis* and anti-*Prevotella intermedia* mean activities of ginseng extract were lower than that of propolis extract by 1.32 and 0.17 AU, respectively. The results of the current study support the usage of ginseng extract as an adjuvant to periodontal therapy after proper *in-vivo* studies are performed.

Keywords: *Prevotella intermedia*; *Porphyromonas gingivalis*; Propolis Extract; Ginseng Extract; Antibacterial

Introduction

Periodontal diseases are complex infections initiated by subginigival plaque biofilm harbouring Gram-negative, anaerobic or microaerophilic bacteria. Periodontal damage results mainly from the immune response induced by virulent types of periodontal pathogens [1]. Strong evidence showed that black pigmented Gram-negative anaerobes such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Tannerella forsythia* are considered as virulent periodotopathogens, which intensely contribute to chronic periodontitis pathogenesis [2]. Antibiotics have been prescribed with conventional mechanical debridement for periodontitis treatment [3]. However, the emergence of antibiotic resistant pathogenic bacteria has become a serious threat to public health [4]. Besides, most of the prescribed antibiotics exhibit a wide range of side-effects such as nausea, vomiting, diarrhoea, and gastrointestinal problems [5].

In recent years, special attention has been paid to the use of natural materials for periodontal treatment with minimal adverse effects. Propolis, a resinous substance produced by honey bees (*Apis melifera*), has been investigated for its antibacterial activities against oral pathogens [6,7]. The chemical composition of propolis varies according to the season of collection by the bees and the regional vegetation [8]. Furthermore, The antimicrobial activity

exerted by propolis is dependent on the synergism between flavonoids, phenolic acids and their esters but not a single propolis compound [6,9].

Panax ginseng has been used in traditional medicine for years. The biological properties of ginseng include antibacterial [10-12], antifungal [13], antitumor [14], and antioxidative activities [10]. Moreover, ginseng exhibited a potential antibacterial activity against the crucial etiologic agent of dental caries, *Streptococcus mutans* [15]. Ginseng has been reported to have several bioactive compounds including flavonoids, phenolics, alkaloids, triterpenes, vitamins, polyacetylenes, and minerals [16]. The major active components of ginseng are kaempferols and ginsenosides [16].

To our knowledge there are no reports on the susceptibility of *Porphyromonas gingivalis* and *Prevotella intermedia* isolated from patients with periodontitis to ginseng. The objectives of this research were to evaluate the susceptibility profile of *Porphyromonas gingivalis* and *Prevotella intermedia* to ginseng and to compare its activity with propolis.

Materials and Methods

Media and chemicals

Bacteriological media used throughout this work were prepared according to the instructions of the manufacturer before autoclaving at 121°C for 15 min. The following media were used throughout the present work: VMGA III transport medium [17], Brucella blood agar with hemin and vitamin K1 (BBAHV, Becton Dickinson GmbH, Germany) [18], tryptone soya broth (TSB, Biolife, Italy), and Müller-Hinton agar (MHA, Biolife, Italy). All chemicals used in the current study were of analytical grade (HiMedia Laboratories, India).

Preparation of propolis extract and ginseng extract

Propolis was purchased from Hanse Honig INH. Philipp Von Rath, Germany and ginseng roots were purchased from the local market. One g of propolis or ginseng was dispersed in 100 ml absolute ethyl alcohol at 37°C for 18 hours in a rotary evaporator. The obtained solution was filtered through Whatman No. 1 filter paper and centrifuged for 10 minutes at 10,000 rpm. The clear supernatant was diluted with distilled water to give 40% ethanol concentration.

Sample collection and isolation of *Porphyromonas gingivalis* and *Prevotella intermedia*

The research protocol was approved by the Ethics Committee of the Faculty of Dentistry at Pharos University in Alexandria, Alexandria, Egypt. Thirty patients diagnosed with moderate to severe chronic periodontitis were enrolled from the patients attending the Clinic of Faculty of Dentistry, Pharos University in Alexandria after obtaining informed consents. All selected patients had at least 3 non-adjacent pockets with minimum 3 mm clinical attachment loss and had no history of periodontal treatment or antibiotic therapy during the previous 1 month. After supragingival plaque had been removed from the teeth, subgingival plaque samples were obtained from multiple pockets per patient (pool samples) after isolation using cotton rolls. Paper points were inserted to the depth of the periodontal pocket until resistance was felt and were kept in site for 10 seconds (Figure 1) [19]. The paper points were immediately transferred to 10 ml autoclaved screw capped test tubes containing 5 ml of VMGA III transport medium [17]. Each tube was vortexed vigorously and an aliquot of 1 ml was inoculated on sterile BBAHV agar medium, a selective medium for the isolation of *Porphyromonas gingivalis* and *Prevotella intermedia* [18]. After anaerobic incubation using the Oxoid Gas-Pak system (Oxoid Ltd, UK) at 37°C for 5 days, the bacteria grown were purified by streaking on BBAHV agar medium. *Porphyromonas gingivalis* and *Prevotella intermedia* were distinguished from other black-pigmented, anaerobic, Gram-negative rods by UV fluorescence [20].

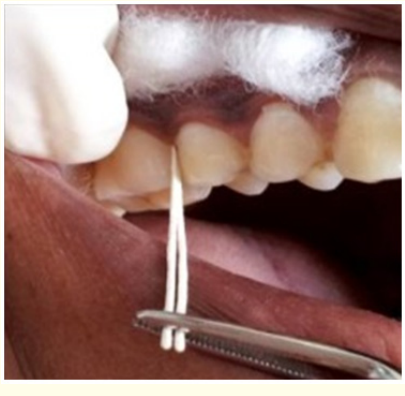

Figure 1: Paper points inserted into the depth of periodontal pocket.

Identification of the obtained bacterial isolates

The obtained *Porphyromonas gingivalis* and *Prevotella intermedia* isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The spectrum produced by MALDI-TOF MS was compared against the reference spectra using Bruker MALDI-TOF Biotyper software to achieve a confidence score for the most related species [21].

Determination of propolis and ginseng extracts MIC and antibacterial activities against *Porphyromonas gingivalis* and *Prevotella intermedia*

The agar diffusion method [22] was adopted to examine the antibacterial activity of propolis and ginseng extracts. A loopful of either *Porphyromonas gingivalis* or *Prevotella intermedia* pure culture was inoculated in TSB and incubated overnight anaerobically at 37°C. A seed culture of either *Porphyromonas gingivalis* or *Prevotella intermedia* (10^5 cfu/ml) was inoculated and spread on the surface of MHA medium. By using a sterile cork borer, one well of 6 mm diameter was punched in each plate. An aliquot of 10 µl of propolis or ginseng extract was placed at the center of each well. After incubation at 37°C for 24h, the diameters of inhibition zones were measured by Vernier calliper. The experiment was carried out in triplicate and the average absolute unit (AU) of inhibition zones was calculated according to Lotfy., *et al*. [4]. The MIC values of propolis extract and ginseng extract against *Porphyromonas gingivalis* and *Prevotella intermedia* were determined as reported in Matar and Lotfy [23].

Statistical analysis

Statistical analysis was performed using paired *t*-test and *P* values ≤ 0.001 were considered statistically significant.

Results

Characterization and identification of *Porphyromonas gingivalis* and *Prevotella intermedia* isolates

Samples were collected and cultured as described previously under the Materials and Methods section. After the incubation period on BBAHV, 24 Gram-negative black pigmented colonies were presumptively identified as *Porphyromonas gingivalis* or *Prevotella intermedia*. Presence of UV fluorescence [20] was used as a rapid taxonomic test to confirm the identification of *Porphyromonas gingivalis* and *Prevotella intermedia*. MALDI-TOF MS proteomic identification was used to compare the cellular protein profiles of the isolated bacteria to reference spectra. According to the results obtained from this analysis (Table 1), the 24 isolates recorded high score values ranged from 2.41 to 2.82 which indicated a highly probable identification of *Porphyromonas gingivalis* and *Prevotella intermedia* (Table 1).

Determination of propolis and ginseng extracts MIC against *Porphyromonas gingivalis* and *Prevotella intermedia*

The MIC results for propolis extract and ginseng extract are listed in Table 2. The 12 isolates of *Porphyromonas gingivalis* had greater sensitivity to propolis extract and ginseng extract than the 12 isolates of *Prevotella intermedia*. Propolis extract showed the lowest MICs, 256 and 512 µg/ml against *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively. On the other hand, all the isolates of *Porphyromonas gingivalis* and *Prevotella intermedia* were susceptible to ginseng extract with MICs of 512 and 1024 µg/ml, respectively.

Bacterial isolate	Best match	Score value*
1	*Porphyromonas gingivalis*	2.75
2	*Porphyromonas gingivalis*	2.45
3	*Porphyromonas gingivalis*	2.69
4	*Porphyromonas gingivalis*	2.81
5	*Porphyromonas gingivalis*	2.44
6	*Porphyromonas gingivalis*	2.51
7	*Porphyromonas gingivalis*	2.41
8	*Porphyromonas gingivalis*	2.59
9	*Porphyromonas gingivalis*	2.53
10	*Porphyromonas gingivalis*	2.77
11	*Porphyromonas gingivalis*	2.59
12	*Porphyromonas gingivalis*	2.81
13	*Prevotella intermedia*	2.49
14	*Prevotella intermedia*	2.58
15	*Prevotella intermedia*	2.68
16	*Prevotella intermedia*	2.74
17	*Prevotella intermedia*	2.82
18	*Prevotella intermedia*	2.56
19	*Prevotella intermedia*	2.49
20	*Prevotella intermedia*	2.50
21	*Prevotella intermedia*	2.61
22	*Prevotella intermedia*	2.58
23	*Prevotella intermedia*	2.74
24	*Prevotella intermedia*	2.69

Table 1: MALDI - TOF MS analysis of the isolated bacteria. *2.300 - 3.000, highly probable species identification; 2.000 - 2.299, probable species identification; 1.700 - 1.999, probable genus identification; and 0.000 - 1.699, not reliable identification.

Extract	Microorganism	MIC (µg/ml)
Propolis extract	*Porphyromonas gingivalis*	256
	Prevotella intermedia	512
Ginseng extract	*Porphyromonas gingivalis*	512
	Prevotella intermedia	1024

Table 2: Susceptibility of 24 isolates of *Porphyromonas gingivalis* and *Prevotella intermedia* to propolis extract and ginseng extract.

Comparison between the antibacterial activities of propolis and ginseng extracts against *Porphyromonas gingivalis* and *Prevotella intermedia*

The data illustrated graphically in Figures 2 and 3 showed a comparison between the antibacterial activity (AU) of ginseng extract versus propolis extract against *Porphyromonas gingivalis* and

Prevotella intermedia. The anti- *Porphyromonas gingivalis* activities of propolis extract ranged from 15.73 to 24.67 AU whereas, ginseng extract showed activities ranged from 14.2 to 23.16 AU. On the other hand, the anti-*Prevotella intermedia* activities of propolis extract ranged from 12.51 to 13.67 AU whereas, ginseng extract showed activities ranged from 12.35 to 13.48 AU. Furthermore, the mean activity of ginseng extract was lower than that of propolis extract by 1.32 and 0.17 AU with respect to *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively (Table 3).

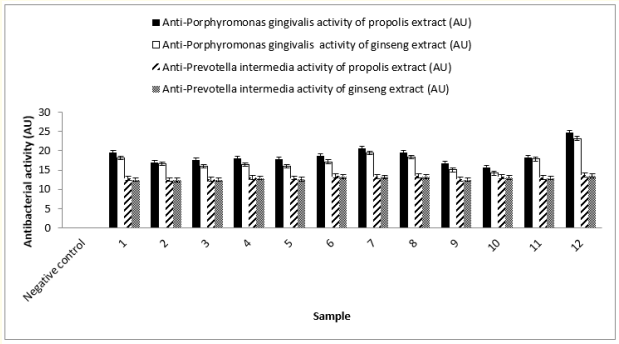

Figure 1: The antimicrobial efficacy of propolis extract compared with ginseng extract on *Porphyromonas gingivalis* and *Prevotella intermedia*.

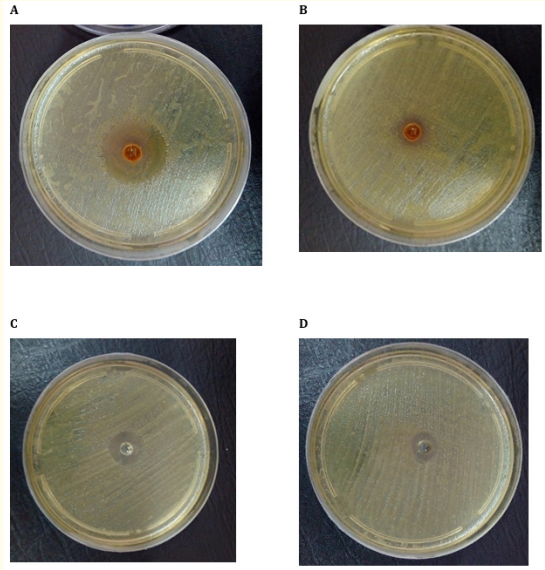

Figure 3: Inhibition zone of propolis extract against *Porphyromonas gingivalis* (A) and *Prevotella intermedia* (B) versus inhibition zone of ginseng extract against *Porphyromonas gingivalis* (C) and *Prevotella intermedia* (D).

Statistical parameter	Anti-*Porphyromonas gingivalis* activity of propolis extract (AU)	Anti-*Porphyromonas gingivalis* activity of ginseng extract (AU)	Anti-*Prevotella intermedia* activity of propolis extract (AU)	Anti-*Prevotella intermedia* activity of ginseng extract (AU)
Mean	18.67658356	17.35196759	13.07039931	12.89931134
Variance	5.288952622	5.484136945	0.137399378	0.146843949
Observations	12	12	12	12
Pearson Correlation	0.980835415	--	0.984789248	--
Hypothesized Mean Difference	0	--	0	--
df	11	--	11	--
t Stat	10.05643886	--	8.856503496	--
P(T<=*t*) one-tail	3.49425×10^{-7}	--	1.22622×10^{-6}	--
t Critical one-tail	4.024701038	--	4.024701038	--
P(T<=*t*) two-tail	6.98849×10^{-7}	--	2.45245×10^{-6}	--
t Critical two-tail	4.436979338	--	4.436979338	--

Table 3: Statistical analysis using paired *t*-test for the effects of propolis and ginseng extracts on *Porphyromonas gingivalis* and *Prevotella intermedia.*

As shown in table 3, the statistical analysis using paired *t*-test for the effects of propolis and ginseng extracts on *Porphyromonas gingivalis* and *Prevotella intermedia* revealed a significant difference between the sample means of the two groups as the *p*-value is lower than 0.001.

Discussion and Conclusion

Porphyromonas gingivalis and *Prevotella intermedia* are regarded as major etiologic agents for periodontitis [2,24]. The current study was undertaken to evaluate the antimicrobial action of the ethanolic extract of ginseng against the periodontopathic bacteria *Porphyromonas gingivalis* and *Prevotella intermedia* and to compare its activity to propolis extract. To the best of our knowledge, no single report has been reported on the susceptibility of *Porphyromonas gingivalis* and *Prevotella intermedia* isolated from patients with periodontitis to ginseng extract. As an orientation step, 24 Gram-negative black pigmented bacteria were isolated from 30 patients with moderate to severe chronic periodontitis. The identification of these isolates was confirmed by MALDI-TOF MS as *Porphyromonas gingivalis* and *Prevotella intermedia*.

The antimicrobial activities of propolis extract against *Porphyromonas gingivalis* and *Prevotella intermedia* were demonstrated in this study to be compared to that of ginseng extract. These results confirmed other studies on propolis extract [6,7]. Propolis extract showed MICs of 256 and 512 µg/ml against *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively. Our results are in good agreement with that reported by Yoshimasu., *et al*. [9] but higher than the findings of Santos., *et al*. [6]. This difference could be explained by the diverse composition of propolis [8], and different methodologies in the preparation of propolis extracts. The antimicrobial action of propolis is complex and depends on the synergistic mechanism between different propolis constituents [6,9]. The compounds in propolis known to have antimicrobial action are mainly flavonoids, phenolic acids and their esters [6,9].

Susceptibility of *Porphyromonas gingivalis* and *Prevotella intermedia* to ginseng extract was comparable to propolis extract with MICs of 512 and 1024 µg/ml, respectively. However, the MIC values obtained for propolis extract were lower than ginseng extract. Our results are relatively close to the results reported by Son., *et al*. [15] on the MIC of ginseng extract against the oral cariogenic *Streptococcus mutans*. The major bioactive compounds of ginseng are ginsenosides and kaempferols [16]. Moreover, Lee., *et al*. [25] reported that a high uronic acid containing polysaccharide isolated from the root of ginseng inhibited the ability of *Porphyromonas gingivalis* to agglutinate erythrocytes.

Our results showed that both propolis and ginseng extracts presented *in-vitro* antimicrobial activities against all of the 24 isolates of *Porphyromonas gingivalis* and *Prevotella intermedia*. The mean antimicrobial activity of propolis extract was 1.08 and 1.01 fold that of ginseng extract with respect to *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively. Consequently, the antimicrobial activity observed for the ginseng extract suggests its usage as an adjuvant to periodontal therapy. However, it is important to clarify that *in-vitro* tests do not simulate the real environment found in the periodontal pockets as they do not consider biofilm formation. Therefore, *in-vivo* tests should be carried out to verify its efficacy within the subgingival environment.

Conflict of Interest

The authors have declared no conflict of interest.

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