

## Original Article

# Antimicrobial Activity of Aqueous Garlic Extract (*Allium sativum*) Against *Porphyromonas gingivalis*: An In-Vitro Study

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## ABSTRACT

**Background and Aim:** Oral hygiene is important against the development of chronic periodontitis. There are concerns about bacterial resistance to antibiotics. The current study aimed to determine the antimicrobial activity of aqueous garlic extract (*Allium sativum*) against *Porphyromonas gingivalis* (*P. gingivalis*).

**Materials and Methods:** Aqueous garlic extract was prepared, and the inhibitory effect of the extract was tested against *P. gingivalis*. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against the control group (0.2% chlorhexidine) were also determined.

**Result:** Significant differences were observed concerning the MIC ( $1.21 \pm 0.37 \mu\text{l}$ ) and MBC ( $1.44 \pm 0.67 \mu\text{l}$ ) against *P. gingivalis* between the aqueous garlic extract and control groups ( $0.29 \pm 0.1 \mu\text{l}$ ;  $P < 0.001$ ). There was a significant difference in the inhibitory zone against *P. gingivalis* between the aqueous garlic extract group ( $20.1 \pm 1.4 \text{ mm}$ ) and the control group ( $27.3 \pm 1.8 \text{ mm}$ ); the inhibitory zone was larger in the control group ( $P < 0.000$ ).

**Conclusion:** The results suggested that although chlorhexidine exhibited better antimicrobial activity against *P. gingivalis*, the aqueous garlic extract also showed acceptable results. Further research using different extraction methods and concentrations is suggested.

**Keywords:** Microbial Sensitivity Tests, Plant Extracts, Garlic, *Porphyromonas gingivalis*

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## Introduction:

The accumulation of bacterial plaque at gingival margins is the primary etiological factor in the development of chronic periodontitis.<sup>(1)</sup> The oral cavity is inhabited by different bacterial species. Supragingival plaque shows the accumulation of Gram-positive cocci while subgingival plaque mainly comprises *Aggregatibacter actinomycetemcomitans* (*Aa*),

*Prevotella intermedia*, *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia*, and *Fusobacterium*.<sup>(2)</sup> *P. gingivalis* is an important Gram-negative anaerobe oral bacterium, which is considered one of the major etiological factors in progressive periodontitis.<sup>(3)</sup> Reduction of the bacterial load and mechanical methods for the removal of plaque are the

main current treatments for gingivitis and periodontitis.<sup>(4)</sup>

Although mechanical treatment decreases subgingival microorganisms, it is not able to eliminate all pathogens. Systemic antibiotics can be used with conventional therapy to delay bacterial proliferation; however, their prolonged use can lead to the development of resistant strains.<sup>(5)</sup> There is significant interest in finding new antibacterial agents considering the increased bacterial resistance to antibiotics.<sup>(6)</sup>

The *Allium* is the main representative genus of the Alliaceae family that is mostly cultivated in the northern hemisphere. Since ancient times, garlic (*Allium sativum* L.) has been used as a common remedy for many diseases. The first citation of this plant can be found in the Codex Ebers (1550 BC), which is an Egyptian medical papyrus that has reported numerous therapeutic effects of garlic, including antidiabetic, anti-atherosclerotic, anti-thrombotic, anti-hypertensive, anti-hyperlipidemic, anti-inflammatory, antioxidant, and anticancer activities.<sup>(7,8)</sup> There has been increasing awareness of garlic medicinal properties. Several reports have suggested that garlic is a rich source of flavonoids and sulfur-containing compounds that have antimicrobial and antioxidant properties.<sup>(9)</sup> Garlic has been documented as a valuable spice and a popular medicine for various disorders.<sup>(8)</sup> Garlic is a strong antibacterial agent and inhibits both Gram-positive and Gram-negative bacteria, including *Escherichia*, *Salmonella*, *Streptococcus mutans* (*S. mutans*), *P. gingivalis*, *Staphylococcus*, *Klebsiella*, *Proteus*, and *Helicobacter pylori*.<sup>(10-13)</sup>

Alliin, which is a volatile molecule, is one of the main ingredients of freshly crushed garlic. It causes the pungent smell of garlic and is a chemically unstable molecule.<sup>(10)</sup> It is reported that aqueous extract of garlic (25, 50, and 75 µl) has shown 16-, 20-, and 25-mm zones of inhibition against *P. gingivalis*, respectively.<sup>(11)</sup> The garlic extract (57.1% (w/v) containing 220 mg/ml of alliin) inhibits the growth of *P. gingivalis*. Garlic extract also inhibits the trypsin-like (92.7%) and total protease activity (94.88%) of *P. gingivalis*.<sup>(14)</sup> It has been suggested that the de-

velopment of resistance to alliin is more difficult compared to certain antibiotics.<sup>(14)</sup> Therefore, the current study aimed to determine the antimicrobial activity of aqueous garlic extract (*Allium sativum*) against *P. gingivalis*.

## Materials and Methods

This in-vitro experimental study was performed to examine the antimicrobial effect of aqueous garlic extract (*Allium sativum*) on *P. gingivalis*.

Garlic bulbs from the Northern region of Iran were dried at room temperature far from sunlight seven months after being harvested. Garlic powder (10 g) was blended in 100 ml of distilled water filtered using cotton wool and ultra-filtered under reduced pressure using a Buchner funnel and a side-arm flask. By subtracting the weight of insoluble material from the weight of the original cloves, the final concentration of garlic extract in the solution was determined to be 16.5% (w/v). The garlic extract was stored at -20°C and used for antibacterial testing.<sup>(3)</sup>

Bacterial culture:

*P. gingivalis* (ATCC 33277) was purchased from the Microbiology Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Bacterial suspensions were prepared (0.5 McFarland), and 100 µl of each bacterial vial was added to 5 ml of sterilized Brain Heart Infusion (BHI) broth and incubated at 37°C for 18 hours. After adjusting the cell concentration to an optical density (OD) equal to seven [ $1.5 \times 10^8$  colony-forming units (CFU)/ml], 50 µl of this suspension was added to each well.<sup>(3)</sup>

Minimum inhibitory concentration (MIC):

This study comprises two groups: garlic aqueous extract (case group, n=10) and chlorhexidine (control group, n=10). One ml of the standard bacterial suspension was mixed with 60 ml of sterile Müller-Hinton agar medium and poured into Petri dishes. The agars were left to set, and each of the plate cups (10-mm diameter) were cut using a sterile cork borer, and agar discs were removed. Garlic extracts were prepared in a series of increasing concentrations.

The bottom of each plate was marked off into eight segments. The procedure involved preparing two-fold dilutions of the antimicrobial agent. Serial dilution from 100%, 50%, 25%, and 12.5% to 10% was used. Fifty  $\mu$ l of extracted garlic was introduced into the wells using automatic microliter pipettes, and all plates were incubated at 37°C for 24 hours. A liquid growth medium was poured into tubes to a minimum volume of 2 ml (macro-dilution) or smaller volumes (micro-dilution) in a 96-well plate. Then, each tube or well was inoculated with a microbial inoculum prepared in the same medium after dilution of the standardized microbial suspension (0.5 McFarland). After mixing, the inoculated tubes and the 96-well plate were incubated. Chlorhexidine (0.2%; C9394, Sigma-Aldrich Inc., Missouri, USA) was used as a positive control. The negative control was distilled water. The sensitivity of *P. gingivalis* and the control was determined by measuring the diameter (mm) of the zone of inhibition. The MIC is the lowest concentration of the antimicrobial agent that completely inhibits bacterial growth.<sup>(11)</sup>

Minimum bactericidal concentration (MBC): To determine the MBC, the MIC dilution tubes with no growth along with the control tube were subcultured and anaerobically incubated for 24 hours at 37°C. The colonies were counted on the next day. The microorganism growth in the control tube was compared to that in the MIC test tubes. A similar number of colonies indicated bacteriostatic activity while a reduced number of colonies indicated a partial or slow bactericidal activity. If there is no microorganism growth, the garlic extract is known to have a bactericidal effect. However, the garlic extract is known to have no bacteriostatic effect if microorganism growth is detected.<sup>(1)</sup>

Statistical analysis:

The data were analyzed using t-test ( $P < 0.05$ ).

### Result:

The MIC and MBC of aqueous garlic extract against *P. gingivalis* are presented in Table 1. According to the results, significant differences were observed regarding MIC ( $1.21 \pm 0.37 \mu$ l) and MBC ( $1.44 \pm 0.67 \mu$ l) between the aqueous garlic extract and chlorhexidine groups ( $0.29 \pm 0.1 \mu$ l) against *P. gingivalis* ( $P < 0.001$ ).

Table 1. Minimum bactericidal concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous garlic extract against *Porphyromonas gingivalis* (*P. gingivalis*)

Groups	Mean $\pm$ SD	CV (%)	
Aqueous garlic extract	MIC ( $\mu$ g/ml; n=10)	1.21 $\pm$ 0.37	30
	MBC ( $\mu$ g/ml)	1.44 $\pm$ 0.67	46
Chlorhexidine	MIC ( $\mu$ g/ml; n=10)	0.29 $\pm$ 0.1	
P-value	0.001		

SD=Standard Deviation, CV=Coefficient of Variation

There was a significant difference in the inhibitory zone against *P. gingivalis* between the aqueous garlic extract ( $20.1 \pm 1.4$  mm) and chlorhexidine ( $27.3 \pm 1.8$  mm) groups; the inhibitory zone was greater in the chlorhexidine group ( $P < 0.000$ ; Table 2).

Table 2. Inhibitory zone (mm) of aqueous garlic extract against *Porphyromonas gingivalis* (*P. gingivalis*)

Groups	Mean $\pm$ SD	CV (%)
Aqueous garlic extract (n=10)	20.1 $\pm$ 1.4	6
Chlorhexidine (n=10)	27.3 $\pm$ 1.8	7
P-value	0.000	

SD=Standard Deviation, CV=Coefficient of Variation

### Discussion:

*Allium sativum*, as a medical plant, exhibits antibacterial, antifungal, antiviral, and antiprotozoal effects, thereby acting as a natural antibiotic.<sup>(12-17)</sup> However, few studies have attempted to determine its role in periodontitis.

In the current study, we tried to determine the antimicrobial activity of aqueous garlic extract (*Allium sativum*) against *P. gingivalis*. According to the results, significant differences were observed regarding the MIC ( $1.21 \pm 0.37 \mu$ l) and MBC ( $1.44 \pm 0.67 \mu$ l) between the aqueous garlic extract and chlorhexidine groups

(0.29±0.1 µl) against *P. gingivalis* (P<0.001).

There was a significant difference in the inhibitory zone against *P. gingivalis* between the aqueous garlic extract (20.1±1.4 mm) and chlorhexidine groups (27.3±1.8 mm); the inhibitory zone was larger in the chlorhexidine group (P<0.000). Numerous garlic extract preparation techniques have been shown to exhibit a wide spectrum of antibacterial activity against clinical isolates of Gram-negative, Gram-positive, and acid-fast bacteria. Essential oils of garlic bulbs have antimicrobial activity against *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Escherichia coli* with inhibition zones of 14.8, 21.1, and 11.0 mm, respectively.<sup>(12)</sup> Our results were dissimilar to this report; differences might be related to bacterial strains. It seems that the extraction method affects the antimicrobial activity of the obtained extract. It is reported that aqueous extracts of garlic (25, 50, and 75 µl) have caused 16-, 20-, and 25-mm zones of inhibition, respectively, against *P. gingivalis*.<sup>(1)</sup> Our result was similar to this report.

Different concentrations of garlic extract (5, 10, 20, and 100%) have similar effects against *S. mutans*, *Streptococcus sanguis* (*S. sanguis*), *Streptococcus salivarius* (*S. salivarius*), *P. aeruginosa*, and *Lactobacillus* spp.<sup>(13)</sup> In the evaluation of the inhibitory effect of garlic against *P. gingivalis* and Aa, it has been revealed that garlic extract may have therapeutic effects on periodontitis<sup>(1)</sup>. Aqueous garlic extract did not show any inhibition zone against Aa in the well diffusion method but it showed inhibitory activity against Aa. This difference may be related to the binding of constituents of garlic to those of the agar medium, thereby limiting the diffusion. Therefore, the MIC obtained using the broth dilution method is considered more reliable.<sup>(18)</sup> In addition, the MIC for Aa was 125 µl/ml, which was much higher than the concentration used for well diffusion (25, 50, and 75 µl/ml), which may have swayed the results related to the inhibitory effect of garlic on Aa.<sup>(18)</sup> Of the Gram-negative species tested in the cited study, *Leptotrichia buccalis* (*L. buccalis*) was the least sensitive to garlic, with a MIC of 35.7 mg/ml, which approached the MICs of the oral Gram-positive species. Although *L. buccalis* has a Gram-negative cell wall structure,

it also possesses membranous, scale-like folds that cover its external surface.<sup>(19)</sup> In the assessment of the antibacterial effect of garlic extract on erythromycin- and methicillin-resistant bacteria isolated from an operating room, Ataee et al reported that 70 strains (100%) in the agar well diffusion method were sensitive to 4-12 µg/ml of garlic extract (MIC=8 µg/ml).<sup>(3)</sup> In addition, the antibacterial effect of garlic extract has been reported against *S. aureus* strains in hamburgers.<sup>(19)</sup> It is reported that the inhibition zones of different concentrations of garlic extract were not significantly different for *S. mutans*, *S. sanguis*, *S. salivarius*, *P. aeruginosa*, and *Lactobacillus* spp. Additionally, the inhibition zones of 5%, 10%, and 20% concentrations were not significantly different.<sup>(20)</sup> Similar results were found in our study. Aqueous extract (16.5%) at serial dilution (10, 12.5, 25, 50, and 100%) exhibited no significant difference with 0.2% chlorhexidine gluconate.<sup>(17)</sup> The MIC of the aqueous extracts of garlic in the cited study was 10%.<sup>(17)</sup>

The main antimicrobial ingredient of garlic is an oxygenated sulfur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is known as allicin.<sup>(16)</sup> Several bacterial strains are sensitive to pure allicin.<sup>(16)</sup> When the garlic is crushed, allicin is formed rapidly by the action of the alliinase (alliin lyase; EC 4.4.1.4) on alliin (S-allyl-L-cysteine sulphoxide).<sup>(14)</sup> Allicin reacts with free thiol groups; its key mechanism of antimicrobial activity is through interaction with thiol-containing enzymes, such as cysteine proteases and alcohol dehydrogenases.<sup>(14)</sup> Since these enzymes are necessary for bacterial nutrition and metabolism, it has been suggested that the development of resistance to allicin is more difficult compared to certain antibiotics.<sup>(14)</sup>

Mouthwashes containing garlic extract have good in-vivo antibacterial activity against salivary *S. mutans*.<sup>(15)</sup> Garlic extract mouthwash inhibits the formation of biofilms by *Staphylococcus epidermidis* at lower MIC levels.<sup>(15)</sup> Allicin complexes with blood proteins and reduces bleeding at periodontal sites.<sup>(14)</sup>

Extensive use of antibiotics has led to antibiotic resistance; therefore, alternative agents that are effective against pathogens but do not disturb the normal flora are of particular interest.<sup>(16)</sup>

Aqueous garlic extract has antibacterial activity at room temperature. Garlic extract could be stored at 4°C. Excessive warming should be avoided.<sup>(21)</sup> This heat stability is very useful when the antimicrobial peptides are used as a food preservative.<sup>(21)</sup> It seems that garlic extract contains antibacterial peptides or proteins, which show stable antibacterial activity at high temperatures less than 80°C with a broad antimicrobial spectrum.<sup>(21)</sup>

An investigation has shown the effect of aqueous garlic extract on *Candida albicans* (*C. albicans*) in the mouth.<sup>(22)</sup>

The results of the cited study showed that the aqueous extract of garlic was able to inhibit the growth of *C. albicans* but its effect was less than that of nystatin.<sup>(22)</sup> Further research using different extraction methods and concentrations is suggested

### Conclusion:

The results suggested that although chlorhexidine exhibited better antimicrobial activity against *P. gingivalis*, the aqueous garlic extract also showed acceptable results. Garlic seems to be safe with the potential for broader applications.

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