**Efficacy of *Psidium guajava* and *Allium sativum* Extracts as Antimicrobial Agents against Periodontal Pathogens**

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**Background:** The accumulation and maturation of bacterial plaque at the gingival margin is widely recognized as the primary etiological factor in the development of chronic periodontitis. With the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection. **Aim:** The aim of this study was to evaluate the efficacy of *Psidium guajava* (guava) and *Allium sativum* (garlic) on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. **Materials and Methods:** Aqueous guava extract (AGvE), ethanolic guava extract (EGvE), aqueous garlic extract (AGE), and ethanolic garlic extract (EGE) were prepared. The inhibitory effects of these extracts for the periodontal pathogens were tested by agar well diffusion method. Minimal inhibitory concentration (MIC) of the aqueous and ethanol extracts of guava and garlic was determined by macrobroth dilution method. Minimum bactericidal concentration (MBC) was done to observe the bactericidal effect of the guava and garlic extracts against the organisms. **Results:** Of the AGE, 25, 50, and 75 μL showed 16, 20, and 25 mm zone of inhibition, respectively, on *P. gingivalis*. The AGE showed greater bacteriostatic activity against the *P. gingivalis* with MIC determined at 16.6 μL/mL. MIC determined for AGvE and EGvE was at 75 μL/mL concentration for *P. gingivalis*, whereas EGvE showed the activity at 75 μL/mL on *P. gingivalis*. MIC determined for AGvE was at 50 μL/mL, whereas MIC determined for EGE was at 3.12 μL/mL for *A. actinomycetemcomitans*. **Conclusion:** *P. guajava* and *A. sativum* displayed a significant antibacterial effect. *A. sativum* was found to be most effective against *P. gingivalis*, whereas *P. guajava* showed the highest efficacy on *A. actinomycetemcomitans*. **KEYWORDS:** *Aggregatibacter actinomycetemcomitans, Allium sativum, antimicrobial, garlic, guava, Porphyromonas gingivalis, Psidium guajava*

**INTRODUCTION**

Periodontitis is a complex disease, which expresses the interaction of the biofilm with the host inflammatory response and subsequent alterations in the bone and connective tissue metabolism. The etiology is multifactorial with periodontal pathogens having a major role in the initiation and progression of the disease.
antimicrobial resistance. There exists a need to develop some innovative strategies that act against periodontal pathogens without any side effects. One such suitable alternative would be to explore the abundantly and economically available phytoplants in nature. The “naturally occurring” active ingredients in plant medicines restore health, with minimal harmful effects and maximum efficiency with minimal side effects.[2]

Psidium guajava (guava) is a phytotherapeutic plant commonly known as guava, which is proven for its antimicrobial, antiparasitic, hepatoprotective, antioxidant, antigenotoxic, antitumagenic, anti-allergic, anticancer, and antihyperglycemic effects.[3] The antibacterial activity against cariogenic bacteria, L. acidophilus, is reported to be as similar to that of chlorhexidine.[4]

Allium sativum (garlic) has been used as medicine since ancient times and has long been known to have antibacterial, antifungal, and antiviral properties.[5,6] It has been suggested that the development of resistance to allicin arises one thousandfold less easily than it does to certain antibiotics.[7]

Though P. guajava and A. sativum are naturally available and have been proven for its antibacterial property, the kind of literature on its effect on periodontal pathogens is scanty. Hence, the study was aimed to evaluate the efficacy of guava and garlic extracts as antimicrobial agents against periodontal pathogens mainly Porphyromonas gingivalis and Aggregatibacter actinomyctetemcomitans.

Materials and Methods

Preparation of guava and garlic extract

Aqueous guava extract (AGvE), ethanolic guava extract (EGvE), aqueous garlic extract (AGE), and ethanolic garlic extract (EGE) were prepared similar to the earlier studies reported in the literature.[8,9]

Bacteria and growth condition

Stock culture of periodontal pathogens P. gingivalis and A. actinomyctetemcomitans were used in this study. Kanamycin blood agar was used to isolate, and oxoid anaerobic jar was used for cultivating P. gingivalis. Dentaid agar was used to isolate, and candle jar technique was used to cultivate A. actinomyctetemcomitans.[8,9]

Agar diffusion procedure

Of the 500 µL/mL of test aqueous extract of the test material (garlic/guava) 75, 50, and 25 µL, and 500 mg/mL of ethanolic extract of test material were added into the respective wells prepared on each plate. The plates were incubated within 15 min of compound application for 18–24 h at 37°C anaerobically. Plates were read only if the lawn of growth was confluent or nearly confluent. Diameter of inhibition zone was measured to the nearest whole millimeter by holding the calipers.

Minimal inhibitory concentration procedure

The minimal inhibitory concentration (MIC) of the aqueous and ethanolic guava and garlic extract was determined by macrobroth dilution method. The guava extract solutions were serial diluted, and concentrations at 500, 250, 125, 62.5, 31.25, 16.6, 8.3, 4, and 2 mg/mL and 500, 250, 125, 62.5, 31.25, 16.6, 8.3, 4, and 2 µL/mL for EGvE and AGvE were obtained, respectively. Similarly, garlic extract solutions were serial diluted and concentrations at 500, 250, 125, 62.5, 31.25, 16.6, 8.3, 4, and 2 mg/mL and 500, 250, 125, 62.5, 31.25, 16.6, 8.3, 4 and 2 µL/mL for EGE and AGE were obtained, respectively. The tubes were then inoculated with 0.1 mL of cultures (107 cells). The MIC was defined as the lowest concentration of the extract that completely inhibited the growth of the organisms.

Minimal bactericidal concentration procedure

The minimal bactericidal concentration (MBC) was tested to observe the bactericidal effect of the garlic and guava extract against the organisms. If there were no growth of microorganisms then the extract was known to have bactericidal effect, and if there were growth of microorganisms then the extract was known to have bacteriostatic effect.

Statistical analysis

The data on various study parameters were obtained and summarized using appropriate statistical measures. The continuous variables in the study such as zone of inhibition and number of colonies were summarized using mean, median, and standard deviation. All the analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 20.0 (IBM, Armonk, New York), and the statistical significance was tested at 5% level.

Results

Antibacterial activity of guava extracts by well diffusion method

Of the AGvE, 75 µL showed 10.4 + 0.54 mm, and 75 µL of EGvE showed 15.4 + 0.54 mm on P. gingivalis. However, no zone of inhibition was seen for lower concentrations of both aqueous and ethanolic extracts of guava on P. gingivalis [Table 1].

Of the AGvE, 50 and 75 µL showed 18.2 + 0.83 and 22.8 + 1.48 mm zone of inhibition, respectively.
on *A. actinomycetemcomitans*. Of the EGvE, 75, 50, 25, 12.5, 6.25, and 3.12 μL showed 20.2 ± 0.83, 18.0 ± 1.00, 15.0 ± 0.70, 13.4 ± 0.54, 11.8 ± 0.44, and 11.6 ± 0.54 mm zone of inhibition, respectively, on *A. actinomycetemcomitans* [Table 2].

**Antibacterial activity of garlic extracts by well diffusion method**

Of the AGE, 25, 50, and 75 μL showed 16.2 ± 0.83, 20.2 ± 0.83, and 25.2 ± 0.83 mm zone of inhibition, respectively, on *P. gingivalis*. However, no zone of inhibition was seen for EGE on *P. gingivalis*. However, 25, 50, and 75 μL of AGE and EGE did not show any zone of inhibition on *A. actinomycetemcomitans* [Table 3].

**Inhibitory effect of aqueous and ethanolic garlic extracts**

The antibacterial activity testing of the AGE and EGE by macrobroth dilution revealed MIC and MBC at different concentrations of the garlic extract. The AGE showed MIC at 16.6 μL/mL. EGE did not show the desired result in comparison to the aqueous extract, the MIC was higher at 62.5 mg/mL for ethanolic extract. In MBC, the AGE showed greater bacteriostatic activity against the *P. gingivalis* [Table 5]. *A. actinomycetemcomitans* showed greater resistance to both the extracts. The MIC for the aqueous extract was determined at 62.5 μL/mL, whereas *A. actinomycetemcomitans* was completely resistant to all the concentrations of the ethanolic extract used in the study. In MBC, the AGE showed greater bacteriostatic activity than EGE [Table 5].

**Table 1: Antibacterial activity of aqueous guava extract and ethanolic guava extract on *Porphyromonas gingivalis***

| Extracts | Zone of inhibition (mm) | Mean ± SD (mm) |
|----------|-------------------------|----------------|
|          | 1 | 2 | 3 | 4 | 5 |               |
| AGvE (μL) |   |   |   |   |   |               |
| 25        | 0 | 0 | 0 | 0 | 0 | 0              |
| 50        | 0 | 0 | 0 | 0 | 0 | 0              |
| 75        | 10 | 11 | 10 | 10 | 11 | 10.4 ± 0.54   |
| EGvE (μL) |   |   |   |   |   |               |
| 25        | 0 | 0 | 0 | 0 | 0 | 0              |
| 50        | 0 | 0 | 0 | 0 | 0 | 0              |
| 75        | 15 | 16 | 15 | 15 | 15 | 15.4 ± 0.54   |

SD = standard deviation, AGvE = aqueous guava extract, EGvE = ethanolic guava extract

**Table 2: Antibacterial activity of aqueous guava extract and ethanolic guava extract on *Aggregatibacter actinomycetemcomitans***

| Extracts | Zone of inhibition (mm) | Mean ± SD (mm) |
|----------|-------------------------|----------------|
|          | 1 | 2 | 3 | 4 | 5 |               |
| AGvE (μL) |   |   |   |   |   |               |
| 25        | 0 | 0 | 0 | 0 | 0 | 0              |
| 50        | 18 | 19 | 18 | 17 | 19 | 18.2 ± 0.83   |
| 75        | 23 | 21 | 23 | 25 | 22 | 22.8 ± 1.48   |
| EGvE (μL) |   |   |   |   |   |               |
| 3.12      | 12 | 12 | 11 | 12 | 11 | 11.6 ± 0.54   |
| 6.25      | 12 | 12 | 11 | 12 | 12 | 11.8 ± 0.44   |
| 12.5      | 13 | 14 | 13 | 14 | 13 | 13.4 ± 0.54   |
| 25        | 15 | 15 | 16 | 14 | 15 | 15.0 ± 0.70   |
| 50        | 18 | 19 | 17 | 17 | 19 | 18.0 ± 1.00   |
| 75        | 20 | 21 | 20 | 19 | 21 | 20.2 ± 0.83   |

SD = standard deviation, AGvE = aqueous guava extract, EGvE = ethanolic guava extract
**DISCUSSION**

Periodontal disease is an infectious immunomodulatory disease ranging in severity from mild gingivitis to advanced loss of connective tissue attachment and supporting bone. The successful treatment of periodontitis requires suppression or elimination of the subgingival periodontal pathogens. Antimicrobial resistance, considerable side effects, and the emergence of previously uncommon infections are the results of improper usage of synthetic antimicrobial agents. Instead, plant chemicals are one of the most powerful and safe alternative chemotherapeutic agents to control many infections if they are supported by scientific-based evidence. Garlic is known to have antibacterial, antifungal, and antiproteolytic activity; it is also reported to be effective against periodontal pathogens.

| Table 3: Antibacterial activity of aqueous garlic extract on *Porphyromonas gingivalis* |
|------------------------------------------|
| AGE (μL) | Zone of inhibition (mm) | Mean ± SD (mm) |
| 25 | 16 | 17 | 16 | 15 | 17 | 16.2 ± 0.83 |
| 50 | 20 | 19 | 20 | 21 | 21 | 20.2 ± 0.83 |
| 75 | 25 | 26 | 26 | 24 | 25 | 25.2 ± 0.83 |

SD = standard deviation, AGE = aqueous garlic extract

| Table 4: Minimal inhibitory concentration and minimal bactericidal concentration of guava extracts on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* |
|------------------------------------------|
| MIC of guava on *Porphyromonas gingivalis* |
| Guava (μL) | 500 | 250 | 125 | 62.5 | 31.25 | 16.6 | 8.3 | 4 | 2 | Control |
| AGvE | S | S | S | S | S | S | S | R | R | R |
| EGvE | S | S | S | S | S | S | S | S | R | R |
| MBC of guava on *P. gingivalis* (CFU/mL) |
| AGvE | NG | NG | NG | NG | NG | NG | 12 | 23 | 36 | 63 | 102 |
| EGvE | NG | NG | NG | NG | NG | NG | 38 | 49 | 58 | 72 | 96 |
| MIC of guava on *Aggregatibacter actinomycetemcomitans* |
| Guava (μL) | 500 | 250 | 125 | 62.5 | 31.25 | 16.6 | 8.3 | 4 | 2 | Control |
| AGvE | S | S | S | S | S | S | S | R | R | R |
| EGvE | S | S | S | S | S | S | S | S | S | S |
| MBC of guava on *A. actinomycetemcomitans* (CFU/mL) |
| AGvE | NG | NG | NG | NG | NG | NG | 86 | 102 | 136 | 148 | 162 |
| EGvE | NG | NG | NG | NG | NG | NG | NG | NG | 46 |

S = susceptible, R = resistant, MIC = minimal inhibitory concentration, MBC = minimal bactericidal concentration, AGvE = aqueous guava extract, EGvE = ethanolic guava extract

| Table 5: Minimal inhibitory concentration and minimal bactericidal concentration of garlic extracts on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* |
|------------------------------------------|
| MIC of garlic on *Porphyromonas gingivalis* |
| Garlic (μL) | 500 | 250 | 125 | 62.5 | 31.25 | 16.6 | 8.3 | 4 | 2 | Control |
| AGE | S | S | S | S | S | S | S | R | R | R |
| EGE | S | S | S | R | R | R | R | R | R | R |
| MBC of garlic on *P. gingivalis* (CFU/mL) |
| AGE | 150 | >300 | >350 | >400 | >450 | >500 | >500 | >500 | >500 | >500 |
| EGE | 300 | 400 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| MIC of garlic on *Aggregatibacter actinomycetemcomitans* |
| Garlic (μL) | 500 | 250 | 125 | 62.5 | 31.25 | 16.6 | 8.3 | 4 | 2 | Control |
| AGE | R | R | R | R | R | R | R | R | R | R |
| EGE | R | R | R | R | R | R | R | R | R | R |
| MBC of garlic on *A. actinomycetemcomitans* (CFU/mL) |
| AGE | 300 | 350 | M | M | M | M | M | M | M |
| EGE | M | M | M | M | M | M | M | M | M |

S = susceptible, R = resistant, M = >5 × 10<sup>9</sup>, MIC = minimal inhibitory concentration, MBC = minimal bactericidal concentration, AGE = aqueous garlic extract, EGE = ethanolic garlic extract
It is also reported that herbal medicines can be beneficial in oral diseases such as periodontitis and gingivitis. This study assessed the activity of guava and garlic, particularly against putative periodontal pathogens. Collectively, the putative periodontal pathogens tested were *A. actinomycetemcomitans* and *P. gingivalis*.

In this study, the AGE showed better zone of inhibition on *P. gingivalis* when compared to EGE and both aqueous and ethanolic extracts of guava. The EGvE showed better zone of inhibition on *A. actinomycetemcomitans* when compared to AGvE and both aqueous and ethanolic extracts of garlic. The data obtained revealed that *P. gingivalis* was very much susceptible to AGE, and *A. actinomycetemcomitans* was very much susceptible to EGvE. This may be due to the structural differences between the microorganisms.

Garlic extract though showed bacteriostatic activity in this study, it did not show any bactericidal activity. Garlic extract showed bacteriostatic activity on *P. gingivalis* and *A. actinomycetemcomitans*, but *A. actinomycetemcomitans* was resistant to EGE. The AGE did not show any zone of inhibition for *A. actinomycetemcomitans* in well diffusion but AGE showed inhibitory activity on *A. actinomycetemcomitans* in broth dilution assay, that is, in MIC. This difference probably may be indicative of constituents of garlic binding to constituents in the agar medium, limiting the diffusion. So MIC values obtained using the broth dilution method were considered more reliable. Also, MIC values shown for *A. actinomycetemcomitans* were at 125 mg/mL, which was much higher than the concentration taken for well diffusion, that is, 25, 50, and 75 mg/mL. This must have influenced the results of inhibitory effect of garlic on *A. actinomycetemcomitans*.

In this study, MIC values of garlic extract were lower in *P. gingivalis* compared to those in *A. actinomycetemcomitans*, which are similar to the findings of a study by Bakri and Douglas. However, it is in contrast to the study by Bachrach et al., which showed lower MIC values for *A. actinomycetemcomitans* compared to those for *P. gingivalis*. MIC values of guava extract were lower in *A. actinomycetemcomitans* compared to those in *P. gingivalis* in this study.

Biswa et al. concluded from their study that the guava plant extracts have no antibacterial effect on the gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*), but show antibacterial effect on gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*). In contrast, Gitika and Kumar reported that guava extract showed antibacterial effect on both gram-positive (*Micrococcus luteus, Bacillus subtilis, S. aureus*, and *Streptococcus sp.*) and gram-negative bacteria (*E. coli, Pseudomonas aeruginosa*, and *Salmonella typhimurium*). However, in this study, both aqueous and ethanolic extract of guava showed bacteriostatic and bactericidal activity against the gram-negative microorganisms (*A. actinomycetemcomitans* and *P. gingivalis*).

In this study, the AGE was more potent than the ethanolic extract, similar to the observations of a study by Roy et al. and El-Mahmood and Amey, but in contrast with that of Debnath. The difference in the inhibitory activity between AGE and EGE may be due to the evaporation of volatile constituents of garlic in ethanolic extract. Aqueous and ethanolic extracts of guava were more efficacious on *A. actinomycetemcomitans* compared to those on *P. gingivalis*. It could be due to the neutralization effect of guava extract on *A. actinomycetemcomitans* leukotoxin, which is one of the major endotoxins causing periodontal disease. The efficacy of ethanolic guava leaf extract was found to be better than that of aqueous guava leaf extract. Ethanolic extract contains tannins as well as flavonoids, whereas aqueous extract contains tannins but not flavonoids. This difference in the composition of ethanolic and aqueous extract can be attributed to the difference in the solubility of various components of guava leaves in water and organic solvents.

However, further studies and clinical trials need to be undertaken to explore the efficacy of guava and garlic in humans. Combination of guava and garlic extract should be tried and evaluated.

**Conclusion**

From this study, it can be concluded that there is a supportive evidence for the antimicrobial activity of *P. guajava* and *A. sativum* against periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis*. Hence, these extracts can be used as economical and suitable adjuvant to synthetic drugs and can be a potential therapeutic agent for periodontal diseases.

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**Conflicts of interest**

There are no conflicts of interest.

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