

## In vitro Evaluation of Antibacterial Efficacy of Pineapple Extract (Bromelain) on Periodontal Pathogens

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### Abstract:

**Background:** Periodontitis is an inflammatory disease resulting in the destruction of periodontal tissues. Various treatment modalities have been tried in the form of mechanical therapy and surgical therapy. Antimicrobial agents have been used as a monotherapy and as an adjunct with mechanical debridement. Various plant extracts have been used as antibacterial agents. Pineapple extract (bromelain) is one such agent. Hence this study was conducted to assess the antibacterial efficacy of bromelain on both aerobic and anaerobic periodontal microorganisms. The aim was to assess the antibacterial efficacy of bromelain on both aerobic and anaerobic periodontal microorganisms.

**Materials and Methods:** Minimum inhibitory concentration (MIC) of bromelain was tested on isolated strains of *Streptococcus mutans*, *Enterococcus faecalis* *Aggregatibacter actinomycetemcomitans* (Aa), and *Porphyromonas gingivalis* (Pg) using serial dilution broth method.

**Results:** *S. mutans* showed sensitivity at the lowest concentration of 2 mg/ml as compared to *E. faecalis* (31.25 mg/ml) while *P. gingivalis* showed sensitivity at the lowest concentration of 4.15 mg/ml as compared to Aa (16.6 mg/ml).

**Conclusion:** Bromelain exerts an antibacterial effect against potent periodontal pathogens; hence, it may be used as an antibacterial agent. However, further trial has to be conducted to validate this result.

**Key Words:** *Aggregatibacter actinomycetemcomitans*, bromelain, *Enterococcus faecalis* periodontitis, *Porphyromonas gingivalis*, *Streptococcus mutans*

### Introduction

From time immemorial, periodontal diseases have been considered as one of the major health problems affecting humans. Epidemiologic studies have shown that destructive periodontal diseases in the form of periodontitis with significant bone loss have affected mankind in the ancient times also.<sup>1</sup> Pathogenic microorganisms in dental plaque, cause an abnormal host tissue response resulting in periodontal disease. Periodontal disease is a chronic condition which starts with gingival inflammation and progressively develops toward hard and soft tissue destruction and tooth loss.<sup>2,3</sup> Though there are various etiological factors for the development of periodontal disease, the main etiology is microbiological insult to the periodontal tissues.<sup>4,5</sup> Wide array of microorganisms have been associated with periodontal disease, out of which *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg) and *Streptococcus mutans* have been predominantly associated with periodontal diseases. The treatment of periodontal disease has always been inclined toward the disruption of these microbial floras either through mechanical therapy or by the use of antimicrobial agents. In this regard, various agents have been tried and tested for their antimicrobial properties. One such agent being an enzyme extract from pineapple.

Pineapple or ananas comosus belongs to the family of bromeliaceae.<sup>6,7</sup> It has been widely used as a therapeutic plant in several resident cultures and these therapeutic qualities of pineapple are accredited to bromelain, which is an elementary extract from pineapple that contains, along with other compounds, various proteinases. Bromelain has shown to exhibit various fibrinolytic, antiedematous, antithrombotic, and anti-inflammatory activities both *in vitro* and *in vivo*.<sup>6</sup> Ever since bromelain was known chemically, it has been used as a phytomedical agent.<sup>8</sup> To confirm its ubiquitous nature, various clinical studies have been conducted, and the results have shown that bromelain may help in the treatment of several diseases such as cardiovascular disease, osteoarthritis, diarrhea, and cancer. It has been widely used in debridement of burns, coagulation, and immunogenicity.<sup>6</sup> It has been widely used as an anti-inflammatory agent, however, its use as an antibacterial agent is yet to be studied. Hence, this qualitative *in vitro* analysis was conducted to evaluate the antibacterial

efficacy of bromelain tablets (500 mg) on potent periodontal pathogens.

### Materials and Methods<sup>9</sup>

Preparation of bromelain solution: Bromelain tablet (500 mg) was dissolved in the dimethyl sulfoxide solution. 400 µl of this solution was used to test the MIC.

### Bacterial strains

MIC was tested for isolated strains of aerobic organisms, *Enterococcus faecalis* (ATCC. No. 35550), *S. mutans* (ATCC. No. 25175), and isolated strains of anaerobic organisms *P. gingivalis* (ATCC. No. 33277), Aa (ATCC. No. 29523).

The minimum inhibitory concentration (MIC): First, the given organisms were grown in pure form. The MIC for bromelain was tested using microdilution broth method. A volume of 400 ml of the prepared bromelian solution was taken in the first tube. 200 ml of brain heart infusion (BHI) broth was added from 2<sup>nd</sup> tube to the last tube. A volume of 200 ml of melatonin was diluted from 2<sup>nd</sup> to the last tube and then 200 ml of each organism was added in the tube. The tubes were incubated in an anaerobic jar for 24-48 h and then checked for the turbidity.

The lowest concentration at which bromelain restricted the growth of microorganisms was considered as the MIC. Solution was taken in the first tube. A volume of 200 ml of BHI broth was added from 2<sup>nd</sup> tube to the last tube. A volume of 200 ml of bromelain was diluted from 2<sup>nd</sup> to the last tube and then 200 ml of each organism was added in the tube. The tubes were incubated in an anaerobic jar for 24-48 h and then checked for the turbidity.

### Results

According to the results of the present study, bromelain showed antibacterial efficacy against all the isolated strains of both aerobic and anaerobic microorganisms (Tables 1 and 2). *S. mutans* showed sensitivity at the lowest concentration of 2 mg/ml as compared to *E. faecalis* (31.25 mg/ml) while Pg showed sensitivity at the lowest concentration of 4.15 mg/ml as compared to Aa (16.6 mg/ml).

### Discussion

The management of periodontal disease has been focused toward the disruption of plaque microflora which involves the mechanical therapy and use of antimicrobial agents. Wide range of antibiotics have been tried and tested against periodontal pathogens. However, due the side effects of various antimicrobial drugs and development of various antibacterial resistant strains of microorganism its use has been abridged. Hence, to overcome this problem and to increase patient acceptance, a wide array of herbal products and plant extracts have been tried and tested. Pineapple extract is one such product. Pineapple is the universal name of ananas comosus. It belongs to the member of the family bromeliaceae, which is grown in a number of subtropical and tropical countries including, India.<sup>6</sup> The medicinal property of pineapple is due to bromelian which is a rough aqueous extract from stem and fruit of pineapple.<sup>6</sup> Bromelain mainly comprised of various mixtures of thiolendopeptidases and other compounds such as carbohydrates glycoproteins phosphatases, glucosidase, peroxidases, cellulases, and several protease inhibitors.<sup>10</sup> It has been used in dentistry as an anti-inflammatory and analgesic drug. However, its use as an antibacterial agent is yet to be tested. Therefore, this qualitative analysis *in vitro* analysis was conducted to test the antibacterial activity of bromelain against isolated strains of *S. mutans* (ATCC. No. 25175), *E. faecalis* (ATCC.No. 35550), Aa (ATCC. No. 29523) and Pg (ATCC. No. 33277).

Broth dilution/serial dilution method was used to test the MIC (MIC) of the bromelain on aerobic and anaerobic periodontal pathogens. Broth dilution method can be either macro or microdilution.<sup>11</sup> Due to its simplicity and accuracy, broth dilution method is a reliable technique to test the efficacy of any antimicrobial agent *in vitro*.

In this present study, *S. mutans* was sensitive to bromelain at a minimum concentration of 2 mg/ml, Aa at 16.6 mg/ml and Pg at 4.15 mg/ml, respectively (Tables 1 and 2) which is contradictory to the study conducted by Khosropanah *et al.*<sup>7</sup> Enterotoxins liberated by certain intestinal pathogens such as vibrio cholera and *Escherichia coli* cause diarrhea in animals and in studies conducted by Mynott and Chandler, it has been

Table 1: MIC against aerobic organisms.

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	16.6 mg/ml	8.3 mg/ml	4.15 mg/ml	2 mg/ml	1 mg/ml
<i>S. mutans</i>	S	S	S	S	S	S	S	S	S	R
<i>E. coli</i>	S	S	S	S	S	R	R	R	R	R

*S. mutans*: *Streptococcus mutans*, *E. coli*: *Escherichia coli*, MIC: Minimum inhibitory concentration

Table 2: MIC against anaerobic organism.

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	16.6mg/ml	8.3 mg/ml	4.15 mg/ml	2 mg/ml	1 mg/ml
Pg	S	S	S	S	S	S	S	S	R	R
Aa	S	S	S	S	S	S	R	R	R	R

Pg: *Porphyromonas gingivalis*, Aa: *Aggregatibacter actinomycetemcomitans*, MIC: Minimum inhibitory concentration

shown that bromelain exerts effects against these intestinal pathogens.<sup>11,12</sup> This effect may be due to bromelain's interaction with intestinal secretory signaling pathways, which includes the adenosine 3':5'-cyclic monophosphatase, guanosine 3':5'-cyclic monophosphatase and calcium-dependent signaling cascades. Bromelain also has antiadhesion property which prevents the bacteria from adhering to specific glycoprotein receptors located on the intestinal mucosa.<sup>13</sup> Therefore, with the results of the present study, we can hypothesize that bromelain may prevent the attachment of bacteria, thereby exerting antibacterial action. However, since this is a preliminary study furthermore trials have to be conducted to validate this hypothesis.

### Conclusion

Plant extracts are widely used as antimicrobial agents. Bromelain is one such agent which has been widely used as anti-inflammatory drug in the field of medicine and dentistry. The results of the present study show its antibacterial efficacy also, however, more clinical trials have to be conducted in order to validate this hypothesis.

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