**Enzymatic Activity of Bromelain Isolated Pineapple (Ananas comosus) Hump and Its Antibacterial Effect on Enterococcus faecalis**

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**ABSTRACT**

**Background:** Enterococcus faecalis is the frequent cause of oral infections, such as periodontitis, infected root canals, and peri-radicular abscesses. Pineapple (Ananas comosus) fruit contains bromelain, one of proteolytic enzymes associated with several health benefits. Bromelain has been shown to promote healthy digestion, stimulate the immune system, improve cardiovascular conditions, and accelerate wound healing. Bromelain compounds possess anti-inflammatory and anticancer properties and exhibit antibacterial activity. **Objectives:** To analyze the enzymatic activity of bromelain extracted from pineapple hump and investigate the antibacterial effect of bromelain against *E. faecalis*.

**Methods:** Pineapple hump was dried and extracted with maceration technique. Further purification was obtained by ammonium sulfate fractionation, dialysis and ion exchange chromatography. Minimum inhibitory concentration (MIC) test using diffusion and dilution techniques tested the antibacterial activity of the bromelain extract towards *E. faecalis*. A one-way analysis of variance (ANOVA) test analyzed the significance of the differences in the *E. faecalis* inhibition zones after treatment with a range of bromelain extract concentrations. Differences were considered statistically significant if \( p < 0.05 \).

**Results:** The specific activity of bromelain in the crude extract was 62.89 U/mg. Furthermore, bromelain activity using ammonium sulfate fractionation was 50.99 U/mg, dialysis was 54.59 U/mg, and ion exchange chromatography was 152.38 U/mg. The bromelain extract showed effective inhibitory and bactericidal activity against *E. faecalis*. The results of the inhibition test using a bromelain extract purified by ion exchange chromatography demonstrated that a concentration as small as 12.5% was effective in inhibiting the growth of *E. faecalis* (\( p < 0.05 \)).

**Conclusion:** The highest enzymatic activity of bromelain was found after purification with ion exchange chromatography. Bromelain exerted an antibacterial effect against a potent endodontic pathogen, but further studies are needed to explore this effect.

Keywords: ammonium sulfate, bromelain, *Enterococcus faecalis*, ion exchange chromatography, pineapple hump.
Background

The primary etiologic agents of endodontic infections are bacteria, 90% of which are anaerobic. One of the pathogenic bacteria found in infected root canals is Enterococcus faecalis, a facultative anaerobic, Gram-positive, coccus-shaped bacteria with antibiotic resistance. Previous studies have shown that 63% of failed root canal treatment are caused by E. faecalis. While E. faecalis is only occasionally identified in primary endodontic infections, it is frequently detected in cases where the endodontic therapy has failed. E. faecalis is the most commonly isolated bacterial species in oral infections, including marginal periodontitis, infected root canals, and periradicular abscesses.

In-vitro studies have shown that an E. faecalis virulence factor may be related to the bacteria’s enhanced ability to invade dentinal tubules and adhere to collagen in the presence of human serum. Up to 40% of the E. faecalis cell wall consists of polysaccharides and peptidoglycans that protect the bacteria from the high osmotic pressure of the cytoplasm against the cell wall. Additional virulence factors allow E. faecalis to produce toxins, compete with other bacteria, and resist the host’s defensive mechanisms. The intrinsic resistance of enterococci to many commonly used antimicrobial agents may have given them a cumulative advantage in the acquisition of genes encoding resistance to aminoglycosides, penicillin, tetracycline, chloramphenicol, and vancomycin. By using fermentation, this bacteria can catabolize a wide spectrum of energy sources, including carbohydrates, glycerols, lactates, malates, and citrates. The process enhances the ability of E. faecalis to survive under the difficult nutritional conditions of an infected root canal.

Endodontic treatments are frequently administered to address root canal infections. The most commonly used irrigation solutions are sodium hypochlorite (NaOCl) and chlorhexidine (CHX). However, the recurrence of root canal infection remains high. Alternative therapies, such as those using herbs and fruits should be developed. Pineapple (Ananas comosus) is a common Indonesian fruit that thrives in tropical climates, especially in the equatorial region. Data from the Indonesia Commodity Futures Trading Regulatory Agency shows that in 2005 Indonesia was the second largest pineapple exporter in the world. Data from 2009 indicate that the country’s pineapple production was 1,558,196 tons. Desserts, canned foods, drinks, and jams are processed from the pineapple fruit, but the pineapple’s stems, leaves, outer skins, and humps have not been optimally developed.

Pineapple hump contains a proteolytic enzyme called bromelain that promotes healthy digestion, stimulates the immune system, improves cardiovascular conditions, and accelerates wound healing. Bromelain compounds have anti-inflammatory and anticancer properties and exhibit antibacterial activity. Sulphydryl and phosphoric acid groups are prevalent in bromelain compounds, and there is a frequent association with organic calcium. Bromelain includes peroxidases and protease inhibitors, and belongs to the peptidase class of hydrolases. Studies have shown that bromelain exerts an antibacterial effect against potent periodontal pathogens, such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. In the present study, ammonium sulfate fractionation was first used to increase enzyme concentration. Ammonium sulfate was selected because it has a high solubility in water, does not contain toxic substances, and has antibacterial properties. Ammonium sulfate also has protein solubility properties that allow it to polarize water molecules, engage in ionic interactions with salts, and repulse proteins of the same ion state. However, bromelain’s antibacterial effect on E. faecalis still needs to be explored. This study analyzed the enzymatic activity of bromelain in a crude pineapple hump extract and investigated the antibacterial effect of bromelain against the oral pathogen E. faecalis.

Material and Methods

The pineapple plants were analyzed by the Herbarium Bogoriense, Botanical Field of the Biological Research Center - LIPI Cibinong and confirmed to be Ananas comosus (L.) Merr. Syn. Ananas comosus (L.) Merr. f. sativus Schult. f. Mez. of the family Bromeliaceae. The species identification of the pineapple plants used in this study took place at the Aromatic and Medicinal Plant Research Center (BALITRO) in Bogor, Indonesia.
identification was based on a whole pineapple specimen with skins, fruit, humps, leaves, and roots. The extraction took place at the Botanical Field of the Biological Research Center - LIPI Bogor. The fractionation and chromatography took place at Laboratory of Industrial Engineering Development of Agro and Biomedicine (LAPTIAB), Puspitek Serpong, Indonesia.

An enzyme purification technique was needed to extract the bromelain from pineapple hump samples. The most common enzyme purification methods are ammonium sulfate fractionation, dialysis, ion exchange chromatography.

**Extract of Pineapple Hump by Maceration**

The pineapples for this study were obtained from the Ciapus plantation in the Curug Nangka area of Bogor Regency, Indonesia. A total of 100 pineapples with a gross weight of 30 kg were peeled, and the humps were collected. After peeling, the total weight of the humps was 7 kg. The humps were dried in an oven for three days at 40°C. After drying, the extract weight was 700 g. The hump was then macerated with water for 24 hours at 4°C and filtered to obtain 131.1 g of crude extract. During the maceration process, a solvent was used three times over the course of 24 hours. The extract was filtered off to determine the yield, and the remaining solvent was evaporated using a rotary evaporator (Buchi Rotavapor R-124) and a heater with a temperature of 40°C. The crude maceration extract was stored in the refrigerator (4°C).

**Ammonium Sulfate Fractionation**

Ammonium sulfate (5.35 g, 20%) (Merck, Germany) was gradually added to 50 mL of the crude pineapple hump extract that had been placed on an ice container. The mixture was shaken until the ammonium sulfate was completely dissolved. Then the solution was deposited overnight in the refrigerator (4°C). The next day, it was centrifuged at 3800 rpm at 4°C for 30 minutes. The supernatant and pellet were examined for enzymatic activity and protein content. As both the supernatant and pellet showed enzymatic activity and the presence of protein, the ammonium sulfate concentration was gradually increased. The procedure was repeated with ammonium sulfate concentrations of 30%, 40%, 50%, and 55%.

**Dialysis**

The pellet of ammonium sulfate fractionation (20% - 55% concentration) was collected and then diluted 20x with PBS with a total sample volume of 20 mL. Samples were inserted into the dialysis membrane bound together at both ends. Then, the membrane samples were inserted into 500 mL Erlenmeyer tube and 600 mL of buffer solution was added into tube and incubated for 18 hours in cold room (15°C), in orbital shaker with stirrer. After incubation, sample was put into a 50 mL tube and centrifuged 3800 rpm, 4°C for 30 minutes. Subsequently, each supernatant and pellets was tested using Amano. All experiment was done by duplicate.

**Ion Exchange Chromatography**

Bromelain has an optimum pH of 7, which is below the isoelectric pH of 9.5, so it is a positive ion (cation) that will bind to acid. A carboxymethyl cellulose (CMC) cation exchange column matrix was used to purify bromelain from the ammonium sulfate and dialysis samples.

The working phase settings of the ion exchange chromatography (GE AKTA Prime Plus, Minessota USA) were as follows:

1. Column Matric : CMC
2. Flow rate : 1 mL/mins
3. Fraction : 1 mL
4. Sample : 2 mL
5. PBS : 2 mL
6. Set of equilibration : 5 mL (column filled with buffer)
7. Set of sample : 2 mL
8. Set of wash 1 and 2 (PBS) : 5 mL
9. Set of eluent (NaCL in PBS) : 20 mL
10. Fraction tube : 25 tubes
The enzymatic activity of the ammonium sulfate, dialysis and ion exchange chromatography extracts was analyzed using the Amano method. A control microtube was prepared with 625 μL Trichloroacetic Acid (TCA) (Merck, Germany) and incubated at 37°C for 2 minutes. The bromelain extract (125 μL) was added to the microtube, and it was re-incubated at 37°C for 10 minutes. Casein (625 μL, 0.6%) (Calbiochem, US and Canada) was added to the mixture, and the microtube was re-incubated at 37°C for 10 minutes. The sample was centrifuged 11,000 rpm at 4°C for 5 minutes to separate a pellet from the supernatant. The filtrate (300 μL) was removed from the supernatant, and 750 μL of 0.55 M Na₂CO₃ (Merck, Germany) and 150 μL Follin (Merck, Germany) were added to the remaining pellet in the microtube. After another 10-minute incubation at 37°C, the absorbance was measured at 660 nm.

The enzymatic activity of the samples was measured by preparing microtubes as follows. Casein (625 μL, 0.6%) was incubated at 37°C for 2 minutes. Then 125 μL of bromelain extract was added, and the mixture was re-incubated at 37°C for 10 minutes. Next, 625 μL TCA was added to the microtube, and it was re-incubated at 37°C for 10 minutes. The sample was centrifuged at 11,000 rpm at 4°C for 5 minutes, separating a pellet from the supernatant. Filtrate of 300 μL was removed from the supernatant, and 750 μL of 0.55 M Na₂CO₃ and 150 μL Follin were added to the pellet. The microtube was again incubated at 37°C for 10 minutes. The absorbance was measured at a wavelength of 660 nm.

To prepare a standard for the enzymatic activity measurement, 300 μL tyrosine (10 μg/mL) (Merck, Germany), 750 μL 0.55 M Na₂CO₃, and 150 μL Follin were added to a microtube that was then incubated at 37°C for 10 minutes. The absorbance was measured at a wavelength of 660 nm. To prepare the blank for the enzymatic activity measurement, 300 μL 0.1 M HCl, 750 μL 0.55 M Na₂CO₃, and 150 μL Follin were added to a microtube, which was incubated at 37°C for 10 minutes. The absorbance was measured at a wavelength of 660 nm. The enzymatic activity tests on the control, sample, standard, and blank were performed in duplicate.

The enzymatic activity was calculated according to the following formula:

\[
\text{Protease Activity (U/mL)} = \frac{A1 - A2}{A3 - A4} \times 3 \times \frac{1.375}{0.3} \times \frac{1}{10} \times \frac{\text{Dm}}{\text{Ve}}
\]

- \(A1\) : Sample absorbance
- \(A2\) : Control absorbance
- \(A3\) : Standard absorbance
- \(A4\) : Blank absorbance
- \(3\) : Quantity of tyrosine per 0.3 mL standard solution of tyrosine (µg)
- \(\frac{1.375}{0.3}\) : The final volume of the reaction mixture
- \(10\) : Reaction time
- \(\text{Dm}\) : Dilution factor
- \(\text{Ve}\) : Enzyme volume (mL)

**Minimum Inhibitory Concentration (MIC) Test**

**Bacterial Culture**

*E. faecalis* ATCC 29212 was inoculated into brain heart infusion (BHI) broth and incubated for 24 hours at 37°C under anaerobic conditions using a gas pack jar system. The suspension was diluted to achieve an optical density (OD) of 0.25 (10⁷ CFU/mL).

**Dilution Technique**

Bromelain extract purified from the ion exchange chromatography (1 g, 1000 mg/mL) was diluted into 100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125% concentrations using phosphate buffer saline (PBS) and placed into a 5 mL tube in the ratio of 1 mL extract: 1 mL of bacteria (10⁷ CFU/mL). CHX (0.2%) was used as the positive control, and a tube without bromelain extract was used as the negative control. All the tubes were vortexed and incubated for 24, 48, and 72 hours at 37°C; then the turbidity was observed spectrophotometrically. The MIC results were determined by evaluating the lowest bromelain concentrations that could inhibit bacterial growth.
Aliquots of *E. faecalis* (10^7 CFU/mL) were removed from the culture suspension using a 10 μL sterile micropipette, poured onto a petri dish containing agar medium with sucrose, and flattened using a spreader. In each petri dish, 6 wells were created using a perforator with a diameter of 6 mm. Each well was filled with 50 μL of bromelain extract purified from the ion exchange chromatography at different concentrations (100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125%). PBS was used as negative control, and chlorhexidine (CHX) was used as positive control. Subsequently, all petri dishes were placed in the anaerobic jar and incubated for 72 hours at 37°C in an anaerobic atmosphere using the gas-pack jar system. After 72 hours, the inhibition zone was measured using a digital caliper (with a length of 0.01 mm) to define the antibiotic activity. Each experiment was performed in triplicate.

**Statistical Analysis**

The one-way ANOVA test was used to determine the significance of the differences in the *E. faecalis* inhibition zones after treatment with the pineapple hump extract at different concentrations and treatment times. The differences were considered statistically significant if *p*<0.05. The statistical calculations were performed using the SPSS Statistics for Windows software version 20 (IBM, USA).

### Table 1. Results of the enzymatic activity test on the crude bromelain extract with dilutions of 40x, 30x, 20x, 10x, and 5x.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Sample (µL)</th>
<th>PBS (µL)</th>
<th>Average ± SD</th>
<th>Control</th>
<th>Activity test (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40x</td>
<td>25</td>
<td>975</td>
<td>0.48 ± 0.01</td>
<td>0.31</td>
<td>16.59</td>
</tr>
<tr>
<td>30x</td>
<td>33.33</td>
<td>966.7</td>
<td>0.54 ± 0.00</td>
<td>0.48</td>
<td>5.84</td>
</tr>
<tr>
<td>20x</td>
<td>50</td>
<td>950</td>
<td>0.71 ± 0.01</td>
<td>0.54</td>
<td>7.19</td>
</tr>
<tr>
<td>10x</td>
<td>100</td>
<td>900</td>
<td>1.37 ± 0.06</td>
<td>0.74</td>
<td>62.89</td>
</tr>
<tr>
<td>5x</td>
<td>200</td>
<td>800</td>
<td>2.55 ± 0.01</td>
<td>2.43</td>
<td>12.49</td>
</tr>
</tbody>
</table>

**Result**

### Amano Result from Crude Extract

In the enzymatic activity test, the highest value for the crude pineapple extract was 62.89 U/mL (10x dilution), while the lowest value was 5.84 U/mL (30x dilution). The 20x dilution provided the most appropriate OD value for a test using a spectrophotometer set at 660 nm, that is 0.71 with an enzymatic activity of 7.19 U/mL (Table 1).

### Amano Result from Ammonium Sulfate Fractionation

The results of the enzymatic activity test on the ammonium sulfate fractionation samples showed the highest activity in the pellet that precipitated at the 20% ammonium sulfate concentration, indicating that the protein bound to the precipitate. The lowest activity value occurred at the 55% ammonium sulfate concentration, indicating that most of the protein had already precipitated; thus the enzymatic activity in the pellets and supernatant decreased (Table 2).

### Amano Result from Dialysis

The enzymatic activity test results after dialysis showed that in supernatant tube the enzymatic activity was 2.29 U/mL, while in pellets tube the enzymatic activity was found to be 54.59 U/mL (Table 3).
**Table 2.** The results of the enzymatic activity test on the various concentrations of the ammonium sulfate fractionation (sn = supernatant, p = pellet).

<table>
<thead>
<tr>
<th>Concentrate</th>
<th>Solution (mL)</th>
<th>Ammonium sulfate (g)</th>
<th>Average ± SD</th>
<th>Control</th>
<th>Activity test (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% sn</td>
<td>50</td>
<td>5.35</td>
<td>0.953 ± 0.001</td>
<td>0.744</td>
<td>20.89</td>
</tr>
<tr>
<td>20% p</td>
<td></td>
<td></td>
<td>0.548 ± 0.001</td>
<td>0.238</td>
<td>50.99</td>
</tr>
<tr>
<td>30% sn</td>
<td>48</td>
<td>2.688</td>
<td>0.856 ± 0.003</td>
<td>0.656</td>
<td>19.99</td>
</tr>
<tr>
<td>30% p</td>
<td></td>
<td></td>
<td>0.693 ± 0.001</td>
<td>0.299</td>
<td>41.38</td>
</tr>
<tr>
<td>40% sn</td>
<td>46</td>
<td>2.622</td>
<td>0.537 ± 0.006</td>
<td>0.307</td>
<td>23.04</td>
</tr>
<tr>
<td>40% p</td>
<td></td>
<td></td>
<td>0.481 ± 0.002</td>
<td>0.227</td>
<td>25.44</td>
</tr>
<tr>
<td>50% sn</td>
<td>44</td>
<td>2.596</td>
<td>0.424 ± 0.001</td>
<td>0.270</td>
<td>15.44</td>
</tr>
<tr>
<td>50% p</td>
<td></td>
<td></td>
<td>0.343 ± 0.003</td>
<td>0.293</td>
<td>4.99</td>
</tr>
<tr>
<td>55% sn</td>
<td>42</td>
<td>1.5</td>
<td>0.341 ± 0.001</td>
<td>0.311</td>
<td>2.99</td>
</tr>
<tr>
<td>55% p</td>
<td></td>
<td></td>
<td>0.296 ± 0.002</td>
<td>0.256</td>
<td>3.99</td>
</tr>
</tbody>
</table>

**Table 3.** The results of the enzymatic activity test after dialysis.

<table>
<thead>
<tr>
<th></th>
<th>Average ± SD</th>
<th>Control</th>
<th>Activity test (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>0.345 ± 0.001</td>
<td>0.322</td>
<td>2.29</td>
</tr>
<tr>
<td>Pellets</td>
<td>0.652 ± 0.002</td>
<td>0.406</td>
<td>54.59</td>
</tr>
</tbody>
</table>
Amano Result from Ion Exchange Chromatography

The enzymatic activity test results after the ion exchange chromatography showed that tube 24 had the highest enzymatic activity at 152.38 U/mL, while the lowest activity was found in tube 22 with a value of 60.69 U/mL. The activity for tube 23 was 118.98 U/mL, and the value for tube 25 was 131.39 U/mL (Table 4).

Results of the MIC Dilution Technique

The results of the bacterial growth inhibition test showed a negative concentration of *E. faecalis* in the tubes with 100%, 75%, 50%, 25%, and 12.5% of the bromelain extract for the 24 to 72-hours incubation period. A positive concentration was seen in the 12.5% extract after 72 hours of incubation, while the 3.125% solution was positive after 48 hours of incubation (Table 5).

**Results of the MIC Diffusion Technique**

The bromelain enzymes showed effective inhibitory and bactericidal activity against *E. faecalis* at low concentrations. The inhibition test used the bromelain extract from *Ananas comosus* with an enzymatic activity value of 61.50 U/mL and a specific activity of 6.60 U/mg. The results showed that a concentration as small as 12.5% was effective in inhibiting the growth of *E. faecalis*, generating an inhibition zone of 8.8 ± 0.02 mm (Fig. 1, 2).

<table>
<thead>
<tr>
<th>Tube</th>
<th>Average ± SD</th>
<th>Control</th>
<th>Activity test (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>0.755 ± 0.003</td>
<td>0.148</td>
<td>60.69</td>
</tr>
<tr>
<td>23</td>
<td>1.314 ± 0.001</td>
<td>0.124</td>
<td>118.98</td>
</tr>
<tr>
<td>24</td>
<td>1.679 ± 0.014</td>
<td>0.155</td>
<td>152.38</td>
</tr>
<tr>
<td>25</td>
<td>1.537 ± 0.002</td>
<td>0.223</td>
<td>131.39</td>
</tr>
</tbody>
</table>

**Table 4.** The results of the enzymatic activity test after ion exchange chromatography of the bromelain extract.

<table>
<thead>
<tr>
<th>Concentrate (%)</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>75</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.5</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6.25</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3.125</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Control +</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control -</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

**Table 5.** The results of the antibacterial assay dilution with various concentrations of bromelain extract purified from the ion exchange chromatography (100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125%), a positive control (chlorhexidine), and a negative control (PBS).
Figure 1. The inhibition zones (mm) of *E. faecalis* after treatment with different concentrations of bromelain extract purified from the ion exchange chromatography (100%, 50%, 25%, 12.5%, 6.25%, and 3.12%).

Figure 2. The results of the bacterial growth inhibition diffusion test showing *E. faecalis* cultures after treatment with different concentrations of pineapple hump extract after 72 hours of incubation. (A) concentration of 100% (21.82 ± 1.24 mm), (B) concentration of 50% (17.67 ± 0.97 mm), (C) concentration of 25% (13.78 ± 0.62 mm), (D) concentration of 12.5% (8.84 ± 0.02 mm), (E) concentration of 6.25% (0 mm), (F) concentration of 3.125% (0 mm), (G) positive control (32.3 ± 1.54 mm), and (H) negative control (0 mm).
Discussion

This study analyzed the enzymatic activity of bromelain in a crude pineapple hump extract. The highest enzymatic activity of bromelain was found after purification with ion exchange chromatography. The first extraction step using maceration technique. Maceration with water-based solvents is commonly used to obtain crude extracts from plants. Natural materials are preferred in many health-related fields due to their efficacy and safety. Each natural substance has an active component that is responsible for its beneficial effect. Solvents are often required to isolate and purify these active components, maximizing the compound’s benefits and minimizing any side effects.24

At temperatures as low as 4°C, enzyme proteins interact to form clumps and precipitates, protecting them from being denatured. This is an optimal temperature for protein purification with ammonium sulfate fractionation. Any excess ammonium sulfate salt can be separated through protein dialysis. The salt molecules diffuse out of the dialysis pouch because their small molecules tend to diffuse into solutions with lower concentrations.25 In the present study, the specific activity value obtained after ion exchange chromatography was 152.38 U/mg, higher than the specific activity after ammonium sulfate fractionation. The chromatography purification removed residual salts and other small molecules, purifying the proteins and increasing the enzymatic activity.

Bromelain proteases tend to chelate metal ions. Previous research has indicated that this chelation can be controlled, increasing enzymatic activity. As metal ions can be released into the mouth from orthodontic treatments and oral prostheses,26 the potential oral use of bromelain should consider that the presence of metal ions may decrease its enzymatic activity.

The present study used ion exchange chromatography as the primary purification method. This technique separates proteins based on the molecular load, using pH, optimum temperature, and acidity levels to differentiate proteins with similar positive and negative ions or isoelectric pH (pl). Positive ions (cations) will bind to acid molecules, while negative ions (anions) will bind to basic molecules. Proteins that have a pH above the pl value are designated as negative ion (anions) and can be purified with an anion exchange matrix, i.e., diethyl aminooethyl (DEAE) cellulose and DEAE sephadex. Proteins that have a pH below the pl value are positive ions (cations) and can be purified with a cation exchange matrix, i.e., carboxymethyl cellulose (CMC).27

This study investigated the antibacterial effect of bromelain against E. faecalis as the root canal pathogen. The results of the dilution test revealed the lowest bromelain extract concentrations that inhibited E. faecalis growth (i.e., where the microtube remained clear when observed under the light). Concentrations of 100%, 75%, 50%, and 25% that were incubated with bacteria for 24, 48, and 72 hours remained clear. The bromelain extract showed the ability to inhibit E. faecalis growth at concentrations as low as 25% over a 72-hour incubation period and as low as 12.5% over incubations of 24 and 48 hours.

Many studies have focused on the antimicrobial effects of herb and fruit extracts. Bromelain has been shown to effectively inhibit the growth of Staphylococcus aureus, Trichurismuris, Heligmosomoidespolygyrus, Escherichia coli, and Vibrio cholera.28-31 In vitro studies have demonstrated that bromelain also has the ability to inhibit the growth of Candida albicans.21 As another example, anthocyanins in grape skin (Vitis vinifera L. var. Alphonse Lavallee) have inhibited the growth of Bacillus cereus, Micrococcus luteus, and Escherichia coli.32 Other studies have reported that strawberry juice has an antibacterial effect against biofilms of E. faecalis and P. gingivalis.33

The results of the diffusion assay showed that bromelain can inhibit E. faecalis growth at a range of concentrations. The minimum concentration that inhibited the growth of E. faecalis, indicated by the formation of a clear zone during the 72-hour incubation period, was 12.5%. Bromelain is believed to act by disrupting the peptidoglycan and polysaccharide components of bacterial cell membranes, causing an instability in the membrane’s electrostatic forces.34 Bromelain also denatures membrane proteins by forming complex compounds with them, disrupting their chemical structure, causing protein...
coagulation, and cell membrane lysis. Bromelain remains a promising antibacterial candidate against oral pathogens. Further stability and sensory studies of the enzymes in this extract are needed.

Bromelain is a sulfhydryl proteolytic enzyme that can hydrolyze bacterial membrane proteins. Bromelain’s enzymatic activity is not affected by protein chemicals or colored chemicals, thus it has stable protein bonds. In medicinal applications, bromelain has shown therapeutic use in inhibiting platelet aggregation, improving cardiovascular conditions, boosting the immune system, aiding in digestion, and accelerating healing from injuries. It has anti-inflammatory, antitumor, antibacterial, and immune modulating properties. Bromelain has the potential to reduce plaque formation in the teeth. This study showed that bromelain also has antibacterial activity against *E. faecalis*.

**Conclusion**

Bromelain was isolated from crude pineapple hump extract by ammonium sulfate fractionation and ion exchange chromatography. The highest bromelain enzymatic activity was obtained after purification by ion exchange chromatography. Purified bromelain had an antibacterial effect on the oral pathogen *E. faecalis*. Further studies are needed to explore this effect on other oral bacteria.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

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