Frankincense (*Boswellia serrata*) Extract Effects on Growth and Biofilm Formation of *Porphyromonas gingivalis*, and Its Intracellular Infection in Human Gingival Epithelial Cells

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OBJECTIVE

Traditional antibiotics have been commonly used to treat several infectious diseases for decades, however, misuse and over-prescription have been escalating antibiotic resistance episodes. *Boswellia serrata* extract, derived from Boswellia trees prevalent in the Middle East, Africa, and Asia, offers multifaceted therapeutic benefits, including anti-cancer, anti-inflammatory, and anti-microbial effects. Periodontitis affects 20-50% of the global population, targeting the gingival tissues, supporting structures around teeth, and the alveolar bone. *Porphyromonas gingivalis* and *Fusobacterium nucleatum* are two prominent bacterial culprits in this pathogenesis. The objective of this study was to explore the potential effects of *B. serrata* extracts against *P. gingivalis* and *F. nucleatum* growth in vitra and infection in human epithelial oral cells and determine whether *B. serrata* could be a promising alternative for treating periodontitis without relying on antibiotics.

METHODS

Minimum Inhibitory Concentration

Freshly grown *P. gingivalis* or *F. nucleatum* were added to wells in 96-well plates at a final concentration of 5 x 10^5 CFU/mL. The bacteria were incubated under anaerobic conditions with serial dilutions ranging from 512 ug/mL to 0.25 ug/mL of *B. serrata* extract. After 48 hours, the growth and turbidity were assessed by measuring the optical density at 550 nm.

Biofilm Formation and Reduction Assays

Biofilm formation of *P. gingivalis* or *F. nucleatum* was assessed using the crystal violet staining assay. *B. serrata* extract was added to all wells in serial dilutions (ranging from 128 ug/mL to 0.25 ug/mL) in 24-well plates. Bacteria were added at a concentration of 1 x10^7 CFU/mL. Positive controls used were 100 U/mL of penicillin and 100ug/mL of streptomycin. Bacteria broth was used as a negative control. After 48 hours of anaerobic incubation, the supernatant was removed and non-adherent bacteria was rinsed, and adhered biofilms were fixed with methanol, stained with crystal violet, and dissolved with methanol. Optical density values at 560 nm were measured using a microplate reader.

Lactate Dehydrogenase Quantification

Cell viability was assessed by lactate dehydrogenase levels measured spectrophotometrically. After following the manufacturer's instructions, absorbance values were recorded at 490nm and 680nm.

Antibiotic Protection Assay

GECs were seeded in 6-well plates and infected with *P. gingivalis* at an MOI of 100, for 2 hours, at 37°C, 5% CO_2. Cells were washed and treated with metronidazole and gentamicin for 1h. After incubation, antibiotics were removed and cells were washed followed by an incubation with or without *B. serrata* at different concentrations (ranging from 16 – 0.25ug/mL). Sterile distilled water was added into wells to lyse the cells and 50uL of each cell lysate was plated onto*Brucella* Blood Agar Plate. Plates were incubated for 10 days, then CFUs were quantified.

Immunostaining for *P. gingivalis*

GECs were seeded onto coverslips in 24-well plates and infected for 2h. Then, the cells were treated with antibiotics for 1h, then incubated for 21h. Infected cells were washed and fixed using absolute cold methanol for 10min. Then, cells were permeabilized and blocked at 4°C overnight. Cells were incubated with primary rabbit polyclonal antibody anti-*P. gingivalis* at 4°C overnight. After washes with PBS, secondary goat anti-rabbit IgG was added into the wells for incubation for 2h. Coverslips were washed, counterstained, and mounted on a slide and images were acquired to be used to quantify the level of infection with immunostained *P. gingivalis*.

RESULTS

B. serrata Extract Differentially Impacts the Growth of P. gingivalis and F. nucleatum

Organisms	MIC (µg/mL)
P. gingivalis ATCC 33277	32
F. nucleatum ATCC 22586	>512

MIC, Minimum inhibitory concentration.

Table 1. Results show an average of n = 4 experiments.

B. serrata Extract Inhibits P. gingivalis and F. nucleatum Biofilm Formation

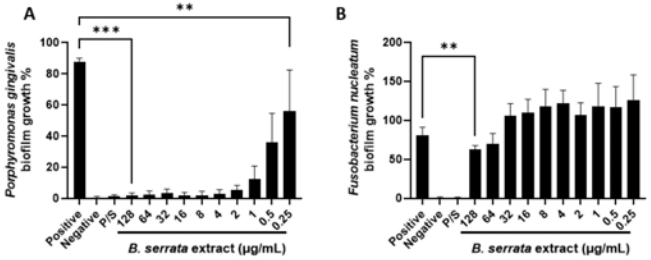

Figure 1. Graphs show biofilm growth percentage for (A) *P. gingivalis* and (B) *F. nucleatum*. Results were normalized to the positive control (only bacteria without treatments). Negative control: only media. P/S: penicillin/streptomycin. $n = 4$. ** $p \leq 0.01$, *** $p \leq 0.001$.

B. serrata Extract Induces P. gingivalis Biofilm Reduction

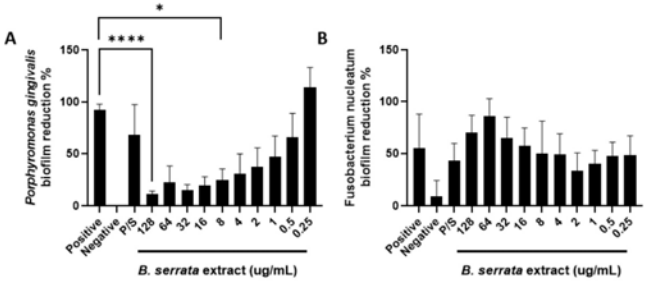

Figure 2. Graphs show biofilm growth percentage for (A) *P. gingivalis* and (B) *F. nucleatum*. Results were normalized to the positive control (only bacteria with no treatments). Negative control: only media. P/S: penicillin/streptomycin. n = 3. * $p < 0.05$, **** $p < 0.0001$.

B. serrata Extract at Low Doses Is Not Toxic to Human Gingival Epithelial Cells

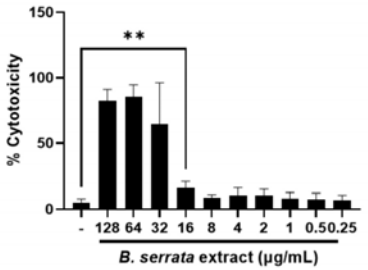

Figure 3. Graph shows cytotoxicity percentage, normalized to the positive control (untreated cell incubated with lysis buffer). n = 3. ** $p < 0.001$.

B. serrata Extract Decreases Intracellular P. gingivalis Infection in Human Gingival Epithelial Cells

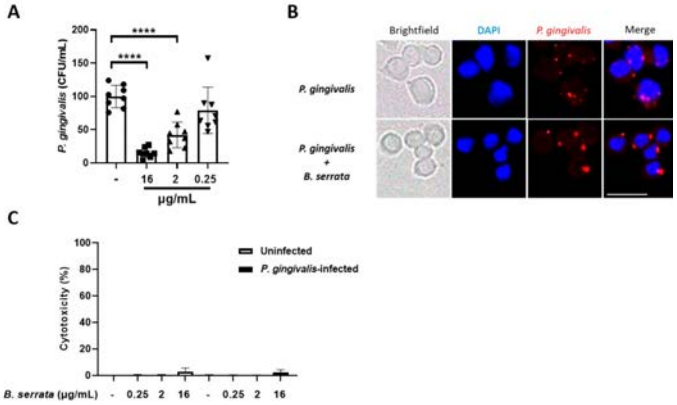

Figure 4. (A) After 21 h incubation with B. serrata extract, cells were used for detection of P. gingivalis by fluorescence microscopy (B), and the supernatant was used for LDH quantitation (C). n = 3. Scale bar = 100 µm. **** $p < 0.0001$.

CONCLUSION

We found the extract to be more effective at inhibiting biofilm growth in P. gingivalis than *F. nucleatum*. We also found that the extract was better at reducing biofilm formation in P. gingivalis than *F. nucleatum*. We also found *B. serrata* extract significantly reduced *P. gingivalis* infected human GECs without harming the host cells.