

Determining The Effects of Fulvic acid on Biofilm/Planktonic *Streptococcus Mutans* Growth **Mark Botros**¹, and Richard L. Gregory²

¹Purdue School of Science, Indiana University-Purdue University Indianapolis; ²Departments of Oral Biology/Pathology and Laboratory Medicine, Indiana University Schools of Dentistry and Medicine,

Fulvic acid, a major organic compound extract of Shilajit has been the focus of dental research for the past few years. Shilajit, a sticky tar-like substance of dark brownish color, was used during the ancient times, thousands of years ago and continues to be the traditional method today in India to aid with curing bone/cartilage diseases. Shilajit has also been proven to have anti-inflammatory and pain suppressing effects. This experiment determined the minimum inhibitory concentration (MIC), which is the lowest concentration of fulvic acid, an active component of shilajit that inhibits the visible growth of S. mutans. This experiment also determined the minimum bactericidal concentration (MBC) which is the lowest concentration of fulvic acid that kills S. mutans. A 3-day procedure to determine the growth vs inhibition of the S. mutans was conducted and bacterial readings were recorded using a spectrophotometer after treating S. mutans with 10% formaldehyde, crystal violet stain, and iso-propanol with 30-45 minute incubations between each. The experiment determined that very high concentrations of fulvic acid killed S. mutans, while less concentrated fulvic acid inhibited the growth of S. mutans bacterial cells. A solution comprised of a 5% concentration of fulvic acid killed all of the S. mutans; 5.00%, 2.50%, and 1.25% fulvic acid concentrations had bacterial absorbance of 0.000, 0.009, and 0.027, respectively, as compared to the control group's normal bacterial growth absorbance of 0.254. Additionally, solutions ranging from a two-fold dilution of fulvic acid to six-fold dilution of fulvic acid inhibited the growth of S. mutans. A similar trend was also observed in planktonic and biofilm formation. For all of the above, in the seventh and eighth dilution (0.078% and 0.039% respectively) of the fulvic acid, the growth of S. mutans bacteria was similar to the control group due to the level of dilution. Overall it was observed that fulvic acid is able to kill bacteria in strong concentrations. Additionally it is able to inhibit further growth of bacteria in lower concentrations, but once the solution becomes too dilute, it does not have an effect on bacterial growth. This contributes greatly to the field of oral health because this data can be utilized for further research on oral bacterial growth inhibitors. Furthermore, the data collected here is a significant starting point for research on the specific minimum concentrations necessary to inhibit oral bacteria growth, because this can be used to determine the smallest amounts of fulvic acid, the bacteria the human body can handle.

Mentor: Richard L. Gregory, Department of Oral Biology, Indiana University School of Dentistry