An *In Vitro* Study on The Potential Antiplaque Effect of *Piper Betle* and *Psidium Guajava*

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This study was carried out in an attempt to seek for new agents from plant extracts for use in plaque control. Scientific data gathered on the properties of the extract towards oral microbes were used in designing experimental approaches to elucidate the potential of the extracts as antiplaque agents. The emphasis of this research was directed at the initial stage of dental plaque formation, specifically the adherence of bacteria, referred to as the early plaque colonizers, to the acquired pellicle on the supragingival tooth surface. *Streptococcus sanguis*, *Streptococcus mitis* and *Actinomycetes* sp. were identified as the predominant bacteria involved in this initial stage and were used as test organisms throughout the study. The aqueous extracts of six local plants were systematically screened for their antimicrobial activity and two, namely *Piper betle* Linn. and *Psidium guajava* L. were selected for further testing based on their positive antimicrobial activities. Both extracts were shown to have MIC values within the range of 2.61 to 4.69 mg/ml, with *Actinomycetes* sp. at the lower and *S. sanguis* at the higher ends. They were found to have LC30 (3.6 mg/ml for *Piper betle* and >5 mg/ml for *Psidium guajava*) and EC50 (not determinable for *Piper betle* and 51.5 µg/ml for *Psidium guajava*) that were well above the concentration level considered to be toxic. *Piper betle* and *Psidium guajava* extracts were shown to have fluoride content of 9.25 ppm and 11.5 ppm, respectively. Some aggregating activity was also displayed by the extract of the latter.

The anti-adherence activity of *Psidium guajava* was shown to be slightly more potent than that of *Piper betle*. At 4 mg/ml (MIC range), the former was able to reduce the adhering capacity of the experimental pellicle to *S. sanguis*, *S. mitis* and *Actinomycetes* sp. by 28.1%, 48.8% and 40.2%, respectively compared to the latter (26.5%, 14.8% and 36.2%, respectively). Both extracts also exhibited the ability to reduce the cell surface hydrophobicity of each of the early colonizers which may render them to become less adherent. With less capacity to bind to the experimental pellicle and surface hydrophobicity of its cell reduced, the colonization of the early colonizers during the early phase of plaque formation may be minimised.

*Piper betle* and *Psidium guajava* extracts also influenced the growth profiles of the bacteria by firstly, extending their generation times and secondly, by reducing their specific growth rates. The generation time of *S. sanguis* and *S. mitis* were increased by 12.0- and 10.4-fold respectively, in the presence of *Piper betle*, and by 1.8- and 2.6-fold respectively, in the presence of *Psidium guajava*. The effect on the growth of *Actinomycetes* sp. was at a much lower magnitude. These findings were reaffirmed by the electron micrographs obtained from the ultrastructural studies using the SEM. Under the effect of the *Piper betle* extract, most of the cells had maintained their normal sizes but their population was greatly reduced. Under the effect of *Psidium guajava* extract however, the population was not very much affected but the sizes of most of the cells were greatly diminished. An addition to that *Psidium guajava* extract also showed aggregative effect on the cells causing them to clump in aggregates.

Extension of the generation time may have been accentuated by the presence of fluoride in the extracts which may have interfered with the energy-generating system of the bacteria. For *Psidium guajava*, the extended generation time may have also been due to the aggregating activity of the extract.

As a consequence of the suppressed growth, the bacterial cells were unable to divide or grow successfully, hence the bacteriostatic effect of the extracts.

All the properties exhibited by the plant extracts towards the early plaque colonizers may potentiate their antiplaque activities.

Expression of Telomerase and Evaluation of hTERT, p53 and pRb Expression in Oral Carcinogenesis

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Oral carcinogenesis is a multi-step process involving distinct genetic alterations. Alterations in tumor suppressor genes, namely *TP53* and *Rb*, have been implicated in oral carcinogenesis. Also, telomerase which is a ribonucleoprotein complex with core units of human telomerase RNA (hTR) and human telomerase reverse transcriptase (hTERT) has been found activated in oral precancer and cancer. Their role as potential tumor markers still remains unclear.