6. SUMMARY

Oral health is important to general health and wellbeing of human beings. Dental caries, an infectious microbiologic disease causing teeth’s calcified tissue localized dissolution and destruction affect peoples of all ages throughout the world. Although these diseases have affected human beings since time immemorial, now a day its prevalence is greatly increased depicting them as a pending public health problem. Natives of India well exploited the therapeutic potential and medicinal efficacy of its plant wealth greatly for curing oral diseases and maintaining oral health. The dental caries preventing ethnomedicinal plants can be used for these difficult to eradicate disease causing organisms. Biofilm specific inhibitory methods and pharmaco-chemical studies of these plants may lead to identifying better drugs of high potential and very specific to the caries treatment and oral health maintenance.

Hundred (42 males and 58 females) samples were collected during one year collection period between Sep 2009 and Sep 2010 from caries infected patients attending outpatient ward of Government Head Quarters Hospital, Erode Tamilnadu, India. From them, 189 isolates were isolated which were identified presumptively as Streptococcus spp (28%), Lactobacillus spp (21%), Staphylococcus spp (16%), Klebsiella spp (12%), and Kurthia spp (8%). They were analyzed for the dental caries causative’s primary requirement of biofilm production by three different methods. The ability to form biofilm by Christensen's test-tube method, colonies slime production by Congo red agar method, quantification by micro-titer plate assay and are ranked accordingly. All these three methods yielded frequent positive results for most of the dental cariogenic isolates. From this, high biofilm producing five virulent isolates were selected and subjected to taxonomic characterization and species level identification. These isolates were identified as Streptococcus mutans, Lactobacillus casei, Staphylococcus aureus, Klebsiella pneumoniae and Kurthia gibsonii. The two dominant Lactobacillus casei, Staphylococcus aureus, and the previously not reported Kurthia gibsonii were submitted to MTCC, Institute of Microbial Technology, Chandigarh, India. They confirmed the identity by phenotype characterization. The monomeric sugar composition of their exopolysaccharides were determined using HPLC which revealed that the glucose and sucrose are the primary sugars the Streptococcus mutans EPS. EPS of Lactobacillus casei
contains glucose and lactose where as the EPS of *Staphylococcus aureus* composed of sucrose and glucose. *Kurthia gibsonii* EPS contains predominantly lactose and glucose. Glucose and sucrose are found to be the main sugars of *Klebsiella pneumoniae* biofilm exopolysaccharide. The antimicrobial susceptibility determined by Kirby Bauer-disk diffusion method as per the recommended CLSI standard revealed the antimicrobial resistance of these selected cariogenic biofilm forming organisms. The epifluorescence microscope image also exhibits the *in vitro* biofilm forming nature of these isolates. It also justifies the rationale of the study searching efficient anticaries and biofilm inhibiting compound from less explored ethanomedicinal plant.

Ethnobotanical survey was conducted among traditional healers of Malayalies tribals living in medicinal plant rich Kolli hills, Namakkal District, Tamilnadu, Southern India who was trained by traditional ways between January 2011 and March 2011. This survey identified the use of fifteen plants from twelve families for dental diseases treatment and general oral healthcare maintenance. They were collected and the parts used were successively extracted with hexane, chloroform, ethyl acetate and methanol solvents. They were screened for their anticaries activity against the selected cariogenic biofilm forming bacterial isolates. They exhibit anticaries activity in the order of *Azadirachta indica* > *Lawsonia innermis* > *Mimusops elengi* > *Psidium guajaya* > *Tephrosia purpurea* > *Achyranthes aspera* > *Acacia arabica* > *Argemone mexicana* > *Ficus bengalensis* > *Solanum incanum* > *Albizia lebbeck* > *Solanum nigrum* > *Mangifera indica* > *Mentha arvensis* > *Mimosa pudica*. Amongst them, the voluminous work reported *A. indica* and *L. innermis* were excluded, *M. elengi* (leaves) were chosen for further detailed investigation. The highly active ethyl acetate extract was subjected to bioactivity guided fractionation which led to the isolation of their active principles. Upon physical and spectroscopic investigation (UV, IR, $^1$H NMR, $^{13}$C NMR, 2D NMR (HMBC) and GC-MS analysis, the purified compounds were identified as chondrillasterol and myricetin-3-O-α-L-rhamno-pyranoside.

Anticaries activity of the two isolated compounds and their determined MIC revealed that chondrillasterol exhibits high inhibitory activity on *L. casei*, *S. mutans* and *S. aureus* followed by *K. gibsonii* and *K. pneumoniae*. The myrecitin-3-O-α-L-rhamnopyronoside exerted the similar pattern of inhibition but, they are more active even
in low concentration in comparison with chondrillasterol. The high \textit{in silico} binding energies obtained indicates their efficient interaction with various targets of cariogenic organisms and their role in pain reduction.

The performed microtiter plate analysis and modified gradient-plating methods revealed their antibiofilm activity, concentration dependent inhibition and interference on the exopolysaccharide synthesis and biofilm formation. 25 \textmu g/ml of chondrillasterol and 12.5 \textmu g/ml of myricetin-3-O-\alpha-L-rhamno-pyranoside inhibit 80-94\% of biofilm formation. The epi-fluorescence microscopy studies revealing \textit{in vitro} biofilm and bacterial adherence inhibition also confirmed the bioactivity of these compounds.

Although the leaves of \textit{M. elengi} have been studied extensively for its other biological activity, according to our knowledge, this is the first of kind reporting the cariogenic inhibitory potential of its compound through biofilm specific studies. Hence, further studies may broaden the application of these compounds in developing new approaches and applications for the prevention and controlling diseases associated with microbial colonization of the teeth. Moreover our findings, also supports scientific validation of tradition knowledge and use of these ethnomedicinal plants for oral care maintenance. However, these findings emphasize a need for future studies examining the effect of chondrilasterol and myricetin 3 rhamnoside on global gene expression against bacterial behaviors.