5. DISCUSSION

Dental biofilm, the pale yellow plaque that develops naturally on the teeth are the cause of all dental diseases including caries. The ability to form biofilm is essential for oral bacteria to cause disease, and growth as a biofilm affords many advantages to bacteria. Most importantly in the oral cavity, failure of the bacterium to attach and grow as a biofilm will result in rapid clearance. Hence like any other environment, the bacteria in the highly dynamic oral environment also trying to attach them to the tooth's smooth surface and colonize them as a means of adaptation to an oral environment. These attached bacteria forms biofilm, live and develop in communities which are an essential property for dental plaque formation (Zambori et al., 2012). It is believed that an irritating matter between teeth is the source of dental diseases and dental plaque which is naturally pathogenic (Berchier et al., 2008). It is by and large accepted that oral hygiene maintenance through regular removal of dental plaque and food deposits are dental caries and periodontal disease preventing indispensable factor (Rasingam et al., 2012).

Toothbrush is the very much essential and most often applied tool for everyday dental care. They are habitually used for several weeks or even months, leading to colonization with the user’s own oral bacterial flora comprising facultative pathogenic bacteria. The retention and survival of microorganisms on toothbrushes pose a recontamination threat particularly for those patients who are at risk for systemic infections originating from the oral cavity, e.g., after T-cell depleted bone marrow transplantation. Continuous re-exposition by tooth brushing may maintain persisting oral infections or lead to (auto-) reinfections. Superior cleaning efficiency of electric toothbrushes makes them as more frequently used one for mechanical reduction of dental plaque. However, their mechanical efficiencies of the rotation/oscillation based, or the sonic-based technical approaches are not evidently demonstrated so far (Zautner et al., 2013). As an accessory to clean and maintain the aesthetics and health of teeth, tooth pastes are widely used. They believed to promote oral hygiene by serving as an abrasive that aids in removing the dental plaque and food from the teeth, assists in suppressing halitosis, and delivers active ingredients. Noteworthy reductions in caries prevalence and incidence have been achieved by incorporating fluoride, chlorhexidine, triclosan and other chemotherapeutics (Ten Cate, 2006) into these tooth pastes. So now most of the
commercial toothpastes also contain fluorides and other antimicrobials including triclosan
and zinc citrate (Allaker and Douglas, 2009). Then again they are found to be associated
with a number of other unwanted side effects. Hence, despite the presence of several
chemoprophylactic anti-dental biofilm agents on the market, the search for an effective
agent still continues because of the undesirable side effects associated with these agents
(Kim et al., 2011). Number of recent reports revealed the potentiality of the natural
products as an alternative to the chemoprophylactic agents for controlling dental biofilms.

Even though toothbrushes and toothpastes have found wide usage, natural
methods of teeth-cleaning using chewing sticks selected and prepared from the twigs,
stems, or roots from various plant species have been practiced for thousands of years in
Asia, Africa, the Middle East, and the Americas (Wu et al., 2001). Still many cultures do
not use plastic-bristle brushes (Sohaibani and Murugan, 2012). Instead, herbal chewing
sticks taken from plants, shrubs, or trees with high antimicrobial activity (Hobbs, 2008)
are used for relieving dental problems. Many in vitro studies have demonstrated that the
aqueous extracts of these chewing sticks including miswak inhibit the growth of several
oral microorganisms (Al-Otaibi et al., 2004). They are, therefore, helpful in fighting oral
bacteria and maintaining oral health. Many in vivo studies also already demonstrated their
efficiency. Darout et al. (2000) proved that the miswak use for oral hygiene was found to
be comparable (or slightly better) than toothbrush use with respect to the periodontal
status of miswak users in a Sudanese population. Several studies are available on the
antibacterial activity, and associated role of Asian (Almas, 2001), African (Ndukwe et al.,
2004; van Vuuren and Viljoen, 2006), Ethiopian (Kassu et al., 1999), Indian (Hebbar et
al., 2004), and Nigerian (Fadulu, 1975) natural tooth stick in maintaining oral hygiene.
There is also a report which is not favoring the use of chewing stick. Increased plaque
formation and gingival bleeding was also observed among individuals using chewing
sticks in comparison with toothbrush-users. These chewing sticks achieve antiplaque
effects due to the combined effect of mechanical cleaning, enhanced salivation and
leaching-out of antimicrobial substances (Wu et al., 2001). Chewing sticks come from
different species of plants and, within one stick; the chemically active component may be
heterogeneous. Furthermore, there is potential for deriving active compounds having
specific effects on the biofilm-forming, dental caries-causing organisms from these
widely used chewing stick and other oral health care maintaining medicinal plants
Among the several approaches used for dental biofilm control, the use of antibiofilm agents having dual functionalities like reducing the cariogenic bacteria viability and controlling their colonization on the tooth surface could be more effective (Murugan et al., 2013). Detection of new natural, Generally Recognized As Safe (GRAS) compounds is very active hitherto towards for the successful development of alternative approaches with one goal to reduce or prevent caries (Gazzani et al., 2012). Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as suitable alternatives to synthetic chemicals (Palombo, 2009). In recent times, their phytochemicals are recognized as a worthy substitute to synthetic chemical substances meant for caries prevention (Morgan et al., 2001). India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. However, contrasting China, India is not competent enough to capitalize this herbal wealth by promoting its use in the developed world in spite of their renewed interest in herbal medicines (Kamboj, 2000). Hence this is the right time to look into the rich traditional knowledge and herbal medicine heritage of India. In an effort to raise the profile of Indian medicinal plant in the international scenario of oral health care maintenance especially in caries control and to do researcher’s part for the exploration of the available rich biodiversity for nations wealth, this present research was undertaken.

The ecological imbalance within the oral biofilm leads to the dissolution of tooth hard tissues (enamel) resulting in dental caries. As a prerequisite to survive in the oral environment, all oral microorganisms should have the ability to form biofilm, hence the formation and development of plaque, the dental biofilm is implicated as the main etiological agent of dental caries. In fact, father of microbiology Antonie van Leeuwenhoek (1632–1723) himself studied his own dental plaque sample; hence these dental plaques are the first biofilm microorganism made available for study. They are defined as “communities of sessile microorganisms that are encased within an
extracellular polymeric substance (EPS) that has been generated by the microorganisms themselves” (Williams et al., 2011). During the current study 189 bacterial isolates comprising Lactobacillus spp., Streptococcus spp., Kurthia spp., Staphylococcus spp., Klebsiella spp., Pseudomonas spp., Escherichia spp. and others were isolated from 100 samples. Among them, the Streptococcus spp. Staphylococcus spp. and Lactobacillus spp. were found as predominant isolates. The species belonging to the streptococci group, Streptococcus mutans and S. sobrinus role in the onset of dental decay is well established by a number of authors. They are the primary cariogenic bacterium in human beings. Other bacteria found in actively progressive carious lesions are considered to be secondary invaders, probably commensal with S. mutans with regard to their physiological activities (Hamada and Slade, 1980). The epidemiological surveys and the caries lesions genetic-based bacterial composition determination studies have well established the oral cavity Lactobacillus presence role in dental caries progression (Yang et al., 2010). Several studies reported the isolation of Staphylococcus spp. bacterium from the oral region including the root carious lesions, but their role in caries initiation and development is not well established (Kouidhi et al., 2010).

The cariogenic isolates Lactobacillus casei, Streptococcus mutans, Kurthia gibsonii, Staphylococcus aureus and Klebsiella pneumoniae determined as strong biofilm producing organisms. According to Socransky and Haffajee (2000) many bacteria prefers this biofilm mode of growth due to the number of advantages it offers. It includes protection from competing microorganisms; environmental factors for instance host defense mechanisms, potentially toxic environmental substances like lethal chemicals or antibiotics. It also facilitates processing and uptake of nutrients, cross-feeding, removal of potentially harmful metabolic products as well as the development of an appropriate physicochemical environment. Oral bacteria are often associated with dental plaque, forming highly organized microbial communities. The ability to form biofilm has been considered as one of the virulence factors for many oral pathogenic species and often depends on its growth status. Bacteria within a biofilm are invariably less susceptible to antimicrobial agents than their planktonic counterparts (Liu et al., 2011). All these L. casei, S. mutans, K. gibsonii, S. aureus and K. pneumoniae isolates were found to be resistant to ampicillin, metronidazole and penicillin G and intermediate susceptibility to a number of other antibiotics. Controlling this cariogenic biofilm forming organism’s
growth and once formed biofilm can be a significant challenge due to their less sensitive to antimicrobial agents (Landini et al., 2010). The biofilm lifestyle is associated with a high tolerance to exogenous stress, and hence treatment of biofilms with antibiotics or other biocides is usually ineffective at eradicating them. Therefore, its formation is a significant problem in many fields (Rendueles et al., 2013). This greater resistance has been attributed to the difficulty of antimicrobial agents in penetrating the biofilm, short replication time of bacteria in this biological state, appearance of modified microenvironments within the biofilm, or to the antibiotic tolerance (Moscoso et al., 2009; Simoes et al., 2010).

Tooth brushes play a vital role in the modern world to keep the tooth strong and healthy. Our ancestors have very strong teeth even after seventy to eighty year old; the only reason behind this secret is natural products, used by the elderly people. The ethnobiological scientific knowledge reciprocally relates human beings with their environment. The primary concerns of ethnobiology include adaptation of locally available resource sustainable utilization and their conservation methods (Rasingam et al., 2012). The leaves are widely used for medicinal preparation due to their easy access and active role in photosynthesis and production of metabolites (Ghorbani, 2005). In most of the situation, these tribal healthcare medicine preparation and application for disease treatment are accompanied by elaborate rituals and music as previously observed in the case of Mikirs of India (Borthakur, 1981). Ethnobotanical uses of plant species may vary from country to country and from culture to culture. Screening of herbal resources having medicinal value with the indigenous knowledge behind their use and their sustainable utilization of such plant materials is of paramount important for the conservation of herbal resources for the mankind (Rasingam et al., 2012). The present finding listed 15 plants having been used by the malayali tribals of Kolli hills. It is interesting to note that, many of the plants documented in the present study are in usage for similar utility in other parts of the state/country/world. A voluminous work has reported *A.indica* antibacterial activity; hence the plant was not taken up for further investigation during this study. Moreover, the use of neem in dental care has been well established. Number of biologically active principles were isolated from different parts of the plant which include azadirachtin, melanin, gedunin, nimbinin, nimbidin, nimbolides, salanin, nimbin, valassin, etc. (Bhandari, 1990; Ruskin, 1991; Said et al., 1996; Almas, 1999; William
Charles Evans, 2002; Mondal and Mondal, 2012). Numbers of these products are already found use in commercial applications.

In the same way, for *Linnermis* it was already established that lawsone (a naphtha-quinone derivative) etc. has already been proved to be the active principle, so they were also not taken up for further investigation. Number of chemical studies have shown that the aerial parts of *Linnermis* contains lawsone, lawsoniaside, lalioside, syringinoside, daphneside, lupeol, 30-nor-lupan-3β-ol-20-one, betulin, betulinic acid, lawnermis acid, lawsaritol, stigmasterol, β-sitosterol, lacoumarin, apigenin-7-glucoside, apigenin-4-glycoside, luteolin-7-glucoside, luteolin-3-glucoside, linalool, α ionone, and β ionone (Bharadwaj et al., 1976; Atal et al., 1978; Gupta et al., 1992; Handa et al., 1997; Kirkland and Marzin, 2003) which shown to be responsible for their bioactivity already. Hence, though they have shown marked activity against the tested cariogenic isolates, they were not taken up for further study.

Biofilm microorganisms secrete multiple EPS in contrast to their planktonic counterparts, which can make up to 50–90% of the total organic matter of biofilms. The polysaccharides are the typical components of the EPS; then again proteins, nucleic acids, (phospho) lipids etc. have also been identified, at times in considerable amounts. The structural and functional integrity of microbial bio-films are mostly determined by EPS which also contributes significantly to the biofilm community organization. They are implicated in the formation and maintenance of a three-dimensional, gel-like, highly hydrated and locally charged (often anionic) biofilm matrix, in which the microorganisms are more or less immobilized (Denkhaus et al., 2007). These exopolysaccharides that make up a crucial part of the EPS aids to cement whole bacterial populations to a surface rather than enclosing individual cells. The function of the exopolysaccharide (EPS) in pathogenesis has been studied in many organisms. The surface-associated EPS (exopolysaccharides) plays an important function in both extracellular and intracellular adherence of cariogenic organisms during their conversion from planktonic to biofilm mode and protects them from environmental as well as host factors (Bales et al., 2013), the current research interests focus on the secreted exopolysaccharides, particularly the high molecular weight exopolysaccharides that are believed for form the “backbone” of the EPS to which proteins, nucleic acids, and capsular polysaccharides adhere (Bales et
Streptococcus mutans are the important contributor of the extracellular polysaccharide (EPS) matrix in dental biofilms. The chemical compositions and detailed structures of EPS can provide more insight into their in situ physical properties and acidogenicity of S. mutans as part of the biofilms its cariogenicity (Li et al., 2012). For controlling and eliminating the S. mutans biofilms, it is necessary to understand the physiology of S. mutans. Number of previous studies look into the relationship between the cariogenic nature and surface roughness of S. mutans, and also tied the surface-cell adhesion of the surface polysaccharides to the different mutations of S. mutans cells (Liu et al., 2013). Glucans, synthesized from dietary sucrose by its glucosyltransferases (GTFs) have been recognized as virulence factors in the etiology and pathogenesis of dental caries. The formed glucans promote the adherence and accumulation of cariogenic streptococci on the tooth surface and play an essential role in the development of pathogenic dental plaque related to caries-forming activity. The structural analyses of S. mutans EPS showed that the insoluble glucans are predominantly (α1→3) linked, whereas the soluble glucans are comprised basically of (α1→6)-linked glucose residues (Li et al., 2012). Lactobacillus sp. exopolysaccharides are divided into two types, the HePSs and HoPSs as other bacterial EPS. HoPSs are composed of one kind of monosaccharide (hexose) whereas; several types of monosaccharides constitute HePSs. The molecular weights HoPSs range between 4.0 × 10^4 and 6.0 × 10^6 Da. HePSs are produced at higher concentrations than HoPSs secreted by Lactobacillus sp. contain glucose or fructose as sole monosaccharide and are classified as glucans and fructans respectively. Several strains of L. casei showed variation in the EPS monosaccharides composition according to the culture media carbon sources (Badel et al., 2011). The surface exopolysaccharides (K-antigens) of more than 80 serovars K. pneumoniae has been studied in detail. However, there have only been a few attempts to isolate the exopolysaccharide associated with biofilm EPS. Ratto and colleagues used an ethanol extraction protocol to isolate EPS exopolysaccharide from two similar K. pneumoniae strains and found each contained, 60% mannose, 20% galactose, and 17% GalA. While Bales et al. (2012) found that two strains of K. pneumoniae have a high percentage of mannose as well as significant amounts of galactose and Gal A, they also possessed considerable rhamnose as well as minor fractions of arabinose, fructose, GlcA, GlcNAc, and xylose, depending on the strain. The results of the current study also revealed the differential composition of the exopolysaccharides isolated from the biofilm forming
cariogenic *L. casei*, *S. mutans*, *K. gibsonii*, *S. aureus* and *K. pneumoniae*. As a result, it is understood that the extraction/purification methods followed during this research is effective at separating the high molecular weight EPS exopolysaccharide from the biofilm of cariogenic organisms. Further characterization of these cariogenic organisms EPS could lead to easy identification and design of more effective anti-biofilm therapeutic agents.

Compound 1 was identified as chondrillasterol. It analyzed for C_{29}H_{48}O (M+, m/z = 412) melting point of 168°C and α [D] = -2°C. It answered Lieberman-Burchard test for steroids. The IR spectrum showed peaks for hydroxyl group (OH stretching) (3435, 1035 cm^{-1}), gem dimethyl (1385 cm^{-1}) and tri sub double bond (1640, 832 cm^{-1}) and trans olefinic linkage (972 cm^{-1}). ^1H NMR showed H-18, most up field at δ 0.53. H-19 together H-29 appeared as 6 protons multiplite at δ 0.77 – 0.79. H-21 appeared most downfield at δ 1.01 as three proton doublet (J=6.8Hz). H-26 and H-27 each appeared as three proton doublets (J=7.2 and 6.4Hz respectively) at δ 0.81 and 0.83. H-3 appeared as 1 proton multiplite at δ 3.58. H-7 appeared downfield at δ 5.11 as being singlet. The trans olefinic protons H-22 and H-23 appeared as double doublets at δ 5.08 (J=15.2 and 8.4Hz) and 5.00 (J=15.2 and 8.8Hz). The ^13C NMR spectrum also confirmed the structure of compound 1 to be chondrillasterol. The presence of Δ^7 bond was prodded by the chemical shift values of C-7 and C-8 (117.46 and 139.57). The olefinic carbons at C-22 and C-23 appeared at δ 138.18 and 129.43. C-3 appeared at δ 71.07 as in sterols with 3β-OH.


Compound 2 was identified as myricetin-3-O-α-L-rhamno-pyranoside or 3, 3^1, 4^1, 5, 5^1, 7-hexahydroxy – flavone – 3 – O-α-L– rhamnopyranoside. It answered for phenol, flavonoid and glycoside. It was obtained as pale yellow amorphous powder. It analyzed for C_{21}H_{20}O_{12} formula and 464 of m weight. The IR spectrum showed peaks for
chelated hydroxyl (3401 cm⁻¹), flavonoid carbonyl (1651 cm⁻¹) and aromatic system (1609, 1508, 960, 823 cm⁻¹). The UV spectrum showed maxima at 266, 355 nm. The spectral shifts on addition shifts reagents viz. sodium acetate (NaoAc), sodium acetate (NaoAc) plus boric acid (H₃BO₃), aluminium chloride (AlCl₃), AlCl₃ plus hydrochloric acid (HCl) and sodium methoxide (NaoMe) showed the presence of OH groups at 5, 7 and 4¹ with orthodihydroxy group in ring B, it also suggested O-glycosylation at C-3 position. The UV spectrum and the effect of shift reagents also confirmed the structure of the compound 2. The compound showed at 266, 355 nm. Addition of sodium acetate made band II to move 274 nm (Δλ = 8 nm) which suggested the presence of 7-OH group. Addition of H₃BO₃ made band I also show a bathochromic shift from 356 to 371 nm (Δλ = 15 nm). This showed presents of orthodihydroxy group in ring B. Addition of aluminium chloride (AlCl₃) caused a bathochromic shift band I from 355 to 417 nm (Δλ = 62 nm), however, addition of HCl made band I to move to 402 nm (Δλ = 47 nm). The shift of 47 nm with AlCl₃ plus HCl suggested presents of 5-OH and sugar attached at 3OH (3 Hydroxyl). This confirmed along with other spectral data the compound 2 is myricetin-3-O-δα-L-rhamno-pyranoside. Addition of sodium methoxide (NaoMe) caused band I to show a bathochromic shift of only 28 nm (355 to 383 nm) with decreasing intensity due to alkaline degradation of the compound otherwise a shift of about 40-60 nm without decrease intensity of band I are expected for 4¹ OH.

¹H NMR spectrum showed H-6 and H-8 as meta coupled (2H₂) doublets at δ 6.20 and 6.37. H-2¹ and H-6¹ being equivalent appeared as two proton singlet at δ 6.89. The chelated hydroxyl at C-5 appeared at δ 12.68 as a broad singlet exchangeable with D₂O. The anomeric hydrogen of rhamnose appeared as a narrow doublet (J = 1.2 Hz) at δ 5.20 suggesting α linkage. The rhamnose methyl appeared as 3 proton doublet (J = 6.0 Hz) at δ 0.84. The other sugar protons appeared in the region δ 3.18 – 3.98. The ¹³C NMR spectrum also confirmed the structure of compound 2 to be myricetin-3-O-α-L-rhamno-pyranoside. The flavonoid carbonyl carbon C-4 appeared at δ 177.72. C-3 which is attached to rhamnose as O – glycoside appeared at δ 134.21. The anomeric carbon C-1¹¹ of the rhamnose unit appeared at δ 101.85 its confirmed glycosylation of the 3OH. C-6 and C-8 appeared at δ 98.63 and 93.44 as in 5, 7 – dihydroxy flavones. The HMBC correlation is given in Table.15. All the assignments are in accordance with the correlation found the anomeric hydrogen H-1¹¹ (δ 5.20) shows a correlation with C-3 (δ
and C-2\(^{11}\) (δ 70.0) thus confirm the position of \(\alpha\) – L-rhamnose at C-3. The assignment corresponded to those reported in literature (Subramanian et al., 1972; Arot et al., 1996).

When compared Gram-positive organisms need more concentrations of the compounds to kill them than Gram negative organisms. It is believed that Gram negative bacteria are more resistant to plant based antimicrobials than Gram positive bacteria. This is because the Gram negative bacteria have an effective permeability barrier, comprised of outer membrane, which restricts the penetration of antimicrobial compounds, which extrude plant extracts across this barrier. The single membrane of Gram positive bacteria is considerably more accessible to permeation by plant extracts in a region where these bacteria have limited protection (Chanda and Kaneria, 2011).

The present study found that the MICs of compound 1 and 2 were indicating that myricetin-3-O-\(\alpha\)-L-rhamno-pyranoside and chondrillasterol, the most abundant flavonoids components in these two fractions, are the bioactive compounds against oral pathogens. Flavonoids have been widely cited as the main bioactive compounds in many natural products, such as propolis, grape, green tea, oolong tea, Rubus ulmifolius and cranberry. Flavonoids can exhibit various biological effects and have anti-oxidant, anti-carcinogenic, cardio-protective, chelation and antimicrobial properties. Martini et al. (2004) investigated the antimicrobial activity of seven flavonoids (including kaempferol, myrecitin and quercetin) isolated from Combretum erythrophyllum against Gram-positive and Gram-negative bacteria. They found that tested flavonoids exhibited similar activities against individual microorganisms, possibly due to similarities in structures. It has been postulated that flavonoids suppress a wide array of microorganisms probably due to their ability to form complexes with extracellular soluble proteins, which can then binds to bacterial cell walls (Guan et al., 2012).

During the last few years, efforts have been directed towards developing preventive strategies that can be used to disarm microorganisms without killing them. An innovative approach is the use of biocide-free antibiofilm agents with novel targets, unique modes of action and proprieties that are different from those of the currently used antimicrobials. In addition, one of the main advantages of plant derived compounds with potential pharmaceutical and medical applications is the lack of shared pathogens.
between plants and mammals like alkaloids, terpenoids, flavonoids and coumarins, peptides, glycosides, nucleosides and polyphenols. They may act in a variety of ways: antibiotics, allosteric regulators, catalysis, catalytic cofactors, regulatory activities at level of DNA, RNA and protein, pigments, mutagens, antimutagens, receptor agonists, antagonists, signal molecules, siderophores, detergents, metal complexing/transporting agents, pheromones, toxins and other interesting activities (Villa and Cappitelli, 2013).

Anti-adhesion agents reduce the total mass of causative microorganisms but do not affect the viability of the oral bacteria, thereby abolishing the selection of resistant strains and occurrence of secondary infections. Thus, application of anti-adhesion agents appears to be a very promising approach in oral hygiene (Stauder et al., 2010). Hence, both the myricetin-3-O-α-L-rhamno-pyranoside and chondrillasterol inhibiting the adherence and biofilm formation hold the potential to be developed into anticaries agent.

The most important virulence factors the primary etiologic agent *S. mutans* are their ability of producing glucosyltransferase (GTF) and synthesizing the water insoluble glucans from sucrose. GTF is the main enzyme that catalyses the introduction of a glucose moiety from sucrose to the adhesive glucans. They also permit bacteria to adhere firmly to the tooth surface and contribute to the formation of dental plaque. These cariogenic streptococci might produce at least three types of GTFs: one synthesizes 1,3-linked water insoluble glucans, the second one produces noteworthy amounts of both water-soluble and insoluble glucans, and the third one incorporates glucose in α-1-6-linkages into soluble glucan products. Therefore, GTFs are essential for dental caries progression (Figueiredo et al., 2010). The high binding scores of *Mimusops elengi* natural ligands myricetin-3-0-α-L-rhamnopyronoside and chondrillasterol with the *Streptococcus mutans* glycosyltransferase (3AIC) indicates the possibility of interfering glucosyltransferase function thereby exoploysaccharide synthesis. These glycosyltransferases (GTs) also catalyzes the synthesis of the numerous glycoconjugates including lipopolysaccharides. Hence, the molecular interaction between GT4 family glycosyltransferases (2IW1) involved in LPS biosynthesis with the myricetin-3-0-α-L-rhamnopyronoside and chondrillasterol natural ligands from *M. elengi* indicated their possible role in reducing the virulence of the cariogenic organisms.
The Streptococcus mutans antigen I/II (AgI/II) is a cell surface-localized protein that adheres to salivary components and extracellular matrix molecules. They are also called as P1, B, SpaP, or PAc) of S. mutans. They are studied for the previous as a target for protective immunity against dental caries. Apart from adherence, they also influences biofilm formation, promotes platelet aggregation, collagen-dependent bacterial invasion of dentin, and cariogenicity. Now are identified in members of the Group A and Group B streptococci, suggesting a role for this adhesion in a variety of species (Larson et al., 2011). Myrecitin-3-0-α-L-rhamnopyronoside showed the binding energy -8.59 and the chondrillasterol showed the binding energy -8.1 when interacts with Streptococcus mutans cell surface-localized salivary component and extracellular matrix molecule adhering antigen I/II (AgI/II) (3QE5). Hence they may also involve in preventing the cariogenic organisms adherence.

Communication between neighboring bacteria via quorum sensing is social behavior that enables interactions within mono and mixed bacterial communities. Such systems are found in both Gram-positive and Gram negative micro-organisms. The stimuli signal molecules or auto inducers are produced at a basal constant level, and their concentration thus is a function of microbial density (Scheie and Petersen, 2004). Quorum sensing requires production and release of chemical signal molecules called autoinducers that increase in concentration as a function of cell density but can also depend upon physiological conditions. The quorum-sensing system allows bacteria to express specific genes in a coordinated fashion. Quorum sensing has been shown to play an important role in the development of biofilms (Elias and Banin, 2012). Some bacteria in biofilm release EPS-degrading enzymes to maintain structural heterogeneity. This interesting phenomenon is a quorum sensing regulated one. Hence these cell-cell communications must be considered as a morphogenetic mechanism. By sensing cell-produced compounds QS signal cells recognize the local cell density and react by switching on or off particular sets of functional genes (De Beer and Stoodley, 2006) mostly virulence ones. Two types of quorum-sensing systems are recognized in bacteria: intra-species communication and inter-species communication. Gram-negative bacteria usually use acyl homoserine lactone (AHL) as signal molecules, while gram-positive bacteria utilize small peptides (Dufour et al., 2012). The high binding energies of both Myrecitin-3-0-α-L-rhamnopyronoside and chondrillasterol Gram-positive bacteria Rap phosphatase quorum-
sensing receptors (4I1A) showed that these two compounds may interfere the QS communication between the cariogenic organisms and may act as quorum quenching compounds.

The obtained results shown that the bioactive compound myrecitin-3-0-α-L-rhamnopyronoside and chondrillasterol of *M. elengi* has preferential anti cariogenic activity against the biofilm forming cariogenic clinical isolates tested. Its inhibitory effect on the cariogenic organism’s growth and biofilm formation suggested that myrecitin-3-0-α-L-rhamnopyronoside as well as the chondrillasterol might have a potential to become an important ingredient caries controlling agents including tooth paste, mouth wash and other clinical application in treating dental caries by inhibiting or interfering the cariogenic mechanisms of these cariogenic organisms within dental plaque.