2. REVIEW OF LITERATURE

In this chapter, the ecology of oral cavity and etiopathology of dental caries will be introduced concisely with more emphasis on the biofilm forming nature of dental caries causing organisms along with the role of biofilm in initiation and progression of this disease. It will be followed by a thorough review of literature taking into account the currently available and novel antimicrobial strategies for dental plaque control. The preceding part of this chapter had focused more on recent advances in anti-plaque agents, including the chemoprophylactic, natural antimicrobial agents; their mode of action along with the role of the bioinformatics tools in their development would be discussed.

2.1 Oral environment

Oral cavity, the primary gateway to the human body comprises teeth, saliva, gingival sulcus, tongue, cheeks, hard and soft palates, tonsils, gums, inner cheek linings (Olsen, 2006; Cho et al., 2010; Zarco et al., 2012), making it as a unique ecosystem. It can be considered as a planet full of different surfaces and environments. It offers a home for a vast diversity of species that it has been called the tropical rainforest of the body (Olsen, 2006). It is possibly the most complex and heterogeneous microbial habitat in the human body (Cho et al., 2010). It is commonly sterile at birth. However, within 6 to 10 h after birth, microbes from the mother and to a lesser extent from the environment establish themselves in the oral cavity (Marcotte and Lavoie, 1998). Greater than 500 species of bacteria at $10^8$–$10^9$ bacteria per mL saliva or mg dental plaque inhabits human oral cavity (Rasooli et al., 2008). The majority of them are either facultative or obligate anaerobes (Cho et al., 2010). A complex and dynamic moist environment always exist in human mouth owing to its anatomic and functional features (Sreenivasan and Gaffar, 2008), an ideal one for biofilm.

The dual vital physiological fluids (saliva and gingival crevicular fluid) continual oral surface bathing sustains oral ecosystems by supplying water, nutrients, adherence, and antimicrobial factors (Marcotte and Lavoie, 1998). 500–1,500 ml/day is saliva produced and mouth contains 0.77–1.07 ml (Sreenivasan and Gaffar, 2008). It comprises several proteins, minerals, antimicrobial enzymes, water, carbohydrates, glycoproteins, proteins, amino acids, gases, several ions, including sodium, potassium, calcium, chloride, bicarbonates well as phosphate. It also comprises innate (e.g. lysozyme,
lactoferrin, sialoperoxidase, antimicrobial peptides, etc.) and adaptive immunity (e.g. sIgA) components be capable of directly inhibiting several exogenous microbes. Thus saliva is vital to the oral cavity since it performs an important function of maintaining homeostasis and defending from disease (Marcotte and Lavoie, 1998; Marsh, 2003; Olsen, 2006; Zarco et al., 2012). Many microorganisms subsist there since the continuous bathing of mouth with saliva maintains optimal growth conditions such as temperature (warm between 34 and 36°C) and moist with close to neutrality pH between 6.75 and 7.25 (Marcotte and Lavoie, 1998; Marsh, 2003). The serum-like gingival crevicular fluid (GCF) is an exudate comes from plasma which passes through the gingival (junction epithelium) to reach the gingival crevice and flows along teeth. GCF contains host defense components (antibodies, phagocytes) and glycoproteins/proteins, supplying nutrients for bacteria in the gingival crevice (Olsen, 2006). The saliva baths supra gingival environment, while the gingival crevice (the subgingival environment) is bathed mainly by the GCF (Marcotte and Lavoie, 1998). The microorganisms inhabiting the oral cavity are quite able to spread to other sites of the body (Zarco et al., 2012).

2.1.1 Oral microorganisms

The recent technological advancement applying molecular techniques for the identification and analysis of complex bacterial communities have demonstrated the oral microbiota diversity and the presence of numerous strains not described before (Silva et al., 2012). At present, available reports estimates more than 700 types of organisms in the mouth (Sreenivasan and Gaffar, 2008; Tong et al., 2010; Zarco et al., 2012), out of which around 60% of them were identified. The exact composition of oral biofilms/ ecological niche varies on distinct anatomical surfaces due to the prevailing physical and biological properties of each site (Silva et al., 2012). Tongue contains Streptococcus salivarius; soft tissue contains S. sanguinis and S. australis. The organisms like Rothia dentocariosa, Actinomyces sp, S. sanguinis, S. gordonii and Abiotrophia were found on the teeth (Olsen, 2006). The oral microorganism’s list also includes S. mutans, S. mitis, S. pneumonia, S. pyogenes, Lactobacillus spp, Staphylococcus aureus, S. epidermidis, Enterococcus faecalis, Clostridium sp, C. tetani, Pseudomonas aeruginosa, Escherichia coli (Marcotte and Lavoie, 1998; Song et al., 2006; Islam et al., 2007; Allaker and Douglas, 2009; Cho et al., 2010; Ogunshe and Odumesi, 2010; Tong et al., 2010; Prasanth, 2011; Wong et al., 2012; Yim et al., 2013), Mycobacteria, Candida albicans, Actinomycetes sp, A.
odontolyticus, Neisseria sp, Arachnia, Bacteroides, Bifidobacterium, Eubacterium, Fusobacterium, Leptotrichia, Peptococcus, Peptostreptococcus, Propionibacterium, Selenomonas, Treponema, and Veillonella (Devi and Ramasubramaniaraja, 2009; He et al., 2013). Spirochetes (Marcotte and Lavoie, 1998; Lauten et al., 2005; Yanti et al., 2008; Prasanth, 2011; Rosas-Pinon et al., 2012; Zarco et al., 2012). Normally, the human oral microflora stays alive in a healthy balanced state with the host because of environmental constraints. Hence, oral infection is a result of alteration in the local environment resulting from imbalance in the oral microflora. It may be due to diet, a reduction in host immune responses, or the effects of an administered therapeutic drug, such as an antibiotic. On the whole, the oral infection implicated microorganisms are opportunistic pathogens which are harmless and cause disease only during favorable condition (Williams et al., 2011).

Several bacteria depend on the metabolic co-operation to develop into oral microbial communities. Numbers of examples available evidences the existence of a web of metabolic exchanges found in the oral cavity. The Gram-negative, anaerobic cocci Veillonella species are part of the pioneer oral community after birth and found abundantly in the plaque. Lactic acid formed as a result of Streptococci growth is their favored substrate; hence their removal accelerates the glycolysis rate in streptococci, which is an end-product (lactate) inhibition event. The diffusible signal formed during the co-culture of S. gordonii and V. atypica upregulates S. gordonii amylase gene, the amyB leading to more fermentable glucose production and thus added lactic acid, a condition more favorable for V. atypica (Wright et al., 2013). At the same time, S. gordonii materializes its gain through A. naeslundii interaction. Co-culturing of them leads to differential the expression of a number of S. gordonii arginine biosynthetic genes which potentially increases its arginine biosynthesis efficiency. The degradation of salivary mucins also emphasizes the benefits of interspecies cooperation. Individually, Streptococcus species alone are not certainly produces all requisite mucin hydrolytic enzymes, but their co-operation enables more efficient utilization of the mucin oligosaccharides for growth (Wright et al., 2013). “The importance of community interactions became apparent when the ‘defined microbial community’ concept was exploited to determine the role played by individual species” (Marsh, 2003). However, the early pioneering colonizer like streptococci colonization enhances the growth and
virulence of potentially pathogenic bacteria such as *P. gingivalis* and *T. denticola*. Hence, the mitis-group streptococci e.g. *S. gordonii*, *S. oralis* are called as oral cavity accessory pathogens (Wright *et al.*, 2013). Variety of factors including temperature, pH, oxidation-reduction potential, nutrient availability and water, oral structure anatomy, salivary flow, and antimicrobial substances influence the oral cavity, the selection of oral microorganisms and supports the equilibrium maintenance amongst bacterial populations (Marcotte and Lavoie, 1998). The competition between ‘good’ bacteria as well as other microbes with their ‘nefarious cousins’ keeps the pathogens under control in healthy mouths. The change in conditions makes the pathogenic microbes ‘gang up’ themselves against beneficial species and seize the control of the mouth’s surfaces (Olsen, 2006). The establishment of oral diseases by these pathogens not only pave the way for irreversible damage and unnecessary pain, but also dental anxiety, general health problems, depression, low self-esteem, lost school time and poor quality of life (ADA, 2004). These oral diseases, including dental caries, gingival inflammation, periodontal disease and tooth loss upset the overall health very much (Tsai *et al.*, 2007). Nowadays, oral disease became a major public health problem all over the world and dental caries is on the whole widespread oral disease (Li *et al.*, 2013).

### 2.1.2 Human tooth and its anatomy

Teeth are hardest structures of the human body located on the upper or lower jaw. They are used for chewing or mastication of ingested food. Teeth also provide shape to the face and assist in the process of speaking clearly. Man has two dentitions: “the primary (deciduous, baby, milk or first teeth) one, which is fully erupted approximately at the age of two, and the permanent one, which replaces the primary dentition when the person is between 6 and 13 years old. Deciduous incisors, canines and molars are eventually all replaced by their respective permanent counterparts. Moreover, permanent dentition has 12 additional molars. Consequently, there are 20 deciduous and 32 permanent teeth. The visible part of the tooth is called the crown, while the part covered by the gum is called root (Fig 2.1). The bulk of the tooth is composed of dentin, which is surrounded by a thin layer of enamel in the tooth” (Fattibenea and Callens, 2010).

The human tooth is made of both calcified hard and noncalcified or soft tissues. The hard tissues include enamel, dentin, and cementum whereas the soft tissue comprises
pulp, the center of the tooth that contains nerves, blood vessels and connective tissue. The visible part of the teeth is called anatomical crown which is in general covered by enamel. The enamel is the hard-calcified tissue covering the dentin in the crown of the tooth. It is made up of mineral crystallites of hydroxyapatite called prisms or rods which are grouped in clusters with hexagonal cross-sections. These clusters are bound together by interprismatic enamel, in which the crystallites are oriented in a direction different from that in the prisms (Fattibenea and Callens, 2010). Since it contains no living cells, it can’t repair damage from decay or from wear. Soft tissues that cover and protect the roots of the teeth and cover teeth that have not yet erupted are known as gums or gingiva. Pulp chamber is the space occupied by the pulp-the soft tissue at the center of the teeth containing nerves, blood vessels and connective tissue. The connecting area which connects the crown with the root is neck. The tooth portion which is underneath enamel and cementum is called as dentin. It is much rigid than bone, on the other hand softer than enamel. About 70% of dentin is mineral matter (Fattibenea and Callens, 2010). It comprises small hollow tubes or canals which cause sensitivity upon exposure to heat and cold or acidic or sticky foods. The jaw portion which encircles the teeth roots are called as alveolar bone or jawbone. The root canal is the part of the pulp cavity inside the tooth root; the chamber within the tooth root contains the pulp. The hard connective tissue covering the tooth root, giving attachment to the periodontal ligament is known as cementum. Those systems of collagenous connective tissue fibers that connect the root of a tooth to its socket are called as periodontal ligament. Hence the tooth has a number of ecological locales (Olsen, 2006).

![Fig. 2.1 Anatomy of teeth](Courtesy: Nesiama and Sinn, 2010).
2.2 Dental caries

Caries is a Greek word meaning destruction or decay. Dental caries, also known as tooth decay or a cavity is one of the most prevalent transmissible infectious (Tsai et al., 2007) diseases in humans, second only to the common cold (Islam et al., 2007). Dental caries is a supragingival condition (Palombo, 2009). “A global increase in the prevalence of dental caries is predicted to be a pending public health crisis which affects children as well as adults, primary as well as permanent teeth, and coronal as well as root surfaces” (Bagramian et al., 2009). They are nevertheless a main oral health problem in most industrialized countries, albeit remarkable global population oral health achievement. They affect mainly school children (60-90%) along with a huge number of adults globally. They are a most prevalent oral disease in several Asian and Latin-American countries (WHO, 2013). The World Health Organization global burden of disease: 2004 update report estimated that out world population (000) 1116985.088, 175.113128662109 deaths occurred in WHO member states due to oral diseases. Amongst, the dental caries alone caused 88.4702682495117 deaths. In India, 27538 deaths occurred due to oral diseases in which dental caries alone accounted for 16653 deaths (WHO, 2008).

Dental caries was first described by Miller’s chemoparasitic theory of 1890 (Touger-Decker and van Loveren, 2003). It is a bacterial disease of dental hard tissues and is characterized by a localized, progressive, molecular disintegration of the tooth structure. It could also be seen on oral ecosystem exposed root surfaces as a consequence of gingival recession (Marcotte and Lavoie, 1998; Xiao et al., 2012). The carbohydrate metabolic product of oral microorganisms destroy dental hard tissue and dissemble organic substance resulting in tooth damage thus causes caries (tooth infection), a primary step of oral diseases (Williams et al., 2011; Khan et al., 2012; He et al., 2013; Yim et al., 2013). Enamel demineralization is enhanced to a great extent by organic acids produced due to these biofilm organisms’s carbohydrate metabolism (Steinberg et al., 2004). As it progresses, widespread enamel and dentin destruction occurs, followed by cavitation, inflammation of pulp and periapical tissue, even tooth loss (He et al., 2013). During the manifestation of this multifactorial infectious disease, the diet, nutrition, microbial infection, and the host response altogether play important roles (Tsai et al., 2007). The clinicians and researchers considered mostly the following categories of dental caries which include smooth-surface caries, pit and fissure caries, enamel caries, dentinal caries,
secondary caries, early childhood caries and root caries (Devi and Ramasubramaniaraja, 2009). Although the differences of opinion varies concerning the cause of dental caries raise, returning to the past successful public health strategies is the well-known remedy (Bagramian et al., 2009).

Dental caries is forms by way of an ordered sequence of events, resulting in the formation of tooth surface biofilm, a structurally- and functionally-organized, species-rich microbial community of microorganisms embedded in a matrix of polymers (Marsh, 2006; Song et al., 2006; Almeida et al., 2012; Spratt et al., 2012). The dental biofilm composed of complex matrix having host and bacterial origin, including microbial extracellular products and salivary compounds (Marcotte and Lavoie, 1998; Islam et al., 2007).

2.2.1 Symptoms, pathogenesis and associated risks

Dental caries, the well-known as tooth decay are the main cause of oral pain and tooth loss. Though, they begin as minor surface changes can persist until there are lesions in the dentin (Zarco et al., 2012.) The symptoms of dental caries includes tooth pain, bad breath (produced due to sulfur-based odorous waste products), fever, chills, foul taste, abscess, cervical adenopathy, trismus (Devi and Ramasubramaniaraja, 2009; Cho et al., 2010). In recent times, oral microbiologists gave an account on the relationship between cardiovascular system and gum diseases. They identified specific types of gum disease bacteria that are most damaging one for the cardiovascular system. Thus, it is no surprise that oral health is being increasingly linked along with other conditions such as heart disease (a heart attack), pregnancy and stroke (Cho et al., 2010). Earlier, Needleman (1998) observed that subjects with periodontitis have 25 per cent increased risk of coronary heart diseases. Poor oral hygiene was also associated with an increased risk (Needleman, 1998).

2.2.2 Periodontal diseases

Periodontal diseases are a common term describing the inflammatory pathologic state of teeth supporting tissues (Marcotte and Lavoie, 1998). Characteristically, in these diseases, the junctional epithelial tissue at the base of the gingival crevice migrates down the root of the tooth with the result of the formation of a periodontal pocket (Spratt et al., 2012). In these subgingival disease conditions, anaerobic Gram-negative bacteria such as
Porphyromonas gingivalis, Actinobacillus sp, Prevotella sp and Fusobacterium sp were implicated. The infection at or below the gingival crevice triggers a cellular inflammatory response of the gingival and surrounding connective tissues. These inflammatory responses can manifest as the very common gingivitis, seen as bleeding of the gingival or gum tissues or periodontitis or as the inflammatory response causes loss of collagen attaching the teeth to the bone and loss of bone (Palombo, 2009) like alveolar bone. Both these oral dental caries and periodontal diseases are manifested as a result of ecologically driven imbalances of oral microbial biofilms. They are caused by micro-organisms belonging to the resident oral microflora, rather than by classic microbial pathogens (Scheie and Petersen, 2004).

2.2.3 Periodontitis

Periodontitis includes the destruction of the connective tissue attachment and the adjacent alveolar bone. The initiation and development of periodontitis, the disease of periodontal tissue destruction is a complex process which involves plaque accumulation, release of bacterial substances and the host inflammatory response. In periodontitis, the gingival crevice is deepened to form a periodontal pocket due to the apical migration of the junction epithelium along the root surface (Marcotte and Lavoie, 1998). There is a strong association between severe periodontal diseases and poor oral health (Palombo, 2009).

2.2.4 Gingivitis

Gingivitis is defined as an inflammation of gingival tissues which does not affect the attachment of teeth (Olsen, 2006). It can be described as a nonspecific inflammatory process of the gingivae (gums) without destruction of the supporting tissues. It is the most widespread form of periodontal disease. The dental plaque accumulation at gingival margins due to inadequate dental hygiene leads to the inflammation of the gingiva (Spratt et al., 2012). Though bacterial tissue invasion is a rare event, their released products penetrate into the gingiva and cause tissue destruction, either directly through the action of enzymes and endotoxins, or indirectly via inflammation induction. The host bacterial antigen inflammatory response exhibits both protective and destructive functions in periodontal diseases. The phagocyte's lysosomal enzymes release causes tissue damage. Cytokine production stimulates connective tissue cells to release metalloproteinase
(including collagenases) or activate bone resorption (Marcotte and Lavoie, 1998). The total number of cultivable bacteria recoverable from a healthy subgingival crevice is comparatively less ($10^3$ to $10^6$ CFU/crevice). It seems that the gingival crevice microbiota are an extension of the supragingival plaque. *Porphyromonas gingivalis, P. endodontalis, Prevotella melaninogenica, P. intermedia, P. loescheii, and P. denti* like Gram-negative black-pigmented rods are seldom isolated from a healthy gingival crevice (Marcotte and Lavoie, 1998). Normally, gingivitis is associated with a general increase in the biofilm mass on the gingival margin (Olsen, 2006). It is a reversible condition in which a return to precise dental hygiene practices will restore gingival health (Spratt et al., 2012).

### 2.3 Dental plaque

A tooth in the oral cavity offers a persistent humid environment with adherent surfaces prompting the attachment of colossal deposits of microorganisms. Hence, the dental plaque is a natural occurrence on the teeth surface mostly where there is a reduction of salivary flow mediated mechanical removal and at stagnant sites, for example, in the interproximal regions or fissures of teeth. 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 generally believed that an irritating matter between teeth is the source of dental diseases and dental plaque which is naturally pathogenic. It is generally accepted that bacterial plaque is an important etiological factor of periodontal diseases (Berchier et al., 2008). The approximal surfaces on the mesial (anterior) and distal (posterior) surfaces of the tooth are also disposed to plaque formation, and these surfaces are prone to both the decay and periodontal disease. The occlusal surfaces, which are the chewing surfaces of molar and premolar teeth, are traversed by developmental grooves or fissures that are colonized by a scant flora relative to the smooth and approximal surfaces. These fissures, as well as developmental pits on the smooth surfaces, are most caries prone sites on the teeth. The incisal surfaces on the top edge of anterior teeth are not colonized by appreciable numbers of bacteria and are normally carries-free (Loesche, 1986). The tongue owing to its papillary surface offer colonization sites which are all well secluded from mechanical removal. The gingival crevice, an area between the junction epithelium of the gingival and teeth too make available a characteristic colonization site that includes both hard and soft tissues (Marcotte and Lavoie, 1998). Dental plaques are generally divided into two forms:
subgingival and supragingival plaque. The plaque accumulates on the crown, the teeth’s portion above the gingival tissue is called the supragingival plaque (Kreth et al., 2009).

Recent technological developments allow the assessment of sizable numbers of bacterial species in substantial numbers of plaque samples obtained from a range of subjects. A light and electron microscopy examination of plaque portions reveal a remarkable degree of order in colonization patterns. Early supragingival plaque demonstrated the columnar arrangement of morphologically distinct bacterial species from the tooth surface to its outer surface (Socransky et al., 1998). The supragingival plaque consists mainly of Gram positive facultative anaerobic (streptococci) and the subgingival plaque of anaerobic Gram-negative bacteria (Zambori et al., 2013). The properties of dental plaque are biofilm in nature, like other biofilms found in the body and the environment. Dense mushroom-like structures originate from the enamel surface, interspersed with bacteria-free channels used as diffusion pathways by these biofilm formers. These channels are possibly filled with EPS matrix produced by the bacteria. Biofilm bacteria use signaling molecules for their communication (quorum-sensing), optimizing their virulence factors and survival. Since bacteria in a biofilm mode have a “physiology different from that of planktonic cells, live under nutrient limitation and often in a dormant state and respond differently to antibiotics and antimicrobials highlight why the study of bacteria in the oral cavity is now taken on by studying the biofilms rather than individual species” (ten Cate, 2006).

Biofilm can’t be simply defined as they differ to a great extent in structure and composition from one environmental niche to another. Technological advancement contributes new discoveries and substantial knowledge on microbial biofilms regularly. So the definitions of biofilm continuously evolved and are now defined in a variety of ways. The following are a few among them. Microbial biofilms are extremely complex microbial ecosystems consisting of microorganisms attached to a surface and embedded in an organic polymer matrix of microbial origin (Percival et al., 2011). Costerton et al. (1998) defined a biofilm as “a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface”. According to Donlan (2002) “biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily
polysaccharide material”. Elder and colleagues described a biofilm in more cooperative terms as “a functional consortium of microorganisms organized within an extensive exopolymer matrix,” whereas Carpentier and Cerf simplify the concept as “a community of microbes embedded in an organic polymer matrix, adhering to a surface” (Costerton et al., 1999; Dunne, 2002). According to Gurenlian (2007) “biofilms are organized to maximize energy, spatial arrangements, communication and continuity of the community of microorganisms”. Behlau and Gilmore (2008) defined biofilms as a “collection of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface”.

More recently Jain et al. (2011) defined biofilm as “a microbially-derived sessile community characterized by cells that attach to a substratum or interface or to each other, with the help of gelatinous extracellular polymeric substances.” These special gelatinous extracellular adhesive are known as “biofilm matrix”. According to Percival et al. (2011) biofilms are “microbial cells immobilized in a matrix of extracellular polymers acting as an independent functioning ecosystem, homeostatically regulated”. Biofilms have been metaphorically dubbed “city of microbes”, and the extracellular biopolymers, in which exopolysaccharide predominates, as the “house of the biofilm cells”. ‘Microbial or bacterial origin’ therefore ignores infections where bacteria interact with host molecules and receptors to attach, replicate, and aggregate. Therefore, Hall-Stoodley et al. (2012) gave a more comprehensive definition for clinically relevant biofilm as ‘aggregated, microbial cells surrounded by a polymeric self-produced matrix, which may contain host components’. Definitions of biofilms also include ‘embedded in an extracellular polymeric matrix of microbial origin.’ However, ‘extra-microbial’ host-derived components are particularly important in complex host environments such as dental plaques (Hall-Stoodley et al., 2012).

### 2.3.1 Dental biofilm

Understanding oral biofilms as a complex bacterial structure will have the potential to impact significantly on clinical practice since most antimicrobial products that showed good in vitro results did not show similar effects under clinical evaluations (Silva et al., 2012). Dental plaque is defined ”as the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and host origin” (Xie
et al., 2008). A dental plaque sample from human was the first biofilm microorganism made available for study. They are defined as “communities of sessile microorganisms that are encased within an EPS matrix that has been generated by the microorganisms themselves” (Williams et al., 2011). In the seventeenth century by Antonie van Leeuwenhoek (1632–1723), the father of microbiology described ‘animalcules’ living within his own dental plaque. The inventor of microscope Anton van Leeuwenhoek observed smears he prepared from his own dental plaque to test his newly invented microscopes, and was able to observe bacteria, giving way to the discovery of microorganisms (Moscoso et al., 2009). The description of dental biofilms in his words,

“... Tho my teeth are kept usually very clean, nevertheless when I view them in a Magnifying Glass, I find growing between them a little white matter as thick as wetted flower . . . to my great surprise . . . contained very many small living Animalcules, which moved themselves extravagantly . . . hence I conclude, that the Vinegar with which I washt my Teeth, kill’d only those Animalcules which were on the outside of the scurf, but did not pass thro the whole substance of it . . . The number of these Animalcules in the scurf of a man’s Teeth, are so many that I believe they exceed the number of Men in a kingdom.”

Anthony van Leeuwenhoek.

It is reasonable to suggest that this early study of dental plaque was the first documented evidence of the existence of microbial biofilms (Percival et al., 2011). He can be credited with the discovery of microbial biofilms.

Ever since many studies have evaluated the composition of plaque using light and electron microscopy, cultural techniques and more recently immunologic or DNA probe techniques. Each and every one of these techniques emphasizes Leeuwenhoek's initial observation that subgingival plaques are comprised of a large complex mixture of bacterial species (Socransky et al., 1998). The medical importance of biofilms has been recognized only after a century of Anthony van Leeuwenhoek observation even though in 80% of bacterial infections biofilms are implicated (Behlau and Gilmore, 2008). After their extensive study on dental plaque and sessile communities in mountain streams Costerton et al. (1978) hypothesized the mechanisms by which microorganisms adhere to
living and non-living materials and derive benefit from this ecologic niche (Donlan, 2002).

The impact of bacterial biofilms on various aspects of our day-to-day lives has led to an increased number of biofilm-related studies in the past decade (Elias and Banin, 2012). The concept that “bacteria live preferentially in matrix enclosed communities attached to surfaces has emerged gradually from scientific observations over an extended period of time, but the pace at which this concept has advanced has accelerated sharply during the past two decades” (Costerton and Wilson, 2004). The peculiar features of these cell aggregate biofilms did not allow them to be explained by Koch’s postulates (Moscoso et al., 2009). Miller, who worked in Berlin, put forth the chemico-parasitic theory which tried to explain the etiology of caries based on the bacteriological approach. According to him “the dental caries was caused by bacteria that fermented carbohydrates in foods and produced acids to destroy the hard tissues of teeth”. Although his theory gets along with many points of the current concept, there were some insufficiencies in some important issues (Hamada, 2002). Anyhow “Oral biofilms formation is an important event associated with the initiation of most common infections in the oral cavity such as caries, gingivitis, and periodontal diseases” (Ali et al., 2012).

2.3.2 Adaptive features of dental biofilm organisms

Bacteria are extra-ordinarily well in adapting their survival at “feast and famine”, and also adjusting their needs to accommodate highly diverse environments. Scientific inquiry has discovered a number of the microbial characteristics that facilitate the way bacteria adapt to changing environments. Their capacity of forming and maintaining biofilms is key to these adaptations.

The unique environment oral cavity includes many dissimilar surfaces (hard, soft, natural and artificial) which share the same ecological niche. Hence, to survive in this ‘open growth system’ and to resist shear forces, bacteria need to adhere either to soft or hard tissues (Shemesh et al., 2010). Noteworthy numbers of indigenous microorganisms comprising bacteria, spirochaetes and yeasts are added through periodic nutrition in the form of diet (Sreenivasan and Gaffar, 2008). They must adopt themselves to the more changeable temperature found on the mucosal and tooth supra gingival surface. Microorganisms colonizing these sites are exposed to extremely variable hot and cold
meals during food intake, and hence they must adapt to them (Marcotte and Lavoie, 1998). Also, these oral ecosystems are influenced by age-related changes comprising those due to teeth eruption, changes in dietary habits, hormones, salivary flow, the immune system, or other factors. Puberty and pregnancy are shown increased levels of plasma steroid hormones and later in the crevicular fluid and saliva with an increase in gingival inflammation which accompanies an increase in gingival exudates (Marcotte and Lavoie, 1998). The mouth allows the growth of all aerobic, facultative anaerobic and anaerobic bacteria due to its varied oxidation-reduction potentials (Marcotte and Lavoie, 1998). Generation of low pH due to frequent consumption of high-sucrose diets enhanced fermentation of sucrose into lactate (Marcotte and Lavoie, 1998). The fall in pH and eH (with a thickening of the plaque) aids highly fermentative facultative species by providing a suitable ecological niches deep within the plaque (Walsh and Tsang, 2008).

The oral cavity inhabiting microorganisms should exhibit adaptive characters both to the mechanical and antimicrobial action of saliva. The constant flow of saliva flushes not only the food debris but also bacteria away from the oral cavity into the gut. It also has many antimicrobial factors that not only inhibits adhesion of bacteria but also protects the oral environment from harmful bacterial products (Sammons, 2003). Apart, it’s resting, and stimulated flow, pH, fluoride, calcium and bicarbonate levels and its antibacterial properties (antibodies, lysozyme, lactoferrin, peroxidase) (Walsh and Tsang, 2008) influence the oral microorganisms. It also acts as a buffering agent during the continuous acid production in the oral cavity which protects from bacterial caries action which evidenced from more susceptibility of dry mouth syndrome individuals to dental caries (Islam et al., 2007). Floating of more than 60 saliva proteins not only provides bacterial growth nutrients but makes the bacteria stick together in such large clumps that prevent them from adhering to tooth surfaces (Olsen, 2006). These groups of salivary proteins, lysozyme, lactoferrin and peroxidases act together with other salivary components limits the growth of bacteria or kill them directly (Marcotte and Lavoie, 1998).

The pH or hydrogen ion concentrations are also an essential oral microbial ecology parameter. Though individual bacteria possess specific molecular strategies enabling them to adapt rapidly to sudden changes in pH (Marsh, 2003), pH affects
microorganisms as well as their enzymes directly and also influences the dissolution of many molecules that eventually influence microorganisms (Marcotte and Lavoie, 1998). The low-pH environment facilitates the diffusion of calcium, phosphate, and carbonate out of teeth, which usually protect the enamel from oral pathogens (Zarco et al., 2012). There has been a broad consensus that quantity and frequency of consumption of sucrose-containing foods has significance on caries incidence (Hamada, 2002). Acidogenic and aciduric bacteria especially mutans streptococci (such as Streptococcus mutans and S. sobrinus) and lactobacilli are able to metabolize dietary sugars to acid rapidly, creating a low pH in the vicinity (Marsh, 2006). These organisms grow and metabolize optimally at low pH; under such conditions they become more competitive, whereas most species associated with enamel health are sensitive to acidic environmental conditions (Marsh, 2003). Amongst the exogenous dietary components, carbohydrates and proteins shows the highest influence on the oral microbiota composition (Marcotte and Lavoie, 1998). In the mouth, endogenous proteins and glycoprotein’s are the main sources of carbon and nitrogen for the resident oral microflora. Endogenous proteins and glycoproteins of the mouth are the main carbon (C) and nitrogen (N) sources for the resident microflora. Pure cultures of oral bacteria can metabolize such molecules only poorly or not at all. The synergistic action of species, each of them having complementary enzymes enables catabolism of these host molecules (Olsen, 2006).

### 2.3.3 Dental biofilm formation

The bacterial collections around the teeth and gums as a sticky, creamy colored mass are known as plaque which serves as a biofilm (Devi and Ramasubramaniaraja, 2009). These dental bacterial plaque or biofilm communities are complex and dynamic structures like other biofilms that accumulate through sequential and orderly colonization of multiple oral bacteria (Kolenbrander et al., 2002). They are found adhering tenaciously to tooth surfaces, restorations, and prosthetic appliances. Mechanisms governing biofilm formation have generated considerable interest in the general biofilm field and also in dental-related biofilms. The formation of dental biofilm, the dental plaque, comprises a series of steps that begin with the initial pellicle colonization to the complex formation of a mature biofilm. It can be divided subjectively into following distinct phases (Silva et al., 2012).
Formation of conditioning film through adsorption of host and bacterial molecules to the tooth surface

Passive transport of oral bacteria to the pellicle-coated tooth surface and their attachment

Coaggregation (coadhesion) of later colonizers to already attached early colonizers

Production of confluent growth through multiplication of attached microorganisms

Detachment of active bacteria from surfaces (Silva et al., 2012).

2.3.3a Formation of conditioning film through adsorption of host and bacterial molecules to the tooth surface (Silva et al., 2012)

Pellicle forms immediately following eruption or cleaning and directly influences the pattern of initial microbial colonization (Silva et al., 2012). The proteinaceous film present on the tooth surface to which the highly diverse oral microbial community constantly interacting is known as the pellicle. These pellicles which provide adherence, for the low number of a small group of organisms (mostly Streptococci and Actinomyces sp like primary colonizers) is either mammalian or bacterial derivative of sources including salivary proteins and exoenzymes (Xiao et al., 2012). Normally the salivary proteins of saliva adsorb strongly onto the teeth, protecting enamel against acid dissolution. This adsorbed protective layer pellicle formation is a rapid process occurring within seconds of a clean tooth surface being exposed to a salivary conditioning film (Williams et al., 2011). The initial attachment of bacteria begins with pellicle formation. The so-called acquired pellicle, the saliva film containing albumin, glycoproteins, acidic proline rich proteins, mucins, sialic acids and other compounds which covers the tooth surface offers receptors for the initial colonizers (Kreth et al., 2009). The primary colonizing bacteria, the ‘early colonisers’ make contact with pellicle receptors following either passive transport in saliva or by active motility of microorganisms to the tooth surface (Williams et al., 2011).

2.3.3b Passive transport of oral bacteria to the pellicle-coated tooth surface and their attachment (Silva et al., 2012)

Once the pellicle formed, bacteria initiate their attachment to the outer surface of the pellicle. The non-specific reversible phase involving physico-chemical interactions among salivary bacteria and acquired enamel pellicle generate a weak area of net
attraction enabling reversible adhesion. Subsequently, strong, short-range interactions between adhesins, the particular bacterial cell surface molecules and complementary receptors in the pellicle develops resulting in irreversible attachment (Lamont and Jenkinson, 2000). Many oral bacteria possess more than one type of adhesion on their cell surface (Silva et al., 2012). Microscopic observation of pellicle coated surfaces shown single cells mostly of Gram-positive coccoid cells collectively with a few rod-shaped organisms, following 2-4 h of plaque formation (Marsh and Bradshaw, 1995).

2.3.3c Co-aggregation of later colonizers to already attached early colonizers and microcolony formation

The dental plaque micro-colony formation starts once the attached bacteria covers the tooth surface. The biofilm grows primarily through cell division of the adherent bacteria, rather than through the attachment of new bacteria. The universal feature of all biofilm bacteria, the microbial co-aggregation (Rickard et al., 2003), enables rapid colonization of surfaces. All oral bacteria display their ability to adhere to at least one other species of oral bacteria and usually to multiple species (Silva et al., 2012). This inherent co-aggregation characteristic of many species of oral bacteria, their ability of recognizing and attaching to genetically distinct bacterial cells has been associated with biofilm formation as well as maturation of dental plaque (Kolenbrander and Palmer, 2004). This cell-to-cell adherence, known as co-aggregation (Socransky and Haffajee, 2000), the most important process provides more attachment sites for later colonizers including Gram-positive and Gram-negative bacteria. The succession of biofilm development involves co-aggregation and co-adherence of oral bacteria, and if undisturbed develops into a stratified, complex biofilm (Kreth et al., 2009). Hence, as a consequence of co-aggregation, a second wave of bacterial colonizers adheres to bacteria that are already attached to the pellicle leading to the formation of a complex array of different bacteria as suggested (Fig 2.2). This co-aggregation also involves specific interbacterial adhesion-receptor interactions (Kolenbrander et al., 2000). The glucan-binding lectin (GBL) produced by many bacteria plays an imperative role in glucan-dependent cell aggregation (Limsong et al., 2004). It also makes the functional organization of dental plaque possible (Bradshaw et al., 1998). Single cells, mainly of Gram-positive coccoid cells can be seen on pellicle coated surfaces by microscopy, together with a few rod-shaped organisms, after few hours (2-4 h) of plaque formation.
(Silva et al., 2012). Usually the members of oral Streptococci (S. sanguis, S. oralis and S. mitis) are the predominant pioneer species (Silva et al., 2012). Although the oral streptococci initially predominate in plaque, and can constitute up to 80% of early plaque population, the Actinomyces naeslundii, and some other haemophili are also recognized as significant colonizing species (Rosan and Lamont, 2000).

Fig. 2.2 Dental biofilm development and the role of co-aggregation (Courtesy: Rickard et al., 2003)

2.3.3d Multiplication of attached microorganisms resulting in confluent growth

The attached microorganism’s cell division leads to confluent growth and ultimately, the spatially and functionally organized 3D mixed-culture mature biofilm. The resultant dental plaque performs as a real microbial community shows more properties than the aggregate of the component species (Marsh, 2004; Silva et al., 2012). Thus inside the dental plaque biofilm, the inhabiting bacteria occupies a varied range of ecological niches (“habitats”). They exist not as isolated species have complex physical and metabolically synergistic relationships with other species (Walsh and Tsang, 2008). Further metabolism of microbes affects the spatial distribution of bacteria leading to the formation of various gradients across biologically significant factors. The main factors affecting the microbial growth (nutrients, pH, O₂, etc.) exhibits gradient development. This would lead to vertical and horizontal stratification of the plaque biofilm and produces a mosaic of micro-environments (Marsh, 2000).
Microorganisms modify physical and chemical properties of their surroundings through their metabolic activity. During biofilm formation metabolism by early colonizers (facultative anaerobic bacteria) depletes oxygen (O$_2$), produces carbon dioxide (CO$_2$) and hydrogen (H$_2$). This lowers the redox potential and promotes growth of late colonizers, many of which are anaerobes. As caries progresses, the lesion penetrates the enamel and reaches the dentine. This makes protein available for cariogenic bacteria (Olsen, 2006). In periodontitis, the junctional epithelium at the base of the gingival crevice migrates down the tooth root to form the periodontal pocket. Simultaneously, gingival crevice fluid (GCF) production increases as a result of increased biofilm accumulation in the periodontal pocket. This new environment would act as a select one for periodontal pathogens (Olsen, 2006). These gradients are not linear and such heterogeneity may explain the co-existence of organisms with contradictory requirements (e.g. in terms of atmosphere, nutrition) and influence on the antimicrobial agent’s activity at diverse biofilm locations (Marsh, 2003). Mature dental plaque on teeth contains about $10^9$ bacteria per gram and up to c.200 microbial species or phylotypes (Wright et al., 2013).

![Fig. 2.3 The temporal arrangement in the dental biofilm](image)

**Fig. 2.3 The temporal arrangement in the dental biofilm** (Courtesy Rickard et al., 2003).

**2.3.3e Detachment active bacteria from colonized surfaces**

The attached bacteria react to the environmental signals and detach themselves from the surfaces, which allow the colonization of cells elsewhere (Silva et al., 2012) and...
their dispersal as well as colonization on new surfaces. The dispersal of biofilm cells may be either by shedding of daughter cells from actively growing cells, detachment as a result of nutrient levels or quorum sensing, or shearing of biofilm aggregates (continuous removal of small portions of the biofilm) because of flow effects. However, the mechanisms underlying the process of shedding by actively growing cells in a biofilm are not well understood (Donlan, 2002).

### 2.4 Biochemical events during caries formation

Normally diagnosis has been defined as “the analysis of the underlying physiological/biochemical cause(s) of a disease or condition.” In the presence of the biofilm, every single sucrose catabolism generates an acidic environment in the immediate surrounding areas of the tooth leading to an initiation a sequence of random mineral loss and mineral gain biochemical reactions. Under normal circumstances, the loss and gain of mineral contents are in stable condition because of dynamic equilibrium maintenance between the tooth-oral fluid and the biofilm microbial content (Carounanidy and Sathyanarayanan, 2009). Dental caries development is associated with a sequence of events in the tooth surface biofilm, a bacterial interaction place with diet (Hayacibara et al., 2005). According to the ecological plaque hypothesis, the disease is a result of a shift in the balance of the resident microflora due to a response to a change in local environmental conditions (e.g. sugar rich diet, low saliva flow, immune suppression) leading to significant ecological pressure. These conditions disrupt the natural homeostasis present in plaque during health, leading to an environment of organisms that can cause disease. For example, a sucrose-rich diet produces prolonged conditions of acidic pH, an environmental change favoring the growth of dental plaque acid-tolerating bacteria capable of demineralizing enamel, at the expense of healthy associated species (Dufour et al., 2012).

There is a general consensus that the frequent consumption of carbohydrates mainly sucrose, can result in the emergence of cariogenic microorganisms (Hayacibara et al., 2005) and selection of bacteria that are acidogenic (capable of producing acid from carbohydrates) as well as aciduric (capable of tolerating acid) in tandem with a low-pH environment. These conditions favor the solubilization of tooth minerals, a process called as demineralization. The critical pH at which demineralization commences range between
pH 5.0 and 5.5 (Marcotte and Lavoie, 1998; Xiao et al., 2012). The low pH environment in the biofilm matrix causes dissolution of the tooth surfaces and initiates dental caries (Kim et al., 2011). Thus, the acidogenic and aciduric Gram-positive bacteria, primarily the mutans streptococci (Streptococcus mutans and S. sobrinus), lactobacilli and actinomycetes, are the main organisms involved in dental caries development. They catabolize sucrose to teeth’s calcium phosphate dissolving organic acids (Palombo, 2009). These acids dissolve the hard, crystalline structure of the teeth, resulting in carious lesions (Khan et al., 2012). The primary acid-tolerant bacteria associated with the plaque are S. mutans, S. salivarius, S. sanguis, S. anginosus, S. constellatus, S. gordonii, S. intermedius, S. mitis, S. oralis, S. cricetus, S. rattus, Lactobacillus spp, staphylococci, micrococcii, Enterococcus faecalis (Marcotte and Lavoie, 1998; Song et al., 2006; Islam et al., 2007; Allaker and Douglas, 2009; Cho et al., 2010; Ogunshe and Odumesi, 2010; Tong et al., 2010; Prasanth, 2011; Wong et al., 2012; Yim et al., 2013).

2.4.1 Caries balance

The diet and nutrition would upset the tooth demineralization and remineralization balance through numerous ways. The plaque bacteria metabolize sugars and other fermentable carbohydrates provided by the diet into acids (Touger-Decker and van Loveren, 2003). The resultant low pH favors the growth of the acidogenic and aciduric bacteria (mutans streptococci) (Touger-Decker and van Loveren, 2003). Thus whenever a fermentable dietary substrate diffuses into the plaque and is converted to acid end products, some degree of subsurface demineralization occurs. Then, between meals or snacks, the pH in the plaque returns to neutrality and calcium and phosphate ions in the plaque, driven by the supersaturated concentration gradient, diffuse into the lesion, promoting remineralization (Loesche, 1986; Berg, 2006; Devi and Ramasubramaniaraja, 2009; Ali et al., 2012; Spratt et al., 2012). Also, diet having low added sugars and fermentable carbohydrates and high calcium-rich cheese might favor remineralization (Touger-Decker and van Loveren, 2003). These demineralization-remineralization cycles can be documented in the incipient lesion as characteristic zones. Demineralization which progresses to cavitation occurs, if the frequency and magnitude of acid production overwhelm the repair process (Fig 2.4). Frequent eating or repair process compromise by a reduced salivary flow lead to this situation. Plaque acid production restriction results in the domination of remineralization as occurs with the ingestion of low sucrose diets or the
use of sugar substitutes for between-meal snacks (Ehrlich et al., 2008). Fluoride also promotes remineralization (Loesche, 1986; Berg, 2006; Devi and Ramasubramaniaraja, 2009; Ali et al., 2012; Spratt et al., 2012).

Fig. 2.4 Showing caries process as regular flux of demineralization and remineralization (Courtesy: Selwitz et al., 2007).

2.4.2 Caries mechanism

From the above, it can be understood that dental caries is a simple process in concept. The biochemical events of caries mechanism can be summarized as follows (Devi and Ramasubramaniaraja, 2009).

1. Oral plaque acidogenic bacteria ferment carbohydrates in the mouth and produces organic acids, including acetic, formic, lactic and propionic acid.
2. These acids during their travel, diffuses into the enamel, dentin, or cementum dissolves their mineral crystals partially.
3. This mineral (calcium and phosphate) diffuses out of the tooth, leading eventually to cavitations upon continuation.
4. Diffusion of calcium and phosphate collectively with fluoride into the tooth and deposition of a new crystal remnant in the non-cavitated lesion reverses demineralization.

5. When compared with the original carbonated hydroxyapatite mineral, the new mineral crystal surfaces are found to be much more acid resistant.

6. The process of demineralization and remineralization occurs numerous times daily, leading either to cavitation, to repair and reversal, or to maintenance of the status quo.

In root caries, the same mechanism occurs as outlined above, initially causing demineralization and exposure of the collagen fibrils. Once the collagen is exposed, it is open to breakdown by bacterially derived enzymes, leading to rapid cavitation and breakdown of the dentin in the tooth root (Devi and Ramasubramaniaraja, 2009).

2.5 Structure of dental biofilm

Oral microbial biofilms are the three-dimensional, structured, solid surface like the enamel of the teeth, the surface of the root or dental implants attached bacterial communities. Listgarten and co-workers (1975) unraveled tooth attached biofilm architecture through light and electron microscopy studies on epoxy resin crowns and extracted teeth. They highlighted the complex nature of oral biofilms (Zijnge et al., 2010). Normally biofilms are made up of non-randomly distributed micro-colonies of bacterial cells (15-20% by volume) in a shaped matrix or glycocalyx (75-80%) volume (Saini et al., 2011). Since the structure can influence transport resistance, it is a significant determinant in the activity of the biofilm (De Beer and Stoodley, 2006). The shapes of these microcolonies differ widely since they are governed by shear forces due to the passage of fluid over the biofilm. If shear force are low, the colonies are shaped liked towers or mushrooms, at the same time the colonies are elongated and capable of rapid oscillation at high shear force (Socransky and Haffajee, 2000; Behlau and Gilmore, 2008). Each micro-colony is a tiny independent community comprising thousands of compatible bacteria showing its own customized living environment. They are characterized by swift formation of visible layers of micro-organisms owing to the wide-ranging bacterial growth accompanied by excretion of copious amount of extracellular polymers. The layer above is a highly irregular loose layer extending into the surrounding
medium (Fig 2.5). They also have voids or water channels between the micro-colonies that allow the passage of nutrients and other agents throughout the biofilm (Saini et al., 2011). These dental Biofilm if found on hard surfaces of the oral cavity, harbors cariogenic bacteria, cell free enzymes, polysaccharides and host constituents (Steinberg et al., 2004). Cariogenic biofilms are encompassed of varied microbiota in vivo, all of are usually potent acid producers or acid-tolerants bound by a common matrix; which creates a highly adhesive, cohesive, and acidic milieu (Xiao et al., 2012).

**Fig. 2.5 Structure of dental biofilm** Courtesy (Nield-Gehrig and Willmann, 2003)

### 2.5.1 Bacterial life in biofilms

Many and perhaps most species of bacteria prefer this biofilm mode of growth. It offers a number of advantages to colonizing species, including 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 (one species providing nutrients for another), removal of potentially harmful metabolic products as well as the development of an appropriate physicochemical environment (Socransky and Haffajee, 2000), etc.

Microbes when they change their planktonic way of living to organized sessile surface-associated community, the biofilm, upon colonization on surfaces available in nature (Ruzicka et al., 2011), undergoes precisely programmed developmental changes. The environmental signals like nutritional content, temperature, osmolarity, pH, iron, oxygen, etc., advocate (Behlau and Gilmore, 2008). When they lead biofilm mode of life, they become less susceptible to anti-microbials and more resistant to immune defense mechanisms. They require 10-1000 times increased concentration of an agent which kills planktonic micro-organisms to have the same efficacy (Scheie and Petersen, 2004). The biofilm and planktonic cells contrast considerably in their physiology, gene expression
pattern, and even morphology. Since they are less sensitive to antimicrobial agents, controlling their growth could be a significant challenge once it forms (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). The following (Table 2.1) are the major differences found among biofilm and planktonic bacteria (Behlau and Gilmore, 2008).

### Table 2.1 Differences between planktonic and biofilm bacteria (Behlau and Gilmore, 2008)

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Differences in mode, cultural, susceptibility and gene expression</th>
<th>Planktonic Bacteria</th>
<th>Biofilm Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Suspended cells; single cells</td>
<td>Aggregated cells; multiple cells at an interface</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Little capsular matrix</td>
<td>Surrounded by EPS matrix</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Physiologically homogeneous and active</td>
<td>Physiologically heterogeneous and more dormant cells</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Intracellular signaling not critical for cell division</td>
<td>Intracellular signaling critical for growth and higher-order architecture formation</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Antibiotic-susceptible physiologically active cells</td>
<td>10-1000 times increased antibiotic resistance</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Individual cells recognized and effectively targeted by host immune response</td>
<td>Matrix-embedded cells are inaccessible to host response and resistant to the antimicrobial agents</td>
<td></td>
</tr>
</tbody>
</table>

### 2.5.2 Biofilm resistance

Bacteria living in biofilms exhibit 100- to 1,000-fold increase in their tolerance to antibiotics in comparison to their free-swimming counterparts (Moscoso et al., 2009; Dufour et al., 2012). The antibiotic resistance common mechanisms including efflux pumps, modifying enzymes, and target mutations could not be accounted for the bacterial protection in biofilm. The possibility of slow or incomplete penetration of the antibiotics, alternation of chemical microenvironment within the biofilm and formation of a subpopulation having unique, and highly protected, phenotypic cell differentiation similar to spore formation are speculated or assumed as possible reasons (Stewart and Costerton, 2001). 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). The strongly charged or highly
chemically reactive agents are unable to reach the biofilm’s deeper zones since the biofilm acts as an ion-exchange resin. In addition, the matrix trapped and concentrated extracellular enzymes such as \( \beta \)-lactamases, formaldehyde lyase and formaldehyde dehydrogenase inactivates susceptible, typically positively charged, hydrophilic antibiotics. The biofilm thickness, hydrodynamics and the turnover rate of the microcolonies will also affect antibiotic effectiveness (Socransky and Haffajee, 2000).

The physiological heterogeneity within a biofilm, actively metabolizing and dividing cells having ready access to nutrients at the surface and mostly dormant internal cells, lead to cells with very much dissimilar antibiotic susceptibilities (Behlau and Gilmore, 2008). Normally antimicrobials express restricted ability to eradicate bacteria deep into biofilms, in part owing to their binding with the biofilm outer layer components (ten Cate, 2012).

The association of molecules like EPS and DNA within the biofilm constitutes a physical barrier to the diffusion of antimicrobial agents (Bordi and de Bentzmann, 2011). It is suggested that EPS can interact with antibiotics in a manner leading to a decline in their antibacterial activity (Tetz et al., 2009). Consequently, the chemotherapeutic agents find difficulty in penetrating the polysaccharide matrix to reach and affect the microorganisms. Thus, the matrix helps to increase the chances of the colony’s survival by protecting bacteria deep inside the biofilm from antibiotics and antiseptics (Gurenlian, 2007). The clinical relevance of biofilms is their low antimicrobial sensitivity while displaying enhanced pathogenicity (pathogenic synergism). The structure of the plaque biofilm might restrict the penetration of antimicrobial agents, while bacteria growing on a surface grow slowly and display a novel phenotype, one consequence of which is a reduced sensitivity to inhibitors (Parsek and Fuqua, 2004; Marsh, 2006; Van Houdt and Michiels, 2010).

Suppression of some resident bacterial populations by the orally or systemically given antibiotics that reach the oral cavity through saliva and gingival crevicular fluid lead to overgrowth of antibiotic resistant bacteria (Marcotte and Lavoie, 1998), thus makes an imbalance in oral ecosystem. Hence the manifestation of characters that make sessile microorganisms more resistance to antimicrobial agents than their planktonic counterparts is the highly human being damaging property of biofilms (Villa and Cappitelli, 2013).
2.5.3 Exopolysaccharides

Biofilm cells, unlike their free-floating planktonic counterparts, are embedded in a self-produced extracellular matrix, the extracellular polymeric substance (EPS) that holds them together (Simoes et al., 2010; Dufour et al., 2012). They are called otherwise as glycocalyx, slime layer, capsule or a sheath (Percival et al., 2011). The EPS accounts (by volume) 80–85% of biofilms, whereas the cells credits only 15–20% (Dufour et al., 2012). The EPS determines the physical properties of the biofilm while the bacterial cells determine its physiological properties (De Beer and Stoodley, 2006). These bacterial origin EPS are polysaccharides synthesized and secreted into the exterior of the cell or are synthesized extra-cellular by cell wall-anchored enzymes (Nwodo et al., 2012).

Many oral biofilms members exhibit ability of both synthesing and degrading EPS (Socransky and Haffajee, 2000). The occurrence of different types of polysaccharides and their production is the species and strain dependent one. The *Pseudomonas aeruginosa* alginate, staphylococcal polysaccharide intercellular adhesion, streptococcal and lactobacilli glucans and fructans are examples of best-known biofilm-associated EPS (Ruzicka et al., 2011). There are well-described differences in the production of water-soluble polysaccharides (fructans) and water-insoluble polysaccharides (glucans) among mutans streptococci group (Ruzicka et al., 2011). Although the EPS may vary in chemical and physical properties, the characteristic constituents of EPS include polysaccharides, proteins, nucleic acid, lipids, etc., (Sihorkar and Vyas, 2001; Dufour et al., 2012). The monomeric composition of EPS lead to the recognition of two groups viz. the homopolysaccharides and heteropolysaccharides (Nwodo et al., 2012).

The attachment, detachment, mechanical strength, antibiotic resistance, and exoenzymatic degradation activities of the biofilm were linked to their EPS (De Beer and Stoodley, 2006). They act as a backbone of the biofilm by binding the biofilms bacteria together in a sticky web of tangled EPS fibers which connects cells as well as anchor them to a surface and to each other (Sihorkar and Vyas, 2001). Extracellular polymeric matrixes (EPM) are a main building constituent of biofilms and are accountable for most of their physical, chemical and biological properties (Ruzicka et al., 2011). These EPM are one of bacterial product in the pellicle and on microbial surfaces provides the early foundation of the EPS-rich matrix (and binding sites), so intensely persuades the virulent
biofilm formation events. The parallel acidification of the EPS-rich matrix due to the fermentation of sucrose and additional fermentable carbohydrates like glucose, fructose, and maltose favors the growth of the acid tolerant microorganisms thus promotes tooth enamel demineralization leading to the clinical onset of cavitation (Xiao et al., 2012).

The EPS matrix serves as the ‘house for biofilm cells’. The matrix plays a role in numerous processes including attachment, cell-to-cell interconnection and interactions between subpopulations, tolerance and exchange of genetic material (Harmsen et al., 2010). Hence, EPM plays a vital role not only their formation but also behavior (Ruzicka et al., 2011). The EPS matrix is one of the characteristic features of antibiotic tolerant microbial biofilm. These biofilm matrixes offer antibiotic protection by physically interfering their diffusion or by their direct binding with the antibiotics. They also act as a chemically active barrier. The anionic EPS matrix could bind and sequester toxic cationic heavy metals, cationic antimicrobial peptides, and positively-charged antibiotics (e.g. amino glycosides) (Dufour et al., 2012). Thereby they delays or prevents antimicrobials from reaching target microorganisms (Simoes et al., 2010). They help the inhabiting microorganisms by acting as a buffer and support them by holding the extracellular enzymes and their substrate thus enhances their substrate utilization (Socransky and Haffajee, 2000). These EPM in addition protects bacterial cells from opsonization and complement system effects. Likewise, they defend cells from phagocytosis (Ruzicka et al., 2011). Hence, they are deemed to be necessary for biofilm lifestyle existence and global expression of bacterial pathogen’s virulence. EPS of cariogenic oral biofilms are not only key components of the matrix but are recognized virulence factors involved in the pathogenesis of dental caries (Xiao et al., 2012).

2.6 Plaque control methods

The History of oral hygiene products are very old one going back over 6000 years (Sammons, 2003). Dental caries causing organism in the plaque lead biofilm mode of life hence have an advantage over their free-floating (planktonic) counterparts. The extracellular matrix keeps the bacteria banded together, so they are not flushed away by the action of saliva and gingival crevicular fluid (Gurenlian, 2007). For controlling these typical biofilm plaque, the methods including (1) mechanical removal, (2) local or systemic use of antimicrobial drugs, (3) cytotoxic or cariogenic products formation
preventing plaque biochemistry, (4) prevention of colonization by preventing bacterial attachment to the tooth surface, (5) modification plaque ecology to a less pathogenic flora are suggested as possible methods (Addy, 1986; Sammons, 2003). Currently, numbers of agents and their application through various routes are under investigation for dental biofilm control which includes probiotics, antimicrobial peptides, interspecies and inter-kingdom signaling interfering agents, mutacins, bacteriocins, quorum-sensing peptides, nanotechnological approaches, natural products, photodynamic therapy, prebiotics, predatory bacteria and ‘designer’ bacteria (ten Cate and Zaura, 2012). Thus the plaque control methods include both physical and chemical methods.

2.6.1 Physical methods of plaque control

Physical methods comprise heat, mechanical scrubbing, brushing, scraping and high pressure spraying (Sandasi et al., 2011). Brushing with toothbrush mechanically removes the supra gingival plaque through reducing the total number of the tooth surface colonizing bacteria and disrupting their biofilm forming process. Since it prevents or hinders the accumulation and maturation of plaque consequently the establishment of anaerobic conditions, reduces the pathogenic organism’s growth (Sammons, 2003). Frequent and efficacious brushing and flossing of plaque mechanical removal processes are the principal means of periodontal diseases prevention and caries risk reduction (Marcotte and Lavoie, 1998). These patient-based strategies like daily tooth brushing, inter dental cleaning along with the application of topical antimicrobial chemotherapeutics are would reduce the bacterial biofilm and helps in preventing periodontal diseases (Gurenlian, 2007). Hence, the dental plaque disrupting mechanical methods, including tooth brushing, inter dental cleaning along with professional scaling procedures, are required regularly for effective disruption and removal of plaque biofilm (Gurenlian, 2007). For effective prevention of caries, the plaque control methods should remove all plaque, reduce plaque levels below the threshold for disease, and alter plaque pathogenicity (Addy, 1986) which is unachievable one currently.

2.6.2 Chemical methods of plaque control

A range of chemotherapeutic agents has been examined for their ability to control oral microorganisms and to affect plaque formation (Emilson, 1994). The usage of these chemical biocides for example, disinfectants, detergents and preservatives for controlling
dental plaque are very common (Sandasi et al., 2011). These antimicrobial agents could assist in dental protection by reducing the bacterial adhesion to the tooth surface, their growth and plaque accumulation, by selective inhibition on oral disease associated bacteria, or by inhibiting their virulence determinants expression like acid production or protease activity (Marcotte and Lavoie, 1998). The antimicrobial agents applied for plaque control can be divided into several groups including antibiotics, enzymes (mutanase, glucanase; amyloglucosidase-glucose oxidase), bisbiguanides (chlorhexidine, hekitidine), quaternary ammonium compounds (cetyl pyridinium chloride), phenolic compounds (triclosan, povidone iodine), natural products (plant extracts: apigenin, Tt-farnesol), essential oils (menthol, thymol, eucalyptol), fluorides, metal (copper, zinc, stannous) ions, oxygenating agents, surfactants (sodium lauryl sulphate, delmopinol), enzymes and other antiseptics (Sreenivasan and Gaffar, 2002; Tsai et al., 2007; Yanti et al., 2008; Islam et al., 2009; Porto et al., 2009; Marsh, 2010; Naidoo et al., 2012).

2.6.3 Vehicles for anti-plaque agents

The various vehicles for anti-plaque agents include toothpastes, mouth-rinses, gels, gums, varnishes and irrigants (Sammons, 2003). Dentifrices are a common vehicle for routine patient-directed oral hygiene. Dentifrices formulation includes a number of antiplaque biocides such as triclosan, metal salts, essential oils and chlorhexidine to provide additional benefits such as reductions in plaque and gingivitis. Triclosan, a broad-spectrum biocide showing effects on many types of bacteria found their way extensively into the formulation of dentifrices (Sreenivasan and Gaffar, 2002). A gel is a thickened version of a mouthwash, often consisting of a water or water–alcohol base with flavor, surfactant and humectants, such as glycerol. A varnish is a polymer-based matrix that slowly releases an agent onto the tooth surface to which it is applied and to saliva.

2.6.3a Mouth rinses

The effectiveness of antibacterial mouth rinses in decreasing tooth surface plaque is well established. In general, mouth rinses may contain fluorides, alcohols, detergents and other antimicrobial substances. Such synthetic antimicrobials include povidone iodine products, chlorhexidine and cetylpyridinium chloride (Allaker and Douglas, 2009). The antimicrobial agents found in mouth rinses also include chlorhexidine, quaternary ammonium compounds, plant extracts, metal ions, and phenolic compounds. These
Antimicrobial agents have been shown to reduce dental plaque formation, caries and gingivitis (Marcotte and Lavoie, 1998). The important requirement of this oral biofilm controlling antiseptics for instance, mouth rinses is that their formulation must ensure their penetration into the plaque matrix and gain access to the pathogenic bacteria (Gurenlian, 2007).

2.6.4 Antibiotic resistance

The antibiotics showing selective activity against anaerobic organisms are the most commonly used ones such as minocycline, combinations of metronidazole amoxicillin or metroxyzole ciprofloxin and penicillin, vancomycin and kanamycin and are administered either systemically or topically (Addy, 1986; Sammons, 2003). Also the other antibiotics, including ampicillin, chlorhexidine, erythromycin, spiramycin, and vancomycin, were found to be effective in preventing dental caries (Yim et al., 2013). “When manual treatments are supplemented with local and systemic antibiotics, the oral cavity experiences a change in composition and abundance of various bacteria. Local antibiotics kill or freeze an array of species at diseased sites in the oral cavity, as well as heal oral lesions and halt plaque accumulation. Systemic drugs target pathogens at sites around the body in addition to the oral cavity, but are limited to the species they target. They can also reduce any bleeding in the periodontium. However, systemic drugs are conventionally used as a last resort in the treatment of periodontal diseases for cost-effective purposes” (Zarco et al., 2012.). The widespread use of these drugs has led to rapid development of drug-resistant strains, which are the leading cause of failure in clinical applications (Termentzi et al., 2011). Although a number of control measures are in place for biofilm control, most of these seem to be almost ineffective due to the increased resistance conferred by sessile cells (Sandasi et al., 2011).

The formation of very high antibiotic concentration tolerant persister cells is another antibiotic tolerance mechanism shown by biofilm population. These specialized persister cells enter into a state of dormancy, which allows them to survive stress conditions and prevents death because antibiotics target cell growth. They are not antibiotic-resistant mutants, but rather phenotypic variants of the wild type that form stochastically in a clonal population of genetically identical cells. Slow-growing biofilms produce substantial numbers of persister cells. According to persister survival model, the
antibiotic tolerance of bacterial biofilms is due to the biofilm persisters which are protected from host defenses by the biofilm matrix. Once the antibiotic treatment has been stopped, the persister cells repopulate the biofilm and the infection relapses. The host immune system also targets and kills only planktonic persisters. These biofilm persisters have a much broader clinical significance than only in the context of biofilm infections (Dufour et al., 2012). Controlling systemic use of antibiotics to avoid the resistant organism buildup and adverse drug reactions is a much need one in dentistry (Sammons, 2003).

2.6.5 Side effects

Though noteworthy reductions in caries prevalence and incidence have been achieved through the introduction of fluoride, chlorhexidine, triclosan and other chemotherapeutics as antiplaque agents, they are associated with a number of unwanted side effects. They act on the hard tissues rather than on the oral bacteria (Ten Cate, 2006), induce a number of hypersensitivity reactions in patients and lead to the emergence of resistant bacterial strains and super infection by resistant organisms such as Candida obviate (Addy, 1986). They also causes tooth and restoration staining (Islam et al., 2009; Palombo 2009; Chen et al., 2012; He et al., 2013), taste sensation alteration, taste change, shortness of breath, desquamation and soreness in the oral mucosa (Islam et al., 2009; He et al., 2013), fluorosis (He et al., 2013), diarrhea (Palombo, 2009; Porto et al., 2009), increasing calculus formation, oral and intestinal flora disarrangement (Porto et al., 2009), taste irritation, oral cavity ecology alteration (Hannig et al., 2008), allergic contact dermatitis, burning sensation (Chen et al., 2012), and lead to the emergence of bacterial resistance (Song et al., 2006; Palombo, 2009) along with promoting the development of multidrug resistant (MDR) strains (Yim et al., 2013).

Mouth rinses often contain surfactant, alcohol, fluoride and antimicrobial agents (Morgan et al., 2001). Alcohol is an important ingredient in mouth-rinses which added to make them biologically active, dissolve the active ingredients and as flavoring agents. However, the use of alcohol has increasing concern since their use may increase the risk of developing oral cancer. There is a report available on increased risk of developing OPC (oropharyngeal cancer) due to use of mouthwashes that contain alcohol (Ciancio et al., 2010). Cetylpyridinium chloride, chlorhexidine, amine fluorides or products
containing such agents, are reported to exhibit toxicity (Palombo, 2009). The carcinogens introduce toxic agents into salivary fluid that damage DNA, cause mutations and damage the integrity of oral cavity. The oral cavity reacts to the toxins with an inflammatory response, which then produces the pathogenic agents that contribute to tumor development, thus maintaining a vicious carcinogenic cycle (Zarco et al., 2012).

2.7 Alternate plaque control agents

The above listed problems and inefficacy of the currently used antiplaque agents warrants a search for new antibacterial substances that are safe for humans and specific for oral pathogens. Microorganisms, animals, and plants have been investigated for the possession of components having these desirable characters (Yim et al., 2013). Emergence of biofilm mediated conventional antimicrobial resistant clearly shows that new biofilm control strategies are required (Simoes et al., 2010) to control dental caries plaque and the causative biofilm organisms. Among the several approaches used for biofilm control, the use of anti-biofilm 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). Most treatments are now aimed at either elimination of this bacterium or suppression of its virulence (Koo et al., 2003; Limsong et al., 2004; Hayacibara et al., 2005; Islam et al., 2007; Wong et al., 2012). Therefore, a meaningful strategy for preventing dental caries would be to control both the growth and virulence factors (Song et al., 2006). One approach to reduce the incidence of caries is to develop therapeutic agents with antimicrobial and/or anti-adherent properties aimed to prevent the bacterial proliferation on the tooth surface (Nostro et al., 2004). Apart, the current strategy aims at targeting other virulence and pathogenic properties of the causatives. The manifestation of dental caries depends mainly on the causative organism’s acid production and acid tolerance. Hence the reduction or elimination acid production and acid tolerance physiological abilities of dental biofilms organisms like S. mutans would lead to acidification potential of dental biofilms and subsequent dental caries formation decrease (Kim et al., 2011).

While developing the anti-caries and anti-gingivitis agent, the biology of the oral bacterial communities must be taken into account. The new antiplaque agents under development are designed to (1) prevent adhesion of the target organisms, (2) prevent
biofilm formation, (3) remove established biofilm, (4) disrupt existing biofilms, (5) prevent coaggregation by the target organisms, (6) prevent further biofilm growth, (7) kill specific organisms in the biofilm (Sbordone and Bortolaia, 2003; Spratt et al., 2012). A good antiplaque agent should have the following qualities: safe, selective and specific effects on pathogenic microorganisms, pleasant/neutral odour and taste, retain antimicrobial efficacy at low concentrations, fast action, non-toxic, non-allergenic, non-irritant, good oral retention, less or not at all disruptive to the oral microbial ecology, global regulatory authorities approval, substantive, compatible with toothpaste ingredients, chemically defined and stable, physical stability, cost effective and easy to use (Morgan et al., 2001; Sammons, 2003; Palombo, 2009). Detection of new natural, Generally Recognised 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), which may in the future result, in fruitful outcome.

2.8 Plants as alternate source for antiplaque compounds

Natural compounds have been and continue to be, an important source of new leads for many pharmaceuticals and agrochemicals (Chanda and Kaneria, 2011). Natural drugs could represent an attractive approach to limit the emergence and the spread of the difficult to treat antimicrobial resistant organisms. Recently, there is an upsurge in the scientific interest on studying plant materials as sources of new compounds for their processing into therapeutic agents (Nostro, 2006). Herbal products have been used since time immemorial in folk medicine, encompassing both Eastern and Western medical traditions. The pharmaceutical companies have shown interest in exploring plants as sources for new efficient, safe, and quality phytotherapeutic agents over the preceding years (Groppo et al., 2008).

Screening natural products to identify a reservoir of antimicrobial materials for use is against these infections either lonely or in combination is a pressing demand. These natural products use might decrease drug resistance development in microorganisms as they are surrounded by a group of antimicrobial chemicals acting together (Wong et al.,

So far, thousands of phytochemicals have been shown to have antimicrobial activity (Allaker and Douglas, 2009). Though medicinal plants were used for centuries in folk medicines and numbers of studies have been carried out on them deciphering the scientific basis of their effectiveness, only a small proportion of plant species have been investigated thoroughly for their medicinal properties, and undoubtedly there are other many biologically active new compounds to be discovered (Nostro, 2006). The recent year’s revival of interest in the use of medicinal plants in developed as well as developing countries is due to the fact that medicinal plant’s compounds were proven to an effective source of chemotherapeutic agents without undesirable side effects (Khan et al., 2010).

Thus, there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Chanda and Kaneria, 2011). Plants are a significant likely source of new bioactive bacterial virulence disrupting compounds hence; they may supply alternatives to the commonly used dental biofilm chemicals. Presence of antioral pathogens properties among a number of plant metabolites is well established. Henceforth, natural products are still significant sources of innovative therapeutic agents which could be useful for the development of alternative or adjunctive anticaries therapies (D'Angelis et al., 2012). Plant-derived compounds found to inhibit peptidoglycan synthesis, damage microbial membrane structures, modify bacterial membrane surface hydrophobicity and modulate quorum sensing, all of which could influence biofilm formation (Rasooli et al., 2008). Natural products such as epicatechin, kaempferol, chitosan and funoran, isolated from natural products, also have been investigated for their efficacy against oral microbial pathogens (Rosas-Pinon et al., 2012). A literature survey carried out on determining the feasibility of using available natural products as preventive measures for dental caries showed promising results (Cho et al., 2010; Yim et al., 2010). The main reasons recorded for the use of medicinal plants are their accessibility and cost (Yim et al., 2010). Plants represent a virtually inexhaustible and sustainable source of biocide-free antibiofilm agents with novel targets, unique modes of action and proprieties with potential for utilization in a plethora of medical, agricultural as well as industrial fields. On the other hand, realization of this possibility has been hindered so far due to the insufficient fundamental research carried on understanding comprehensively the ecologically relevant functions of plant-derived compounds in the real natural environments (Villa and Cappitelli, 2013).
2.9 Medicinal plants and their oral care agents

Now there is wide recognition that the goal of caries treatment is to reverse or halt disease progression, eventually via manipulation of the microbial composition using chemotherapeutics (Filoche et al., 2008). Developing therapeutic agents with antimicrobial and/or anti-adherent properties preventing the bacterial proliferation on the tooth surface is also another one line of attack for reducing the incidences of caries (Nostro et al., 2004). Hence, regular control of dental plaque, a natural biofilm, formed on tooth surfaces and compacted at gingival margins is the most significant aspect of good oral health (Gilbert et al., 2007). Though active ingredients in oral care products, such as dentifrices and mouthwashes, have the potential to compensate for shortfalls by having significant effects on the vitality of dental biofilm, but they are not apt replacement for mechanical cleansing (Gilbert et al., 2007). Number of plants and their products people use worldwide for oral care maintenance might have potential to be developed into anticaries agent.

In recent times, phytochemicals are recognized as a worthy substitute to synthetic chemical substances meant for caries prevention (Morgan et al., 2001). The flow microcalorimeter `studies on natural' putative antimicrobial obtained from the extracts from Celastrus scandens, Chamaebatia foliolosa, Digitaria sanguinalis, Ginkgo biloba, Juniperous virginiana, Anacardium occidentale L. and Ilex paraguayensis St. Hil., or mate tea, were found to be exhibit effective inhibition against S. mutans (Morgan et al., 2001). Extract of Helichrysum italicum G. Don a compositae family member widespread in the Mediterranean regions found to affect some of the cariogenic properties of S. mutans for instance surface hydrophobicity, adherence and cellular aggregation. It's flavonoidic components viz. apigenin, luteolin, gnaphaliin; naringenin, pinocembrin and tiliroside are believed to be responsible for its S. mutans antiadherence property (Nostro et al., 2004). Filoche et al. (2005) believed that plant essential oils might have a role in the development of novel anticaries treatments. They came to this opinion based on their comparative study on antimicrobial effects of essential oils alone and in combination with chlorhexidine digluconate against planktonic and biofilm cultures of S. mutans and L. plantarum. They studied the antimicrobial potency of essential oils from cinnamon, tea-tree (Melaleuca alternifolia), manuka (Leptospermum scoparium), Leptospermum morrisonii, arnica, eucalyptus, grapefruit, the essential oil mouth rinse cool mint listerine
and two of its components, menthol and thymol. They found that amount of chlorhexidine required to achieve an equivalent growth inhibition against the biofilm cultures was reduced 4–10-fold in combination with cinnamon, manuka, *L. morrisonii*, thymol, and listerine. Novel mouth rinse (IND 61,164) containing essential oils and extracts from four plant species, tea tree oil’ (*Melaleuca alternifolia*) or manuka (*Leptospermum scoparium*) and botanical extracts such as calendula (*Calendula officinalis*) or green tea extract (*Camellia sinensis*) have become popular ‘natural’ products believed to be antimicrobial/anti-inflammatory agents. Mouth rinses and dentifrices containing these ingredients purport to have antiplaque and anti-gingivitis properties (Lauten et al., 2005). The quercetin-3-O-α-L-arabinopyranoside (guaijaverin), a biologically active compound obtained from crude methanol extract of *P. guajava* via bioautography-directed chromatographic fractionation inhibits *S. mutans* growth and thereby demonstrated their high potential antiplaque capacity (Prabu et al., 2006). Song et al. (2006) reported the methanol extract from the root of *Polygonum cuspidatum* (MEP) reduced the production of glycolytic acid by *S. mutans* and *S. sobrinus*. Lectins from seeds of *Canavalia ensiformis*, *Canavalia brasiliensis*, *Dioclea violacea*, *Dioclea grandiflora*, *Cratylia floribunda* and *Vatairea macrocarpato*, all members Leguminosae family, inhibit the in vitro adherence of five streptococci species to acquired pellicle suggesting their usefulness in anti-adhesion therapeutics (Teixeira et al., 2006). Hammad et al. (2007) found that the aqueous extract of thyme (*Thymus vulgaris*) was found to have good potential as plaque control agent with less adverse effects and may be more acceptable to consumers and the regulatory agencies in comparison to synthetic chemical compounds.

Though a remarkable achievement has attained through bactericidal approach, the search continues for active ingredients that could prevent dental plaque formation without affecting the oral cavity biological equilibrium. Besides, dental biofilm controls insist not only bacteriostatic but also anti-adhesive actions in order to detach microbial deposits or to inhibit the initial bacterial adherence (Furiga et al., 2008). Number of studies reported the anticariogenic effect of polyphenols extracted from different types of plants during the last twenty years (Xie et al., 2008). *Mentha piperita* and *Rosmarinus officinalis* essential oils significantly decreased bacterial adhesion and affected bacterial viability in biofilms. They are also capable of affecting biofilm formation significantly (Rasooli et al., 2008). *Curcuma xanthorrhiza* Roxb. xanthorrhizol and nutmeg macelignan showed in vitro
antibacterial activity against both *S. mutans* biofilm and planktonic cells. Their antibiofilm activity is due to reduction of cell surface hydrophobicity (Yanti *et al.*, 2008). The mulberry (*Morus alba*) is chewed during toothache to avoid further destruction or cavitation of the tooth traditionally. Its purified compound 1-deoxynojirimycin (DNJ) was learned to inhibit *S. mutans* biofilm (Islam *et al.*, 2008). Rasooli *et al.* (2009) showed that essential oils of *Eucalyptus camaldulensis* and *Mentha spicata* are capable of disturbing biofilm formation. They considerably reduced bacterial adhesion and upset bacterial viability in biofilms. The lectins isolated from *Canavalia ensiformis*, *Trigonella foenumgraecum*, *Triticum aestivum*, *Arachis hypogaea*, *Cajanus cajan*, *Phaseolus vulgaris*, and *Pisum sativum* found to inhibit the initial biofilm formation by *S. mutans*. The glucose/mannose-specific lectin altered the adhesion arrangement of the bacteria on the saliva-coated surfaces (Islam *et al.*, 2009).

A novel antibiofilm active compound (4aS, 5R, 8aS) 5, 8a-di-1-propyl-octahydronaphthalen-1-(2H)-one, exhibiting antibiofilm activity against *S. mutans* with a high potential to be used as a therapeutic agent against dental caries could be obtained from *Trachyspermum ammi* (Khan *et al.*, 2010). Numbers of natural substances like plant extract components including tannins and other polyphenols were shown to have GTFs and sucrose-dependent *S. mutans* colonization inhibitory activities (Khan *et al.*, 2010). The traditional Chinese medicine Asian common fruit, *Prunus mume*, the Japanese Apricot inhibits total of 15 oral pathogens including *Streptococcus mutans*, *S. sobrinus*, *S. mitis*, *S. sanguinis*, *Lactobacillus acidophilus*, *P. gingivalis*, *Aggregatibacter actinomyctemcomitans* (Seneviratne *et al.*, 2011). *Cucurma longa* essential oil has been shown to inhibit the bacterial growth, acid production, adherence to HAs, and biofilm formation of *S. mutans* (Lee *et al.*, 2011). The medicinal plant's active components, for example, carvacrol and dimer chalcons combination may be exploited to develop dental diseases antimicrobial gels (Seneviratne *et al.*, 2011). The traditional Chinese medicine modified in Japan kampo's crude drug and flavoring agent liquorice both alone and, as liquorice/Ca(OH)$_2$ mixture showed a potent bactericidal effect against root canal environment *Enterococcus faecalis* and retained compatibility with fibroblasts in tissue culture compared to the commonly used root canal medicament Ca(OH)$_2$ (Badr *et al.*, 2011). Yim *et al.* (2013) believed that the herbal medicines Notoginseng Radix and
Perillae Rhizoma reduce the synthesis of water-insoluble glucans by inhibiting the enzymatic activity of the *S. mutans* GTase rather than by inhibiting bacterial growth.

The data presented above call attention to the potential role of plant extracts/phytocompounds in developing new antimicrobial agents. However, even if a lot has been published by now, there is still many more to be done so that these substances can be used in the future (Nostro, 2006). Also, the above mentioned and number of other studies show the way for the acceptance of traditional medicine and natural products as an alternative form of oral health care (Prabu *et al.*, 2006) particularly potential source for the development of anticaries agents.

Even though the toothbrushes and toothpastes are used widely, natural methods of tooth cleaning using chewing sticks selected and prepared from the twigs, stems or roots from a variety of plant species have been practiced for thousands of years in Asia, Africa, the Middle East and the Americas (Wu *et al.*, 2001). Peoples in southern India use mango leaf for cleaning teeth. Mangiferin, a compound present in mango leaves showed a noteworthy antibacterial property against certain strains of Pneumococci, Streptococci, Staphylococci, and *Lactobacillus acidophilus* (Singh and Purohit, 2011). In many parts of the world, traditionally people use the roots and stems of plants having dense group of minute fibers, natural fiber chewing stick which can be frayed into natural tooth brush for cleansing the teeth. This natural tooth brushes while cleaning mechanically like their synthetic counterparts, deemed to show soothing on the gums. In addition to mechanical cleaning, the bioactive compounds leaching from them are expected to exhibit number of reactions in the oral environment including their antimicrobial action on pathogens, mouth refreshment by imparting fragrance in the mouth, bad odor elimination, salivary gland and stimulation of taste bud sensation. The usage of these plant products achieve and maintain good tooth and gum health naturally. World health organization (WHO) also advices researchers to explore the possible use of natural products such as herb and plant extracts with the aim of overcoming the side effects (Al-Bayaty *et al.*. 2010) antimicrobial already in use. Though clinical medicine utilized herbs for thousands of years, now only the researchers have been able to employ scientific methods to prove their efficacy and offer a better understanding on their mechanisms of action (Badr *et al.*, 2011).
2.9.1 Antibiofilm potential of plants

Since the cells in a biofilm are more resistant to antimicrobial agents compared to free-floating or planktonic cells hence most plant extracts are not showing antibiofilm activity (Sandasi et al., 2011). Hence the literature on the antibiofilm activity of plant extracts is currently minimal (Park et al., 2011). If they have antibiofilm activity, the anticaries metabolism of natural antimicrobial agents can be classified into two mechanisms. One is to destroy the integrity of the cell wall and the other is to inhibit bacterial adhesion instead of killing the bacteria (Kim et al., 2008). Numerous studies have investigated the ability of cranberry juice or cranberry constituents to prevent adhesion of oral pathogens to surfaces and related phenomena, such as the production of glucans and fructans, and the formation of biofilms. Exposure of oral streptococci to 25% cranberry juice for as little as 10 s has been shown to inhibit adsorption of cells to saliva-coated hydroxyapatite beads by between 61.8% and 95.1%, with the exception of S. sobrinus for which reduced adsorption was seen after 10 min. In addition, cell surface hydrophobicity of some of the bacteria was reduced and a preparation of high molecular weight cranberry juice constituents inhibited biofilm formation (Palombo, 2009).

Sohaibani and Murugan (2012) evaluated the various extracts of the miswak plant Salvadora persica for growth inhibition and antibiofilm effects on cariogenic Streptococcus mutans isolates. They showed that the bioactive, dual-function, antibiofilm agents in S. persica not only inhibit growth, but also control the colonization and accumulation of caries-causing S. mutans. They also showed the interference of bacterial communication quorum-sensing (QS) by the phytochemicals through Ligand Fit docking protocols. Murugan et al. (2013) evaluated the use of ethanomedicinal herb Achyranthes aspera in caries management. They showed that these plant extracts inhibits the growth and biofilm formation of cariogenic isolate Streptococcus mutans. Their study concluded that anticaries bioactive compounds of A. aspera with higher QS response regulator binding energy, low toxicity and optimal pharmacokinetic properties could be used for caries and as an alternative means of quorum quenching. These studies indicate the availability of plant phytochemical repositore for controlling biofilm formation. 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).

2.10 Plant active biomolecules and ethnobotany

Many different clues can be obtained from the ethno-botanical survey of medicinal plants for the development of drugs towards treating human diseases (Ghosh, 2003). There is an upsurge in ethno-medical research interest over the last 50 years which is in fact, episodic in nature. During the second half of the last century, there has been a more tension between modern drug discovery approaches comprising combinational chemistry, \textit{in silico} drug design, functional genomics proteomics, etc., and traditional approaches based discovery of novel bioactive molecules of plant origin (Alan Cox, 2005). If truth be told, maximum numbers of the plant compounds employed in modern medicine were first discovered during the course of ethno-botanical investigation (Gurib-Fakim, 2006). Many commercially proven drugs used in modern medicine were initially used either in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity. Fabricant and Farnsworth (2001) listed 86 important drugs developed from the ethno-medicinal base with active compounds and their clinical use.

2.10.1 Ethno-botanical studies

Herbal medicines are presumed to have immense importance in the primary health care of individuals and communities in many developing countries (Ghosh, 2003). Historians from all around the world have produced evidence to show that apparently all primitive people used herbs often in a convenient way (Gilani and Atta-ur-Rahaman, 2005). Extensive studies revaluing the traditional medicine’s research results on different plant species and their therapeutic principles are going on throughout the world nowadays (Scartezzini and Speroni, 2000). 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. There is an imperative need to inventory and record all ethno-
biological information among the diverse ethnic communities before the traditional
cultures are completely lost (Lenin Bapuji and Venkat ratnam, 2009).

2.10.2 Indian and Tamilnadu tribal populations

The tribal population of India who are recognized as Scheduled Tribes by the
Government of India includes a total of 104,281,034 peoples representing 8.6 percent of
the country’s population, out of which 10,461,872 lives in urban and 93,819,162 in rural
(Ministry of Tribal Affairs, 2013) forest dominated villages, and there are about 573
communities among them (Fig 2.6). They mainly are spread across the country mainly in
the forest and hilly regions. The essential characteristics of these communities are
primitive traits, geographical isolation, distinct culture, shy of contact with community at
large and economically backward (Ministry of Tribal Affairs, 2013). Importance of
forests in tribal economy is well known as they are a source of subsistence and live hood
for the tribal communities. In India 187 districts have been identified as tribal districts
and are generally rich in forest cover and hence forest resources (SRF, 2001). Tamilnadu
is the 11th largest state in India having 794,697 ST populations accounting 1.1 percent of
total and comprising 36 types of tribal communities who are distributed among the forests
and adjoining areas of various districts. Tamilnadu has a great tradition of preserving its
forests wealth and concern for the environment, which has taught us to respect nature and
understand the complex inter-relationship between living and non-living things. The
state’s forest ecosystem includes a variety of flora and fauna representing remarkable
biodiversity essential for the environmental stability and water conservation thereby
creating food security for survival of present and the future generations. In Tamilnadu,
the Western Ghats comprise the Nilgiris, Anamalasis, Cardamon hills, Palani hills and
Tirunelveli hills; the Estern Ghats comprise Javadu malai, Shevaroyan kundrugal, Palani
hills, Kolli hills and the eastern coastal plains provide various habitats and niches suitable
for a variety of flora. Some tribal communities such as Malasars, Malamalasars,
Malayalis, Irulas, Gonds, Koysd, Konda reddis, Valmikis, Koyas, Chenchus, Lambadis,
Jatapus, Savaras, Bagatas, Kammaras, Khondas, Nukadoras, Porjas, Jatapus, Paliyar,
Kanikar, Todas, Kotas, Kattunayakas, Apatani and Chellipale (Ministry of Tribal Affairs,
2005-2006).
2.10.3 Medicinal plants exploration by traditional knowledge systems

Medicinal plants value to the human being is very well established one. It is appraised that 70 to 80% of the world population depend mainly on traditional health care system and mostly on herbal medicines (Lenin Bapuji and Venkat ratnam, 2009). Since the poor people have limited alternatives, they depend on traditional health care system.

Fig. 2.6 Distribution of tribal populations across the various Indian states (Courtesy: Ministry of tribal affairs, 2013).
The biodiversity wealth of India is utilized by the native communities in several forms of medicine. Medicinal plants have been used for thousands of years in folk medicine for various applications including maintaining oral hygiene. Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz et al., 2004). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine (Lakshman Raju Badugu, 2012). Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Park and Pezzutto, 2002). Medicinal plant’s phytochemical or the bioactive compounds serve as a novel source or as an alternative to synthetic drugs for the management infectious diseases. These phytochemicals could be come from all parts of the plant like bark, leaves, roots, fruits, seeds, fruit rind, flowers, stem, whole plant, etc. and many reports are available on their antimicrobial activity. However, leaf is usually the most preferred part for therapeutic purpose (Chanda et al., 2010; Maji et al., 2010).

2.10.4 Botanical explorations of Malayali tribal at Kolli hills

Ethno-botanical research can provide a wealth of information regarding both past and present relationships between plants and the traditional societies. Ethno-botany is not new to India because of its rich ethnic diversity. Kolli hills, Namakkal district, Tamilnadu is a ‘Naturalists Heaven’ a treasure trove of medicinal plants, and the native home of traditional hill country and people. The forest types of Kolli hills range from evergreen to moist deciduous and dry deciduous. Ever since time immemorial Kolli hills have always been famous for its medicinal plants. An extensive range of medicinal plants and herbs used in Ayurvedic, Siddha and Unani are natured, cultivated, gathered and sent from here (Elavarasi and Saravanan, 2012). Dwarakan and Ansari (1992) study lead to listing the less known use of 30 plant species by the local Gounder, Malayali and Veduvan tribals inhabiting in Kolli hills of Salem (Namakkal) districts. Another ethnobotanical survey carried out by Dwarakan and Alagesaboopathi (1999) in Kolli and Shevaroy hills of Salem (Namakkal) district among the local people, who have the rich indigenous
knowledge on crude drug resources of medicinal plants identified 25 species of plants used against human and animal diseases. The ethnobotanical survey study conducted by Udayan et al. (2006) at Shevaroy hills of Salem (Namakkal) district provided further information on 30 plants used by the folk medicinal practitioners of Malayali tribal community. From the above, it can be well understood that ethanobotanical documentation of Kolli hills is incomplete. Only limited ethnobotanical studies were available on medicinal plant rich Kolli hills.

2.11. *Mimusops elengi* L.: A Medico-ethnobotanical survey

2.11.1 Scientific classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<td><em>M. elengi</em></td>
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<tr>
<td>Binomial name</td>
<td><em>Mimusops elengi</em> L. (Raghunathan and Mitra, 2000, 2001; Baliga et al., 2011; Gami et al., 2012; Kadam et al., 2012)</td>
</tr>
</tbody>
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2.11.2 Synonyms & vernacular names

| English  | Bullet wood, Indian Medlar, Spanish cherry. |
| Hindi    | Mulsari, Sinha kasaraka, Molchari, Maulsiri, Bakula |
| Sanskrit | Bakula, Kesara, Madhugandha, Gokui, Anangaka, Bakula, Chirapushpa, Dhanvi, Gudhpushpa, Kantha, Karuka, Kesha, Madhupushpa |
| Marathi  | Ovalli, Bakhor, Bakula, Barsoli, Ovalli, Owli, Vavoli, Wovali, Wowl |
| Kannada  | Karak, Bakula, Pagade, Ranjal, Konkani, Omval. |
| Telugu   | Bogada, Bogada-manu, Singhali, Minn-mal, Muhulla, |
Muhuna, Telgu Kesari, Pogada, Vagula, Magadam

Tamil : Vagulam, Magadam, Muhunain, Alagu, Ilangi, Kesaram, Kosaram, Magil, Magilam, Nakum, Magizham

Magizhamboo

Gujarati : Babhuli, Bolsari, Varsoli, Vovoli

Oriya : Kira kauli, Baula Bengali, Bakul.

Malayalam : Bakulam, Elengi, Ilanni, Iranni, Ilenji, Makuram

Punjabi : Maulsari, Maulsiri

Bengali : Bakal, Bakul, Bohl, Bukal

Nepalese : Bakulapuspa

Sinhalese : Munemal

German : Affengesict

French : Karanicum

Unani : Moolsari

Burmese : Kaya

Malaysian : Enengi

Urdu : Molsari, Kirakuli

Burmese : Khaya

Malay : Tanjong

Myanmar : Kha-Yay

Sinhalese : Munnamal, Muhula, Muhuma

Thai : Pikul (Raghunathan and Mitra, 2000, 2001; Baliga et al., 2011; Gami et al., 2012; Kadam et al., 2012).

2.11.3 Medicinal and pharmacological activities of *Mimusops elengi* L

*Mimusops elengi* L. an evergreen tree is found application in traditional medicine for numerous medicinal purposes. The bark, flower, seeds, fruits and leaves have immense therapeutic value in traditional Indian medicine system. The water mixtures of the fruits are supposed to promote delivery during childbirth. Ripened fruits ease urination; alleviate burning micturition and helps in urinary calculi removal (Nadkarni, 1976; Mitra, 1981). It has medicinal properties including antinociceptive, diuretic effects, gastroprotective, antibacterial, antifungal; anticariogenic, free radical scavenging, antihyperglycemic etc. are well documented. The traditional practitioners of Southern
India use the powdered dried flower as a brain tonic. It corrects the assimilation process of the digestive system and hence is used for curing diarrhea, dysentery and intestinal worms (Gami et al., 2012). It’s fruit and flower lotion is believed to be effective in healing sores wounds and ulcers (Baliga et al., 2011). Its snuff is trusted to be a neurotonic and found use to release headache and cephalalgia. Natives of Southern India use water distillate of flower both as a stimulant medicine and as a perfume. The decoction of the flowers is deemed to be useful against heart diseases, to act as antidiuretic in polyuria, as an antitoxin, to treat leucorrhoea and menorrhagia. The fruits are believed to be effective preventers of chronic dysentery and constipations. They are surmised to prevent premature ejaculations, to possess antidiuretic effects and are also useful as an anti-toxin. The ripe fruit is supposed to be a general tonic and decrease the vitiated pitta dosha. A leaf extract of different solvent found application in curing suppuration and earache and if given orally cures internal pains (Baliga et al., 2011). The bark methanol extract of M. elengi in the form of ointment showed considerable healing activity in all three types of wound models on mice: the excision, the incision and dead space wound model as comparable to those of a standard drug (Gupta et al., 2011). The decoctions of the leaves are also supposed to be useful in cleaning dermal wounds, ulitis and ulemorrhagia. It is also reported possessing anti-ulcer effects and fertility increase in women (Baliga et al., 2011).

2.11.4 Antioxidant effects

The leaf methanol extract exhibited significant antioxidant activity compared to the reference antioxidant ascorbic acid in a dose dependent manner (Saha et al., 2008), but Ganu et al. (2010) observed inferior activity of the same solvent bark extract. Boonyuen et al. (2009) observed higher antioxidant capacity of the immature fruit’s crude extract than that of either the mature or the ripe fruit. Purnima et al. (2010) showed crude methanol extract’s significant antioxidant activity. The alcohol extracts of bark showed more potent antioxidant activity than its petroleum ether and chloroform extract (Ashok et al., 2010). The leaf and fruit possess antioxidant effects and the ripe fruits could also be a potential source of natural antioxidant (Ganu et al., 2010). Shaik et al. (2011) showed leaf extract’s promising quenching impact on the extent of lipid peroxidation, along with enhancement of antioxidant defense system in pancreas tissues. The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical, nitric oxide, ABTS radical and hydroxyl radical assay
of chloroform extract of barks found to exhibit significant potential to be used as a natural antioxidant agent (Rao et al., 2011).

2.11.5 Antidiabetic, diuretic and hypotensive activities

The methanolic extract of *M. elengi* is shown to possess hypotensive activity in anesthetized rats (Dar et al., 1999). The bark aqueous extract shown to decreased blood glucose and glycosylated hemoglobin, increased serum insulin levels and rectified some glucogenic enzymes and thus have hyperglycemic effects in alloxan induced diabetic rats (Jerline et al., 2009). Ganu et al. (2010) have shown the stem bark methanol extract’s antidiabetic effects in allaxon-induced model in mice. The ethyl acetate, ethanol, methanol and aqueous extracts of the bark possess diuretic effects. Among them, the water extract was shown to be best (Katedeshmukh et al., 2010).

2.11.6 Cytotoxic activity

The brine shrimp lethality bioassay of bark methanolic extract exhibited good cytotoxic activity with LC50 value of 40µg/ml (Nasrin et al., 2010). Ethanol extracts (95%) of *M. elengi* flower exhibited promising cytotoxic activity against the cholangiocarcinoma CL-6 cell line with cell survival of less than 50% at the concentration of 50 µg/ml (Mahavorasirikul et al., 2010). The methanolic extracts of leaf also possess cytotoxic substances (Karmakar et al., 2011). The meristimatic cells of root tips of *Allium cepa* showed the cytotoxic effects of Ethanolic extract of barks (Bhujbal et al., 2011).

2.11.7 Antibacterial activity

Studies conducted on the extracts obtained from all parts of the plant revealed their antibacterial activity. Nair and Chanda. (2007) reported the potential antibacterial activity of Ethanolic extracts than aqueous leaf extracts on medically important bacterial strains. The ethanolic extract of barks showed significant activity against clinical *Staphylococcus* isolates including *S. aureus* (Rangama et al., 2007). Petroleum ether, ethyl acetate and methanol extracts of bark, fruits and leaves, exhibited antibacterial activity against some pathogenic bacteria. When compared the fruit extracts were less potent to those obtained from bark and leaves (Ali et al., 2008). The antibacterial activity of dichloromethane, ethyl acetate, acetone, methanol, ethanol, acetone-water, methanol-water and ethanol-water extracts of stem bark on Gram positive and Gram negative
strains were exposed (Shahwar and Raza, 2009). The antimicrobial activity of acetone and aqueous bark extracts on salivary micro flora were also revealed (Deshpande et al., 2010; Kulkarni et al., 2011). The various extracts (aqueous, petroleum ether, toluene, methanol, ethanol and chloroform) showed a strong antibacterial activity against pathogenic Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae and Streptococcus pneumoniae (Lalitha et al., 2011).

2.11.8 Antifungal activity

The petroleum ether, ethyl acetate and methanol extracts of bark, fruits and leaves exhibited antifungal activities against some pathogenic fungi in the order bark > leaf > fruit (Ali et al., 2008). The aqueous leaf and bark extracts showed significant higher reduction in Sclerotinia sclerotiorum (Lib.) de Bary in vitro radial growth and sclerotial development, and the responsible antifungal compound is found to be heat liable (Niranjan et al., 2009). Satish et al. (2010) investigated this M. elengi leaves aqueous, petroleum ether, benzene, chloroform, methanolic and ethanolic extracts in vitro antifungal effects on a range of phytopathogenic fungi belonging to Alternaria alternata, Drechslera, Fusarium, Aspergillus and Penicillium. Their results indicated that the aqueous, methanolic and ethanolic extracts possess high antifungal activity and its activity guided alkaloid fraction is highly significant. The hexane, ethyl acetate, ethanol and methanol extracts did not show any antifungal activity against caries infected patients isolates Candida albicans (Jebashree et al., 2011). The activity of the extracts may support the folkloric uses of these plants as an agent for management of sores, gonorrhoea, dysentery, wounds and toothache.

2.11.9 Oral care applications and anticaries activity of M. elengi

The powder of the bark skin and the tender stems were used like tooth brush for cleansing the teeth. In Ayurveda, the decoction prepared from M. elengi is recommended for the treatment various dental ailments and gums healthy maintenance. Rinsing the mouth with bark decoction is believed to strengthen the gums, reduce inflammation, prevent bleeding of gums, and to stop bad breath caused by pyorrhea and dental caries (Mitra, 1981). The preliminary screening of the bark chloroform, methanol and water extracts found to exhibit prominent antibacterial activity against different Gram positive, Gram negative microorganisms and organisms isolated from tooth tartar of dental patients
(Murudkar et al., 2007). The cariogenic *Staphylococcus aureus, Streptococcus mutans, S. salivarius, S. sanguis, Lactobacillus acidophilus* and *Candida albicans* are found susceptible to the petroleum ether, acetone, methanol and aqueous extracts (Ajaybhan et al., 2010; Prabhat et al., 2010). Among them, the methanolic extract was observed to be the most potent followed by aqueous; acetone and petroleum ether extracts (Ajaybhan et al., 2010) due to leaching out of more phytoconstituents in it (Prabhat et al., 2010). Jebashree et al. (2011) also observed similar activity against the dental caries isolates *S. mutans* and *C. albicans* by the hexane, ethyl acetate, ethanol and methanol extracts of *M. elengi*. Ethyl acetate extract showed promising anti-caries activity against these dental caries pathogens. The unripe fruit is used as a masticatory and is supposed to be helpful in fixing loose teeth (Baliga et al., 2011).

**2.11.10 Phytochemical constituents**

Bark of *M. elengi* contains tannin, some caoutchouc, wax, coloring matter, starch and ash forming inorganic salts. Taraxerone, taraxerol, betulinic acid, spinasterol, sodium salt of betulinic acid, urosolic acid, Fatty acid esters of alpha-spinasterol, mimusopfarnanol, lupeol, alpha cadinol, tau muurolol, hexadecanoic acid, diisobutyl phthalate, octadecadienoic acid β amyrin, lupeo, α- sitosterol glycoside, quercitol and β-sitosterol were isolated from the bark (Mishra and Mitra, 1967; Hart et al., 1968; Misra and Mitra, 1968; Jahan et al., 1995; Sharma et al., 2000; Jahann et al., 2001; Akhtar et al., 2009; Manjeshwar et al., 2011). Stem contains Alpha cadinol, tau muurolol, hexadecanoic acid, diisobutyl phthalate, octadecadienoic acid, gallic acid esters and phenyl propanoxyl gallate (Ruikar et al., 2009; Akhtar et al., 2010). Fruit showed, moisture (79.27 %), protein (1.29%), fat (2.76 KCal), reducing sugar (8.9%), non-reducing sugar (6.3%), total sugar (15.2%), fiber (1.13%), vitamin C (3.27 mg/100 gm), mineral content (0.32%), iron (0.59 mg/100 gm), sodium (5.16 mg/100 gm), potassium (98.54 mg/100 gm) (Nazarudeen et al., 2010). Flowers contain D-mannitol, sitosterol and β-sitosterol-β-D-glucoside. Flowers also yielded quercitol, ursolicacid, dihydroquercetin and a triterpene. A leaf contains hentriacontane, carotene, lupeol, steroidal saponin, 5 alpha-stigmast-9 (11) en-3-obeta-D-glucopyranosyl and (1-5)-o-beta-dxylofuranoside (Misra and Mitra, 1967, 1968; Saxena et al., 1988).
Seed known to have quercitol, ursolic acid, dihydroquercetin, quercetin, β-d glucosides of-β-sitosterol, α-spinasterol, mimusopsin pentacyclic triterpenes, mimusopgenone, mimusopin and mimusopsin, mimusin, taxifolin, α-spinasterol glucoside, miglycoside1, gallic acid protobassic acid and mimugenone (Mishra and Mitra, 1967; Jahan et al., 1995; Sahu et al., 1995; Sen et al., 1995; Lavaud et al., 1996; Sahu, 1996; Sahu et al., 1997; Boonyuen et al., 2009). Sahu et al. (1997) have reported the isolation of triterpenoid, saponins, mimusin [3 – O - (β – D - glucopyranosyl) - 2β, 3β, 6β, 23-tetrahydroxyolean-12 – en – 28 - oic acid 28 – O – α – L – rhamnopyranosyl - (1-3) – β –D – xylopyranosyl - (1 - 4) – α – L – rhamnopyranosyl - (1-2) – α – L - arabinopyranoside], mi - saponion A and 16α - hydroxyl Mi - saponion A from the seeds. Sen et al. (1995) have isolated two new pentacyclic triterpenes, mimusopgenone and mimugenone from the same and characterized as 2β, 3β, 23 – trihydroxy – 28 noroleana - 5, 12 - dien-16 - one and 3β, 23- dihydroxyoleana - 5, 12 – dien - 1 6 - one, respectively, based on their spectroscopic properties. Lavaud et al. (1996) have isolated two saponins 3 – O - (β – D - glucuronopyranosyl) 28 – O - (α – L - rhamnopyranosyl (1 - 3) β – D - xylopyraosyl (1 - 4) and α – L – arabinopyranosyl - protobassic acid from the seed kernels. Hazara et al. (2007) reported the isolation of antibacterial compounds, 2, 3 – dihydro - 3, 31, 41, 5, 7-pentahydroxy flavones and 3, 31, 41, 5, 7-pentahydroxy flavones from the seed.

2.12 In silico interaction studies predicting the mode of action of phytochemicals

Scientific and technological advances including genomics and informatics extend the limits of our knowledge concerning human health challenges. Recent advances in bioinformatics placed tools like virtual screening (VS) as an alternative strategy for drug development and finding out the potential targets (Bielska et al., 2011) while reducing the extremely high therapeutic drug discovery-associated costs and risks. Moreover, the innumerable number of phytochemicals and the technical limitations of elucidating their synergism specify the scope for the application of omics technologies towards determination of potential global effects of interactions in mixtures. According to Amadasie et al. (2007) an agreed approach, combining different docking tools and scoring functions based on different concepts, should allow a more reliable analysis of the inclusion mechanism, overcoming errors and approximations of each single molecular modeling tool.
According to Valerio et al. (2007) the computational methods can be useful when experimental data are insufficient, unreliable, unavailable or inconsistent between studies. This approach reduces animal testing, facilitates, the review process and also has applicability for the evaluation of chemically identified individual components of botanical mixtures or chemicals of natural origin that have not been subjected to \textit{in vivo} testing. The failure of reckoning true synergy between specific isolated phytochemicals within a herb or herbal extract owing to plenty of uncharacterized phytochemicals in whole plants and many herb extracts and the possibility of many complex interactions, provides the explanation for the net activity, as other unknown constituents may have a contributory role (Jordan et al., 2010).

As Schilter et al. (2007) stated the interactions between constituents will be extremely important when data for highly purified preparations are used to assess relatively unpurified material, prediction their interaction is significant. Already Yang et al. (2004) have discussed the possibility of using \textit{in silico} methods for the investigation of simple chemical mixtures. Most of these interaction data are derivatives of \textit{in vitro} studies. Naturally, such studies should be followed up with \textit{in vivo} analysis. This is not at all times practical due to the wide complexity of herbs and their extracts phytochemicals (Rietjens et al., 2008) and is limited by the complex combinations of plant chemicals (Jordan et al., 2010). Hence assessment of potential interactions between classes of compounds, or the primary compounds involved may give an answer to the dearth of information on potential matrix effects in medicinal herbs (Rietjens et al., 2008), and the use of omics technologies to determine potential global effects of interactions in mixtures (Jordan et al., 2010). To dock the compounds towards the active site of the protein, the docking simulation was carried out by using AutoDock 4.0 suites. Already number of authors reported its suitability for performing docking of ligands to their macromolecular receptors (Cosconati et al., 2010).