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MEDICINAL PHYTOCHEMICALS TARGETING TOBACCO-INDUCED ORAL DYSBIOSIS AND BIOFILM FORMATION: MECHANISMS, THERAPEUTIC POTENTIAL, AND FUTURE PERSPECTIVES

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ABSTRACT:
Tobacco consumption is a major modifiable risk factor for oral diseases, particularly periodontitis, owing to its profound effects on the oral microbiome and biofilm development. Exposure to tobacco smoke disrupts microbial homeostasis, resulting in oral dysbiosis characterized by enrichment of pathogenic species such as Porphyromonas gingivalis, Fusobacterium nucleatum, and Streptococcus mutans. These microbial alterations enhance bacterial adhesion, quorum sensing, extracellular polymeric substance production, and biofilm maturation, thereby increasing resistance to host immune responses and conventional antimicrobial therapies. Recent advances have highlighted medicinal plants as promising adjunctive strategies for preventing and managing tobacco-associated oral biofilm infections. Plant compounds like flavonoids, polyphenols, terpenoids, alkaloids, catechins, and phenolic acids possess broad-spectrum antibacterial, antioxidant, anti-inflammatory, and antibiofilm properties owing to their ability to affect different phases of biofilm formation such as bacterial adherence, extra-cellular matrix synthesis, quorum sensing, and virulence factors. This review comprehensively examines the molecular mechanisms underlying tobacco-induced oral dysbiosis and biofilm formation while critically evaluating current evidence on plant-derived antibiofilm agents and their therapeutic potential in oral healthcare. In addition, emerging technologies such as nanotechnology-based drug delivery systems and microbiome-targeted approaches are discussed as strategies to improve the clinical translation of phytotherapeutics. Despite encouraging preclinical evidence, significant challenges remain, including variability in phytochemical composition, limited bioavailability, lack of standardized formulations, and insufficient high-quality clinical trials. Future research should focus on mechanistic investigations, optimized delivery platforms, and well-designed clinical studies to validate medicinal plants as evidence-based therapeutics for tobacco-associated oral biofilm diseases. Collectively, integrating phytochemicals with modern precision oral healthcare may provide a promising avenue for restoring microbial homeostasis and improving periodontal health in tobacco users.

KEYWORDS: Tobacco; Oral microbiome; Oral dysbiosis; Biofilm formation; Medicinal plants; Phytochemicals; Periodontitis; Quorum sensing; Antibiofilm therapy.

1. INTRODUCTION

1.1 Overview of tobacco consumption (smokers vs non-smokers)

Tobacco smoking is a prevalent public health issue in the world, and one that contributes greatly to the burden of disease and early death. Even though there have been numerous campaigns on its adverse health effects, tobacco smoking remains prevalent. [1] There are more than 1.1 billion people who smoke (>1/7) in the world today, and over 8 million people die each year due to smoking1. Smoking is one of the top five risk factors for the global burden of disease, leading to several illnesses such as cancer, heart disease, lung disease, and gum disease [2]. Periodontal disease, otherwise called gum disease, refers to different polymicrobial infections (including gingivitis and periodontitis) that attack supporting structures of the teeth, such as the gingival, alveolar bone, and periodontal ligament. It is considered the main reason for losing one's teeth, as well as one of the causes of systemic illnesses. The use of tobacco has been established as one of the key risk factors for periodontitis and has an impact on the prevalence, severity, progression, and treatment of the disease, second only to dental plaque. Several epidemiological reports have reported an elevated risk for periodontal disease among smokers than nonsmokers, with the degree of risk depending on the length and frequency of smoking [3,4,5]. Smokers possess a clinically unique susceptibility to developing periodontitis, with deeper probing depths, broader and worse attachment loss, higher rates of bone resorption, and more tooth loss [6,7,8]. In addition, cigarette smoke is shown to have a negative impact on the treatment results of nonsurgical and surgical

procedures as well as implant insertion [9,10].Given that there are already documented detrimental consequences of smoking on periodontal health, it is essential to comprehend the mechanisms behind the association, although they are poorly understood. The periodontal microbiome and host immune response are considered to be crucial in initiating and perpetuating periodontal disease development [11].
The smoke produced after smoking tobacco is a dynamic composition of over 5000 compounds, which are either cytotoxic, mutagenic, carcinogenic, or antigenic [12]. One of the active components that has high addictive potential is nicotine, a parasympathomimetic alkaloid. It is the most recognized compound for being associated with dependence and is the reason for extensive smoking and inability to quit the habit [13]. For these reasons, nicotine and one of its primary metabolites, cotinine, have been extensively studied to determine the effects of smoking on periodontal microorganisms [14, 15]. In addition, the cigarette smoke extract (CSE) and cigarette smoke condensate (CSC) solutions are also representative solutions for the biological study in vitro, where the non-nicotine compounds are taken into account [16,17].

1.2 Oral Microbiota

For the oral cavity and other bodily systems to remain healthy, the oral microbiome is essential. A highly active microbial ecosystem consisting of bacteria, fungi, viruses, and archaea that coexist peacefully with their host organism is the oral microbiome. The oral microbiome thrives in different areas in the mouth, such as the tongue, teeth, gingival sulcus, and oral mucosa, where it ensures that the immune system is modulated, nutrients are metabolized, and pathogens do not penetrate the system. The mouth is one of the main points of contact between the body and the external environment. An alteration in this delicate microbial balance is referred to as dysbiosis, which not only affects dental caries and periodontal disease but can have major consequences for overall health.[18,19]. Dysbiosis is a significant disruption of the microbial equilibrium that can result in systemic diseases as well as oral conditions including periodontitis and tooth caries. It has been discovered that dysbiosis and increased pathogenicity are directly linked to inflammatory diseases like diabetes, rheumatoid arthritis, and systemic lupus erythematosus. Patients with diabetes had higher levels of Porphyromonas, Capnocytophaga, and Pseudomonas bacteria, while those with autoimmune illnesses had higher levels of Prevotella and Selenomonas. These conditions cause the mouth to become more inflamed, which leads to the loss of alveolar and periodontal bone. Additionally, oral pathogens have been discovered in distant organs, suggesting that the oral cavity may be a source of systemic infection [20,21]. Due to complex interactions between the immune system and the pathogens, the oral microbiome has an impact on systemic health. Chronic inflammation is brought on by the immune system's overreaction to dysbiotic bacteria populations. Both oral and systemic inflammatory illnesses have been found to have high levels of cytokines such as interleukin-17 (IL-17). It has been shown that inhibiting IL-17 effectively reverses the pathogenic activity of oral bacteria [20,21]. Additionally, oral cavity microbes may enter the bloodstream or gastrointestinal tract and cause systemic inflammation in conditions such pancreatic cancer, liver cirrhosis, heart disease, and neurological disorders [21,22].Growing awareness of the human microbiome as a "superorganism" supports the idea that microbial populations are essential to human physiology. The significance of these microbial populations in regulating host metabolism, immunity, and disease susceptibility has been brought to light by the Human Microbiome Project. In this sense, the oral microbiome has drawn a lot of interest as a crucial target for both illness detection and treatment. It is an excellent option for non-invasive diagnostic methods and treatments because to its accessibility and sensitivity to environmental conditions [19,22,23].

Figure 1: Comparison of the healthy oral microbiome (eubiosis) and tobacco-induced oral dysbiosis, highlighting reduced microbial diversity and increased biofilm-associated pathogenicity in smokers.

2 Importance of oral hygiene and microbial balance

Oral cleanliness is necessary for keeping the oral microbiota balanced, which is made up of numerous microbes that have positive roles in oral and systemic health. In case the oral hygiene is maintained properly, the microbes live in a symbiosis with their hosts, ensuring that there is no presence of harmful species and helping the normal function of the mouth (23, 166). Improper oral hygiene causes an imbalance in the microbiome of the mouth with an increase in pathogenic microbes (18, 41, 165). Dental plaque formation and biofilm formation resulting from poor oral hygiene causes the multiplication of cariogenic and periodontopathogenic bacteria. Oral biofilms are complex microbial communities enclosed in an extracellular matrix that renders them capable of tolerating various stresses and resistants to antibacterial chemicals (73, 128). Periodontal disorders, caries, and oral inflammatory conditions are caused by the overgrowth of microorganisms such Porphyromonas gingivalis, Fusobacterium nucleatum, and Streptococcus mutans (35, 37, 41, 161). Maintenance of microbial equilibrium by effective oral hygiene procedures such as brushing, flossing, and antibacterial mouthwashes is an important strategy that can prevent biofilm formation and the progression of any disease (127, 128, 129). Additionally, there is increasing evidence to show that maintaining oral microbial balance is essential for overall wellbeing.Oral dysbiosis has been linked to a number of systemic disorders, including diabetes, cardiovascular disease, hepatic disease, pancreatic cancer, and others, according to several scientific investigations (18–22, 131). Lifestyle factors such as tobacco usage also affect the oral microbiome balance through the modification of microbial population, increased pathogenic biofilm production, and weakened immunity of the host. Tobacco use has been known to promote the presence of periodontal pathogens, resulting in periodontal disease development (30, 38-40, 66, 99). Thus, it can be concluded that proper oral hygiene is essential for sustaining microbial balance and good health (127, 166).

2.1 Effects of Smoking on the Growth and Virulence of Key Pathogens

In another study, [24] evaluated the influence of nicotine and cotinine concentrations of 400, 100, 25, 6.25, 1.5, and 0.4 μg/mL on the growth of seven types of oral bacteria, including P. gingivalis, where these concentrations corresponded to either similar or greater amounts of nicotine and cotinine compared to the physiological concentration in saliva and gingival crevicular fluid [25,26,27]; however, these compounds failed to change the pattern of growth of the examined bacteria. In addition, exposure to CSE in concentrations of 0.5, 2, and 4 μg/mL nicotine equivalents had no effect on the growth of P. gingivalis, F. nucleatum, and F. alocis [28,29]. The adaptation to environmental fluctuations and, as a result, the ability to survive efficiently has been ensured by the complex structure of the bacterial cells [30]. The effect of CSE resulted in phenotypic alterations in P. gingivalis in terms of increased FimA gene expression, up-regulation of the RagA and RagB protein production, inhibition of capsule production, and pro-inflammatory signaling suppression caused by TLR2 [28].However, most of these effects were reversed upon subculturing of CSE-stressed bacteria into fresh medium lacking CSE. Therefore, it can be said that P. gingivalis exhibits reversible adaptation to CSE as environmental stress. CSE also exhibited strain-specific regulation on cell-associated Kgp and RgP gingipains expression (inhibitory effect in P. gingivalis ATCC33277 strain while stimulatory effect in P. gingivalis W83 strain) [31]. Further, CSE inhibited the pro-inflammatory ability of P. gingivalis biofilms (TNF-a, IL-6) [32]. All these findings imply that periodontal bacteria are able to survive in the combination of toxins produced during smoking through changes in their virulence factors The potential mechanisms involved in the detrimental effect of smoking on subgingival microflora Subgingival microflora constitutes a diverse population adhering to the surface of teeth as biofilms. It is difficult to have a full grasp of the mechanisms involved in smoking affecting these biofilms owing to the few studies conducted so far. This is due to the fact that, apart from the direct effects of smoking on biofilm development and evolution, there is also an indirect effect, mostly involving the host's immune response. The creation of biofilm in the mouth involves four steps, and biofilm formation involves two important stages; these are the adhesion of bacteria and colonization of the bacteria[33]. The effect of smoking on important bacteria (as discussed under the heading “In vitro Effect of Smoking on Important Periodontal Bacteria”) has demonstrated that the presence of CSE may change the expression of many virulence factors of P. gingivalis. These changes will help in forming biofilm by enhancing the expression of FimA and outer membrane protein (RagA, RagB, and putative lipoprotein) and decreasing the expression of capsular polysaccharide [28,34].The fimbrial antigen FimA is an important element for P. gingivalis and acts as a key factor during the adhesion of the bacteria to the periodontium. This occurs through binding the bacteria to salivary proteins, extracellular matrix, eukaryotic cells, and bacteria of the same or different species [35]. The up-regulation of FimA not only helped in the enhanced formation of biofilms of P. gingivalis but also facilitated binding to Streptococcus gordonii, which serves as an early colonizer of the biofilm. At the same time, the overexpression of FimA also decreased the host reaction to P. gingivalis by interfering with the proinflammatory reaction to TLR2 bacterial survival. Since P. gingivalis is known for its central position in coordinating the virulence of the biofilm and, as a consequence, the inflammatory reaction (as such, P. gingivalis is referred to as a "keystone" periodontal pathogen; [36], any modification in the virulence of P. gingivalis can have a community-wide impact and facilitate a switch from a healthy subgingival biofilm to a dysbiotic one, which may lead to the development of periodontitis. Fusobacterium, particularly F. nucleatum, is involved in physical interactions between Gram-positive and Gram-negative organisms and is regarded as an intermediate colonizer, connecting the colonization of commensals attached to teeth and epithelial surfaces with true pathogens [37]. Therefore, the increased prevalence of Fusobacterium species in the subgingival microorganisms in cigarette smokers [38,39,40] would be able to act as a transporter for periodontal disease-causing bacteria, hence resulting in biofilm colonization with the pathogens.

3.Tobacco Use and Oral Microbial Environment

The use of tobacco affects the oral microbial ecosystem by creating imbalances within the oral microbiome and promoting microbial dysbiosis. Tobacco exposure results in increased colonization of harmful bacteria like P. gingivalis and F. nucleatum and decreased colonization of harmless bacteria (30, 38, 39, 66). Tobacco increases the development of biofilms, alters the virulence of the bacteria, and compromises the host immune response, making the individual more vulnerable to periodontal diseases and other oral infections (28, 32, 34, 88, 99). Moreover, changes in microbial composition induced by smoking have been associated with chronic inflammation and poor systemic health effects (43, 45, 61, 71). Tobacco use is therefore a major risk factor for the development of oral diseases and oral microbial dysbiosis (3–5, 8, 11).

3.1 Types of Tobacco Users

Age, health, dental hygiene practices, and lifestyle all have an impact on the stability of the oral microbiome [41].Smoking, which may lead to the dysfunction of many body systems, is also one of the key determinants of the oral microbiota, because smoking components can interact with oral microbes in the oral environment [42]. Many research studies were done based on the influence of smoking status on oralmicrobiome structures; however, results have been inconsistent [43,44].It has been shown in research performed among 1204 Americans that there exists some kind of individual microbial composition among the current smokers different from those of non-current smokers [45]. These two groups showed differences in bacterial taxonomic and functional pathways, such as an increase in the relative abundance of eight bacterial genera (including Megasphaera) and 21 pathways (including PWY-7237 and P562-PWY) that are highly prevalent in smokers but not in non-smokers. The oral microbiota may be involved in the impact of cigaret smoking on cardiometabolic disease, as evidenced by the microbial characteristics linked to cigaret smoking that partially explain the variations in cardiometabolic risk variables according to cigaret smoking.[45]

3.3 Variations in Metabolic Pathways Between Smokers and Non-Smokers

Out of the total 36 articles considered in this review, nine were identified where the differences in metabolic pathways have been studied [65, 66, 67, 48, 68, 61, 69, 70, 49]. Wu et al. showed that glycan biosynthesis, metabolism, amino acid metabolism pathways, and xenobiotic biodegradation were over-represented. Aerobic metabolism, which includes the TCA cycle, oxidative phosphorylation, and nitrate reduction pathways in current smokers, is another under-represented metabolic pathway [66, 49]. It was clear from the results of another investigation by Sato et al. that smokers and non-smokers had different routes in the TCA cycle, glyoxylate cycle, and biosynthesis/degradation of other chemicals [70]. According to Jia et al.'s research, smokers had higher levels of acid synthesis, amino acid metabolism enzymes, amino sugars, and nucleotide sugar metabolism [61].According to a recent study, smokers had higher activity in pathways related to amino acids, nucleotides, vitamins, terpenoids, polyketides, and glycan metabolism, but lower activity in membrane transport and lipid metabolism pathways along with xenobiotic biodegradation [67]. Srivastava et al. have discovered that smokeless tobacco users had high levels of cellular processes and signalling, xenobiotic degradation, and amino acid metabolism [48]. High levels of pathways linked to amino acid metabolism, production, and degradation have been demonstrated by another study [65]. Furthermore, chewing tobacco users have a high number of pathways linked to the reductive TCA cycle and pyrimidine biosynthesis, according to Sawant et al. [68].

3.4 Effect of Tobacco on Oral Hygiene

Some of the bacterial genera that are usually found in saliva include Megasphaera, Anaeroglobus, Dialister, Rothia, Atopobium, Actinomyces, Howardella, and Romboutsia. These genera had increased relative abundance in smoking individuals while the genus Johnsonella had reduced relative abundance in comparison with nonsmokers, following adjustment for covariates such as sex, age, education, annual individual income, body mass index, alcohol consumption, tea consumption, and toothbrushing daily. [71]

4.1 Biofilm

A biofilm refers to the collection of microorganisms in which free-floating microorganism cells adhere to the surface [72]. Biofilms often possess an extracellular polymeric substance (EPS) matrix, which is a protective covering for microorganisms and is commonly referred to as slime (although not all slime is biofilm); this matrix is a polymer mass consisting mostly of proteins, extra-cellular carbohydrates, and DNAs [73,74]. Based on the structure of the EPS matrix, biofilms can develop into several unique structures, such as filaments and mushroom-like microcolonies [75].Furthermore, it has been demonstrated that the influences of fluid flow, nutritional content, and quoromone signals regulate the development of biofilms in microcolonies. N-Acyl homoserine lactones (AHLs), which are utilised in bacterial communication, are responsible for quorum sensing. [76, 77]. Biofilms are frequently seen in both natural settings and healthcare facilities, and they can form on both biotic and abiotic surfaces. However, the process of communication between various bacteria is started by the planktonic bacteria. [78, 79]. In the end, bacteria from the aged biofilm disperse due to factors including fluid dynamics and shear force. As a result, daughter cells disperse and migrate to new areas or create new biofilms [80,81].

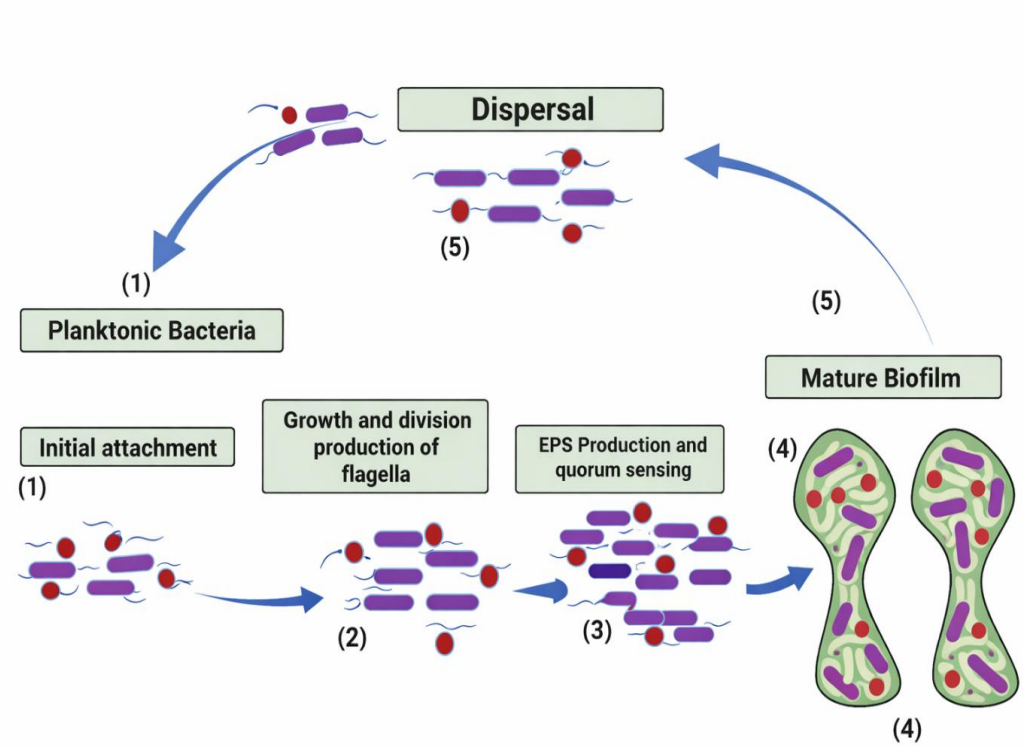

Figure 2.The figure depicts the five stages of biofilm formation along with the key factors involved in biofilm development and quorum sensing.

4.2. Sequential Stages of Biofilm Development

As seen in Fig. 3, the five stages of biofilm formation are explained below. They begin with the first stage, which is the adhesion of planktonic microorganisms to the surface. A variety of physiochemical characteristics that characterise the type of bacterial cell surface interaction with the surface govern the first stage, which is reversible [82].The secondary stage involves specific adherence to the surface, where the bacteria undergo genotypical and phenotypical changes. Maturation occurs at the third stage, when the bacteria irreversibly adhering to the surface interact with organic and inorganic substances [83]. The phenomenon of microcolonization of bacteria is said to be the fourth phase, where the interaction becomes stabilized, and the bacteria begin the process of communication between various bacteria [78,79]. The fifth and final stage is that of dispersal, wherein the factors like the hydrodynamic force and the shearing stress lead to the dispersal of microbes from the old biofilm. This leads to either the displacement of new generations of microorganisms and their movement to new places or the creation of new biofilms. [80,81]

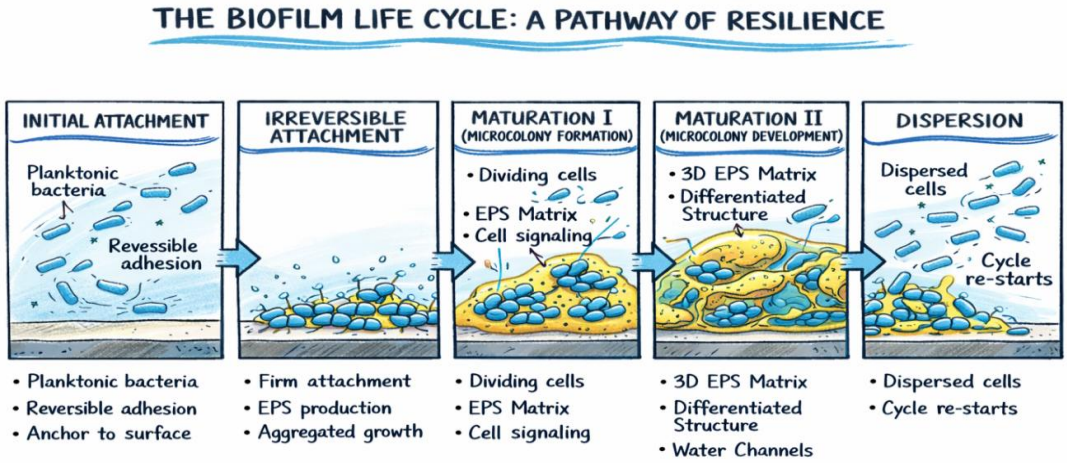

Figure 3.This figure shows the five-stage life cycle of a bacterial biofilm, from initial attachment to dispersion and re-colonization.

4.3 Tobacco Smoke Enhances Biofilm Formation

Environmental conditions including temperature, pH, and iron are significant in influencing the transcriptome of the bacteria. They also have a vital part to play in biofilm formation by the species of Pseudomonas aeruginosa, Klebsiella pneumoniae, Porphyromonas gingivalis, and Burkholderia pseudomallei causing melioidosis [84,85].It may thus come as no surprise that cigarette smoke, comprising thousands of different chemical compounds, can have a significant effect on bacterial physiology and biofilm development. As the table below shows, there is increasing proof that tobacco encourages the development of bacterial biofilms in many disease-causing bacteria.This is especially relevant to the study by Goldstein-Daruech et al. [86], which discovered that a brief exposure to cigaret smoke significantly increased the formation of biofilms in 75% (12 out of 16) of the clinical isolates of different species among smokers with chronic rhinosinusitis, whereas no increase in biofilm formation occurred in 0% (0 out of 18) of the clinical isolates among non-smokers. It is worth mentioning that the increase in biofilm formation can be reversed by eliminating cigarette smoke. [86].

Table 1: Tobacco Promotes Biofilm Development in Diverse Human Pathogens

Bacterium (Gram)	Associated Disease(s)	Tobacco Stimulus	Reference
Klebsiella pneumoniae (−)	Chronic rhinosinusitis isolate	Smoke	Goldstein-Daruech et al., 2011 [86]
Pseudomonas aeruginosa (−)	Nosocomial infections; UTI; respiratory infections including pneumonia	Smoke	Antunes, 2012 [30]; Goldstein-Daruech et al., 2011 [95]
Porphyromonas gingivalis (−)	Chronic periodontitis	CSE (Cigarette Smoke Extract)	Bagaitkar et al., 2009, 2011 [184,88]
Proteus vulgaris (−)	Chronic rhinosinusitis isolate	Smoke	Goldstein-Daruech et al., 2011 [86]
Staphylococcus aureus (+)	Nosocomial infections; endocarditis; osteomyelitis; respiratory infections	CSE (Cigarette Smoke Extract)	Kulkarni, 2013 [94]; Goldstein-Daruech et al., 2011 [86]
Streptococcus pneumoniae (+)	Pneumonia; bronchitis; endocarditis; meningitis	CSE (Cigarette Smoke Extract)	Cockeran et al., 2014 [185]; Mutepe et al., 2012 [186]; Goldstein-Daruech et al., 2011 [86]
Streptococcus mutans (+)	Dental caries	Nicotine, CSE	Li, 2014 [8]; Huang et al., 2012 [187]; Baboni, 2010 [188]

4.4 Tobacco Smoke-Mediated Mechanisms of Biofilm Formation

The impact of tobacco smoke on biofilm formation. Nonetheless, it is evident that attachment to a layer—which could consist of endothelium or epithelial cells, pre-existing bacteria adhered to a surface, or even a layer of proteins or carbohydrates—is the initial stage in the formation of a biofilm. Because tobacco smoke increases the synthesis of the eukaryotic platelet-activating factor receptor (PAF-R), which binds to the phosphocholine in the bacterial cell wall, Streptococcus pneumoniae adheres to the lung epithelial cells [87]. The primary fimbrial protein, FimA, is elevated in P. gingivalis to enhance adherence by binding to the GAPDH surface protein of Streptococcus gordonii [88]. The main nicotine metabolite, cotinine, has been shown to increase the ability of the bacteria P. gingivalis to attach to the layers of epithelial cells [89]. Furthermore, the cigarette smoke extract, CSE, seems to increase the ability of the bacteria A. actinomycetemcomitans, an oral biofilm bacteria that cause one type of periodontal disease, to attach to the layers of epithelial cells [90]. The enzyme sortase A, which is responsible for transporting the correct LPXTGX proteins from the cariogenic bacteria S. mutans and other Gram-positive bacteria to their surface, includes the cell surface protein P1. Among the proteins found on P1 there are salivary agglutinin-binding protein, promoting the growth of biofilm and antigen I/II. It has recently been found that the nicotine, a tobacco alkaloid, enhances the expression of the antigen I/II protein of S. mutans, promoting biofilm formation [91]. The authors [91] state that the enhanced biofilm formation due to nicotine may be the reason why smokers have more carious lesions than non-smokers [92]. Indeed, nicotine has been shown to enhance the dual species S. mutans biofilm formation together with Streptococcus sanguinis [93]. Furthermore, exposure to cigarette smoke leads to an increase in biofilm formation in the prominent human pathogen, Staphylococcus aureus, which can be responsible for causing skin infections, pneumonia, endocarditis, and septic shock [94]. Exposure to cigarette smoke causes the up-regulation of staphylococcal accessory regulator A (sarA) and required for biofilm formation (rbf) genes that produce biofilm enhancer proteins and fibronectin-binding protein A (fnbA) that help in adherence. A class of genes known as accessory gene regulator (agr) has been linked with bacterial dispersal via quorum sensing. Cigarette smoke is known to inhibit the activity of the agrC gene. The pretreatment of S. aureus with the antioxidant N-acetyl cysteine blocks the effect of cigarette smoke on S. aureus biofilm formation [94].Whole

cigarette smoke exposure has been shown to promote biofilm development in other pathogens as well; however, in both cases, there is a lack of insight into the mechanisms involved. Cigarette smoke promotes the expression of some biofilm formation genes of P. aeruginosa (pilF, flgK) and the inhibition of the quorum sensing gene rhlA [95]. In addition, cigarette smoke condensate has been shown to promote the biofilm development of Streptococcus pneumoniae with decreased pneumolysin production [96].

4.5 Tobacco smoke and oral biofilms

The impact of cigarette smoking on biofilms is best understood in cases of biofilms located within periodontal tissue since these sites contain easily obtainable dental plaques which are very common sources of bacterial infection.Oral biofilms are multifarious and colonize supra- and sub-gingival regions in the mouth, and the composition of which has been implicated in the severity of periodontal disease [97]. Periodontitis is a disease caused by and/or promoted by smoking [98]. Effect of smoking on the composition of bacterial population of the dental biofilm has been described in several studies, and several major periodontal pathogens including T. denticola, F. nucleatum and P. gingivalis have been found to be present in greater numbers in cigarette smokers than non-smokers [99,100-101]. Oral biofilms in non-smokers may contain larger number of streptococci and other commensals [102].

Importantly, a reversion toward a healthier biofilm was noticed after 6 months following smoking cessation [103]. The increased presence of P. gingivalis, the "workhorse" pathogen in periodontal research laboratories, has been consistently shown among smokers [104,105]. Moreover, P. gingivalis was detected in higher amounts among smokers, compared to nonsmokers, and the infection is persistent in smokers, rather than in nonsmokers [100,106].

4.6 Significance of biofilms in oral health

Most of these microorganisms protect themselves from the adverse conditions of the environment through various mechanisms of survival like morphogenesis, proteolytic mechanism, demographic heterogeneity, etc. Biofilm formation is considered to be one of the predominant growth forms of the microorganisms existing in 90 percent of the bacterial strains. It is generally believed that the biofilms are heterogeneous communities of microbial cells growing in association on a surface in a matrix of different polysaccharides, proteins, and DNA. Quorum sensing is a process of communication among the bacteria within as well as outside the biofilm matrix.Antibiotic resistance is an adaptive form of resistance in the bacteria in biofilm when compared to their planktonic parts making biofilm-induced ill health conditions and infections difficult to treat all over the world [107,108]. The matrix serves as a physical barrier for the drug and offers a protective ecological environment for the microorganisms. There are various microorganisms such as P. aeruginosa, C. albicans, S. epidermidis, and M. tuberculosis Mycoplasma that can lead to health problems due to the resistance to the antimicrobial drug and immune system response [109]. Therefore, innovative strategies to combat the development of microbial biofilm are needed. According to the research, the innovative strategies to prevent biofilm development and quorum sensing are widely explored, and it shows that natural drugs prove their efficacy in combating drug resistance and antibiofilm disease [110]. P. aeruginosa, C. albicans, S. epidermidis, and M. tuberculosis Mycoplasma [108] are examples of such micro-organisms responsible for causing severe health issues because of resistance to anti-microbial drugs and immune system reactions. New techniques for combating anti-microbial biofilm production have to be discovered. The literature shows that there have been many innovative approaches for the prevention of biofilm and quorum sensing that have been extensively researched and proved the effectiveness of natural medications [110].

Biofilm has been defined as a community of microorganisms wherein planktonic cells associate with each other to form a group [111]. The associated groups of microorganisms are usually surrounded by an extracellular polymeric substance (EPS) matrix produced by themselves and known as slime (but all the slime is not necessarily a biofilm) which consists of a combination of proteins, extracellular saccharides and DNAs [112,113]. The structure of biofilms depends on the nature of the EPS matrix, and may include filamentous streamers and microcolonies resembling mushrooms [114]. It has been shown that the morphological structure of biofilms in microcolonies is governed by fluid flow, nutrient availability and quorum sensing (messenger molecules). N-Acyl homoserine lactones (AHLs) have been found to be involved in microbial communication [115,116].These communities have been known to be incorporated into an extracellular polymeric substance (EPS) that is generated by them, a matrix cell referred to as slime although not all slime qualifies to be termed as biofilm, a polymeric aggregation that is made up of a variety of proteins, extracellular saccharides and DNAs [112,113]. The nature of EPS matrix enables biofilm to develop a variety of structures including filamentous streamers and mushroom-like microcolonies [114]. The morphogenesis of biofilms in microcolonies is influenced by the flow of fluids, nutritional aspects and the messenger molecules known as quoromones. Bacteria use the N-acyl homoserine lactones (AHLs) in communicating with each other [115,116]. Biofilms may develop on both living and non-living substrates and are common in the environment and health care settings. Mutation capability is higher than the older planktonic cells [117,118]; this has led to the development of antibiotic resistance of biofilm.Each element is needed for the construction of the biofilm structure, Pel and psl polysaccharides are responsible for the formation of a solid surface for the biofilm, while alginate is responsible for the surface adhesion.In case of starvation, eDNA may act as a nutrient source [119,120]. The main reason why the bacteria become resistant to the antibiotics, which destroy the biofilm, is their ability to mutate in comparison with the old planktonic [117,118]. All elements are needed for the biofilm construction. Alginate provides the surface adhesion, while pel and psl polysaccharides provide the solid surface for the biofilm. In case of starvation, eDNA may act as a nutrient source [119,120].

4.7 Quorum Sensing and Its Association with Biofilm Development

It consists of a number of phases, ranging from four to five, including phase one, which is biofilm formation and characterized by rigid development of the biofilm, being the first versible attachment of planktonic bacteria; the second phase involves the formation of colony; the third involves bacterial growth; the fourth includes extracellular matrix production, while the fifth and final phase entails the maturation of biofilm leading to the reversible detachment of cells from biofilm previously formed [121,122]. Exopolysaccharide (EPS) is made up of many vital nutrients, including Aap protein attached to the cell surface with G-5 domain, which acts as an adhesive component of the cells; upon infection in Gram-negative bacteria, Sortase A (SrtA), a transpeptidase, causes extracellular localization and binding of cell surface proteins [included that The G-5 domain of the Aap protein attached to the cell surface and responsible for cell adhesion is among many essential nutrients in the exopolysaccharide (EPS).In the infection process in Gram-negative bacteria, Sortase A (SrtA), which is a transpeptidase, causes extracellular localization and can bind cell surface proteins; inhibitors to this adhesion can increase the antibiofilm activity [123,124]. Therefore, it may be [125]. A chemical signal molecule, known as quorum sensing (QS) is vital for cell-to-cell communication [126]. A chemical signal molecule, known as quorum sensing (QS) is vital for cell-to-cell communication [126]. In the infection process in Gram-negative bacteria, Sortase A (SrtA), which is a transpeptidase, causes extracellular localization and can bind cell surface proteins; inhibitors to this adhesion can increase the antibiofilm activity [123,124]. One of the various essential nutrients that form part of the exopolysaccharide (EPS). A transpeptidase known as Sortase A (SrtA) can bind cell surface proteins and cause extracellular localization in the infection process in Gram-negative bacteria; inhibitors to this adhesion can increase the antibiofilm activity [123,124]. As such, it may be [125]. Communication between cells depends on a chemical signal molecule known as quorum sensing (QS) [126].

5. Oral Biofilms: From Dental Caries to Systemic Diseases

It is estimated that 3.5 million people across the globe suffer from chronic and progressive oral diseases; despite their preventability, these public health problems may affect poor people disproportionately, and thus may affect their longevity and quality of life, which are linked to social and economic inequity [127]. Although these public health problems are preventable, poor people may be affected disproportionately by them, which may influence their longevity and quality of life, which are related to social and economic inequity [127]. As has been noted earlier, the complex interplay between the microbiome and oral microenvironment, as well as the pathogens and particular host features leads to the development of such conditions as dental caries, periodontal diseases, as well as other disorders associated with the oral cavity; these problems have common risk factors [128,129]. The features of bacterial biofilm, i.e. complex microbial consortia interacting with each other and triggering the immunological response and action of antimicrobial compounds are directly related to the manifestation and development of the above disorders [130].

Inflammation of the mouth, as well as inflammation elsewhere in the body, could result from the action of the dysbiotic microbiota by releasing toxins or metabolites into the bloodstream, as supported by an increasing body of research. The adverse impact of the effect on other bodily systems is therefore contingent upon the synergy between the two forms of inflammation [131].

Higher numbers of P.gingivalis were observed in patients suffering from both oral and esophageal squamous cell carcinoma [132]. In fact, there is evidence from a meta-analysis suggesting that individuals suffering from periodontal disease have a greater risk of developing oral cancer [133]. Another research carried out by Michaud et al. [134] also offers evidence that periodontal disease is associated with the increased risk of developing oral, lung, and pancreatic cancers. The occurrence of oral, lung, and pancreatic tumors is positively associated with periodontal disease, as shown by a recent study by Michaud et al. [134]. It appears that patients suffering from esophageal and oral squamous cell carcinoma have higher numbers of P. gingivalis [132].

6.1 Plant Extracts and Phytochemicals Which Inhibit the Adhesion of Oral Bacteria

The ability of plant extracts and phytochemicals to stop cariogenic bacteria from adhering to surfaces was examined in some of the aforementioned investigations. Crude or whole plant extracts' anti-adhesion properties. The cranberry, Vaccinium macrocarpon (Ericaceae), has been shown to have positive impacts on human health, such as reducing urinary tract infections by inhibiting Helicobacter pylori from adhering to the stomach mucosa and Escherichia coli from adhering to cells [179,180]. The possible effects of the consumption of cranberry juice or the cranberry substances on the inhibition of the oral pathogens' adherence to surfaces and related processes such as the production of glucans and fructans and formation of biofilms have been the focus of many studies. Except for S. sobrinus, where there was less adsorption after 10 minutes, it has been shown that exposing the oral streptococci to 25% cranberry juice for 10 seconds results in the reduction of the cell adherence by 61.8% to 95.1% [181].Additionally, a preparation of high molecular weight cranberry juice contents prevented the formation of biofilms and decreased the hydrophobicity of certain of the bacteria's cell surfaces.According to these findings, cranberry juice may stop the formation of dental plaque by blocking the first stage of biofilm formation. It was also demonstrated that a high molecular weight cranberry preparation decreased the activity of glucosyltransferase and fructosyltransferase [182].

6.2 Biological Properties of Plants

Since the effectiveness of medicinal plants in treating many diseases, the traditional knowledge of these plants which have medicinal value for different purposes has received great attention from the scientific world [135]. Bioactive compounds are also known as bionutrients and are highly present in medicinal plants. These bioactive compounds can

be located in the seed, root, leaves, flower, and sometimes in the whole plant, making them important sources of compounds that have similarities with food additives and flavors among others [136].
Bioactive substances are secondary metabolites that can be categorised according to their chemical structure, composition, or synthesis mechanism. Alkaloids, terpenoids, and phenolic compounds are the three primary classes of bioactive molecules that make up approximately 90% of all secondary metabolites [137]. Saponins, lipids, carbohydrates, ketones, and other substances are among the minor categories of secondary metabolites [138,139]. Due to their great variability in chemical and structural features as well as biological activity [140,141], phenolics belong to the most studied group of natural products at present. This biological activity might include antioxidant, antimutagenic, antitumor, antiallergenic, anti-inflammatory, antiviral, antiulcerogenic, antidiarrheal, anthelmintic, antihepatitic, and antiproliferative properties [142,143]. Numerous phenolic compounds with antibacterial qualities found in medicinal plants offer defence against harmful substances. In order to tackle drug-resistant bacteria, several of these substances can provide long-term treatments [144]. The compounds belonging to this secondary metabolite class, mostly flavonoids, which are one of the largest and widely studied chemical classes found in plants, can be synthesised via the shikimate pathway and consist of benzene, hydrogen, and oxygen rings [145]. There is proof that flavonoids act effectively against different microorganisms [146]. The anti-microbial effects of these compounds are thought to be influenced by their ability to complex with soluble and extracellular proteins, as well as with the bacterial membranes, making them more permeable and disruptive. Moreover, these chemicals could also inhibit the activities of β-hydroxyacyl-acyl transport protein dehydratase and DNA gyrase [147]; some catechins belonging to this group have shown an inhibiting effect on both Gram-positive and Gram-negative bacteria [148]. On the other hand, apigenin inhibited the GTF and fructosyltransferase proteins of S. mutans without causing any significant damage to the bacteria [149]. By suppressing numerous virulence genes and reducing the production of both water-soluble and insoluble glucans, quercetin derivatives hindered the biofilm formation by S. mutans [150]. However, the breakdown of the bacteria's membrane is considered to be the antimicrobial action mechanism for terpenoid compounds [151,152].Finally, the antibacterial effect of alkaloids, synthesized from amino acids such as tyrosine, is connected with their ability to intercalate into DNA, interfering with cell division, and leading to cell death [153]. In contrast to direct destruction of bacteria, the antimicrobial effects of the plant-based product can be achieved through the influence of several key steps in pathogenics process [154,155]. Various works prove the effectiveness of plant extracts against a wide range of oral cavity microbe infections. The ability of the plant-based products to inhibit the formation of biofilms in the oral cavity, to disrupt and reduce the adherence of the microbial pathogens to different oral surfaces (the first step in the formation of dental plaque and subsequent development of cavities and periodontal diseases), is the theme of some of them. Plant extracts and purified phytochemicals have proven their ability to act as bactericides, blocking one or several stages in the development of dental plaque through inhibiting biofilm adherence, aggregation or formation, or through prevention of cariogenic bacteria to produce glycolytic acid [156].A large number of scientific sources indicate the ability of polyphenols to inhibit the creation and accumulation of dental biofilm and oral biofilm. A wide range of compounds, such as catechins, flavonoids, alkaloids, terpenoids, proanthocyanin oligomers, and other compounds obtained from plants, are able to inhibit GTFs of S. mutans, which constitute an integral part of biofilm matrix and are among the virulence factors of S. mutans. Some compounds capable of biological activity have the potential to suppress infections and prevent diseases as proved by the use of traditional pharmaceuticals [139]. Hence, daily application of plant extracts and their derivatives is a promising alternative to artificial medicine in the treatment of oral diseases.[157]

6.3The role of herbal extracts in dental treatment

Several biologically active agents that have been isolated from natural products, including flavonoids, terpenoids, alkaloids, and phenols, possess antibacterial, wound healing, anti-inflammatory, and antioxidant activity. These properties are very important to defend oneself against periodontal pathogens such as Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and many other bacteria which cause development of periodontal disease [158]. The co-infection of S. mutans and C. albicans causes an etiological factor for dental caries on both in vitro and in vivo basis. The formation of biofilm is better in this interaction than in single species biofilm since C. albicans produces exopolysaccharide and enhances the survivability of S. mutans [159]. The oral streptococci augment the invasive characteristics of Candida albicans whereas S. mutans increases its biofilm development with the help of Candida albicans [160]. The complicated, polymicrobial inflammatory infections known as periodontal diseases are typified by the destruction of the tissues that support teeth. If left untreated, the condition progresses from gingivitis, an acute inflammation of the gingiva, to tooth pockets and ultimately tooth loss. The primary cause of chronic periodontitis is the Gram-negative anaerobe Porphyromonas gingivalis [161]. Gram-negative, anaerobic Prevotella bacteria typically inhabit the mouth cavity. P. dentalis, P. fusca, P. enoeca, P. denticola, P. melaninogenica, P. intermedia 17, and P. intermedia 17–2 are among the species of this genus that cause the generation of inflammatory cytokines, which also contribute to periodontal illnesses [162]. Halitosis, a disorder marked by oral odour, is another dental problem. In this instance, bacteria including Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia, Porphyromonas endodontalis, Fusobacterium nucleatum, and Solobacterium moorei are involved (163). Volatile sulphur compounds (VSCs), which are produced when halitosis bacteria break down proteins, are the cause of this foul breath [164]. The oral microbiota is defined as a collection of bacteria, fungi, protozoa, viruses, and archaea in the mouth cavity. The oral microbiota includes all these microbes as well as up to 1000 more species. Eubiosis or microbial homeostasis is described as the condition in which the microbiota of the healthy hosts is balanced in terms of symbiotic/commensal relations [165]. Dysbiosis is a parasitic/pathogenic relationship state, which leads to periodontitis and dental caries along with other diseases due to some changes or insults including smoking, immunosuppression, and dieting [166;165].

Dental disorders can be diagnosed, prevented, and treated through the development of new drug delivery systems, such as nanosystems [167]. Toothpaste, mouthwashes, and polishing paste are some examples of the dental hygiene products, which can be enhanced using nanomaterials. The unique surface-to-volume ratio makes nanomaterials more available to tissues and cells. In addition, the size of the nanomaterials ranges from 1 to 100 nm [168]. To enhance mechanical, antibacterial, and regenerative properties of the dental material, nanoparticles made of various metals have been added to it. For example, Zn, Ag, Au, Cu, and Ti have antibacterial properties [167].Another method is photodynamic therapy, which creates hazardous oxygen species that interact with and harm pathogens' cellular components by combining a certain wavelength of light with a photosensitiser medication in the presence of oxygen [169]. For oral health, integrating these technologies with natural items has shown encouraging outcomes [170;171]. Because of their calming qualities, herbal treatments are well known for their traditional uses, which have greatly advanced dental practice. Many plant-based products have been found to have significant anti-inflammatory properties. There has been a wide range of research in this field; some studies have examined the effectiveness of more complicated combinations, while others have concentrated on single-ingredient compositions. The potential of herbal medicines to improve dental treatments and patient outcomes is highlighted by this variety of approaches [172]. The effectiveness of Nigella sativa essential oil (NSEO) in improving dental health by addressing oxidative stress, inflammation, and periodontal infections was assessed in a study. Significant antioxidant qualities were shown by NSEO, which had strong free radical-scavenging activity and successfully reduced DPPH radicals. By preventing protein denaturation and stabilising red blood cell membranes, the oil also demonstrated anti-inflammatory properties. Notably, NSEO showed antifungal properties against Candida albicans and antibacterial action against important periodontal pathogens, such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. Its safety for oral care formulations was proven by cytocompatibility testing, which maintained over 82% cell viability at concentrations as high as 100 µg/mL [173]. Another study supported the traditional usage of Multidentia crassa in creating plant-based remedies for dental pain and inflammation by providing the first scientific assessment of the plant's potential advantages for oral health. The study emphasises the analgesic and anti-inflammatory qualities of M. crassa extracts, which may offer practical remedies for oral health problems, particularly in environments with limited resources. 58 active chemicals, including phenols and carboxylic acids, were found using Fourier transform infrared (FT-IR) analysis and gas chromatography–mass spectrometry (GC–MS). Despite significant safety concerns for specific molecules, molecular docking studies showed that stigmastan-3,5-diene exhibits strong binding potential to oral health-related proteins, showing its promise as a natural analgesic and anti-inflammatory drug [174].The effects of green tea extracts on oral inflammation and microbiota balance in mice with acetic acid-induced oral inflammation were examined in this work by Pan et al. The researchers sought to determine whether green tea extract could be used as a natural treatment to lower inflammation and replenish the mouth cavity's microbial diversity. The anti-inflammatory effect of green tea extract was demonstrated by the considerable reduction of inflammatory markers including TNF-α and IL-1β. Additionally, by lowering pathogenic microorganisms and promoting mucosal tissue regeneration, it supported a balanced oral microbiome. These protective benefits were facilitated by green tea's bioactive polyphenols, especially catechins. According to the results, green tea extract may be a promising natural therapeutic agent for treating oral dysbiosis and inflammation, which calls for more clinical research [175]. Another study examined the use of traditional medicinal plants, with an emphasis on their anti-inflammatory qualities, to treat oral disorders. In dentistry, conditions including gum inflammation and tooth discomfort are increasingly being treated with traditional medicine, which is used by 80% of the world's population. The four plants under investigation were These include Azadirachta indica (neem), Terminalia chebula (haritaki), Camellia sinensis (green tea), and Pipernigrum (black pepper). The extracts have been found to possess good protease inhibition, showing their efficacy as anti-inflammatory agents, whereas the molecular docking study indicated that the active ingredients of the extracts could bind well to the trypsin enzyme [176]. The study has also looked into the possibility of using a mixture of natural products (NPM-8) in fluoride toothpaste for its antibacterial and anti-inflammatory activities in response to the increasing prevalence of oral diseases. NPM-8, consisting of eight natural extracts, surpassed the fluoride toothpaste and chlorhexidine in their antibacterial activities against pathogens including Streptococcus mutans and Porphyromonas gingivalis. It has shown its safety and efficacy in use by disrupting the biofilm and reducing pro-inflammatory cytokines. According to the study, NPM-8 might benefit oral health through its anti-inflammatory, antibacterial, and antibiofilm properties in dental products [177].

6.4Natural anti-biofilm agents with clear-cut mechanisms or identified molecular addresses

Numerous natural compounds derived from plants had antibacterial and anti-biofilm properties in vitro. The fundamental processes of anti-biofilm function were found, along with a range of compounds produced from extracts of natural plants or medicinal herbs. The inhibition of polymer matrix formation, suppression of cell adhesion and attachment, disruption of ECM generation, and reduction of virulence factor production are the main mechanisms by which natural products have anti-biofilm effects. These mechanisms prevent the formation of QS networks and biofilms. These antibiofilm compounds isolated from medicinal plants are discussed in the next section.[178]

7 Challenges and Limitations

It's an exciting but difficult journey to integrate natural products and microbiome-based methods into clinical practice. The inadequate capacity to precisely analyse the microbiome is one of the most basic problems. The whole diversity, function, and metabolic activity of microbial communities are still difficult for current analytical techniques to fully capture. Researchers are unable to confidently identify treatment options and evaluate their safety and efficacy because of this gap. Additionally, it delays the creation of trustworthy microbiome biomarkers, which are crucial for clinical

research and individualised therapies. Even diagnostic techniques like microbiome-based IVD testing cannot be completely included into standard healthcare without proven procedures. The disagreement over what constitutes a "healthy" or "dysbiotic" microbiome presents a second difficulty. It is challenging to create uniform criteria since microbial populations differ greatly among individuals. This ambiguity makes it more difficult to establish precise criteria for the development of therapies based on natural products and complicates regulatory control. Because of this, multiple benchmarks may be used by businesses, medical professionals, and researchers, resulting in conflicting assessments of the performance and quality of products. Additional complication is introduced by pharmacokinetic and pharmacodynamic evaluations. In addition to interacting with host tissues, natural chemicals can also be metabolised or altered by local bacteria. These dynamic interactions are not adequately captured by conventional drug evaluation models. This means that clinical reactions are frequently not predicted by preclinical study results. Because each individual may respond differently to the same natural substance or microbial intervention, inter-individual heterogeneity in microbiome makeup adds another level of complexity. Another significant obstacle is still safety concerns. Despite the widespread belief that natural goods are safe by nature, many of them have potent bioactive qualities. Careful investigation is necessary due to potential toxicity, pollution, variations in plant sources, and drug interactions. Thorough screening is crucial since microbiome-derived treatments, such as donor-based products or oral microbiota transplantation, involve extra hazards associated to pathogen transfer. Although personalised microbiome therapies present intriguing opportunities, they also present scientific and regulatory difficulties. Large datasets, sophisticated computational techniques, and well-defined therapeutic frameworks—all of which are constantly evolving—are necessary for creating medicines based on a patient's microbiome. Such customised natural therapies are now difficult to implement since regulatory agencies lack clear procedures for sanctioning them. Another important issue is effectiveness. Even while many natural substances have potent activity in laboratory experiments, their effectiveness in actual human situations may be constrained by low bioavailability, poor stability, or quick oral cavity disintegration. Advanced delivery techniques including nanoencapsulation, slow-release dental coatings, or combinations with probiotics, prebiotics, or postbiotics may be necessary to increase their therapeutic impact.[183]

8 DISCUSSION

In summary, tobacco consumption significantly impacts the composition of microorganisms in the mouth because it causes dysbiosis, increases the virulence of periodontal bacteria, and encourages biofilm production. The impact of such changes does not only increase the destruction of periodontal tissues but also systemic inflammation issues. Current scientific research shows that bacteria caused by smoking, such as Porphyromonas gingivalis, Fusobacterium nucleatum, and Streptococcus mutans, show increased ability to adhere, engage in quorum sensing, and resist antibiotics in biofilm environments.

Plants and drugs derived from phytocompounds have been found to be a viable option in managing diseases related to oral biofilm infections. Bioactive molecules such as flavonoids, alkaloids, terpenoids, catechins, and polyphenols have been found to be very effective against biofilm-forming bacteria due to their antibacterial, anti-inflammatory, antioxidant, and antibiofilm effects on bacterial adhesion, synthesis of extracellular polymeric substances, quorum sensing, and production of virulence factors. Extracts from medicinal plants and green tea have shown positive results in combating oral biofilms.

However, despite these developments, some problems need to be addressed in order for these herbal antibiofilm treatments to become mainstream dental treatments. These include issues of variation in the content of the plants, poor bioavailability of these herbs, inadequate standardization, and lack of validation through extensive clinical studies. Hence, there is a need for future studies to look at more mechanistic studies, novel delivery systems like nanoformulations, microbiome-specific drugs, and clinical trials. Generally, combining these medicinal plants with modern-day dental treatments provides a great approach to treating biofilm diseases associated with tobacco use.

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