Review

Molecular Mechanisms of Inhibition of Streptococcus Species by Phytochemicals

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Abstract: This review paper summarizes the antibacterial effects of phytochemicals of various medicinal plants against pathogenic and cariogenic streptococcal species. The information suggests that these phytochemicals have potential as alternatives to the classical antibiotics currently used for the treatment of streptococcal infections. The phytochemicals demonstrate direct bactericidal or bacteriostatic effects, such as: (i) prevention of bacterial adherence to mucosal surfaces of the pharynx, skin, and teeth surface; (ii) inhibition of glycolytic enzymes and pH drop; (iii) reduction of biofilm and plaque formation; and (iv) cell surface hydrophobicity. Collectively, findings from numerous studies suggest that phytochemicals could be used as drugs for elimination of infections with minimal side effects.

Keywords: streptococci; biofilm; adherence; phytochemical; quorum sensing; S. mutans; S. pyogenes; S. agalactiae; S. pneumoniae

1. Introduction

The aim of this review is to summarize the current knowledge of the antimicrobial activity of naturally occurring molecules isolated from plants against Streptococcus species, focusing on their mechanisms of action. This review will highlight the phytochemicals that could be used as alternatives or enhancements to current antibiotic treatments for Streptococcus species. The scope of the review is limited to inhibitory effects of phytochemicals, mainly polyphenols, against Streptococcus species and where possible, their mechanisms of action against the major virulence factors will be discussed. Due to their major implications on human health, this review has largely focused on four Streptococcus species: (i) S. mutans (ii) S. pyogenes (iii) S. agalactiae and (iv) S. pneumoniae. To explain the potential mechanisms of inhibition of the phytochemicals, S. mutans has been used as the major example.

1.1. Streptococci

Streptococcus species are bacteria belonging to the Firmicutes phylum under the order of Lactobacillales and the family of Streptococcaceae [1]. Three genera exist within the family of Streptococcaceae including Streptococcus, Lactococcus and Lactovum of which Streptococcus is most diverse, containing 79 species [1]. A number of Streptococcus species are pathogenic to humans and animals, with S. pyogenes and S. pneumoniae as the most important pathogens [1]. These Gram positive bacteria generally appear as pairs or chains, are spherical to ovoid in shape, nutritionally fastidious, with fermentative metabolism and many of them form capsules [2].

Streptococcus species are found mostly in the oral cavity and nasopharynx and form a significant portion of the normal microbiota of humans and animals [2,3]. In healthy individuals, normal microbiota are harmless, however, they can cause infection under certain conditions, such as immune
compromised stage [2,4]. *Streptococcus* species (e.g., *S. pyogenes*, *S. agalactiae*, and *S. pneumoniae*) can be classified serologically based on the cell wall carbohydrates into groups A to V [2,5,6]. *Streptococcus* can also be grouped based on morphological differences, type of hemolysis on blood agar, biochemical reactions, cell wall pili-associated protein, and polysaccharide capsule (specific for group B streptococci) [7]. To date more than 85 capsule antigenic types of *S. pneumoniae*, 124 serotypes of *S. pyogenes* and nine CPS (capsular polysaccharide) serotypes of *S. agalactiae* have been proposed [7–9]. The cell wall of streptococci is among the most studied bacterial cell walls [7,10].

### 1.2. Streptococcal Infections and Major Virulence Factors

The diseases caused by streptococci range from non-life-threatening conditions like dental caries, pharyngitis (strep throat) to life-threatening conditions such as necrotizing fasciitis and meningitis (Table 1) [5]. Of all the oral streptococci, *S. mutans* is considered to be the etiological agent of dental caries. According to Petersen et al., industrialized countries spend 5%–10% of their public health expenditures on periodontal disease, dental caries and related dental care [11]. Unquestionably, one of the most common global diseases is dental caries [12].

A more pathogenic *Streptococcus* specie, *S. pyogenes* can be carried asymptomatically by humans but can cause mild to severe diseases, such as pharyngitis, tonsillitis, scarlet fever, cellulitis, erysipelas, rheumatic fever, post-streptococcal glomerulonephritis, necrotizing fasciitis, etc. (Table 1) [13]. It has been estimated that severe *S. pyogenes* infections lead to 517,000 deaths per year globally in addition to 233,000 deaths caused by rheumatic fever disease [14]. In United States alone 1800 invasive *S. pyogenes* disease-related deaths (necrotizing fasciitis and streptococcal toxic shock syndrome) are reported annually [15,16].

Another specie that most frequently has been linked to neonatal infections (early-onset and late-onset) such as sepsis, pneumonia and meningitis is *S. agalactiae* [17,18]. Late-onset neonatal infections (occurring at the age of 1–3 months) put infants at higher risk (as high as 20% even with proper antibiotic treatment) than early-onset neonatal infections of neonates (occurring within the first 24–48 h up to 7 days) [17]. In adults, *S. agalactiae* could cause peripartum choriomamniotitis, bacteremia, pneumonia, endocarditis, osteomyelitis, urinary tract infections, skin and soft tissue infections with immunocompromised individuals at highest risk (Table 1) [18,19].

Other important human pathogenic streptococci, *S. pneumoniae*, claimed the lives of 826,000 children under the age of five in year 2000 [20,21]. Globally, about 14.5 million episodes of invasive pneumococcal disease occur every year however mortality varies at 5%–35% depending on other factors (e.g., comorbidity, age, site of infection) [22]. In USA, annually 4 million episodes of pneumococcal diseases account for 445,000 hospitalizations and 22,000 deaths and *S. pneumoniae* is still the leading cause of bacteremia, meningitis, and pneumonia among all age groups (Table 1) [23].

Streptococci have a variety of potent virulence factors enabling them to cause such diverse infections [5]. Adhesins are one such factor because they play an important role in colonization [5]. Adhesins and virulence factors of streptococci have been reviewed extensively [5,6,24,25]. Carcinogenicity capacity of *S. mutans* is largely dependent on the ability of the bacteria to adhere and produce acid [12]. *S. mutans* glucosyltransferases assist in the adhesion process by synthesizing insoluble glucan from sucrose [12]. On the other hand, *S. pyogenes* produces extracellular proteins that have been shown to give rise to the remarkable virulence of the organism, triggering a nonspecific host immunological response [26]. Specific virulence factors assist *S. pyogenes* to attach to the host tissue, escape phagocytosis, and spread by infiltrating the host epithelial layers followed by colonizing [3,17,27–29]. In the case of *S. agalactiae*, major virulence and pathogenic factors enable the bacterium to stimulate sepsis syndrome, adhere to epithelial surface succeeding invasion, and avoidance of phagocytosis [30]. *S. agalactiae* attaches to host cells via fibronectin, fibrinogen and laminin [30]. For *S. pneumoniae*, a number of proteins, including hyaluronate lyase, pneumolysin, neuraminidases, the major autolysin, choline binding protein A, pneumococcal surface antigen A have been suggested to be virulence associated factors of this bacterium [31]. In addition, polysaccharide capsule is considered to be a key virulence factor [31].
Table 1. Demonstrated virulence factors of streptococci species, disease caused and the associated social and financial cost with the disease.

| Organism    | Diseases                  | Adherence Site                                                                 | Estimated Cases/Costs                                                                                                                                 |
|-------------|---------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| S. mutans   | Dental caries             | Tooth surface, other bacteria present in the biofilm on the surface of the tooth [5] | 500 million visits to dentists and an estimated $108 billion spent on dental services in united states in 2010 [27]                                  |
|             | Dental plaque             |                                                                                |                                                                                                                                                      |
|             | Endocarditis              |                                                                                |                                                                                                                                                      |
| S. pyogenes | Pharyngitis               |                                                                                | 1–2.6 million cases of strep throat, erythromycin-resistant, invasive S. pyogenes causes 1300 illnesses and 160 deaths in united states each year. The total cost (medical and non-medical) of group A streptococcal pharyngitis among school aged children in united states ranges from $224 to $539 million per year [27] |
|             | Cellulitis                | Mucosal surfaces of pharynx, skin [25]                                        |                                                                                                                                                      |
|             | Streptococcal toxic-shock syndrome |                                                                                |                                                                                                                                                      |
|             | Necrotizing fascitis      |                                                                                |                                                                                                                                                      |
|             | Rheumatic fever           |                                                                                |                                                                                                                                                      |
|             | Sequela                   |                                                                                |                                                                                                                                                      |
|             | Erysipelias glomerulonephritis |                                                                                |                                                                                                                                                      |
| S. agalactiae | Neonatal sepsis         | Mucosal surfaces of vaginas and recta of pregnant women, skin [32]            | Clindamycin-resistant S. agalactiae causes an estimated 7600 illnesses and 440 deaths yearly in U.S. 27,000 cases of severe S. agalactiae disease, such as blood infections or meningitis, occurred in 2011, causing 1575 deaths in U.S. [27] |
|             | Meningitis                |                                                                                |                                                                                                                                                      |
|             | Systemic infection in immuno-compromised individuals |                                                                                |                                                                                                                                                      |
| S. pneumonia | Otitis media             | Mucous membranes of the nasopharynx [33]                                      | Cases of resistant pneumococcal pneumonia result in about 32,000 additional doctor visits and about 19,000 additional hospitalizations and costs associated are approximately $96 million in U.S. [27] |
|             | Bacteraemia               |                                                                                |                                                                                                                                                      |
|             | Pneumonia                 |                                                                                |                                                                                                                                                      |
|             | Meningitis                |                                                                                |                                                                                                                                                      |
|             | Bronchitis                |                                                                                |                                                                                                                                                      |
|             | Sinusitis                 |                                                                                |                                                                                                                                                      |
|             | Laryngitis                |                                                                                |                                                                                                                                                      |
|             | Epiglottitis              |                                                                                |                                                                                                                                                      |
1.3. Mechanism of Pathogenicity of Streptococcal Diseases

1.3.1. Adhesion, Plaque, and Biofilm Formation of Streptococcal Species

To cause disease, a bacterial pathogen needs to meet several basic requirements. First, it must be able to adhere to the tissue surface and compete with the normal microbiota present on that surface [5,34,35]. Subsequently, for sustainable attachment, biofilms are developed and this may lead to invasion of the host tissue [6]. To establish biofilm, planktonic bacteria attaches to either inert or coated surfaces and this can be mediated by electrostatic contacts or bacterial surface adhesins [36]. Attachment is followed by proliferation of the primary colonizers and their co-aggregation with other planktonic bacteria, production of exopolysaccharide which stabilizes the architecture, leading to the maturation of the biofilm [36]. Sessile bacteria then could detach and form biofilms at different site [36–38]. Biofilm formation is not an attribute only specific to a few species, but a general ability of all microorganisms. Biofilm formation pathways are species specific, diverse, and dependent on environmental factors [39]. Although diverse, there are common features among all biofilms: (i) cells in the biofilm are glued together by an extracellular matrix made of exopolysaccharides, proteins, and occasionally nucleic acids; (ii) biofilm formation is initiated by environmental and bacterial signals; and (iii) biofilm offers bacteria protection from antibiotics and environmental stresses including immunological responses of the host [39]. Bacterial biofilms can build up on abiotic (plastic, glass, metal, etc.) or biotic (plants, animals, and humans) surfaces [34,38,40]. Mammalian-tissue colonizing species of Streptococcus live within biofilm in the natural environment [6,41,42].

Bacteria increase the expression of their outer cell surface adhesins when environmental conditions allow promoting cell-cell and cell-surface interaction [6,43]. Streptococci owe their success in colonization to their wide range of proteins expressed on their surfaces [5,6]. Surface adhesins facilitate interrelation with salivary, serum, extracellular matrix elements, host cells and other microbes [5,6]. Many of these adhesins are anchored to the cell wall peptidoglycan via their C-terminus or to the cell membrane via their N-terminal lipid (lipoproteins), and other adhesins remain surface localized through non-covalent interactions with other proteins or polysaccharides on the cell surface [6,44].

Most bacterial pathogens, including streptococci, have long filamentous structures known as pili or fimbriae that are also involved in adhesion and biofilm formation [34]. In Gram-positive bacteria, hydrophobic components can be found: (i) covalently bound to cell wall, such as streptococcal M and F proteins, (ii) in the cytoplasmic membrane (e.g., lipoteichoic acid (LTA) of S. pyogenes or sialic acid of S. agalactiae) or (iii) located on the surface, like pili or fimbriae [6,44,45]. Aside from adherence, biofilms are of significant importance because approximately 65% of human bacterial infections involve biofilms [45] including Streptococcus species (e.g., S. mutans, S. pyogenes, S. agalactiae, and S. pneumoniae) [34,40,41,46]. Clinically, biofilms are important because they reduce susceptibility of the bacteria to antimicrobials, prospering resistant bacteria leading to persistent infections [47,48].

The primary cause of dental caries is dental plaque which is a complex biofilm [41]. Broad spectrum of saliva proteins contribute to and initiate adhesion and dental biofilm formation [41,49,50]. Adhesion of S. pyogenes to various host cells is facilitated by the capsule and several factors embedded in the cell wall including M protein, LTA, and F protein [6,25,51]. M protein not only helps bacteria to attach to the host tissue but also inhibits opsonization by binding to host complement regulators and to fibrinogen [52]. A recent study has demonstrated that S. pyogenes pilus promotes pharyngeal cell adhesion and biofilm formation [53]. Altering surface hydrophobicity by sub-minimum inhibitory concentration of penicillin and rifampin reduces the adhesion of S. pyogenes to epithelial cells suggesting that surface-associated LTA will determine the surface hydrophobicity content of S. pyogenes, which consequently affects the bacteria’s interaction with mammalian host cells [54–56].

S. agalactiae produces several virulence factors such as adhesins [6]. These surface proteins and LTA of S. agalactiae bacterial cell wall contribute to the adhesion process mediating the invasion of eukaryotic cells [30]. Non-encapsulated S. agalactiae strains show increased adherence to eukaryotic cells [30]. In vitro studies have shown that S. agalactiae adheres to vaginal, buccal, endothelial and
pulmonary epithelial cells [30]. Many clinical isolates of *S. mutans*, *S. pyogenes*, and *S. agalactiae* have been reported to be hydrophobic while their avirulent counterpart strains lacked this feature [57–60]. Studies have shown that *S. pneumoniae* adheres to abiotic surfaces, e.g., polystyrene or glass, and forms three-dimensional biofilm structures that are about 25 micrometers deep [34]. This three-dimensional structure enables the bacteria to survive for long periods within the bacterial community [34].

### 1.3.2. Proton-Extrusion and Glycolysis of Streptococcal Species

Vital to the survival of bacteria is the regulation of the cytoplasmic pH as cellular activity requires a specific pH range [61]. Cytoplasmic pH is modulated by environmental pH, production, or consumption of internal protons, and transferring acids and bases across the plasma membrane [62]. The function of F-adenosine triphosphatase (F-ATPase) in streptococci is to regulate internal pH by pumping protons out of the cell [62,63]. The physiological role of streptococcal F$_{0}$F$_{1}$-ATPase is to alkalinate the cytoplasmic pH in the acidic pH range and to establish a proton reserve for a variety of secondary transport systems [64–66]. Streptococci are deficient in respiratory chains and are unable to produce a large proton gradient across the membrane, however, they make up for this lack by utilizing a range of basic transport systems [66]. For example, synthesis of a cytochrome-like respiratory chain, formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate by coupling the nicotinamide adenine dinucleotide hydrogen (NADH) oxidation with phosphorylation reaction [66–68]. Generally, ATPase in streptococci does not function as ATP synthase because of lack of a functional electron transport system; thus, it functions as hydrolase for proton movements coupled to ATP hydrolysis that are used for the generation of the proton gradient [66]. Streptococci utilize the glycolytic pathway to metabolize glucose to lactic acid [4,66]. Glucose is taken up, phosphorylated to glucose-6-phosphate through the phosphoenolpyruvate-dependent phosphotransferase system, and then converted to pyruvate, and eventually to lactic acid [66,69].

*S. pneumoniae* and oral streptococci could adapt to different environments and this capability is facilitated by ATPase regulating the intracellular concentration of solutes, including protons, and maintaining the pH homeostasis by proton extrusion [66,70]. Adherence is dependent: (i) on the synthesis of extracellular polysaccharides (mostly glucans) from the disaccharide sucrose through glucosyltransferases (GTFs) for *S. mutans*, and (ii) bacteria’s ability to produce acid by glycolysis and its tolerance to the produced acid [71]. *S. mutans* has the properties of acid production from sugar metabolism causing a drop in pH in dental plaque [72]. Low pH values in the plaque matrix leads to demineralization of tooth enamel, selection of acid-tolerant streptococci and eventually dental caries [72]. The glucans synthesized by GTFs promote the binding and accumulation of *S. mutans* and other bacteria on the tooth surface and contribute to the formation of biofilms [72–75]. *S. mutans* increases the proton-translocation, and F-ATPase activity when the environment’s pH drops, thereby this bacterium could withstand acidification influences [66,76,77]. F-ATPase transfers protons out of cells with the assistance of ATP hydrolysis to maintain its intracellular pH (e.g., more alkaline than the extracellular environment) [76]. F-ATPase enzyme is composed of two domains; (i) F$_{1}$, the cytoplasmic catalytic domain; and (ii) F$_{0}$, the proton-conducting membrane domain [67,78]. *S. mutans* does not produce catalase or cytochromes (thus a heme-based electron transport system) and so does not have oxidative phosphorylation linked to trans-membrane electron transport [66,79].

### 1.3.3. Glucan Synthesis, Aggregation and Quorum Sensing of Streptococcal Species

Glucans interact with surface-associated glucan binding proteins of *S. mutans* to initiate colonization, cell-cell aggregation and the firm adherence of its cells to tooth surfaces [72,80]. *S. mutans* produces three types of GTFs: GTFB, GTFC, GTFD, and each of these enzymes are composed of two functional domains: (i) an amino-terminal catalytic domain (CAT); and (ii) a carboxyl-terminal glucan-binding domain (GBD) [81]. GTFB and GTFC, located on the cell surface, are encoded by *gtfb* and *gtfc* genes and GTFD is encoded by the *gtfd* gene [82]. Therefore, one of the strategies to control biofilm formation and dental caries is to inhibit the activity of GTFs: (i) GTFB (which
synthesizes a polymer of mostly insoluble α, 3-linked glucan); (ii) GTFC (which synthesizes a mixture of insoluble α-1,3-linked glucan and soluble α-1,6-linked glucan); and/or (iii) GTFD (which synthesizes water-soluble glucans rich in α-1,6-glucosidic linkages) [83,84].

Many streptococci use quorum-sensing systems to regulate several physiological properties, including the ability to incorporate foreign deoxyribonucleic acid (DNA), tolerate acid, form biofilm, and become virulent [85–88]. Quorum sensing, a strategy of cell-to-cell communication in a biofilm community, regulates unnecessary over-population and nutrient competition [89,90]. Bacterial activities including virulence gene expression within biofilms is regulated by the occurrence of quorum sensing [91]. This topic as well has comprehensively been discussed in review articles [87,92,93].

1.4. Treatment of Streptococcal Infection

Penicillin or one of its derivatives (e.g., amoxicillin and ampicillin) are the recommended antibiotic treatment for non-allergic patients diagnosed with S. pyogenes and S. agalactiae infections [27]. For allergic individuals, azithromycin and clarithromycin are recommended and in fact, azithromycin is prescribed more commonly than penicillin [94]. For severe S. pyogenes infections like necrotizing fasciitis and toxic shock syndrome, a combination of penicillin and clindamycin are prescribed [95]. S. pyogenes and S. agalactiae are not resistant to penicillin, but over time they have become resistant to clindamycin, tetracycline, vancomycin and macrolides (e.g., erythromycin, azithromycin and clarithromycin) [27]. Clarithromycin, clindamycin and vancomycin resistance among S. pyogenes and S. agalactiae strains are most concerning [27].

1.5. Antibiotic Resistance and Emerging Threats

Antimicrobial resistance is compromising the treatment of invasive infections including severe streptococcal infections [27]. This threat becomes significant in vulnerable patients (e.g., individuals undergoing chemotherapy, dialysis and organ transplants) due to infection-related complications [27]. This puts healthcare providers in the position to use antibiotics that may be more toxic to the patient, and frequently more expensive, leading to an increased risk of long-term disability and lower survival rates [27].

According to Frieden, director of the U.S. Center for Disease Control and Prevention (CDC), antimicrobial resistance is a serious health threat in the 21st century [27]. Infections caused by resistant bacteria are now on the rise and their resistance to multiple types and classes of antibiotics is worrisome [96]. The decrease in the rate of pathogen susceptibility to antibiotics has made it much more difficult to combat the infectious diseases [27]. The CDC’s 2013 report has prioritized drug-resistant S. pneumoniae as a serious threat, and erythromycin-resistant S. pyogenes and clindamycin-resistant S. agalactiae as concerning threats [27].

1.6. Possible Alternatives for Classical Antibiotics

Plants produce diverse secondary metabolites or phytochemicals, most of which are isoprenoids and polyphenols and their oxygen-substituted derivatives such as tannins that could be raw materials for future drugs [97]. Herbs and spices contain useful medicinal compounds including antibacterial chemicals, and researchers have found that many of these compounds inhibit the growth of pathogenic bacteria [97]. Accordingly, experimental observations have shown that herbal preparations are active against many of the pathogens (Table 2).

From the period of 1981 to 2006, 109 new antibacterial drugs were approved for treatment of infectious diseases of which 69% originated from natural products, and 21% of antifungal drugs were natural derivatives or compounds mimicking natural products [98]. Various medicinal plants have recently been tested for their antimicrobial activity and all have proven that phytochemicals, particularly polyphenols, exhibit significant antibacterial activity against Streptococcus species (Table 3).
2. Anti-Streptococcal Attributes of Phytochemicals

Many fruits and plants have shown to possess anti-streptococcal effects (Table 3). Folklore medicinal plants have long been used for the treatment of *S. pyogenes* infections (Table 2) including pharyngitis. For example cashew plant (*Anacardium occidentale*), stickwort (*Agrimonia eupatoria*), mountain daisy (*Arnica montana*), bayberry (*Myrica cerifera*), soft leafed honeysuckle (*Lonicera japonica*), cuajilote (*Parmentiera aculeate*) or baobab (*Adansonia digitata*) [99–104], (Table 2). Particularly more attention has been given to anti-streptococcal effects of phytochemicals against *S. mutans* due to its cariogenic properties. A wide range of commercial and freshly prepared polyphenolic rich extracts (70% propanone) of various teas including green and black tea, lemon, cinnamon, hibiscus, peppermint, grape seed, sloe berry skin, cocoa, blackberry, pomegranate skin, blackcurrant, hawthorn berry skin, red and white wine was tested for their anti-streptococcal activity against oral streptococci (various strains of *S. mutans*, *S. oralis*, *S. gordonii*, *S. salivarius*, *S. sanguis*) [105]. All the tested products exhibited their minimum inhibitory effect at concentrations ranging 0.25–32 mg/mL against *Streptococcus* species [105]. Red grape seed propanone extract was most potent against *S. mutans* and Agro tea extract least effective with minimum inhibitory concentration of 0.5 mg/mL and 32 mg/mL respectively [105]. Phytochemicals, although very limited, also have been shown to hinder the growth of *S. agalactiae* [106–112]. Aqueous, ethanolic and chloroform extracts of bael, Indian gooseberry, moringa, neem, Chinese mahogany exert their minimum inhibitory effects at concentrations ranging from 0.15 mg/mL to 10 mg/mL against *S. agalactiae*, chloroform extract of Chinese mahogany being the most active one [111]. In a study by Nguelefack *et al.* ethyl acetate bark extract of *Distemonanthus benthamianus* at Minimum Bactericidal Concentration (MBC) of 4096 µg/mL was effective against *S. agalactiae* and its phytochemical profile was indicative of presence of flavonoids and phenolics and absence of sterols, triterpenes and alkaloids [113]. Moderate inhibitory effect of wild *Asparagus racemosus* ethanol extract at concentration of 500 µg/disc was also reported for *S. agalactiae* [114].

2.1. Phytochemicals with Inhibitory Activities against Adhesion, Plaque, and Biofilm Formation

Phytochemical-rich extracts and their associated pure compounds have repeatedly shown inhibitory effects against adhesion, plaque, and biofilm formation of streptococcal species (Tables 4 and 5). High molecular weight non-dialysable materials extracted from cranberry juice (NDM) exhibit adhesion reduction activity in a dose-dependent manner at concentrations of 66–1330 µg/mL against *S. sobrinus* [115]. In another study, the ethanolic extract of *Helichrysum italicum* at concentrations of 15–31 µg/mL inhibited the sucrose-dependent adherence of *S. mutans* cells to a glass surface by 90% to 93% [116]. Cranberry juice powder (25%) at 500 µg/mL concentration inhibited the biofilm formations of *S. sobrinus* and *S. sanguinis* significantly [117]. In the same study, cranberry juice powder decreased the cell surface hydrophobicity of *S. mutans* and *S. sobrinus* 6715 by more than 40% [117].
Table 2. Folklore medicine used for Streptococcal diseases or diseases with similar clinical Presentations.

| Folklore Medicinal Plant | Targeted Disease Condition |
|--------------------------|----------------------------|
| Agrimonia eupatoria L.   | Acute sore throat and chronic nasopharyngeal catarrh [118,119] |
| Arnica montana L.        | Inflammation of oral, throat region [99,120,121] |
| Lonicera japonica Thunb. | Erysipelas, pharyngitis, upper respiratory infection [100] |
| Morella cerifera (L.) Small | Cold and sore throat [101,122] |
| Parmentiera aculeata (Kunth) Seem | Otitis media [123] |
| Adansonia digitata L.    | Otitis media [102,124] |
| Anacardium occidentale L. | Sore throat [103,125] |
| Uvaria chamae P. Beauv.  | Sore throat [64,126,127] |
| Annona digitata L.       | Inflamed gums and infected teeth [128] |
| Carica papaya L.         | Toothache [129] |
| Hyoscymus niger L.       | Toothache [130,131] |
| Eucalyptus comaltdulensis Dehn. | Toothache, sore gums [132] |
| Annona reticulata       | Toothache [133,134] |
| Annona squamosa Linn    | Toothache [133,134] |
| Uvaria chamae P. Beauv.  | Inflamed gums [135] |
| Atalation indicum (L.) Sweet, Baliospermum axillare Blume, Blumea lacera (Burm. f.) DC., Canna indica L., Ocimum terumflorum L., Oroxylum indicum (L.) Vent., Polygonum aviculare L., Solanum indicum Linn., Vernonia patula (Aiton) Merrill | For the relief of symptoms of bronchitis, pneumonia, influenza [136] |
| Vigna radiata (L.) R. Wilczek Andrographis paniculata (Burm. f.) Wall. ex Nees | Treatment of sepsis [137,138] |
### Table 3. Inhibitory effects of phytochemicals against selected *Streptococcus* species.

| Species | Strain | Plant | EM | MIC, IZD | Ref. |
|---------|--------|-------|----|---------|-----|
|         |        |       |    |         |     |
| *S. pyogenes* | ATCC 19615 | *Passiflora foetida* L. | EE, ACE | 100–400 µg/mL, 10–20 mm | [139] |
|         |        | *Ageratum conyzoides* L. | AE, EE, ME | 1–2 mg/mL | [140] |
|         |        | *Laggera tomentosa* Sch.-Bip. | | | |
|         |        | *Syzygium guineense* DC. | | | |
|         |        | *Cordia africana* Lam. | | | |
|         |        | *Ferula communis* L. | | | |
|         |        | *Discopodium peninervum* Hochst | | | |
|         |        | *Olea europea* subsp. cuspidate | | | |
|         |        | *Cassia occidentalis* L. | CEE | 5 mg/mL | [141] |
|         |        | *Uvaria chamae* P. Beauv. | CAE, HAE | 9–12 mm, 100 µg/mL | [127] |
|         | CI | *Uvaria chamae* P. Beauv. | CDEE, HEE | 6–21 mm, 100 µg/mL | [127] |
|         | CI | *Cassia occidentalis* L. | EE | 2–6 mm, 0.0005–0.389 µg/mL | [142] |
|         | CI | *Cassia occidentalis* L. | EE | 0.0005–0.44 µg/mL | [142] |
|         | CI | *Cassia occidentalis* L. | HE | 5.5–7 mm | [143] |
|         | CI | *Eucalyptus globulus* Labill. | ME | 32–64 µg/mL | [144] |
|         | CI | *Hibiscus* donum L. | RE, EAE | 10 mm, 512 µg/mL | [106] |
|         | CI | *Prunus armeniaca* L. | CEE, RE | 293 µg/mL | [145] |
|         | ATCC 19615 | *Coffea canephora* Pierre ex Froehner | AE | 15–34 mm | [146] |
|         | ATCC 19615 | *Azlania reticulata* L. | AF | 29 mm | [146] |
|         | CI | *Spathodea axillae* Maire | CHE | 296 µg/mL | [147] |
|         | CI | *Cassia occidentalis* L. | TO | 6.25 µL/mL | [107] |
|         | CI | *Typhina retusa* L. *Spathodea axillae* (L.) Merr. & L.M. Perri | TO | 12.5 µL/mL | [107] |
|         | CI | *Sechium edule* (Jacq.) Sw. | EE | 10–15 mm | [108] |
|         | ATCC 25175 | *Rehmannia glutinosa* Lour. ex Hook. | AEE | 5 mg/mL | [146] |
|         | ATCC 25175 | *Rehmannia glutinosa* Lour. ex Hook. | 75% ME | 14–22 mm, 20, 50 mg/mL | [109], [110] |
|         | MTLC-490 | *Nettle (Urtica dioica)* | | | |
|         | UA139 | *Rehmannia glutinosa* Hook. | HE | 1.25–2.5 µg/mL | [112] |
|         | UA139 | *Cassia occidentalis* L. | HAE | | |
|         | ATCC 700610 | *Eucalyptus globulus* Labill. | EE, EAE | 9–12 mm, 120 µg/mL | [134] |
|         | ATCC 700610 | *Zingiber officinale* Roscoe | ACE, CHE, DEE, EAE, EE, PEE | | |
|         | ATCC 25175 | *Sesame (Sesamum indicum)* | CE, PEE | 40–320 µg/mL | [109] |
|         | MTLC-490 | *Nettle (Urtica dioica)* | Primo, et al. Water, methanol | 12–23 mm | [108] |
|         | UA139 | *Rhodora krisiana* Planch. & Traut. | | | |
|         | ATCC 700610 | *Eucalyptus globulus* Labill. | | | |
|         | ATCC 700610 | *Zingiber officinale* Roscoe | | | |
|         | ATCC 25175 | *Sesame (Sesamum indicum)* | commercial extract | 6 µg/mL | [109, 110] |
### Table 3. Cont.

| Species | Strain | Plant | EM | MIC, IZD | Ref. |
|---------|--------|-------|----|----------|------|
| S. pneumoniae | ATCC 49619, penicillin resistant and sensitive clinical strains | G. rosea var. Engl. (B. Wright) Kuntz ex DC. | EE | 0.001–0.7 µg/mL | [142] |
| | | A. blazei Murill | AE | | [158] |
| | | P. major L. | AE | 0.48 mg/kg | [159] |
| | | ATCC 49619, penicillin resistant and sensitive clinical strains | G. kola Heckel | EE | 0.00008–1.7 µg/mL | [142] |
| | | E. globulus Labill. | ME | 16–32 mg/L | [144] |
| | | S. entomosperma Mill | EO | 2.25 mg/mL | [161] |
| | | T. rugosa L. | EO | 6.25 µL/mL | [107] |
| | | E. globulus Labill. | CAE | 0.7 mg/mL | [162] |
| | | S. zeylanicum (L.) Merr. & L.M. Perr | ME | 12.5 µL/mL | [107] |
| | | S. aromaticum (L.) Merr. & L.M. Perr | ME, AE | 6–11 mm, 60-80 mg/mL | [163] |
| | | L. triloba L. | ME | 1–2 mm/mL | [146] |
| | | S. zeylanicum (L.) Merr. & L.M. Perr | ME | 12.5 µL/mL | [107] |
| | | S. aromaticum (L.) Merr. & L.M. Perr | ME | 12.5 µL/mL | [107] |
| | | S. zeylanicum (L.) Merr. & L.M. Perr | ME | 12.5 µL/mL | [107] |
| | | S. aromaticum (L.) Merr. & L.M. Perr | ME | 12.5 µL/mL | [107] |
| | | S. zeylanicum (L.) Merr. & L.M. Perr | ME | 12.5 µL/mL | [107] |

**Abbreviations:** ACE; Acetone Extract, AE; Aqueous Extract, BE; Butanolic Extract, CAE; Crude Aqueous Extract, CDEE; Cold Ethanolic Extract, CE; Crude Extract, CHE; Chloroform Extract, CEE; Crude Ethanolic Extract, CI; Clinical Isolate, CME; Crude Methanolic Extract, DEE; diethyl ether extract, EAE; Ethyl Acetate Extract, EE; Ethanolic Extract, EM; Extraction Method; EO; Essential Oil, HAE; Hot Aqueous Extract, HE; Hexane Extract, HPLC; High Performance Liquid Chromatography, IZD; Inhibition Zone Diameter, ME; Methanolic Extract, MIC; Minimum Inhibitory Concentration, PE; Petroleum Extract, PEE; Petroleum Ether Extract, Ref.; References.
Table 4. Inhibitory effects of phytochemicals against adhesion, biofilm formation and hydrophobicity.

| Plant/Fruit Name                  | Bioactive Compounds and EM                                                                 | Bacterial Strain            | Concentration and Assay Type                                                                 | Results                                                                 | Ref.  |
|----------------------------------|--------------------------------------------------------------------------------------------|-----------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|-------|
| Maidenhair tree                  | Purified PAC, AE, AEE, ME                                                                  | *S. pyogenes* DSM 2071      | Adhesion reduction at 3 h incubation time                                                      | *P. súdios* 40%                                                        | [164] |
| South African geranium           | Purified PAC, AE, AEE, ME                                                                  | *S. pyogenes* DSM 2071      | Adhesion reduction at 3 h incubation time                                                      | *G. biloba* 100%                                                        |       |
| Cranberry (Vaccinium macrocarpon Aiton) | Purified PAC, AE, AEE, ME                                                                  | *S. pyogenes* DSM 2071      | Adhesion reduction at 3 h incubation time                                                      | *G. biloba* 25%                                                        |       |
| Cranberry (Vaccinium macrocarpon Aiton) | High MW non-dialyzable materials                                                          | *S. mutans* MT 8148R, JC2, Ingbritt, ATCC 10449 | 100–500 µg/mL Inhibition of biofilm formation                                                 | Significant inhibition                                                 | [117] |
| Cranberry (Vaccinium macrocarpon Aiton) | High MW non-dialyzable materials                                                          | *S. mutans* MT 8148R, JC2, Ingbritt, ATCC 10449 | 100–500 µg/mL Inhibition of biofilm formation                                                 | Significant inhibition                                                 | [117] |
| Cocoa (Theobroma cacao L.)       | PP fractions Oligomers: Monomer MW 290                                                     | *S. mutans* NCTC 10449 CI of S. sanguinis LDII | 35 µM Biofilm biomass reduction after 4 h                                                      | In absence of sucrose                                                   | [165] |
| Cranberry (Vaccinium macrocarpon Aiton) | High MW non-dialyzable material, CJ                                                       | *S. sobrinus* 6715          | 1.33 mg/mL Adhesion to glucan or fructan coated hydroxyapatite reduction                      | 95%                                                                    | [115] |
| Red grape (Vitis vinifera L.)    | Red grape marc extract (GME): 20% PP, 3% A,                                               | *S. mutans* ATCC 25175      | 2.0 mg/mL Adhesion to glass surface Inhibition                                                | GME significant inhibition, RWE, PBE effective at >4 mg/mL              | [166] |
| Pine bark                        | Red grape marc extract (GME): 20% PP, 3% A,                                               | *S. mutans* ATCC 25175      | 2.0 mg/mL Adhesion to glass surface Inhibition                                                | GME significant inhibition, RWE, PBE effective at >4 mg/mL              | [166] |
| Blueberry (Vaccinium myrtillus L.)| Small cranberry                                                                             |                              |                                               | S. pneumonia bound to fraction F1 of cranberry and bilberry juices                          | [112] |
| Lingonberry (Vaccinium vitis-idaea L.) | Small cranberry                                                                             |                              |                                               | S. agalactiae bound to bilberry juice and cranberry fractions FII and FIII and to all fractions of cranberry juice and lingonberry | [112] |
| Cloudberry (Rubus chamaemorus L.)| Small cranberry                                                                             |                              |                                               | S. pneumonia bound to fraction F1 of cranberry and bilberry juices                          | [112] |
| Crowberry (Empetrum nigrum L.)   | Blackcurrant                                                                                |                              |                                               | S. agalactiae bound to bilberry juice and cranberry fractions FII and FIII and to all fractions of cranberry juice and lingonberry | [112] |
| Blackcurrant (Ribes nigrum L.)   | Sour cherry (Prunus cerasus L.)                                                             |                              |                                               | S. pneumonia bound to fraction F1 of cranberry and bilberry juices                          | [112] |
| Sour cherry (Prunus cerasus L.)   |                                             |                              |                                               | S. agalactiae bound to bilberry juice and cranberry fractions FII and FIII and to all fractions of cranberry juice and lingonberry | [112] |
### Table 4. Cont.

| Plant/Fruit Name          | Bioactive Compounds and EM | Bacterial Strain        | Concentration and Assay Type                                      | Results                   | Ref.   |
|--------------------------|----------------------------|-------------------------|------------------------------------------------------------------|---------------------------|--------|
| Clove                     |                            |                         |                                                                  |                           |        |
| *Syzygium aromaticum* (L.) Merr. & L.M. Perr | CAE                        | *S. mutans* ATCC 25175  | 20 mg/mL, Percent cell-surface hydrophobicity                      | 0.3% ± 0.1%               | [167]  |
|                          | CAE                        | *S. mutans* ATCC 25175  | 20 mg/mL, Adherence inhibition                                   | 100%                      |        |
|                          | CME                        | *S. mutans* ATCC 25175  | 20 mg/mL, Percent cell-surface hydrophobicity reduction           | 25.2% ± 4.7%              |        |
|                          | CME                        | *S. mutans* ATCC 25175  | 15 mg/mL, Adherence inhibition                                   | 100%                      |        |
| Cocoa                     | Bean husk extract 12.6% PP compounds 30% EE | *S. mutans* MT8148    | 1 mg/mL, Adherence to saliva-coated hydroxyapatite inhibition    | 31%                       | [168]  |
| Guava                     | Quercetin-3-O-alpha-L-arabinopyranoside (guaijaverin) ME | *S. mutans* MTCC1943  | 2 mg/mL, Percent cell hydrophobicity                             | 20%                       | [169]  |
| Cranberry                 | PP fraction                | *S. sobrinus* 6715      | 500 µg/mL, Hydropobicity reduction                               | *S. sobrinus* 6715 90%    |        |
| Devil's horsewhip        | AE, BE, ME, PEE            | CI of *S. mutans*       | 125 µg/mL, Biofilm inhibition                                    | Complete to partial biofilm inhibition | [171]  |
| Meswak                    | ACE, AE, CHE, EE, ME       | CI of *S. mutans*       | 2.6 mg/mL, Biofilm inhibition                                    | significant inhibition    | [172]  |
| Indian gooseberry         | CE, EF                     | *S. mutans* MTCC 497    | 39.04 µg/mL CE, 78.08 µg/mL, Ethanolic fraction, Biofilm inhibition | 50% inhibition            | [173]  |
|                          |                            |                         | 156 µg/mL CE and 312.5 µg/mL Ethanolic fraction, Adherence inhibition | 50% inhibition            |        |
|                          |                            |                         | Hydrophobicity reduction                                         | Partial reduction         |        |
Table 4. Cont.

| Plant/Fruit Name       | Bioactive Compounds and EM                                      | Bacterial Strain       | Concentration and Assay Type                  | Results                 | Ref. |
|------------------------|------------------------------------------------------------------|------------------------|-----------------------------------------------|-------------------------|------|
| Papaya (Carica papaya L.) | Fermented papaya preparation (FPP)                               | S. mutans 25175        | 50 mg/mL, Percent hydrophobicity             | S. mutans: 1.01%        | [174]|
|                        | Alkaloids                                                        | S. mitis 6249          |                                              | S. mitis: 7.66%         |      |
|                        | Flavonoids                                                       |                        |                                              |                         |      |
|                        | Glucosides                                                       |                        |                                              |                         |      |
|                        | Anthraquinones                                                  |                        |                                              |                         |      |
| Curry (Helichrysum Italicum G. Don) | Apigenin                                                        | S. mutans ATCC 35668   | 16–31 µg/mL, Adherence to glass surface inhibition | 90%–93%                 | [116]|
|                        | Luteolin                                                        | S. salivarius ATCC 13419|                                              |                         |      |
|                        | Gnaphaliin                                                      | S. sanguis ATCC 10556  |                                              |                         |      |
|                        | Naringenin                                                      |                        |                                              |                         |      |
|                        | Pinocembrin                                                    |                        |                                              |                         |      |
|                        | Tiliroside                                                     |                        |                                              |                         |      |
|                        | EE                                                              |                        |                                              |                         |      |

Abbreviations: A; Anthocyanin, ACE; Acetone Extract, AE; Aqueous Extract, AEE; Aqueous Ethanol Extract, BE; Butanolic Extract, CAE; Crude Aqueous Extract, CE; Crude Extract, CHE; Chloroform Extract, CI; Clinical Isolate, CJ; Concentrated Juice, CME; Crude Methanol Extract, EE; Ethanol Extract, EF; Ethanol Fractions, EM; Extraction Method, FPP; Fermented Papaya Preparation, GME; Red Grape Marc Extract, HE; Hexane Extract, kDA; Kilodalton, ME; Methanol Extract, MW; Molecular Weight, PAC; Proanthocyanidin, PBE; Pine Bark Extract, PEE; Petroleum Ether Extract, PP; Polyphenol, Ref.; References, RWE; Red Wine Extract.

Table 5. Inhibitory effects of pure phytochemicals against adhesion, biofilm formation, quorum sensing and hydrophobicity.

| Bioactive Compounds        | Bacterial Strain                     | Concentration and Assay Type                  | Results                        | Ref. |
|---------------------------|--------------------------------------|-----------------------------------------------|--------------------------------|------|
| (−)-Epicatechin            |                                      |                                               | (−)-epigallocatechin 15%       | [164]|
| (−)-epicatechin-3-O-gallate|                                      |                                               | (−)-epigallocatechin-3-O-gallate 40% |      |
| (−)-epigallocatechin       | S. pyogenes DSM 2071                 | 30 µg/mL, Adhesion reduction to HEp-2 cells   |                              |      |
| (−)-epigallocatechin-3-O-gallate|                                  |                                               |                              |      |
| Morin                      | S. pyogenes MGAS 6180                | 225 µM, Biofilm biomass reduction             | 50%–60%                       | [175]|
| Ursolic acid (UA)          | S. mutans UA159                      | 1024 µg/mL, Adherence inhibition to tooth surface | Complete inhibition          | [176]|
| Oleanolic acid (OA)        | Actinomyces viscosus ATCC 15987      |                                               |                              |      |
| EGCG                       | ComC-deficient S. mutans             | 0.25 mg/mL, Biofilm inhibition                | 81% Biofilm inhibition        | [177]|
|                           |                                      |                                               | QS inhibition                 |      |

Abbreviations: ComC; competence factor, EGCG; Epigallocatechinate, HEp-2; Human Epithelial Type 2 (Hep-2) Cells, OA; Oleanolic Acid, QS; Quorum Sensing, Ref.; References, UA; Ursolic Acid.
In a different study, anti-adhesion, biofilm inhibition and eradication activity of the two-terpenoids, ursolic acid (UA) and oleanolic acid (OA), were examined. UA and OA showed a Minimum Inhibitory Concentration (MIC) of 256 µg/mL and 1024 µg/mL against *S. mutans* UA159, respectively [176]. The Minimum Bactericidal Concentration (MBC) for UA and OA against the same bacterium were 256 µg/mL and >1024 µg/mL correspondingly [176]. Microtiter plate biofilm assay showed that sub-MIC dose of the compounds inhibited the biofilm formation [176]. Gallic acid at 1–4 mg/mL concentration inhibited up to 70% of *S. mutans* biofilm establishment [178]. Gallic acid, quercetin, and tannic acid all produced significant biofilm inhibition attributes against *S. mutans* however gallic acid was most potent [179]. Methyl gallate at concentrations of 1–4 mg/mL rendered biofilm formation of *S. mutans* to up to 80% [178]. Green and oolong tea contain substantial quantities of gallic acid and epigallocatechin gallate and have exhibited slight inhibition effect on the attachment of *S. mutans* and other oral bacterial to collagen, tooth surfaces and gingival cell line [180]. In the same study, fermented tea with high tannin content opposed to green tea and oolong tea had shown more activity towards attachment of *S. mutans* and other oral bacterial to collagen, tooth surfaces and gingival cell line [180].

Adhesion of *S. mutans* to the tooth surface was hindered after treatment with UA at 256 µg/mL [176]. Sub-MIC dose of UA also affected the adhesion consequently hindering the biofilm formation [176]. UA moreover eradicated the biofilm cells at concentrations of 500–2000 µg/mL [176]. Polyphenolics-rich tea extract at concentrations as low as 1–4 mg/mL prevented the attachment of *S. mutans* to collagen coated hydroxyapatite beads [181].

In another study, the effect of cocoa polyphenol fractions on *S. mutans* biofilm reduction in the absence and presence of sucrose were measured. At 35 µM concentration and after 4 h, biofilm mass was reduced to 68% in the absence of sucrose and to 44% in the presence of sucrose [165]. Biofilm of *S. mutans* on saliva coated hydroxyapatite surface was preformed and then treated (60 s) with purified proanthocyanidin (PAC)-containing fraction of cranberry (various degree of polymerization) [182]. At concentrations of 100 µM (single or combined fractions in 1:1 ratio), confocal 3D images show distorted architecture and deficient biofilm accumulation suggestive of reduced biomass and thickness of adherent bacteria and EPS [182]. Expressions of 119 genes of *S. mutans* within biofilm were altered post exposure to PAC-rich fractions of cranberry [182]. The expression of genes particularly related to adhesion, acid stress tolerance, glycolysis and other cellular activities during biofilm development were downregulated [182]. Structure activity relationship analysis revealed that PAC oligomers with more than eight epicatechin units exhibit higher anti-adhesion effects up to 85% against *S. mutans* however the increase in potency is not proportional [182]. This not only is associated with degree of polymerization but may also be associated with number and location of A-type linkages in the oligomers, and type of interflavan bonds [182].

The anti-adhesive properties of root extract of *Pelargonium sidoides* have been studied against *S. pyogenes* attachment to human epithelial type 2 (HEp-2) cells [164]. Results have shown that after pre-treatment of *S. pyogenes* with methanol insoluble and methanol soluble fractions of the extracts of *Pelargonium sidoides* at concentrations of 30 µg/mL, adhesion of the pathogen to HEp-2 cells was inhibited up to 30% to 35% [183]. To characterize the anti-adhesive constituents of these fractions, comparative chemical studies were performed. The study revealed that the proanthocyanidins content of the fraction was of prodelphinidin nature, and inhibition of the adhesion was in a specific rather than non-specific manner [164,183]. Successful inhibition of adhesion and hydrophobic interactions could reduce and or prevent sore throat caused by *S. pyogenes* [164]. It has been suggested that polymeric flavonoids or other large molecule polyphenols may exhibit higher anti-adhesion effects against streptococci [180]. Coffee high molecular weight fraction nearly completely (91%) hindered the adhesion of *S. mutans* [184].

Similarly, a study on the binding activity of *S. pneumoniae* and *S. agalactiae* to different molecular size fractions (F1, F2, F3) of *Vaccinium* family polyphenols found that binding was highest to wild cranberry (*Vaccinium oxycoccus*) [112]. *S. pneumoniae* cells bound mostly to cranberry juice
low-molecular size fraction (F1) and *S. agalactiae* cells to high-molecular size fraction (F3) [112]. *S. pneumoniae* bound to F1 of bilberry and cranberry juices and *S. agalactiae* attached most actively to F2 and F3 of berry and juice preparations belonging to *Vaccinium* species [112]. Phytochemical analysis has shown that F2 and F3 fractions contain polyphenol macromolecular complexes, including proanthocyanidins and polyhydroxy flavonoids [112]. At sub-MIC level of 2 mg/mL red grape marc extract, composed of 20% polyphenols and 3% anthocyanin, inhibited the adherence of *S. mutans* and *Fusobacterium nucleatum* cells to glass surface [166]. Morin, a flavonol, reduced biofilm biomass of *S. pyogenes* at concentrations exceeding 225 µM up to 65% [175]. Epigallocatechin gallate (EGCG) of *Camellia sinensis* has various physiological effects on *S. mutans* UA159 (Figure 1) and has been proven to inhibit the enzymatic activity of glucosyltransferases, F₁F₀-ATPase, lactate dehydrogenase, biofilm formation and growth [153].

![Figure 1](image-url). Chemical structure of polyphenols with inhibition activity against adherence, biofilm biomass and hydrophobicity. Abbreviations: AI; Adherence Inhibition, BR; Biofilm Biomass Reduction, HR; Hydrophobicity Reduction, MOA; Mode of Action, Ref.; References.

### 2.2. Phytochemicals with Inhibitory Activities against F-ATPase and Glycolytic pH-drop

Phytochemical-rich extracts not only possess anti-adhesion, anti-plaque and anti-biofilm attributes, but also have demonstrated inhibitory effects on streptococcal species F-ATPase and glycolytic...
pH-drop activities (Table 6, Figure 2). Plants and fruits have been studied for their anti-streptococcal effects and fruits such as cranberry (V. macrocarpon), cocoa (Theobroma cacao), babchi (Psoralea corylifolia), mangosteen (Garcinia mangostana) and grape (Vitis vinifera) have shown inhibitory effects on F₀-ATPase and F₁-ATPase, glucosyltransferases (GTFB and GTFC) and acid production activities of S. mutans [80,84,165,185]. The lack of inhibitory activity of monophenolic compounds suggest that the inhibition of F₁–F₀-ATPase by phenolics require two or more phenolic structures [186]. The flavones have also been shown to interact with other ATPases, such as Ca²⁺-ATPase [187] and Na⁺/K⁺-ATPase [188], in addition to their inhibitory effects on F₁–F₀-ATPase [189]. Glycolysis of S. mutans is inhibited by α-mangostin leading to indirect inhibition of respiration by α-mangostin [190]. Glucan production by GTFs and F-ATPase is inhibited by α-mangostin suggesting that S. mutants can be eliminated selectively [190].

![Chemical structure of polyphenols with inhibition activity against F-ATPase, glycolytic enzymes and glycolytic pH-drop. Abbreviations: CI; Clinical Isolate, F-ATPAI; F-ATPase Activity Inhibition, GEI; Glycolytic Enzymes Inhibition, GpHDI; Glycolytic pH-Drop Inhibition, MOA; Mode of Action, Ref.; References.](image-url)

| Compound                                | Strain              | Concentration   | MOA      | Ref. |
|-----------------------------------------|---------------------|-----------------|----------|------|
| α-Mangostin                             | S. mutans UA159     | 31–95 µmol/L    | 50% GEI  | [190]|
| Procyanidin A2                          | S. mutans UA159     | 500 µmol/L      | 29% F-ATPAI | [191]|
| Epicatechin-(4β→8, 2β→O→7)-epicatechin-(4β→8)-epicatechin (A-type proanthocyanidins) [84] | S. mutans UA159 | 500µg/mL | 85% F-ATPAI | [84] |
| Cocoa polyphenol pentamer [165,192]     | S. mutans NCTC 10449 | 500µg/mL | 70% GpHDI  | [165,192] |

Figure 2. Chemical structure of polyphenols with inhibition activity against F-ATPase, glycolytic enzymes and glycolytic pH-drop. Abbreviations: CI; Clinical Isolate, F-ATPAI; F-ATPase Activity Inhibition, GEI; Glycolytic Enzymes Inhibition, GpHDI; Glycolytic pH-Drop Inhibition, MOA; Mode of Action, Ref.; References.
Analysis of low molecular weight cranberry polyphenols against glucosyltransferases, acid production and F-ATPase activity of \textit{S. mutans} UA159 has suggested that compounds like phenolic acids have no inhibitory effect on these virulence factors [84]. Quercetin, quercetin-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-arabinofuranoside, quercetin-3-O-rhamnoside, myricetin, PAC-monomer, PAC-dimer, and procyanidin A2, at the concentrations of 500 $\mu$M inhibited the enzymatic activity of the proton-translocating F-ATPase to some degree [191]. Myricetin, procyanidin A2 and the combination of the two were most effective inhibitors with 32%, 29% and 43% inhibition against F-ATPase activity, respectively [192]. The flavonoids, particularly myricetin, procyanidin A2 and the combination of the two significantly interrupted the glycolytic pH-drop by \textit{S. mutans} cells; however, epicatechin, myricetin-3-O-rhamnoside, caffeic acid, chlorogenic acid had no effect [191]. In presence of cocoa polyphenol pentamer, the terminal pH is increased to 4.67 ± 0.09 within 20 min while in untreated pH was as low as 4.50 ± 0.08 (\textit{S. mutans} converts sucrose to acid and lowers the pH) [165]. These results suggest that 500 $\mu$M cocoa polyphenol pentamer reduced the rate of acid production, at pH 7.0, by 30% [165].

2.3. Phytochemicals with Inhibitory Activities against Glucosyltransferases, Aggregation, and Quorum Sensing

Moreover, phytochemicals-rich extracts have been reported for their inhibitory properties against glucosyltransferases, aggregation, and quorum sensing attributes of streptococcal species (Table 7). Low molecular weight polyphenols of cranberry reduced the glucan synthesis of \textit{S. mutans} cells by GTFB and GTFC [191]. At 500 $\mu$M, the inhibition activities of the tested polyphenols varied from 15%–45% (epicatechin 15%, myricetin-3-O-rhamnoside 20%, procyanidin A2 30%, quercetin-3-O-arabinofuranoside 35% and quercetin-3-O-arabinofuranoside in combination with procyanidin A2 45%) [191]. It is notable that theaflavin of green tea at 10 mM inhibited the GTF activities of \textit{S. mutans} significantly [193].

The effects of fractions (F1, F2, and F3) of juice concentrates of bilberry (\textit{Vaccinium myrtillus}), lingonberry (\textit{Vaccinium vitis-idaea}), cloudberry (\textit{Rubus chamaemorus}), crowberry (\textit{Empetrum nigrum} and \textit{hermaphroditum}), apple (\textit{Malus domestica}), and blackcurrant (\textit{Ribes nigrum}) on anti-coaggregation and anti-aggregation activities of dental plaque bacteria have been tested [194]. Test has been done on the pairs of \textit{S. mutans} IH 113728 with the two strains of \textit{Actinomyces naeslundii} (AHP 28639 and AHP 28651) and \textit{S. mutans} IH 113728 with the two strains of \textit{Fusobacterium nucleatum} (AHN 23952 and AHN 23937) [194]. The anti-aggregation and anti-coaggregation activity was found in F2 and F3 of bilberry, blackcurrant, crowberry and lingonberry juices [194]. Also, F2 and F3 of crowberry at 48 mg/g of Solid Solubles (SS) showed anti-co-aggregation against some of the pairs at 91% and 86%, respectively [194]. The anti-aggregation activity was detected in all bacterial pairs with fraction F2 of bilberry, crowberry and lingonberry juices [194]. The anti-aggregation was mainly achieved with a berry concentration of 48 mg/g of SS [194]. Analysis of composition of the juice fractions showed that F2 and F3 were composed of macromolecular polyphenol complexes, PAC, polyhydroxy flavonoids [194]. Absolute co-aggregation inhibition and anti-aggregation activity were achieved with the F2 of bilberry juice at the concentration of 48 mg/g of SS [194].
Table 6. Inhibitory effects of phytochemicals on F-ATPase activity and glycolytic pH-drop.

| Plant                        | Bioactive Compounds and EM | Bacterial Strain | Concentration and Assay Type | Results                                                                 | Ref. |
|------------------------------|---------------------------|------------------|-------------------------------|-------------------------------------------------------------------------|------|
| Cranberry (Vaccinium macrocarpon Aiton) | FLAV A PAC                | S. mutans UA159  | PAC 500 µg/mL FLAV 125 µg/mL A 200 µg/mL F-ATPase activity inhibition | PAC alone or in combinations >85% FLAV 20% Glycolytic pH-drop 500 µg/mL | [84] |
| Cranberry (Vaccinium macrocarpon Aiton) | Low MW PP                | S. mutans UA159  | 500 µg/mL F-ATPase activity inhibition | Glycolytic pH-drop Significant disruption Myricetin 32% procyanidin A2 29% Myricetin + procyanidin A2 43% | [191] |
| Cocoa (Theobroma cacao L.)    | Oligomeric Monomer MW 290 Dimer MW 578 Tetramer MW 1154 Pentamer MW 1442 HE of PP fractions | S. mutans NCTC 10449 S. sanguinis LDI 1, CI | 500 µM pentamer Glycolytic pH-drop 125 µg/mL F-ATPase activity inhibition 500 µg/mL Glycolytic pH-drop | Significant disruption Significant inhibition | [165] |
| Red wine grape (Vitis vinifera L.) | Gallic acid Catechin Epicatechin Procyanidin B1 Procyanidin B2 Resveratrol Fermented | S. mutans UA159  | 15.6 µg/mL Glycolytic pH-drop 5%–15% Glycolytic enzymes inhibition (GEI) | Decreased ATPase, enolase, lactate dehydrogenase, protease, glucosidase, EPS and acid production activity | [195] |
| Green tea Camellia sinensis (L.) Kuntze | EGCG EE                  | S. mutans UA159  | 15.6 µg/mL Glycolytic pH-drop | Significant inhibition | [153] |
| Methuselah’s beard (Usnea longissima Ach.) | Herbo-metallic preparations | S. mutans  | 5%–15% Glycolytic enzymes inhibition (GEI) | Decreased ATPase, enolase, lactate dehydrogenase, protease, glucosidase, EPS and acid production activity | [196] |
| Purple mangosteen (Garcinia mangostana L.) | α-mangostin EE           | S. mutans UA159  S. rattus FA-1 S. salivarius ATCC 13419 | GEI | Ic50 31 µM Lactic dehydrogenase, 45 µM Aldolase, 95 µM Glyceraldehyde-3-phosphate dehydrogenase inhibition | [190] |

Abbreviations: A; Anthocyanin, EE; Ethanolic Extract, EGCG; Epigallocatechingallate, EM; Extraction Method, EPS; Exopolysaccharide, FLAV; Flavonol, F-ATPase; F-Adenosine triphosphatase, GEI; Glycolytic Enzymes Inhibition, HE; Hexane Extract, IC50; Inhibition Concentration 50%, MW; Molecular Weight, PAC; Proanthocyanidin, PP; Polyphenol, Ref.; References.
Table 7. Inhibitory effects of phytochemicals on glucosyltransferases, aggregation and quorum sensing.

| Plant/Fruit Name | Bioactive Compounds and EM | Bacterial Strain | Concentration and Assay Type | Results | Ref. |
|------------------|---------------------------|------------------|-----------------------------|---------|-----|
| Whortleberry or Bilberry (Vaccinium myrtillus L.) | Molecular size of fractions; F1 <10 kDa, F2 10–100 kDa, F3 >100 kDa CJ | CI of S. mutans IH 113728 A. naeslundii AHP 26639, AHP 28651 F. nucleatum AHN 23952, AHN 23937 | 48 mg/g of SS Inhibition of aggregation and reversal activity | F2 of bilberry juice 100% | [194] |
| Neem (Azadirachta indica A. Juss.) | AE | S. sobrinus ATCC 27607 S. mutans ATCC 25175 S. cristus ATCC 19642 S. sanguis H7PR3 | 250 µg/mL Bacterial aggregation | Microscopically observable bacterial aggregation | [197] |
| Red Wine Grape (Vitis Vinifera L.), and its pomace | Gallic acid Catechin Epicatechin Procyanidin B1 Procyanidin B2 Resveratrol | S. mutans UA159 | 62.5 µg/mL Inhibition of GTF B and C activities | 70%–85% | [195] |
| Green tea and black tea (Camellia sinensis (L.) Kuntze), and polyphenol mixtures | Theaflavin: its mono- and digallates (+)catechin (-)epicatechin and their enantiomers Epigallocatechin (-)gallocatechin HAE | S. mutans OMZ 176 | Theaflavin 1–10 mM Inhibition of GTF activities | significant inhibition | [193] |
| Leaves of Oolong tea (Camellia sinensis (L.) Kuntze) | Oolong tea polyphenol OTF6 (polymeric polyphenol) EE | S. mutans MT8148R | 60–850 µg/mL rGTFs (rGTFB, rGTFD, rGTFC) synthesis inhibition | 50% | [197] |
| Rock cinquefoil (Drymocallis rupestris (L.) Sojak) | PRU2 PRU TAC 155 mg/g TPC 4.6 mg/g TFC 10.2 mg/g | S. mutans CAPM 6067 S. sobrinus CAPM 6070, DSM 20381, doemor CCUG 21020 S. sanguis ATCC 10556 | 0.75–1.5 mg/mL PRU and PRU2 Inhibition of GTF activities | 60% | [198] |
| Apple (Malus domestica Borkh.) | Apple condensed tannins (ACT) Apple PP and apple juice | S. mutans MT 8148 (serotype C) S. sobrinus 6715 (serotype G) | 1.5–5 µg/mL ACT Inhibition of GTF activities | 50% | [80] |
| Hop (Humulus lupulus L.) | High MW PP 36,000–40,000 AEE | S. mutans MT 8148 (serotype C) S. sobrinus ATCC 33478 (serotype G) | 0.1% Inhibition of GTF activities | significant effect | [199] |
| Cranberry (Vaccinium macrocarpon Aiton) | FLAV PAC | S. mutans UA159 | PAC: 500 µg/mL FLAV; 125 µg/mL A; 200 µg/mL Inhibition of GTF B and C activities 2 mg/mL Inhibition of GTF, FTF activities, 1 h incubation | FLAV, PAC or in combination 30%–60% | [84] |
| Cranberry (Vaccinium macrocarpon Aiton) | High MW non-dialysable material (NDM) CJ | S. sobrinus 6715 | 2 mg/mL Inhibition of GTF, FTF activities, 1 h incubation | GTF 20% FTF 40% | [115] |
| Cranberry (Vaccinium macrocarpon Aiton) | Low MW PP | S. mutans UA 159 | 500 µM/L Reduction of glucan synthesis by GTFB, GTFC | Quercetin-3-arabinofuranoside + procyanidin A2 45% | [191] |
### Table 7. Cont.

| Plant/Fruit Name                  | Bioactive Compounds and EM | Bacterial Strain | Concentration and Assay Type | Results                                      | Ref.   |
|-----------------------------------|-----------------------------|------------------|------------------------------|----------------------------------------------|--------|
| Beard lichen (Usnea longissima Ach.) | Herbo-metallic preparations | *S. mutans*      | 5%-15% Inhibition of violacein production | Partial QS inhibition                        | [196]  |
| Indian gooseberry (Emblica Officinalis L.) | Crude and EF | *S. mutans* MTCC 497 | QS inhibition (suppression of comDE), glucan synthesis reduction | [173]  |
| Marupá (Eleutherine americana Merr.) | CE of different extractive solvents | CI of *S. pyogenes* and NPRC109 | 250 mg/mL QS inhibition | Partial to strong inhibition, *R. tomentosa* | [90]   |

Abbreviations: A; Anthocyanin, ACT; Apple condensed tannins, AE; Aqueous Extract, AEE; Aqueous Ethanolic Extract, CE; Crude Extract, CEE; Crude Ethanolic Extract, CI; Clinical Isolate, CJ; Concentrated Juice, comDE; two-component signal transduction system, EE; Ethanolic Extract, EF; Ethanolic Fractions, EM; Extraction Method, FLAV; Flavonol, FTF; Fructosyltransferase, GTF; Glucosyltransferases, HAE; Hot Aqueous Extract, KDa; Kilodalton, MW; Molecular Weight, NDM; High Molecular Weight Non-Dialysable Materials Extracted From Cranberry Juice, PAC; Proanthocyanidin, PP; Polyphenol, PRU; Aqueous Extract Sub-Fraction, PRU2; Diethyl Ether Sub-Fraction, QS; Quorum Sensing, Ref.; References, SS; Solid Soluble, TFC; Total Flavonoid Content, TFC; Total Proanthocyanidins Content, TTC; Total Tannin Content.
Crude extract of *Eleutherine americana* at 250 mg/mL inhibited the quorum-sensing of a clinical isolate of *S. pyogenes*, partially, while at the same concentration *Rhodomyrtus tomentosa* had a stronger inhibition activity [90]. Betulin, oleanane-3,12-dione, benzyl (6Z,9Z,12Z)-6,9,12-octadecatrienoate, and 3-benzylxoxy-1-nitrobutan-2-ol possess great anti-quorum sensing inhibition activities (Figure 3A,B). Few bioactives compounds of *A. aspera* have shown to effectively interact with quorum sensing response regulators of *S. mutans* thus preventing expression of virulence elements [171]. Molecular docking revealed that *A. aspera* bioactive compounds, 3,12-oleandione and betulin, could inhibit quorum sensing by interacting with *S. mutans* OmpR subfamily QS regulatory DNA-binding response regulator and *S. mutans* glycosyltransferase (EPS synthesizing enzyme), respectively [171]. Al-Sohaibani *et al.* performed similar analysis on the bioactive compounds of *Salvadora persica* methanolic extract [172]. Results suggest that benzyl (6Z,9Z,12Z)-6,9,12-octadecatrienoate and 3-benzyloxy-1-nitrobutan-2-ol (Figure 3C,D) are capable of interacting with *S. mutans* OmpR subfamily QS regulatory DNA-binding response regulator thus hindering biofilm formation by this or similar quorum sensing pathway [172].

![Figure 3. Chemical structure of phytochemicals with *S. mutans* quorum sensing inhibition activity. (A): Betulin; (B): Oleanane-3,12-dione; (C): Benzyl (6Z,9Z,12Z)-6,9,12-octadecatrienoate; (D): 3-Benzylxoxy-1-nitrobutan-2-ol.](image)

### 3. Conclusions and Prospects

Each class of classical antibacterial agents (antibiotics) usually targets different sites and processes of pathogenic bacteria. Major antimicrobial actions include disruption of membrane structure, inhibition of protein synthesis, and inhibition of production of folate coenzymes, nucleic acids, and peptidoglycans. Natural antimicrobials like their synthetic counterparts (antibiotics) target different molecules and processes to inhibit the colonization and viability of the bacteria or to inactivate bacterial toxins and or modulate the molecules and processes pre-requisite for bacteria’s metabolic pathways or reduce the rate of protein synthesis. It is worth noting that natural antimicrobial products not necessarily have to be bactericidal to suppress such processes and activities. It is plausible that a compound is likely to be efficient bacterial growth inhibitor if it can deteriorate the cytoplasmic pH, increase the permeability of plasma membrane, prevent extracellular and intracellular microbial enzyme production, interrupt bacterial metabolic pathways, or disrupt plaque and biofilm formation. As observed, there is considerable amount of scientific evidence that phytochemicals exert significant multiple anti-streptococcal effects and apart from their bactericidal effects, their main bacteriostatic strategy is the anti-adhesiveness attribute.

The efficacy of natural products as antimicrobials with fewer or no side effects is likely to depend on the structure of the compound that interacts with the toxin or pathogen and not with molecules of the...
host meaning that their effect is specific. This approach has become the rationale for natural drug design studies as a new field of research. Attempts have been made to understand certain features relating to phytochemical structure and the associated antibacterial activity. High molecular weight and complex phytochemicals exert greater inhibitory effects such as pentamer polyphenolic fraction of cocoa, high molecular weight non-dialyzable material of cranberry and F2 or F3 fractions of crowberry and bilberry. The side effects of the current antimicrobials and the spread of drug-resistant microorganisms have become a significant concern and a threat to successful therapy of microbial diseases. Therefore, there is an urgent demand for the discovery of safe natural compounds with diverse chemical structures and mechanisms of action satisfying both the consumer and the healthcare providers as potential useful therapeutic tools of the post-antibiotic era. Intensive research on such plants could lead to the incorporation of the most potent chemically defined extracts into nutraceuticals or natural health products and becoming a solution to this global concern of evolution of drug-resistant microorganisms.

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