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THE EFFECTS OF VARIOUS SYNTHETIC AND NATURAL ANTIMICROBIAL AGENTS INCLUDING MOUTHWASHES ON ORAL MICROORGANISMS

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A B S T R A C T
Oral cavity harbors large varieties of microorganisms. Some of them are commensals, and others are pathogens. The oral microbial community has a microbial synergism in which some microorganisms play a vital role in creating an environment for the growth and proliferation of other microorganisms that can initiate various oral diseases. Oral hygiene procedures, including regular mouth rinsing and the use of antimicrobial agents in the form of antibiotics administered systemically or for local use, is widespread in dental practice. A non-judicious use of broad-spectrum antibiotics has led to the development of antimicrobial resistance in a wide variety of microorganisms. Its consequence is the ineffectiveness of commonly prescribed antimicrobial agents. The need for natural antimicrobial agents is on the rise. Natural antimicrobial agents have various bioactive compounds with antimicrobial properties. This review paper describes the impact of various synthetic and natural antimicrobial agents on the oral microbiome. It lays out basic framework of treating oral microbial dysbiosis.

1. INTRODUCTION

Human oral cavity consists of hard and soft structures including teeth, gingiva, tongue, hard palate, soft palate, and oral mucosa, major and minor salivary glands. The presence of salivary glands keeps the oral cavity moistened, which is ideal for harboring different microbial community, e.g., bacteria, fungi, protozoa which in terms causes various infectious diseases of the oral structures[1]. The normal flora in the oral cavity provides the defense to the host and prevents colonization of external invaders. When a disturbance occurs in the normal flora of the oral cavity, the oral environment alters and makes it an ideal ambience for the growth and proliferation of the pathogenic microorganisms. Such dysbiosis can cause dental caries, periodontitis, candidiasis and various other oral diseases[2].

Dental caries is a slowly progressive chronic disease characterized by destruction of the enamel surface of the tooth following dentin and pulp tissue by bacterial acid production. *Streptococcus mutans* is believed to be the principal microorganism related to dental caries among all the pathogenic microorganisms present in the oral cavity proper[3]. The tooth decay can occur only if *Streptococcus*

mutans population exceeds 50% of the total bacterial population in the oral cavity. *Streptococcus mutans* produces sticky glucan polymers from sucrose via glucosyl transferase. This glucan helps the bacterium to clinge to the tooth surfaces. Fermentation of fructose produces lactic acid which dissolves tooth enamel, eventually degradation of exposed dentin and pulp tissue occurs[4].

Periodontal disease is called for inflamed structures surrounding the teeth, persistent condition can cause tissue damage. Chronic irritation and bleeding from the gingiva surrounding the tooth is called gingivitis that roots as dental plaque accumulation in the supragingival and subgingival spaces. Eventually, these spaces get blocked, making the environment suitable for the growth of anaerobic bacteria like *Porphyromonas*, *Prevotella* and *Actinomyces*[5]. These bacteria secrete proteases, lipopolysaccharides which degrade the peptides, proteins, amino acids and other nitrogenous compounds present in the subgingival crevice. This biological event induces inflammation of the periodontal tissues[6].

Mechanical control of dental plaque and adjunctive use of antimicrobial agents is essential to maintain microbial homeostasis in the oral cavity, to help in prevention of dental caries and periodontal diseases. Antimicrobial agents and anti-plaque compounds play a distinctive role in mechanical control of dental plaque[7]. However, inappropriate dosage and consumption of antimicrobial agents lead to the development of antibiotic-resistant microbial strains. Regular and non-judicial use of antibiotics in dental infection increases the occurrence of multidrug-resistant pathogens[8].

The aim of this review is to briefly explain the most common synthetic antimicrobial agents such as systemic antibiotics and mouthwashes used in the treatment of oral and dental diseases, emergence of microbial resistance to these synthetic antimicrobial agents and an increasing interest for natural antimicrobial compounds to mitigate the need of traditional antimicrobial agents[9].

Control of dental plaque using conventional antimicrobial agents

The oral cavity is a complex hub of microbial colonies. It constitutes both Gram-positive and Gram-negative aerobes and anaerobes. Some are facultative anaerobes and others are obligate anaerobes. In a microbial culture of dental plaque sample, it contains a varied collection of bacterial species, approximately from 12 to 27 types[10]. Diagram 1 classifies the various microbiota related to dental plaque.

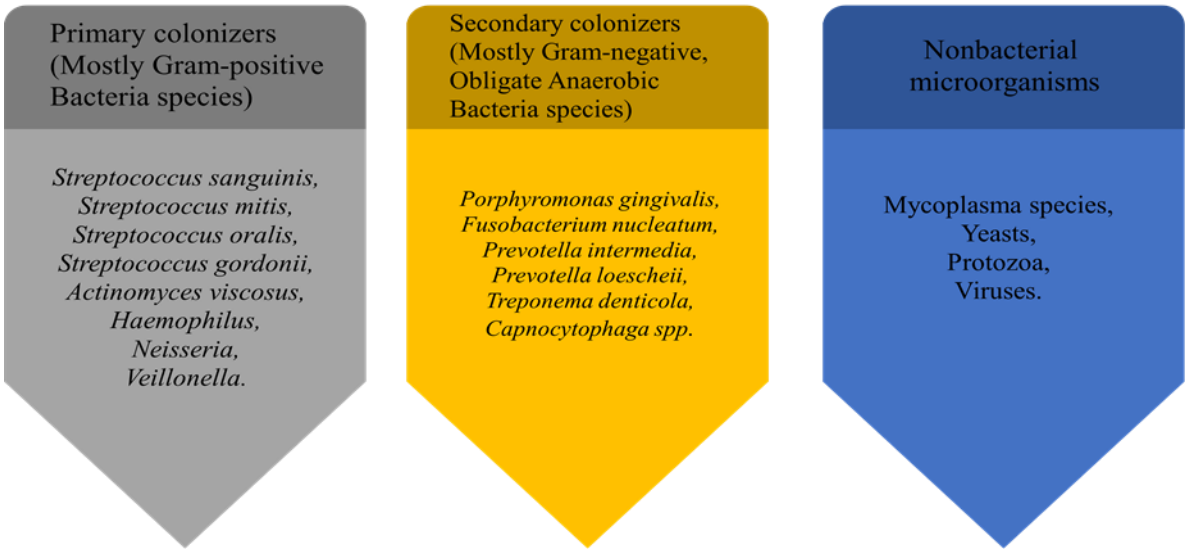


Diagram 1: Various microbiota related to dental plaque[2]

A significant change in the oral environment like change in the pH of the oral cavity tends to microbial shifting resulting in the various oral diseases such as dental caries, gingivitis, periodontitis etc.[2] Diagram 2 depicts the oral diseases resulting from the alteration in the oral environment and the pathogenic microorganisms associated with it.

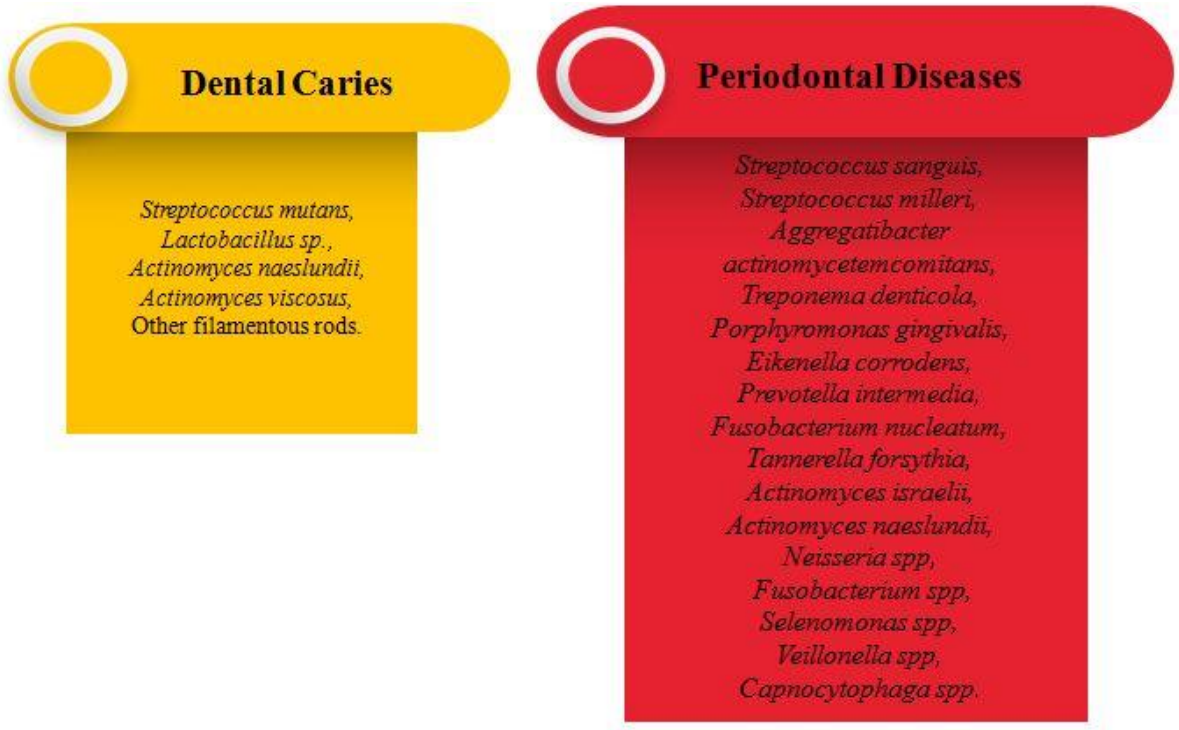


Diagram 2: Pathogenic flora associated with dental caries and periodontal disease

Different antimicrobial agents affect microorganisms residing in dental plaque. As an adjunct to surgical and non-surgical therapy of oral diseases, the effectiveness of antimicrobial agents depends on their potency against pathogenic microflora.[7] Prophylactic use of antimicrobial agents are necessary when there is an ultimate need for prevention of local infection from systemic spreading in patients undergoing oral and dental surgery, where bleeding is inevitable[11]. As antimicrobial agents are adjunctive to dental treatments, odontogenic and non-odontogenic acute or, chronic infections are treated with antibiotics[12]. Immunocompromised patients, patients with congenital heart disease, artificial heart valves, at potential risk of developing infective endocarditis, such systemic infection may rapidly progress with the use of antimicrobial agents as prophylaxis[7].

Antimicrobial agents prescribed for the prevention of oral and dental diseases

A) Systemic Antibiotics

a) Penicillin

Penicillin is a beta-lactam antibiotic used for the treatment of wide variety of oral and dental diseases. It binds irreversibly to the penicillin-binding proteins, thus inhibiting the peptidoglycan layer of the bacterial cell wall synthesis[13].Penicillin is effective against Gram-positive bacteria such as *Streptococci and Staphylococci* and some Gram-negative bacteria[14]. Penicillin-V was used as a prophylactic agent for the prevention and treatment of dental caries[4]. For better efficacy, penicillin can be administered parenterally (I.V. or, I.M.) from 1.2 million international unit (IU) per day up to 24 million international unit (IU) per day[7]. With the extensive use of penicillin, there are some side effects reported. A major drawback is beta-lactam resistance bacteria. Other side effects are nausea,

diarrhea, rash, urticaria, hypersensitivity and neurotoxicity[15]. Numerous studies have reported the resistance to penicillin by oral pathogens. Resistant bacteria produce *mecA* gene which deciphers penicillin-binding protein 2a (PBP2a). It has low binding affinity to beta lactams[16]. The most dominant penicillin-resistant oral pathogens are: *Streptococcus* species, e.g., *Streptococcus oralis, Streptococcus constellatus, Prevotella* species, *e.g., Prevotella intermedia, Prevotella nigrescens, Prevotella melaninogenica*[17].Other resistant pathogens are: *Actinomyces naeslundii, Fusobacterium nucleatum, Eubacterium saburreum, Tannerella forsythia, Bacteroides, Veillonella, Haemophilus, Eikenella, Capnocytophaga,* and *Neisseria sp*[4].

b) Amoxicillin

Because of penicillin hypersensitivity, amoxicillin has gained popularity as an antibiotic of choice for the treatment of oral diseases, specifically for the treatment of periodontal diseases[11]. Amoxicillin can be used alone but the combination of amoxicillin with clavulanic acid is proven to be more effective against anaerobic bacteria in the subgingival crevice[18]. When amoxicillin is administered alone, it is given in the form of 500 mg. capsule 8 hourly or, 1,000 mg. capsule 12 hourly, orally for 5-7 days[7]. When the combination of amoxicillin and clavulanic acid is to be given, a good efficacy can be obtained from 500 mg. to 875 mg. tablet every 8 hourly or, 2,000 mg. tablet every 12 hourly, orally for 5-7 days[19]. According to a study, the minimum inhibitory concentration of amoxicillin is 14.05 µg/ml where the minimum inhibitory concentration of clavulanic acid is 0.40 µg/ml in gingival crevicular fluid observed[20]. Reported side effects are nausea, vomiting, diarrhea, rash, oral thrush, allergic reaction, abdominal pain, yellowish eyes etc[21]. Researches show a resistance of microorganisms to amoxicillin, when enzymatic hydrolysis of the beta-lactam ring takes place[22].

c) Metronidazole

Metronidazole is a nitroimidazole group of antibiotic and is effective against anaerobic organisms such as *Prevotella, Porphyromonas, Fusobacterium, Clostridium, Bacteroides,* and *Peptostreptococcus*[23].It inhibits the nucleic acid synthesis, thus leading to cell death. For oral administration, metronidazole tablet can be taken alone in the form of 500 mg. to 750 mg. tablet 8 hourly[7] or, can be combined with amoxicillin in the form of 250 mg. tablet, each 8 hourly for 8 days or, can be combined with ciprofloxacin in the form of 500 mg. tablet, each 12 hourly for 8 days[11]. For subgingival application, metronidazole gel is used. Adjunct with scaling and root planning, metronidazole gel remains in the crevicular fluid up to 12 hours and after 24 hours the minimum inhibitory concentration (MIC) of metronidazole gel remains almost the same for killing half the count of the major periodontal organisms[24].Various studies have failed to indicate whether metronidazole, tetracycline, or, combination of metronidazole and amoxicillin can be used as a sole therapy or they can be used as an adjunct to mechanical debridement because of the lack of data to validate appropriate dosage and duration of these antibiotics to be used in periodontitis[25].Reported side effects are metallic taste, nausea, loss of appetite, tachycardia, shortness of breath, headaches, flushing of the skin[15]. There were various studies in which bacterial resistance to metronidazole was reported though it is a widely used, antibiotic agent. The mechanisms for resistance are I) Lessened intracellular reduction; and, ii) Slower uptake of the metronidazole. Four genes namely *nimA*, *nimB*, *nimC* and *nimD* can give out to metronidazole resistance[22]. Most predominant metronidazole-resistant oral pathogens are *Aggregatibacter actinomycetemcomitans, Streptococcus mitis, Streptococcus sanguinis, Streptococcus oralis, Streptococcus constellatus, Actinomyces naeslundii, Actinomyces odontolyticus, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter rectus* and

Parvimonas micra[4].

d) Tetracycline

Tetracycline is a broad-spectrum, a bacteriostatic agent that affects both Gram-positive and Gram-negative aerobic and anaerobic species of bacteria. It inhibits bacterial protein synthesis, thus leading to cell death[26]. Tetracycline is used in treating refractory forms of periodontal diseases. The term "refractory" is designated to all forms of destructive periodontal disease which are non-responsive to treatment. It includes localized aggressive periodontitis and refractory chronic periodontitis[27]. Unlike metronidazole or chlorhexidine, tetracycline needs longer exposure time for the initiation of action[28]. For subgingival application tetracycline is found in various systems like powder, gel, irrigating solution, incorporated in non-resorbable fibers. But studies showed that the non-resorbable fibers can maintain their concentration maximum up to 7 days in adjunct with scaling and root planing[29]. Reported side effects are headache, diarrhea, sore mouth or tongue, stomach cramps, vision problems, skin photosensitivity, kidney damage etc[15]. However, the use of tetracycline in pregnant woman causes tooth discoloration of the children during the formation of teeth[4]. Tetracycline resistance in periodontal pathogens was seen in various studies conducted. The mechanisms of resistance demonstrated by bacteria are: i) Bacteria limit the permeability of tetracycline to the ribosomes, thus prevent the drug from reaching the target site; ii) Bacteria prevent binding of tetracycline effectively by altering the ribosome; and, iii) Bacteria produce specific enzymes that inactivate the effectiveness of tetracycline[22]. Vicious tetracycline-resistant species are *Prevotella intermedia*, *Veillonela parvula*, *Parvimonas micra* and *Aggregatibacter actinomycetemcomitans*. Tetracycline resistance has also been examined at the genetic level. The most common *tet* gene identified was *tet*(M), *tet*(W), *tet*(0) and *tet*(Q) which are responsible for tetracycline resistance in oral pathogens[30]. The *tet*(M) gene was present in *Streptococcus intermedius, Streptococcus oralis, Streptococcus sanguinis, Actinomyces* spp., *Bifidobacterium* spp. and *Veillonella* spp. The *tet*(W) gene was present in *Streptococcus, Staphylococcus, Lactobacillus, Prevotella, Veillonella,* and *Neisseria*[4].

e) Azithromycin

Azithromycin belongs to azalides and is a semi-synthetic antibiotic. It is bacteriostatic in nature and has greater efficacy against aerobic and facultative Gram-positive microorganisms, e.g., *Staphylococcus Aureus, Streptococcus Pyogenes*, aerobic and facultative Gram-negative microorganisms, anaerobic microorganisms[30]. It inhibits synthesis of protein by affecting the function of 50S bacterial ribosomal subunits[4]. For better efficacy, azithromycin can be given in the form of 500 mg. tablet, once daily for 3 to 5 days[7]. Reported side effects are nausea, vomiting, abdominal pain, diarrhea, nervousness, skin reactions, anaphylaxis, cholestatic hepatitis, QT prolongation etc[15]. Reported bacterial resistance to azithromycin is induced by target modification or, inactivation by phosphorylase enzymes or, by efflux pumps[22].

f) Clindamycin

Clindamycin is derived from lincomycin and is a semi-synthetic antibiotic. It is bacteriostatic in nature but in higher dosage, it can be bactericidal[31]. Clindamycin is effective against Gram-positive aerobic cocci, e.g., *Staphylococci, Streptococci* and Gram-negative anaerobic bacteria, e.g., *Bacteroides, Fusobacterium, and Prevotella* which are the main organisms of periodontal diseases. It acts by the disruption of ribosomal translocation and inhibition of bacterial protein synthesis[32]. It is an antibiotic of choice for the patients who are allergic to penicillin and where resistance to penicillin is most likely to occur[4]. Refractory periodontitis and rapidly progressing periodontitis can be treated effectively with the adequate dosage form of Clindamycin[27]. For better efficacy, clindamycin can be

given in the form of 300 mg. capsule, 8 hourly for 8 days[11]. Reported side effects are nausea, vomiting, diarrhea, cramping abdominal pain, pseudo-membranous colitis and contact dermatitis[15]. Like other macrolides, reported bacterial resistance to clindamycin is induced by target modification either by methylation or, by mutation of the 23S ribosomal RNA[29].

g) Ciprofloxacin

Ciprofloxacin is a fluoroquinolones group of broad-spectrum antibiotic and is effective against facultative and aerobic Gram negative rods and cocci and a wide range of both Gram-positive and Gram-negative microbiota[33]. It acts on DNA gyrase enzyme, so, the reproduction of bacteria is inhibited. It also inhibits topoisomerase-4 enzyme of microorganisms[29]. For better efficacy, ciprofloxacin can be given in the dosage form of 500 mg. tablet, 12 hourly up to 8 days[11]. According to various clinical studies, ciprofloxacin is highly effective against *Actinobacillus actinomycetemcomitans* which is the notorious microorganism related to aggressive adult periodontitis because it directly got absorbed in the gingival crevicular fluid and periodontal tissue[34]. The mean concentration level of ciprofloxacin in gingival crevicular fluid was observed from 2.5 µg/ml up to 2.7 µg/ml[35]. Reported bacterial resistance to ciprofloxacin is induced by mutation in the gyr A/gyrB subunits of DNA-gyrase and topoisomerase-4, thus alteration of these target enzymes occur[36]. Another mutation occurs in outer-membrane porins which causes reduction in uptake of active drug molecule[37].

h) Minocycline

Minocycline (belongs to tetracycline group of antibiotics) is bacteriostatic in nature. The mode of action of minocycline is inhibition of bacterial protein synthesis by attaching to the 30S ribosomal subunit of bacteria[29]. Best effective dosage form is 100 to 200 mg. capsule, 6 hourly for 21 days[11]. Studies showed that, use of minocycline along with root planning can reduce the number of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans*, spirochetes and motile organisms which are proven to be the periodontal pathogen that causes chronic periodontitis in adults[38]. 2% minocycline ointment has antibacterial activity up to 21 hours when used in subgingival pockets[39].

i) Doxycycline

Doxycycline is a broad spectrum, bacteriostatic agent against bacteria and parasites. The mode of action is inhibition of bacterial protein synthesis, thus leading to cell death[29]. Best effective dosage form is 100 to 200 mg. capsule, 6 hourly for 21 days[11]. Subgingival application of doxycycline polymer in adjunct with root planning showed better improvements in periodontal treatments[40]. However, mechanical debridement could be incorporated alone for better result[41]. Care should be taken during use of doxycycline because of the chance of antibiotic resistance[22].

j) Cephalexin

Cephalexin is from cephalosporin's group of antibiotics and is commonly used to treat periodontal diseases. Cephalexin is effective against Gram-positive oral microorganisms and Gram-negative obligate anaerobes such as *Porphyromonas Gingivalis*, *Prevotella Intermedia*, *Fusobacterium* but has minimum effectiveness against Gram-negative facultative anaerobes[29].

B) Synthetic Mouthwashes/Rinses

a) Chlorhexidine mouth rinse

Chlorhexidine is effective against Gram-positive and Gram-negative bacteria. Also effective against a few varieties of lipophilic viruses, dermatophytes and yeasts[42]. Positively charged chlorhexidine binds

to the negatively charged cell surface of bacteria. It alters the cell membrane integrity of the bacterial cell surface, thus causing leakage of cellular components of bacteria at a low dosage and at instances, severe cell membrane damage in high dosage[43]. Studies showed that various concentrations of chlorhexidine in the form of gel or irrigation in combination with scaling and root planning can cause minor shifting in the subgingival microflora when used for two months consecutively but the effects are temporary and limited[44]. Use of chlorhexidine in conjunction with mechanical debridement within 24 hours is effective because it prevents displacement of bacterial foci from treated to untreated pockets[45]. Inappropriate concentration may decrease its efficacy and sudden decrease in therapeutic concentration occurs due to its high affinity to bind with serum proteins and blood[46]. Some bacteria like *Porphyromonas gingivalis* and other bacteria protect themselves from the bactericidal activity of chlorhexidine by releasing vesicles that bind to and inactivate its activity, thus making them moderately susceptible to chlorhexidine[47]. 2% chlorhexidine mouthwash, three times within 10 minutes, it shows 99% reduction in periodontal pathogens[48]. Chlorhexidine should be used for a limited period only because of various side effects reported in the long term use such as tooth discoloration; slight alteration of taste sensation, delayed wound healing or gingival desquamation if used for a long time[49]. DNA damage in oral mucosal cells, kidney cells and leukocytes may be induced by prolonging use of chlorhexidine[4].

b) Povidone-iodine mouth rinse

Povidone-iodine is effective against Gram-positive bacteria and black-pigmented Gram-negative anaerobic rods. It is also effective against fungi, mycobacteria, viruses and protozoa[50]. It oxidizes hydroxyphenol, thiol and amino groups in nucleotides and amino acids of microbial organelles by penetrating the microbial cell wall and membrane[51]. Studies showed that subgingival pocket irrigation with 0.05% more effective in its antimicrobial effect than 0.2% chlorhexidine mouth rinse solution[52]. Even it is effective against Gram-negative anaerobic rods, spirochetes and motile organisms up to 26 weeks[53]. 10% povidone-iodine solution is much effective as antimicrobial product for the solution of various periodontal problems[54]. Fewer side effects are reported like temporary staining of teeth and tongue and possibilities of thyroid dysfunction[55].

c) Hydrogen peroxide mouth rinse

Hydrogen peroxide mouthwash is effective against both Gram-positive and Gram-negative bacteria e.g., *Streptococcus mutans, Streptococcus salivarius, Actinomyces naeslundii, Actinomyces viscosus, Aggregatibacter actinomycetemcomitans, Haemophilus aphrophilus, Capnocytophaga gingivalis, Mycoplasma salivarium* and *Eikenella corrodens*[43]. Hydrogen peroxide damages cells and delays cell division of the oral pathogens. It releases free oxygen radicals that create a lethal environment for anaerobic bacteria to endure[56]. It is helpful in the treatment of periodontal disease as an adjunct to mechanical debridement of subgingival pockets[57]. Studies suggested that two weeks of subgingival debridement along with an application of 3% hydrogen peroxide can temporarily suppress *Aggregatibacter actinomycetemcomitans* which is one of the main pathogens for the initiation of localized aggressive periodontitis in juveniles and adolescents in comparison to normal saline irrigation subgingivally after root planing[58].

d) Stannous fluoride mouth rinse

According to studies, the effect of stannous fluoride in 0.25% concentration in adjunct with 0.25% americium fluoride subgingival irrigation can temporarily suppress black-pigmented bacteria in comparison to normal saline mouth rinse[59]. 1.64% stannous fluoride mouth rinse can reduce black-pigmented species and spirochetes count a slightly better than normal saline mouth rinse[60].

e) Chlorine dioxide mouth rinse

Chlorine dioxide mouth rinse has a bactericidal effect against oral microflora when it is stabilized[61]. Studies showed that chlorine dioxide oral rinse kills *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Actinomyces odontolyticus*, *Prevotella nigrescens*, *Actinomyces naeslundii*, *Peptostreptococcus micros*, *Streptococcus mutans* and *Actinomyces viscosus*oral bacteria associated with the development and/or progression of oral diseases up to 99% in 10 seconds[62]. In vitro studies showed that unlike chlorhexidine, it is not toxic to the human gingival cells[63].

Antimicrobial resistance (AMR) and growing need for natural antimicrobial agents

From the perspective of recent studies, it has come to light that oral and dental diseases, e.g., dental caries, gingivitis, periodontitis, dento-alveolar abscesses etc. occur when there is a transition of normal flora to pathogenic microbes[11]. It is found that approximately 700 bacterial species can be distinguished in oral microbiota. It has been a difficult task to completely remove oral biofilms despite mechanical interventions, e.g., scaling and root planing[10]. Other than this, microorganisms residing in oral biofilms are 1000 times more resistant to common antimicrobial agents, e.g., amoxicillin, cephalosporin's or chlorhexidine[50]. Resistance to erythromycin, tetracycline, beta-lactamase penicillin is a rising threat for the administration of conventional antibiotics for the treatment of oral and dental diseases as the oral biofilm is a foci of complex microbial colonies[7].

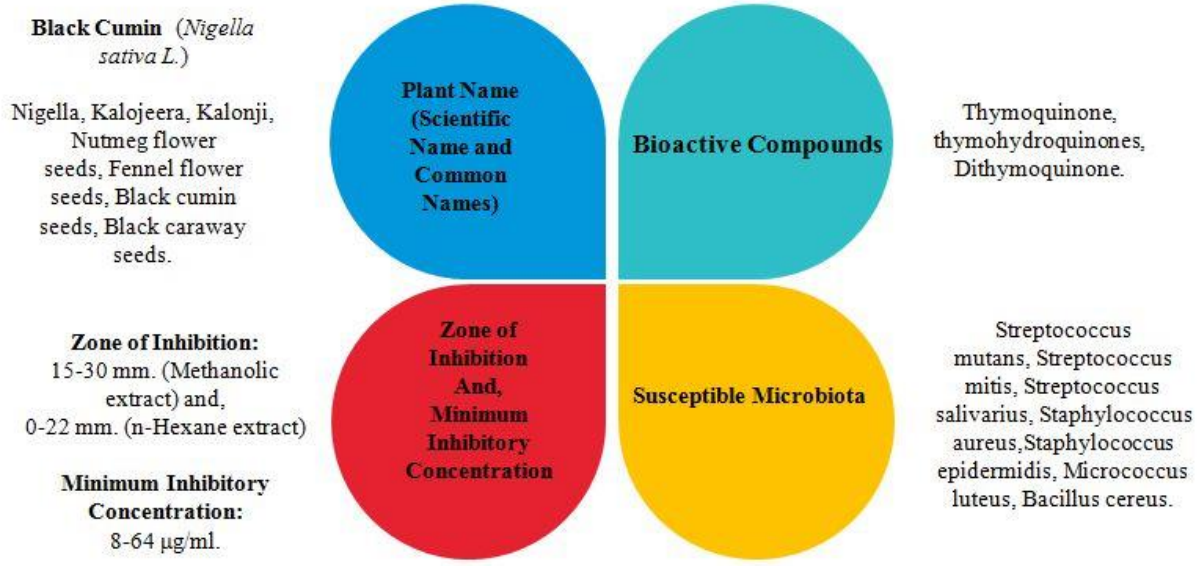
With the increasing risk of development of antimicrobial resistance and ineffectiveness of the conventional antimicrobial agents against oral biofilm mediated oral and dental diseases, the rising demand for natural antimicrobial agents has flourished[64]. Natural antimicrobial agents contain several bioactive compounds which are proven to be effective against pathogenic oral microorganisms[65].

Use of natural antimicrobial compounds for the prevention of oral and dental diseases

A) Natural antimicrobial agents

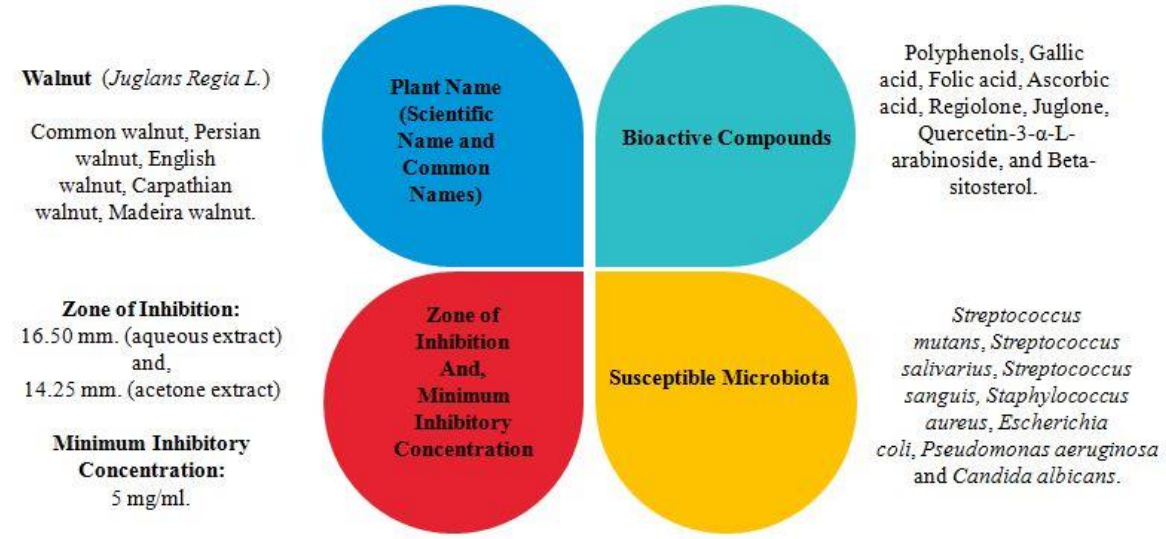
a) Black Cumin (*Nigella sativa L.*) Seed

Black cumin (*Nigella sativa L.*), commonly known as nigella, kalojeera, kalonji is an annual herbaceous plant growing in the Mediterranean Sea sided countries, several African countries, South Asia, and West Asia. Thymoquinone is the bioactive compound found in the volatile oil portion of the seed of the black cumin[66]. Recent *In vitro* study where 16 cariogenic bacteria strain isolated from pediatric patient suffering from dental caries and 11 reference strain were used, showed that thymoquinone is active against *Streptococcus mutans*, *Streptococcus salivarius*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus cereus* species of microorganisms with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 8 to 64 µg/ml consequently in comparison with tetracycline and benzalkonium[67]. In another *in vitro* study, the n-Hexane and methanolic extract of black cumin seeds exhibited 0 to 22 mm. and 15 to 30 mm. diameter of zone of inhibition respectively against *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus mitis*[68]. The mode of action of thymoquinone is "Efflux Pump Inhibition (EPI)" in which bioactive compounds of natural products inhibits the activity of efflux pumps present in the bacterial membranes which eventually limit the removal of intracellular toxic compounds for the microorganisms like antibiotics, antimicrobial peptides, metals and detergents[69].



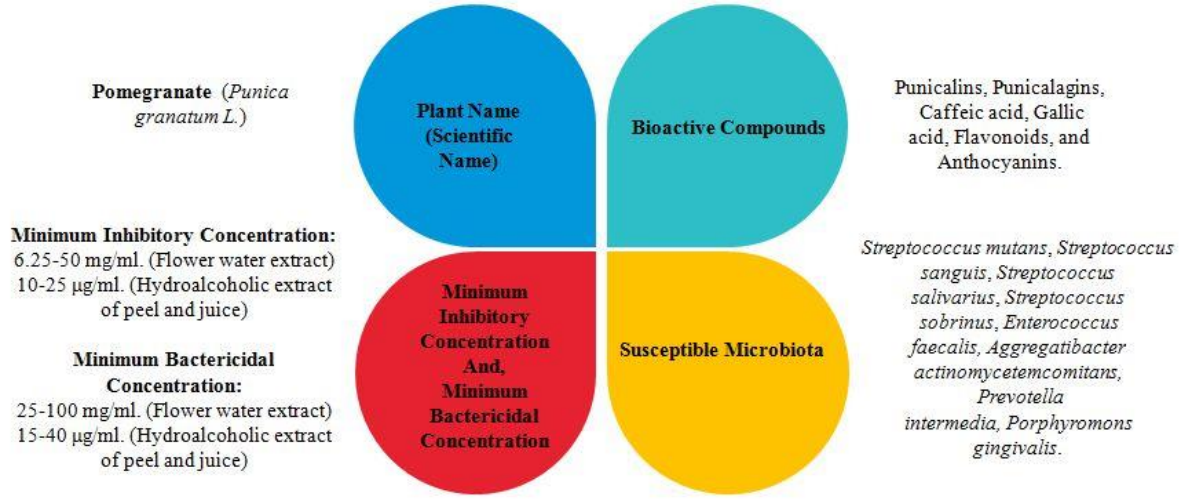
b) Walnut (Juglans Regia L.) Bark

The bark of the green walnut (*Juglans Regia L.*) contains polyphenols, gallic acid, folic acid, ascorbic acid, regiolone, juglone, and beta-sitosterol, which have broad-spectrum antimicrobial activity against *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. *In vitro* study showed that the ethanolic extract of *Juglans Regia L.* is effective against *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, and *Staphylococcus aureus* with minimum inhibitory concentration (MIC) of 5 mg/ml, 2.50 mg/ml, 1.25 mg/ml and 2 mg/ml respectively and it is better than that of the effect of aqueous extract in comparison to erythromycin and tetracycline[70]. In a recent *in vitro* study where saliva samples of fifty carious patients were collected, showed an average of 16.50 mm. diameter of zone of inhibition in aqueous extract, where an average of 14.25 mm. diameter of zone of inhibition in acetone extract against salivary microorganisms when the maximum concentration of *Juglans Regia* used was 300 µg[71].



c) Pomegranate (*Punica granatum L.*)

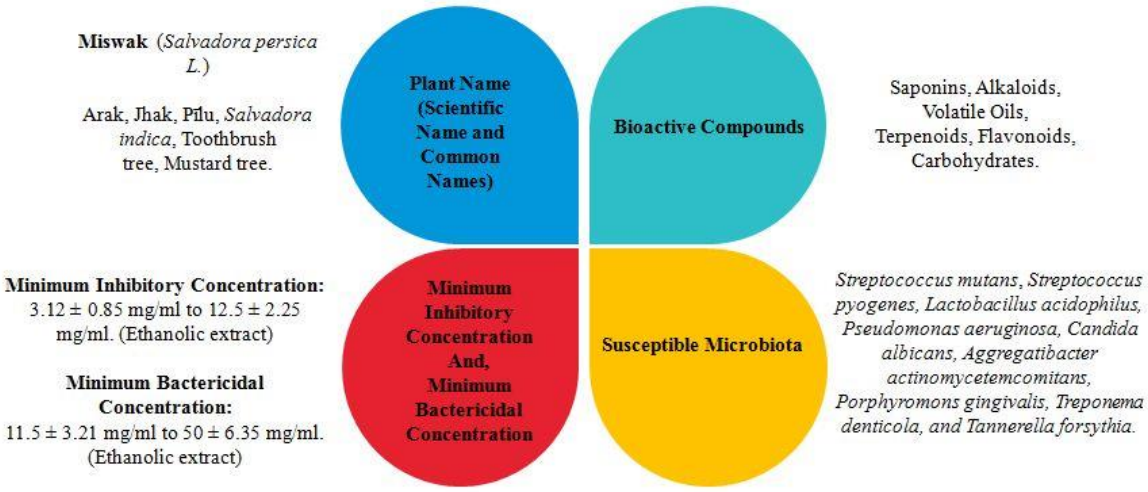
The medicinal parts of the pomegranate (*Punica granatum L.*) plant are its flowers, fruits, seeds, bark, and roots. The pomegranate contains various bioactive compounds which are antibacterial in nature and shows effective antioxidant activity[72]. The bioactive compounds of pomegranate fruit are punicalins and punicalagins which are derivatives of tannin[73]. Other significant bioactive compounds include gallic acid, flavonoids and anthocyanins.[74] Pomegranate's bioactive compound tannin increases bacteriolysis and interferes with bacterial adherence mechanisms onto the tooth surfaces. Thus inhibits the formation of biofilm on tooth surfaces and curtails the progression of oral and dental diseases.[75] According to an *in vitro* study, 15% flower water extract of *Punica granatum L.* is effective against *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus sobrinus*, and *Enterococcus faecalis* with minimum inhibitory concentration (MIC) of 50 mg/ml, 6.25 mg/ml, 25 mg/ml, 25 mg/ml, and 50 mg/ml, respectively and minimum bactericidal concentration (MBC) of 50 mg/ml, 25 mg/ml, 100 mg/ml, 25 mg/ml, and 50 mg/ml, respectively[72]. In another recent *in vitro* study, hydroalcoholic extract of pomegranate juice exhibited minimum inhibitory concentration (MIC) of 25 µg/ml and minimum bactericidal concentration (MBC) of 40 µg/ml against *Streptococcus mutans*; while hydroalcoholic extract of pomegranate peel exhibited minimum inhibitory concentration (MIC) of 10 µg/ml and minimum bactericidal concentration (MBC) of 15 µg/ml against *Streptococcus mutans*, respectively[76].



d) Miswak (*Salvadora persica L.*)

The stem of *Salvadora persica* is used for the maintenance of oral hygiene by the people of Asian subcontinent and the Middle East[69]. The root of the plant is also used by African people[77]. In vitro study revealed that, ethanolic extract of *Salvadora persica* is effective against periodontal pathogens, e.g., *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola, and Tannerella forsythia* with minimum inhibitory concentration (MIC) ranging from 3.12 mg/ml to 12.5 mg/ml; while minimum bactericidal concentration (MBC) ranging from 11.5 mg/ml to 50 mg/ml[78]. The ethanolic extract of *Salvadora persica* had also shown a zone of inhibition of 10 mm. diameter in case of *Aggregatibacter actinomycetemcomitans*, 14 mm. diameter in case of *Treponema denticola*, 15 mm. diameter in case of *Porphyromonas gingivalis* and 19 mm. diameter in case of *Tannerella forsythia*[78]. In another recent *in vivo* study, the aqueous extract of *Salvadora persica* was proven to be effective against *Streptococcus mutans, Lactobacillus acidophilus*, *Staphylococcus aureus*,

Escherichia coli, and *Pseudomonas aeruginosa* than that of hexane and ethanolic extract of *Salvadora persica*[79].



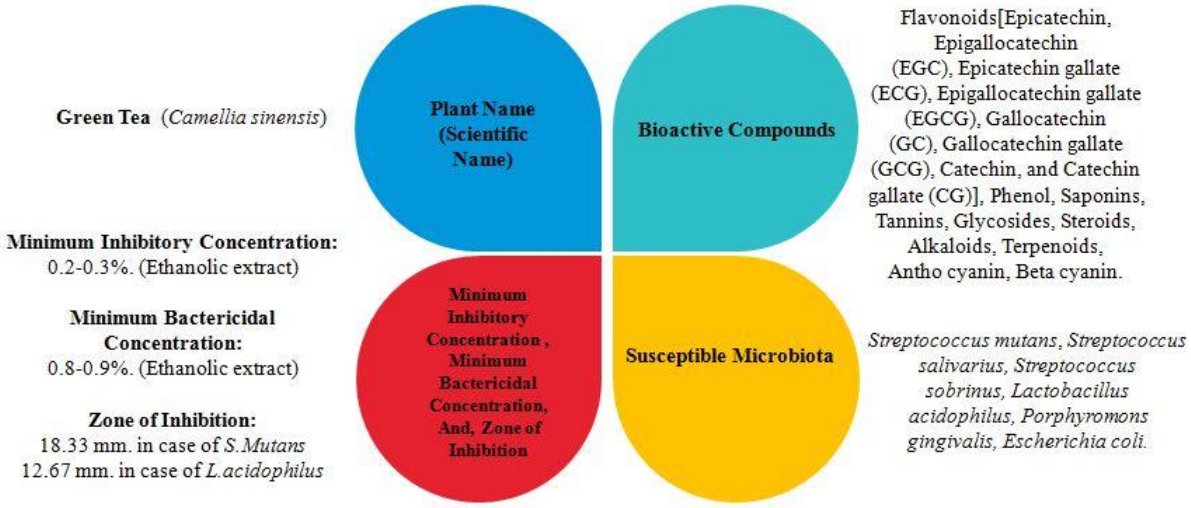
e) Nisin

Nisin is derived from *Streptococcus* and *Lactococcus* species of Gram-positive bacteria and it is an antimicrobial peptide. *Lactococcus lactis* produces 'nisin-A' which has 34 amino acids and is called 'Type-A lantibiotics'[80]. The mode of action of nisin is inhibition of co-aggregation of oral microorganisms. It is shown in various studies that nisin is effective against a wide variety of oral microorganisms such as Gram-positive oral bacteria, e.g., *Streptococcus sanguinis*, *Streptococcus sobrinus*, *Streptococcus gordonii*, Gram-positive opportunistic bacteria, e.g., *Enterococcus faecalis*, Gram-negative oral bacteria, e.g., *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola* and opportunistic yeast, e.g., *Candida albicans*. Nisin and lantibiotics are considered as natural antimicrobial agents as they have potential broad-spectrum effect on complex oral microorganisms and till date, no antimicrobial resistance is reported[81].

B) Natural Mouthwashes/Rinses

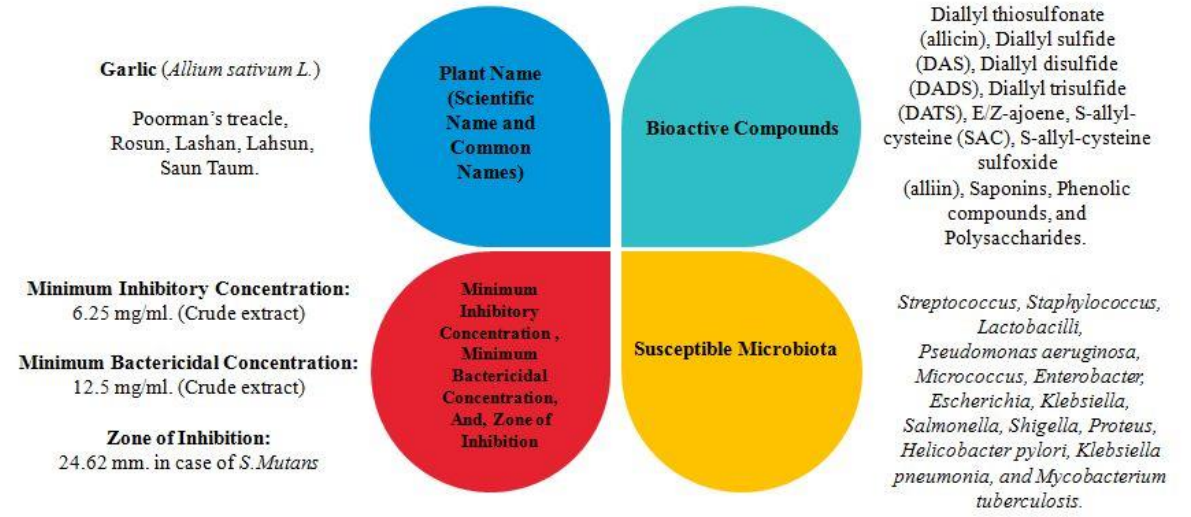
a) Green Tea (*Camellia sinensis*) mouth rinse

Green tea contains polyphenols especially catechins which are the active ingredient for combating the proliferation of bacterial growth. The modes of action of green tea are they interfere with the attachment of the bacterial foci to the enamel surface of the tooth; they act as inhibitors of glucosyl transferase and amylase enzyme which causes caries formation[82]. In an *in vitro* study, ethanolic extract of *camellia sinensis* has shown a mean value of 18.33 mm. diameter of zone of inhibition against *Streptococcus mutans* and a mean value of 12.67 mm. diameter of zone of inhibition against *Lactobacillus acidophilus*. In the same study, the minimum inhibitory concentration (MIC) of 300 µg of ethanolic extract of *camellia sinensis* against *Streptococcus mutans* was 0.2% and against *Lactobacillus acidophilus* was 0.3%; whereas, the minimum bactericidal concentration (MBC) of 300 µg of ethanolic extract of *camellia sinensis* against *Streptococcus mutans* was 0.8% and against *Lactobacillus acidophilus* was 0.9% [83].



b) Garlic *(Allium sativum L.)* mouth rinse

Garlic is a spice commonly found in Central and Southwestern Asia, Northeastern Iran and Middle East. The medicinal part of the garlic is its clove and active ingredients of garlic are allicin and thiosulfinates which has antimicrobial effects against various Gram-positive and Gram-negative bacteria such as *Streptococcus*, *Lactobacilli*, *Staphylococcus*, *Micrococcus* and other species[84]. In an *in vitro* study, 40% concentrations of hydro-alcoholic garlic extract at 60 seconds showed significant reduction of salivary microorganisms mean colony count (941.52) by inhibiting the microbial DNA and protein synthesis partially and complete inhibition of microbial RNA synthesis than that of the 70% concentration of hydro-alcoholic garlic extract at 30 seconds (771.72) [85]. In another *in vitro* study, the crude extract of garlic showed 24.62 mm. diameter of zone of inhibition than that of aqueous and ethanolic extracts with the minimum inhibitory concentration (MIC) of 6.25 mg/ml and the minimum bactericidal concentration (MBC) of 12.5 mg/ml against *Streptococcus mutans*[86].



c) Chitosan mouthwash

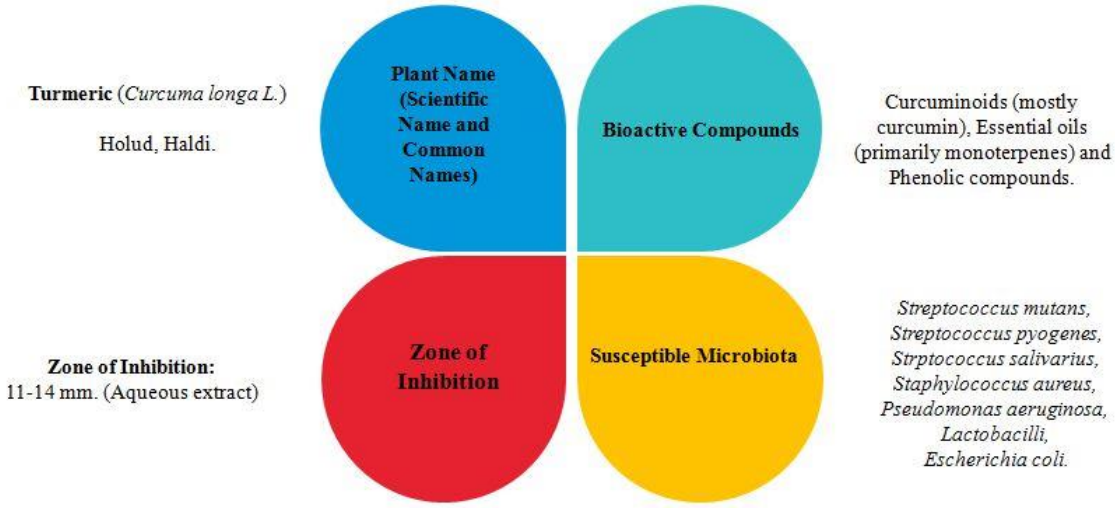
Chitosan is a natural polysaccharide found mainly as a structural unit of the shells of crustaceans, arthropods and fungal cell wall[87]. It is cationic in character and in the presence of low pH, it can interact with the negatively charged bacterial cell surface[88]. Because of its antimicrobial characteristic, in vitro studies showed its efficacy against *Streptococcus mutans*, *Lactobacillus acidophilus, Enterococcus faecium, Prevotella intermedia and Candida albicans* in compared to Chlorhexidine mouth rinse and essential oils mouth rinses[89]. As it is a linear polymer, destabilization of most of the formulation may occur in terms[90].

d) Propolis mouthwash

Propolis is a natural substance acquired from beehives and contains wax, pollen, resin, essential oils, balsams, amino acids, minerals and vitamins[91]. The bio-active compound responsible for its antimicrobial effect is bio-flavonoid, aromatic compounds known as caffeic acid phenethyl ester and phenols[92]. 5% concentration of Brazilian green propolis as an alcohol-free mouth wash has shown significant antibacterial activity against *Streptococcus mutans, Streptococcus sorbinus, Lactobacillus acidophilus, Prevotella oralis*, and *Porphyromonas gingivalis*[55]. Due to its bioactive compound namely caffeic acid phenethyl ester, it is also an effective antifungal agent against *Candida albicans* and an antiviral agent against *Avian influenza virus*[93].

e) Turmeric (*Curcuma longa L.*) mouthwash

Turmeric is a spice and also used for medicinal purpose. The bio-active compound of turmeric is *curcumin* which is a flavonoid[94]. In a randomized controlled trial of sixty individuals, the experiment showed that 0.1% turmeric mouthwash when compared with 0.2% chlorhexidine mouth rinse, was proven to be effective against a wide range of oral microorganisms by reducing plaque index, gingival index and gingival bleeding index[95]. Reported side effects are nausea, gastric irritation, diarrhea, stomach upset and allergic skin reaction and undesired yellowish coloration of the tooth[96].



Conclusion

As the oral cavity lodges normal flora and a microbial synergism between beneficial microflora and pathogenic microorganisms exist, it is crucial to maintain a balance by reducing the number of pathogenic microbes in the oral cavity. Because of the widespread ineffectiveness of the synthetic antimicrobial agents, there is an increasing demand for natural antimicrobials. Natural derived products yield varieties of biochemical compounds which have antimicrobial, antioxidant, anti-

carcinogenic effects. Therefore, some biochemical compounds yet to be experimented for their beneficial or, deleterious effect on the host. Some bioactive compounds of natural products are less effective than that of the synthetic antimicrobials but could be of more effective use if an exact antimicrobial concentration achieved. It can be said that oral microorganisms could develop resistance on natural bioactive compounds in line with synthetic antimicrobial agents. However, experiments should also be carried out to ascertain the target specificity of the natural bioactive compounds, their safety indexes to the host, acceptance of natural products to the patients, and cost effectiveness. Further studies needed to explore the natural products which may have a lucrative impact that can decrease the assertion to use traditional antimicrobial therapeutic agents.

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