The use of plants against oral pathogens

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Dental caries and periodontal diseases in humans have an astonishing impact on the health and welfare of communities. Sick leave, due to oral infections, and the consequent cost of dental treatment results in costing billions of dollars each year [1]. In 2007, the World Health Organization (WHO) stated that 5-10% of public health expenditure was related to dental care. 'Tooth decay and, to a lesser extent periodontal infections, are perhaps the most expensive infections that most individuals have to contend with, during a lifetime' [2].

Natural plant products are surfacing as increasingly popular treatments, even for oral health care. There are over 62 plant species belonging to 29 families documented to treat oral diseases in Burkina Faso, West Africa, alone [3]. Plants and essential oils, such as tea tree oil, are renowned as alternative and sometimes better cures than established drugs. Resistance also develops more slowly with natural products. It is estimated that a quarter of all prescribed medicine in industrialised countries contain one or more components derived from plants [4].

Keywords plants; oral pathogens

1. Introduction

Biofilms (plaque) are formed from the extensive growth of microorganisms, resulting from changes in the oral bacterial ecosystem. Once a biofilm is established it may lead to the formation of dental caries (tooth decay) or even more severe periodontal diseases. Dental caries and periodontal diseases in humans have an astonishing impact on the health and welfare of communities. Sick leave, due to oral infections, and the consequent cost of dental treatment results in costing billions of dollars each year [1]. In 2007, the World Health Organization (WHO) stated that 5-10% of public health expenditure was related to dental care. 'Tooth decay and, to a lesser extent periodontal infections, are perhaps the most expensive infections that most individuals have to contend with, during a lifetime' [2]. Natural plant products are becoming increasingly popular treatments, even for oral health care.

One of the fastest growing sectors in the agribusiness industry is the natural plant products which led to worldwide sales of $23 billion in 2002 alone. In 2008, there were approximately 85,000 medicinally useful plant species; however Africa only contributed 1% to the market even though 75% of the African population still relies on traditional herbal medicine [5].

2. The oral cavity

The oral cavity of humans is a habitat for Gram-positive and Gram-negative bacteria, as well as certain yeasts and fungi, making it one of the most complex microbial habitats in the body. Although saliva contains lysozyme and lactoperoxidase, which are both antibacterial agents, the presence of food particles and shedded epithelial cells, makes the oral cavity a favourable microbial habitat at 37^0C with a neutral pH [6].

In humans, stratified squamous epithelium lines the oral cavity. The tongue is a modification of the squamous epithelium with structures such as teeth and salivary ducts disrupting the continuity. A cuff is formed around each tooth by gingival tissue forming a gingival crevice. A continuous flow of crevicular fluid is released from the gingival crevice. This flow increases during inflammation [1]. Saliva is composed of several functional components which aid in lubrication, enamel remineralisation, digestion, and aggregation and provides oral buffering [7].

Oral commensal bacteria are important as they can regulate the expression of immune mediators, suppress cytokine responses in epithelial cells and prevent colonization by exogenous organisms. *Streptococcus mitis*, *Streptococcus oralis*, *Actinomyces naeslundii*, *Fusobacterium nucleatum*, *Haemophilus parainfluenzae*, *Eikenella corrodens* and certain *Prevotella* species may be oral commensals as they are isolated from healthy but not diseased sites [7].

There are four natural major habitats in the oral cavity, namely the buccal mucosa, dorsum of the tongue, tooth surfaces and crevicular epithelium. Prosthodontic and orthodontic appliances make up a fifth habitat. The diversity of the oral micro flora is intimately linked with the habitats of the oral cavity. Dental plaque is produced by the large masses of bacteria and their products accumulating on the surface of the teeth. There are two main habitats on the tooth's surfaces where plaque accumulate, supragingival and subgingival. Supragingival plaque is further divided into two locations, smooth surface plaque on the crown of the tooth above the gingival tissue and approximal surface plaque (the surface area between teeth). Subgingival plaque occurs below the surface of the gingival epithelium in the shallow crevice surrounding the teeth [1, 2].

Protective calcium phosphate enamel surrounds the living tissue matrix of the tooth, dentin and pulp. Most bacteria found in the oral cavity are facultative anaerobes, which are specifically adapted to grow on teeth and in anaerobic gingival crevices (where food particles accumulate). The gingival crevice is the area where the enamel protrudes from the gingiva (gum). Acidic glycoproteins found in saliva form a thin film on the tooth surface, which allows single bacterial cells to attach. There are only a few bacteria that can attach to the acidic glycoprotein film, namely *Streptococcus sanguis, S. sobrinus, S. mutans, S. mitis* and *Lactobacillus* species. Biofilms are formed due to extensive growth of these acidogenic bacteria and their by-products. Other bacteria such as filamentous *Fusobacterium* species and facultative anaerobic *Actinomyces* species are then able to attach to these areas, forming even larger biofilms [6].

3. Caries

'Caries' is defined as localized destruction of the tissues of the tooth by bacterial fermentation of dietary carbohydrates. First the enamel is demineralised and then the dentin by the acid by-products of microbial metabolism of carbohydrates. However, demineralization is followed by remineralisation. Cavities occur when the demineralization overtakes remineralisation. Streptococci such as *S. mutans* are acidogenic and aciduric (acid tolerant) and reduce plaque pH levels encouraging conditions for other plaque bacteria. Once the pH level falls below 5.5, enamel demineralisation occurs. Fluoride promotes remineralisation and may be one of the mechanisms in which it protects against tooth decay [1, 8]. Two of the main bacterial species responsible for lactic acid production and dental caries are *Streptococcus* (*S. sobrinus* and *S. mutans*) and *Lactobacillus* species [6].

Several stages occur during plaque formation. As dental caries are generally associated with *S. mutans*, the process of biofilm formation of the bacteria on tooth surfaces will be used to clarify the description. Firstly, sucrose and the glucosyltransferases (GTFs) enzymes are required for the accumulation of *S. mutans*. Saliva in the oral cavity produces a film on the tooth surface. This film contains glycoprotein constituents and forms a pellicle on the tooth surface. *Streptococcus mutans* interacts with the α-galactosides in the saliva-derived glycoprotein of the pellicle using an adhesion known as antigen I/II. The cell membrane of *S. mutans* also possesses glucanbinding protein (GBP), serotype carbohydrates and GTFs [9]. This allows for the accumulation of *S. mutans*. Co-aggregation or co-adhesion then takes place as new bacteria attach to those bacteria already attached to the tooth's surface. These steps lead to the formation of a biofilm [1, 9].

Bacteria adherent to surfaces have a higher resistance to clearance by normal cleansing methods as well as to bacteriolytic enzymes and antibiotics. The adherent state is therefore advantageous to survival and a key step in pathogenesis. By preventing microbial adhesion, disease formation can be prevented as well [10].

If plaque is allowed to grow undisturbed calculus may form. Saliva contains calcium and phosphate ions, which may become deposited within deeper layers of undisturbed dental plaque. Bacterial enzymes such as phosphatases and proteases degrade calcification inhibitors, also contained within saliva, which leads to the formation of insoluble calcium phosphate crystals that combines and forms a calcified mass of plaque, termed calculus. Supragingival plaque and calculus contains more Gram-positive organisms, such as *Streptococcus* species and *Actinomyces* species, while subgingival contains more anaerobic Gram-negative species. Considerable amounts of metabolic by-products such as lactic acid accumulate in plaque. The lactic acid produced in plaque leads to caries formation [1, 8, 9]. Sucrose, the favoured carbohydrate substance for oral bacteria, is made available either directly by food ingested or by the action of bacterial or salivary amylases on dietary starch. The trapping of carbohydrates in food particles, remaining in the mouth for considerable periods, is of particular relevance here. Sucrose is required for the last two processes involved in the formation of dental caries [11].

4. Plaque-mediated diseases

In the oral cavity, transition from a predominantly normal Gram-positive facultative microbiota, associated with health, to plaque consisting of obligately anaerobic, proteolytic Gram-negative rods and spirochetes, will give rise to diseases of the soft tissues [7].

The total number of bacteria will increase from 10^2-10^3, normally found in healthy individuals, to 10^4-10^8 organisms during gingivitis and as many as 10^5-10^8 organisms during periodontitis [12].

5. Periodontal diseases

Periodontal diseases are: 'a collective term ascribed to several pathological conditions characterized by degeneration and inflammation of gums, periodontal ligaments, alveolar bone and dental cementum' [13]. Gingivitis is the inflammation of the periodontal ligament that forms the periodontal pocket. Clinical features include redness, swelling and bleeding of the gums. Periodontitis usually develops from untreated gingivitis and can involve loss of bone and tissue decay. The combined activities of microorganisms within the subgingival biofilms and the host responses to

them, lead to the progression of the disease and tissue damage [1, 6, 7]. Periodontopathogens include Gram-negative bacteria such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythus*, *Aggregatibacter actinomycetemcomitans*, *Fusiobacterum nucleatum* and *Capnocytophaga* species.

The pathogenesis of periodontal diseases may fluctuate from slow, chronic progressive destruction of collagen and aggressive tissue degeneration, to brief and acute with varying intensities and durations. Treatment of periodontal diseases includes biofilm control, root surface debridement or root scaling, surgery and the use of antimicrobial agents [1].

6. *Candida albicans*

Candidiasis has become a major public health concern as *Candida* species are opportunistic pathogens associated with immuno-compromised individuals, especially in those affected with the acquired immunodeficiency syndrome (AIDS). Oral candidiasis is most commonly characterized by the development of oral thrush. Up to 90% of individuals with human immunodeficiency virus (HIV) suffer from at least one episode of candidiasis, making candidiasis the leading oral fungal infection in immuno-compromised individuals [4]. It has been determined that isolates of *Candida albicans* are more virulent and genetically altered in HIV-positive patients than those strains encountered in HIV-negative patients [14]. *Candida albicans* also causes denture-associated stomatitis as it is capable of colonizing polymethyl methacrylate materials [7].

The cell wall of *C. albicans* is composed of polysaccharides, mannan, glucan and chitin. There are three virulence factors of *C. albicans*. Namely, the ability of the organism to adhere to oral epithelial cells; secondly, to secrete enzymes such as proteinase and phospholipase that hydrolyse peptide bonds and phospholipids respectively, causing tissue invasion and damage; and lastly to induce a change in phenotypic expression and morphology [15]. The oral cavity has to cope with not only bacterial and yeast infections, but with viral infections as well.

Viruses such as the papilloma virus and herpes simplex virus (HSV) types 1 and 2, as well as other herpes viruses are commonly isolated from oral tissues. It has been suggested that herpes viruses may play a significant role in periodontal diseases as they disrupt host defence mechanisms and thereby facilitate bacterial infection [7].

The human body is capable of responding to and fighting off moderate infection through its adaptable defence system, the innate and adaptive immune response.

7. Host response

The immune response to oral infections may itself damage host tissues; for example, epitopes from certain streptococci stains are cross-reactive to the epitopes present in heart tissue. The antibodies required to combat the infecting bacteria can cause rheumatic heart diseases by binding to the heart tissue and inducing complement-mediated lysis and antibody-dependent cellular toxicity. Deposits of circulating immune complexes in the synovial can cause complementary-mediated joint pain. Other examples include subacute bacterial endocarditis, infected ventriculoarterial shunts, secondary syphilis and gonococcal and meningococcal septicaemia [1]. Investigations in recent years have also implicated oral bacteria as the causal agent of certain systemic diseases, such as pneumonia and cardiovascular disease [16].

8. Treatment

There are several characteristics of an ideal antibiotic. Activity against the microorganisms involved in the infection; good penetration and diffusion at the infection site; it must be well tolerated with few or no adverse effects and should allow for patient compliance. As most infections are not just due to one microorganism, the antibiotic should be active against both Gram-positive and Gram-negative microorganisms, and it is often necessary to use a combination of antibiotics to achieve a spectrum of activity [17].

Antibiotics such as penicillin are narrow-spectrum antibiotics as they are generally active against Gram-positive microorganisms, with the exceptions of ampicillin and amoxicillin which are broad-spectrum and active against Gram-negative bacteria as well. Metronidazole is also a narrow-spectrum antibiotic as it only acts on obligate anaerobes. Tetracycline and ampicillin are broad spectrum antibiotics active against a wide range of Gram-positive and Gram-negative microorganisms. They were often utilized when the causative pathogen was unknown, which lead to the frequent use of the antibiotics resulting in the emergence of resistant pathogens that were once sensitive to the treatment. As yeast and fungi share similarities to human cells, selective toxicity is more difficult to achieve [1].

The over usage and misuse of antibiotics has encouraged alternative methods of treatment, such as treatment delivery vehicles that can release antimicrobial agents directly into the periodontal pocket [8]. Intra-pocket delivery systems place delivery vehicles in or around the periodontal pocket. The delivery vehicles such as fibres, strips, films and injectable gels, are composed of a variety and different combination of drugs such as tetracycline, chlorhexidine,

metronidazole and amoxicillin. More advanced delivery systems include the microparticle, nanoparticle or vesicular system [13].

9. Treatment failure

Drug resistance in microorganisms is becoming a major problem. Mechanisms of antibiotic resistance include inactivation of the drug, altered uptake and modification of the active site of the drug, and acquisition of new genetic material via horizontal transfer or phentotypic variation [1].

Resistant strains of *Candida* are emerging which complicates the treatment process with current antifungal agents. Due to resistance it is not uncommon for a relapse infection to occur. New antifungal agents are required to both treat candidal infections and to curb the growing resistance of these organisms [4].

Chlorhexidine gluconate (CHX) has broad spectrum antimicrobial activity and is generally more effective than either nystatin or amphotericin B in anti-*Candidal* activity [18]. Most mouthwashes contain CHX due to its antibacterial properties. *In vitro* studies have shown that CHX also possesses antifungal activity and anti-adherence against *Candida* species by affecting its structural integrity which leads to fragmentation of the cell wall. However, although CHX reduces the incidence of oral candidiasis, clinical trials have shown that it does not eradicate the oral yeasts. Chlorhexidine gluconate has been shown to be inactivated by food and saliva; it causes taste disturbances and mucosal irritation as well as staining of the teeth and tongue [14, 15, 18].

10. Plants in oral care

Antibiotics appeared to be the cure for most infections; however the in-discriminated use of antibiotics has led to emergence of multidrug-resistant pathogens. Novel therapeutics has always been found in plants. Plants produce small molecule antibiotics, which are generally weaker than those produced by bacteria and fungi; however plants are still able to fight infections successfully. Plants appear to use a concept known as synergy to combat infections. The synergistic interactions of secondary plant metabolites with antibiotics were examined in the treatment of infectious diseases. For example, butylated hydroxyanisole (BHA) green tea in combination with the antibiotic vancomycin was found to be effective against *S. mutans*, non-susceptible *Escherichia coli* and *C. albicans* [19].

Civilizations throughout history have been using plants as traditional medicine to cure various ailments, including toothache. Principle plant parts used in remedies to treat toothache, gingivitis, loose teeth, dental abscesses and general mouth sores, were fresh or dried roots, stems, leaves and bark. Traditional preparation of the plant material is generally a decoction, used to rinse the mouth, gargling or inhalation [3]. Plants can also be used as chewing sticks to form basic toothbrushes and dental floss. Over time, people discovered that chewing the twigs or leaves of certain plants alleviate mouth sores, infections and toothache. The fibrous texture and a palatable taste, combined with antibacterial properties make good chewing sticks [20]. There are over 62 plant species belonging to 29 families documented to treat oral diseases in Burkina Faso, West Africa, alone [3]. Some of these plants used for various tooth problems in various forms are as follows:

10.1Toothache

Plants used to treat toothache include *Acokanthera oppositifolia*; *Albizia adianthifolia* (leaves and roots); *Annona senegalensis* (bark); *Barleria prionitis*; *Carissa bispinosa* (root); *Dicoma anomala* (root); several *Cassia*, *Acacia* and *Ficus* species [3, 20]. *Zea mays*, more commonly known as maize, is part of the staple diet in South Africa; however in Burkina Faso, the decoction made from the flowers is used to treat toothache [3].

10.2 Gingivitis

Plants utilized to treat gingivitis include *Alternanthera pungens* (leaves); *Ceiba pentandra* (bark); *Boswellia dalzielii* (bark and roots); *Maytenus senegalensis* (leaves, bark and roots); *Anogeissus leiocarpus* (bark and roots); *Pteleopsis suberosa* (bark); *Diospyros mespiliformis* (leaves); *Indigofera tinctoria* (leaves and roots); *Ximenia americana* (leaves, bark and roots); *Myrothamnus flabellifolius* (leaves); *Pinus pinaster* (bark); *Bauhinia*, *Acacia* and *Cassia* species [3, 20].

10.3 Chewing sticks

African, Middle Eastern and Asian communities often make use of chewing sticks, or miswak (*Salvadora persica*), as an oral hygiene aid. The World Health Organization is still encouraging this practice for those communities who do not have professional dental care. Various plant species are selected due to availability, long bristle-like fibres and also for pleasant taste. The activity within these natural toothbrushes may often be diverse [21, 22].

Some of the plants used as chewing sticks include *Acacia mellifera* (twigs); *Diospyros lycioides* (roots and twigs); *Jasminum fluminense* (branches), *Salvadora persica* (branches) and *Azadirachta indica* (twigs) [20, 23]. In West Africa, the twigs from citrus trees such as *Citrus aurantafolia*, *Citrus sinensis* and *Cassia sleberlanba* are used as chewing sticks. A popular Nigerian chewing stick is *Fagara zanthoxyloides*. In Kenya, twigs from *Rhus natalensis* and *Euclea divinorum* are preferred. In the United States, *Betula lenta*, *Gaultheria procumbens*, *Liquidambar stryaciflua*, *Sassafras albidum* and *Populus* species are utilized. *Faidherbia albida* bark strips are used in the same manner as dental floss [20, 22].

Several of these chewing sticks have been tested for bioactivity against oral pathogens. A phosphate-buffered saline (PBS) extract from the bark of *Serindeia warneckei* showed activity against *Porphyromonas gingivalis* and *Bacteroides melaninogenicus* exhibiting an MIC of 0.25 µg/ml and 0.5 µg/ml respectively. Aqueous and tannic extracts of the twigs exhibited activity at 2.5% v/v against *C. albicans* [21, 22, 24]. Aqueous and alcohol extracts of *Juglandaceae regia* at 2-8% w/v and 10% w/v respectively, inhibited *in vitro* growth, adherence, acid production and glucan-induced adherence of *S. mutans*. *Rhus natalensis* and *E. divinorum* was shown to inhibit the extracellular peptidase and glycosidase activities of *P. gingivalis*, *P. intermedia* and *Treponema denticola* [9, 22]. When chewing sticks are used effectively, they are at least as efficient as modern toothbrushes [22].

10.4 Chewing gum

Research has shown that some chewing gums containing plant components can treat gingival bleeding and plaque formation. One such example is the chewing gum PYCNOGENOL®, which contains phytochemicals extracted from the bark of the French maritime pine, '*Pinus pinaster'*. PYCNOGENOL® has been shown to be a potent antioxidant-phytochemical, with anti-inflammatory properties. Chewing PYCNOGENOL® reduced gum bleeding by 50% and may suppress biofilm formation [25]. Chewing gum, containing mastic gum, derived from the resin of the *Pistacia lentiscus* tree had significant antibacterial activity against *S. mutans*. It may prove to be a useful adjunct in the prevention of dental caries [26].

10.5 Oral rinses

Oral rinses deliver their therapeutic ingredients and benefits to all accessible surfaces of the oral cavity. Depending on their composition, oral rinses can remain active for an extended period of time. Clinicians often recommend oral rinses to patients to reduce biofilm formation, which aids in controlling gingivitis. Recent studies compared the antimicrobial action of a herbal mouth rinse (The Natural Dentist Healthy Gums Daily Oral Rinse); an essential oil oral rinse (Listerine Cool Mint) and an established 0.12% chlorhexidine gluconate oral rinse (Peridex), against predominant oral bacteria. 'The Natural Dentist' contained extracts of *Aloe barbadensis*, *Echinacea angustifolia*, *Echinacea purpurea*, *Hydrastis canadensis*, *Calendula officinalis* and *Citrus paradisi*. Although 'The Natural Dentist' was found to be less potent than the chlorhexidine gluconate rinse, Peridex, it inhibited the growth of 40 bacterial species tested. When compared to Listerine, 'The Natural Dentist' exhibited significantly lower minimum inhibitory concentrations (MIC's) for *Actinomyces* species, periodontal pathogens *Eubacterium nodatum*, *Tannerella forsythia* and *Prevotella* species, as well as the cariogenic *S. mutans* [27].

10.6 Halitosis

Halitosis is generally caused by Gram-negative anaerobic bacteria such as *P. gingivalis*, *F. nucleatum* and *P. intermedia*. A herbal formulation comprising of herbs, known to contain antimicrobials, such as sage; echinacea; lavender and mastic gum was formulated as a mucoadhesive tablet. The clinical trials showed a significant decrease in halitosis assessments, and may be an effective means of treatment for halitosis [28].

10.7 Antimicrobial activity shown by phyto-derived samples

Plant extracts have been screened for their antibacterial activity against several oral microorganisms, such as *S. mutans*. Garlic juice from *Allium sativum* has shown impressive inhibition of *S. mutans*, considering that this microorganism is resistant to antibacterial agents such as penicillin, amoxicillin, tetracycline and erythromycin (Xavier & Vijayalakshmi, 2007). *Saussurea lappa* is traditionally used in the treatment of halitosis, dental caries and periodontal disease. An ethanolic extract of the roots of *S. lappa*, at a concentration of 1 mg/ml, inhibited the growth of *S. mutans*; the acid production; as well as lowered the adherence of *S. mutans*. It also inhibited the formation of water-insoluble glucan by *S. mutans*, an essential component of biofilm formation [29]. *Myristica fragrans*, known as 'nutmeg', has a wide variety of uses, including being anti-inflammatory and anti-fungal. Macelignan, an isolated compound from *M. fragrans*, exhibited an inhibitory activity of 3.9 µg/ml against *S. mutans*. Macelignan also showed preferential activity against *Streptococcus salivarius*, *S. sobrinus*, *S. sanguis*, *Lactobacillus acidophilus* and *L. casei* with an MIC range of 2-31.3 µg/ml [30]. Four traditional Brazilian plants used in oral care were tested against *P. intermedia*, *P. gingivalis*, *F. nucleatum* and *S. mutans*. The bacteria were susceptible ethanolic extracts of the stem of *Aristolochia cymbifera*;

aqueous extracts of the leaves of *Caesalpinia pyramidalis* and aqueous extracts from the husk fibre of *Cocos nucifera*. Aqueous extracts from the inner bark of *Ziziphus joazeiro* were not as effective [31].

The inhibiting effect of aqueous twig extracts of *Azadirachta indica* (Neem) on the *in vitro* biofilm formation by bacteria was investigated [23]. No inhibition of *Streptococcus cricetus*, *S. sobrinus*, *S. mutans* and *S. sanguis*, was observed in the presence of ≤ 320 μg/ml Neem extract. Pre-treatment of *S. sanguis* with Neem extract resulted in the significant inhibition of bacterial adhesion to saliva-conditioned hydroxyapatite, a composite of bone and enamel. Neem extract also inhibited insoluble glucan synthesis, suggesting that Neem has the ability to reduce the adherence of streptococci to tooth surfaces. *Melaphis chinensis* (Chinese nutgall) extracts are rich in gallotannins. These extracts have anti-adherence properties as well as the ability to inhibit insoluble glucan production among streptococci [23]. A high-molecular weight constituent, isolated from *Vaccinium macrocarpon* (cranberry juice), reduced and even reversed bacterial co-aggregation of *F. nucleatum*, *Actinomyces naeslundii* and *E. coli* in dental biofilms and reduced the enzymatic activity of glucosyltransferase within the biofilm [32]. A clinical trial showed a significant reduction of *S. mutans*. *Vaccinium macrocarpon* macromolecules have been shown to halt lipopolysaccharide (LPS), as well as induced the bacterial production of pro-inflammatory cytokines and proteolytic enzymes by *P. gingivalis*, *T. forsythia* and *Treponema denticola*, thereby aiding in the reduction of periodontal diseases [32].

10.8 Plant-derived substances in oral care products

Oral rinses and toothpaste manufacturers have incorporated plant-derived antimicrobials into their formulations. Miswak extracts, tea tree oil (as Melafresh T96), peppermint, green tea, *Aloe vera*, manuka honey oil (from the manuka tree flowers in New Zealand), and several more essential oils such as eucalyptus, lavandula and rosmarinus are a few of the plants incorporated into oral formulas [33].

These essential oils are active against several cariogenic and periodontopathic bacteria. Tea tree oil and manuka oil inhibited *P. gingivalis* adhesion as well. Listerine™ is a well-known oral rinse containing active ingredients such as thymol, eucalyptol (antimicrobials), methyl salicylate (a cleaning agent) and menthol (a local anaesthetic) [33]. There are several more natural oral care product ranges.

The Dental Herb Company (DHCo), in the United States of America, manufactures a line of natural oral health care products. 'Tooth & Gum Tonic'; 'Under The Gum Irrigant'; 'Tooth & Gum Paste' and 'Tooth & Gum Spritz' formulated from various combinations of red thyme, cinnamon bark, eucalyptus, lavender and peppermint essential oils; as well as echinacea, *Gota kola* and green tea extracts [34].

'LOGONA Naturkosmetik', in Germany, develops oral care products for adults and children known as LOGODENT. Herbal ingredients such as echinacea, anise, green tea, clove oil and a saccharose ester are included to prevent plaque formation and tooth decay. To strengthen the gums and prevent infection, myrrh, witch hazel and chamomile are included [35].

The Natural Dentist range, from the United States of America, formulated for adults and children contains echinacea, goldenseal, calendula, *Aloe vera* gel, blood root and grapefruit seed extract [36]. In India, Himalaya and oral care, HiOra, is formulated and contains extracts of *Symplocos racemosa* (Lodh tree), *Vitis vinifera* (grapes), *Cinnamomum zeylanicum* (cinnamon) and *Carica papaya* (papaya) [37].

Dabur herbal toothpaste has three herbal toothpastes formulated with clove, basil or neem as the main herbal ingredient. Other herbal ingredients include *Anacyclus pyrethrum*, *Acacia arabica*, *S. racemosa*, *Mimuspos elengi* and *Eugenia jambolana*. The benefits stated, indicate that there is an analgesic action for sensitive teeth and it relieves toothache; prevents tooth decay and cavities; removes plaque and refreshes breath. No additives, preservatives, chemicals or fluoride are stated to be present [38].

10.9 Southern African plants in oral care

Of the estimated 3000 medicinal plant species that are regularly used in South African traditional medicine, only 38 indigenous species have been commercialized to some extent [39]. The natural product industry as well as local communities are benefiting from the trade of indigenous medicinal plants. Due to national and international research institutions focusing on South African plants, several phytochemical products have already been marketed. Some plants, such as *Aspalathus linearis* (Rooibos) and *Aloe ferox*, have a greater international status as their trade outside South Africa becomes more prominent [40].

Southern Africa plants used in traditional oral care include *Carpobrotus edulis*; *Dodonaea viscosa* (gargles for oral infections); *Euclea natalensis*; *Securidaca longepedunculata*; *Artemisia afra*; *Dalbergia obovata* and *Warburgia salutaris* (toothache). *Siphonochilus aethiopicus*, *Polygala myrtiflia*, *Glycrrhiza glabra* and *W. salutaris* are used for symptoms of *C. albicans* infections [39-42]. Some of these plants have been reported to have antibacterial activity *in vitro*.

Antimicrobial activity of the methanol and aqueous extracts of *A. afra* and *D. obovata* against *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, *E. coli* and *Klebsiella pneumonia* were determined. Only the methanol extract of *A. afra* showed activity against *S. aureus* and *B. subtilis* [41]. The aqueous extracts of *P. myrtiflia* and *G. glabra* showed activity against *C. albicans* [42].

11. Essential oils

11.1Tea tree oil

Melaleuca alternifolia, a tree indigenous to Australia, produces an essential oil that is more commonly known as tea tree oil (TTO). The tree has been used medicinally for 220 years, but the oil was only commercially produced by steam distillation around 80 years ago. In the 1930's TTO was already recognized as having potential in oral hygiene [43]. Tea tree oil has approximately 100 components and has shown broad-spectrum antimicrobial and anti-inflammatory properties *in vitro* [44].

The essential oil contains around 100 components, most of which are monoterpenes. Uses of TTO range from cuts and insect bites, to acne and tinea, a fungus infection of the keratin component of hair, skin, or nail [44, 45]. Infections caused by viruses, bacteria and fungi respond to clinical treatment with TTO. Scientific evidence has now indicated that a wide variety of oral bacteria are susceptible to TTO [44]. The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of *Actinomyces* species (MIC: 0.1 – 1 %v/v; MBC: 0.1 – 2 %v/v), *C. albicans* (MIC: 0.5 %v/v; MBC: not active), *P. intermedia* (MIC: 0.003 - 0.1 %v/v; MBC: 0.003 - 0.1 %v/v) and *S. mutans* (MIC: 0.25 – 2 %v/v; MBC: 0.25 – 2 %v/v) has been determined [44, 46]. The anti-adhesion capability of TTO has also been determined using *S. mutans*. The safety of TTO has also been determined on cultured human umbilical vein endothelial cells and at a concentration of 0.2% they have little adverse effect on the viability of cells at this concentration [16].

Melafresh T-96, is a toothpaste produced by the Australian company Southern Cross Botanicals, which incorporates TTO into the formula at a concentration of 0.2%. Even after loss of activity during manufacturing it still possess a high potency and a broad spectrum of antibacterial activity of Gram-positive and Gram-negative organisms [47].

11.2 Peppermint essential oil

Mentha piperita L., a perennial herb, is a hybrid between spearmint (*M. spicata* L.) and water mint (*M. aquatica* L.) more commonly known as peppermint. It has been utilized in many foods, cosmetic and pharmaceutical products. *Mentha piperita* essential oil contains the terpenoid, methanol, as its main compound, menthone; isomenthone; 1,8-cineole; menthyl acetate; menthofuran; limonene; β-myrcene; β-carophyllene; pulegone and carvone [48].

Evidence that four *M. piperita* essential oils from various sources, and its components, menthol and methone are active against *Staphylococcus aureus*, *S. epidermis*, *K. pneumoniae*, *E. coli* and *C. albicans*, was found. The antibacterial activity of the essential oils and methanol was 0.63 mg/ml, 2.5 mg/ml, 1.25-2.5 mg/ml and 0.63-2.5 mg/ml for *S. aureus*, *K. pneumoniae*, *E. coli* and *S. epidermis* respectively. Menthone was generally less active than menthol; however against *S. mutans* menthol showed weak activity at 400 μg/ml. An MIC of 0.31-0.63 mg/ml was obtained for the essential oils and methanol against *C. albicans*. However, menthone exhibited an MIC of 2.5 mg/ml against *C. albicans*. Previous investigations of the oil composition were consistent with these results [49].

In vitro activity of *M. piperita* essential oil against *C. albicans* with an MIC of 0.5% v/v was exhibited (Hammer *et al*., 1998). The free radical scavenging capacity of *M. piperita* essential oil has been noted to be stronger than either *M. aquatica* or *M. longifolia*, reducing the radical generator 2,2-diphenyl-1-picrylhydrazyl (DPPH) by 50% with a 50% inhibitory concentration (IC_{50}) value of 2.53 μg/ml. The antiviral activity of HSV-1 and HSV-2 have also been noted when the viruses were pre-treated with the oil prior to adsorption into the host cell, with IC_{50} values of 0.002% and 0.0008% for HSV-1 and HSV-2 respectively [48].

Mentha piperita essential oil is approved for internal as well as external use. Internal uses include treatment of spastic discomfort of the upper gastrointestinal tract and bile ducts and inflammation of the oral mucosa. It can be used externally for myalgia (muscular pain), neuralgia (pain associated with nerves) and as an antimicrobial and antiseptic. Although the oil, leaf extract and aqueous extracts are considered safe, the concentration of pulegone should be limited to 1% [48, 49].

12. Tea

12.1*Camellia sinensis*

In Japan, green tea has been traditionally used to cleanse the mouth. It is also believed that those who consume large amounts of green tea have less tooth decay. Recent studies have shown that tea has potential anti-cariogenic properties [11].

Camellia sinensis (family Theaceae) selectively absorbs fluoride from the soil and stores the fluoride in the leaves as an anion. Acidic soils are conducive to the increased uptake of fluoride in *C. sinensis*. Fluoride levels also increase within leaves and stems of the plant as the plant ages. Leaves store up to 98% of fluoride in plants [50, 51].

Fluoride plays a vital role in bone and teeth mineralization and is therefore an essential element in our diet. It also has inhibitory or stimulatory effects on many soft tissue enzymes and plays a role in dental caries resistance. However, exposure to too much fluoride may result in skeleton and dental fluorosis. The recommended safe daily intake of

fluoride for adults ranges from 1.5–4 mg, while for children and teenagers it ranges from 1.5–2.5 mg [51]. A high level of fluoride exposure leads to tooth discolouration in children and severe osteoarthritis in adults [50].

There are many forms of tea available on the market these days, from green tea and black tea to herbal tea, instant teas and readymade to drink teas. Only green and black tea will be discussed here. Green tea is produced from the bud and young leaves of *C. sinensis*, while black tea undergoes a manufacturing process known as fermentation and possesses a naturally higher level of fluoride [11, 51]. The fluoride concentrations in green tea are lower than those in black. Green tea fluoride concentrations range from 0.59–1.83 mg/l after a five minute infusion. While for black tea the concentrations range from 0.32–2.76 mg/l [51].

Apart from the different fluoride content the main difference between green and black tea is that green tea contains five major catechins. Catechins are polyphenolic (flavan-3-ols) antioxidant plant metabolites which exert antimicrobial and antiviral activity against a variety of organisms such as *Helicobacter pylori*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium tuberculosis* and *C. albicans* [52]. Black tea undergoes fermentation, in which many catechins are oxidised and theaflavins and thearubigins are produced. A few simple catechins, such as epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) are still found in black tea, while catechin (C) and epigallocatechin (EGC) also occur in green tea. An infusion of green tea with hot water contains 0.5–1 g of catechins/l. Black tea prepared in the same manner only produces one-third of the catechins in green tea [11, 53].

Green tea catechins also possess powerful antioxidant properties; better than those of the antioxidant vitamin C. Epicatechin gallate is the most effective antioxidant, followed by EGCG and EGC. These catechins act as nitrogen scavengers and chelating agents to copper (Cu^{2+}) [53].

Green tea interferes with the three processes involved in the pathogenesis of dental caries; namely adherence, glycocalyx formation and acid production. At 100 mg/l, which is less than a ‘cup of tea’ concentration, tea caused inhibition of adherence of *S. mutans* to saliva-coated hydroxyapatite (component of enamel and dentin). Tea catechins also inhibit *S. mutans* and *S. sobrinus* glucosyl transferase activity, with EGCG showing the greatest inhibition. Green tea halts the production of lactic acid by inhibiting bacterial lactate dehydrogenase. Green tea has also been found to inhibit salivary amylase, while black tea inhibits both salivary amylase and the bacterial amylase of *S. mutans*, which may be due to the aflavins found in black tea [11, 53].

The antibacterial activity of tea is somewhat decreased due to catechins affinity for proteins. This property is known as astringency and contributes to the sensation, known as ‘mouthfeel’, experienced when drinking tea. Scientists theorise that the inhibition of amylases and glucosyl transferase and the adherence of *S. mutans* might be due to the interactions of catechins such as EGCG and related compounds with these proteins, ultimately resulting in a loss of function. However no experimental evidence has as yet been produced [11].

Pharmacokinetic studies have revealed that after rinsing the mouth with tea that catechins are found in the saliva for up to 60 minutes; while the rinsing with a tea catechin 0.25% solution had an anti-plaque effect for up to 90 minutes. The enzymatic breakdown of starch in food particles trapped in the mouth was also remarkably reduced [11].

Due to the lower fluoride content and high catechin activity in green tea, it is conclusive to incorporate green tea into a mouthwash or toothpaste, when compared to black tea. It may be worth considering combining a strong antibacterial sample with green tea in toothpastes or mouthwashes, thereby creating a product that contains antibacterial properties as well as being capable of inhibiting bacterial adherence and providing a source of natural fluoride.

13. Conclusions

The use of natural antimicrobial agents may prevent the formation of biofilms and the development of periodontal diseases. The antimicrobial agents of plants therefore, need to be capable of combating periodontal microorganisms and thereby preventing periodontal diseases.

New treatments are required to counter the increasing incidences of resistance and sometimes harsh side effects of treatments, while still combating the microorganisms that cause caries and periodontal diseases. Plants and essential oils, such as tea tree oil, are becoming renowned as alternative and sometimes better cures than established drugs. Resistance also occurs more slowly with natural products. It is estimated that a quarter of all prescriptions in industrialised countries contain one or more components derived from plants [4]. The challenge is to find plants capable of preventing or minimising the effects of dental and other oral diseases. These plants should therefore, contain antimicrobial properties, without being cytotoxic to human epithelial cells.

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