Herbal based therapeutics for tooth remineralization

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ABSTRACT:

Dental caries is considered as the most common polymicrobial oral disease in the world. With the aim of developing alternative approaches to reduce or prevent the decay, non-invasive remineralization strategy is usually preferred in a clinical scenario. A plethora of literature studies reveal the potential of anticaries activity of a number of herbal products, being suggested as alternatives to conventional therapeutic remineralization agents.The aim of this paper is to review the contemporary remineralizing agents available for remineralization therapyand their implementation into clinical practice. A search of articles from "Pubmed", "Medline" and "Scopus" databases with the keywordsRemineralization- demineralization, natural remineralizingagents, herbal dental products, anti-cariogenic plant derivatives was conducted. Major natural products with anticarieseffects are derived from food, beverages, flowersor traditional herbs and the active constituents are mostly polyphenolic in nature. All the herbal agents exhibit antimicrobial and anti-inflammatory activity while someof them are reported to be effective in shifting the de-/remineralizationbalance and collagen biomodification. Future research investigations are warranted to explore the biological validation of these herbal based remineralizing agents through *in vivo* animal and clinical studies prior to commercialization and clinical translation.

KEYWORDS: Dental caries, Remineralization,Traditional herbs, Natural products, Phytotherapeutics

INTRODUCTION:

The world‟s major portion of population is affected by dental caries, irrespective of age, gender and ethnicity. The pathophysiology of dental caries is not a simple and continuous process of tooth mineral loss, but rather a dynamic process with alternate cycles of demineralization and remineralization. The progression or regression of caries lesion depends on the balance between these two processes.[1] Pathological factors such as cariogenic bacteria, fermentable carbohydrates and salivary dysfunction favour demineralization whereas defensive factors like antibacterial agents, saliva and mineralizing ions shift the balance towards remineralization.

Over the years, clinical and scientific evidence have shown the benefits of minimally invasive dentistry (MID). One of the cornerstones of MID concept is the possibility of remineralizing initial enamel caries, also called as white spot lesions (WSLs) by means of topical gels, varnishes, mouthwashes and dentifrices containing several therapeutic agents. These active agents include, fluoride, Casein phospho peptide-amorphous calcium phosphate (CPP-ACP), Bioactive glass (BAG), Hydroxyapatite (HAp) etc., However, the use of these chemical and synthetic agents exhibit systemic side effects such as gastro intestinal disorders, skeletal/dental fluorosis, etc., Other notable drawbacks of these conventional remineralizing agents include, low hardness, lack of strength, high solubility.[2] To overcome the aforementioned limitations, there has been a rising interest in biologically active compounds derived from natural products including food, beverages, flowers and traditional herbs.

Phytotherapy is a field of medicine that uses plants either to treat disease or as health-promoting agents. It is often referred to as herbalism in Western medicine. Traditional use of phytotherapies generally preserves the original composition and integrity of the source plant[3], so that either the whole plant, or a desired percentage of its minimally adulterated components, is used for medicinal purposes. When examined in terms of developments in dentistry, phytotherapy is used because of the broad biological properties, namely, antimicrobial, anti-inflammatory, analgesic, and sedative effects of herbal plants[4].

The active constituent present in most of the herbal agents are polyphenolic in nature. A polyphenol is defined as any compound that contains at least one aromatic ring with one or more hydroxyl groups along with the presence of other substituents[5]. As evident in literature, certain polyphenol compounds could be effective in killing bacteria or inhibiting biofilms[6], while some were able to regulate the de/remineralization of dental hard tissue. In recent years, many studies have been reported on the efficacy of phytotherapeutics on tooth remineralization. The current review article focuses on the various phytotherapeutics, with an emphasis on their properties and the mechanism of remineralization with evidence-based literature.

GALLA CHINENSIS:

Galla chinensis (GC), a traditional Chinese medicine, widely used in China, originates from the abnormal growth of the Rhus leaf tissue in response to secretion of parasitic aphids (family Pemphigidae). It has been used for thousands of years due to its antibacterial, antiviral, anticaries, antioxidative, anticancer, liver-protective, antidiabetic, antidiarrheal, anti-inflammatory and antithrombin activity. GC is rich in gallotannins, comprising nearly, 20% gallic acid (GA) and 7% methyl gallate. GCE is composed of different polyphenol compounds; some of them might form a barrier to prevent calcium ion deposition on the lesion surface. Gallotannins consist of a central glucose core, which is surrounded by several GA units, and further GA units can be attached through depside bonding of additional galloyl residues. In several previous studies, GCE have been found to be effective in inhibiting demineralization and enhancing remineralization [5-7] inferring GCE to be a potentially interesting agent in dental caries prevention. Cheng *et al.* found that GCE had enhanced the efficacy of fluoride in shifting the de-/remineralization of dental enamel. Huang *et al.* also reported that, there was a significant synergistic remineralization effect when GCE was combined with nanohydroxyapatite(n-HAp)[8]. The mechanism of galla chinensis is still unclear, but several hypotheses have been proposed. Chu*et al.* [5] demonstrated that the polyphenolic compounds of GC, namely tannins and gallic acid,interact with calcium ions forming a dissolvable complex, and aid in delivering the ionic calcium to the middle layer of the artificially induced enamel lesions, when tested under dynamic pH cycling conditions. FurtherG. chinensis actually prevents decalcification by inhibiting the translocation of dissolved Ca^{2+} and PO_4^{2-}ions from lesions.Yet another hypothesis is,,enamel organic matrix-galla chinensis-Ca‟ proposed by Zhang *et al.*[9]who evidenced increased deposition of numerous nanosized elliptical apatite particles following GC application resulting in improved surface roughnessof carious enamel surface[10]. The original GCE solution has a low pH, which might dissolve dental enamel. Besides its potential effects in shifting the de-/remineralization balance, GCE have also been proven to be able to inhibit dental biofilms infected with *Streptococcus sanguis, Streptococcus mutans, Actinomyces naeslundii, Lactobacillus rhamnosus*[11]. In an in vitro experiment, Cheng *et al.*[12] investigated the antibiofilm effects of GCE and evidenced that GCE significantly inhibited the acid metabolism of both nascent and mature microcosm biofilms as well as it reduced the bacterial growth.

PROPOLIS

Propolis is a natural resinous mixture produced by honeybees from substances collected from parts of plants, buds, and exudates. The word propolis is derived from Greek, in which pro stands for "at the entrance to" and polis for "community" or "city," implying this natural product is used in hive defence. Bees gather propolis from different plants in different temperate climatic zones[13], hence also known as bee glue. It has attracted increased interest due to its beneficial effect on human health, including antimicrobial activity against a wide range of pathogenic microorganisms. Since ancient times propolis has been extensively employed by man, especially in folk medicine to treat several maladies. Greek and Roman physicians used it as mouth disinfectant and as an antiseptic and healing product in wound treatment, prescribed for topical therapy of cutaneous and mucosal wounds [14]. Propolis was listed as an official drug in the London pharmacopoeias of the 17th century and became very popular in Europe between the 17th and 20th centuries. The first scientific work with propolis was published in 1908 including its chemical properties and composition which was further indexed to chemical abstract [15]. Currently, propolis is being used in many health products in different forms for topical use, in cosmetics or as popular alternative medicine for self-treatment of various diseases. Current applications of propolis include formulations for cold syndrome (upper respiratory tract infections, common cold, and flu-like infections), as well as dermatological preparations useful in wound healing, treatment of burns, acne, herpes simplex and genitalis, and neurodermatitis. Propolis is also used in mouthwashes and toothpastes to prevent caries and to treat gingivitis and stomatitis. It is widely used in cosmetics and in health foods and beverages. It is commercially available in the form of capsules, mouthwash solutions, creams, throat lozenges, powder, and also in many purified products from which the wax was removed. Due to its antimicrobial, antiviral, and antioxidant properties, it is widely used in human and veterinary medicine, pharmacology, and cosmetics, it also poses antitumoral, Hepatoprotective Activity, Antidiabetic activity, Immunomodulatory action, Dental action, etc.,

Propolis is a complex mixture composed of around 50% resins, 30% waxes, 10% essential oils, 5% pollen, and 5% of various organic compounds [13,16,17]. Composition percentages vary with different geographical origin and different climatic conditions[18]. The essential principal compounds responsible for biological activities are polyphenols, aromatic acids, and diterpenic acids, but very few different propolis types have been different in their main bioactive compounds. Different composition is also related to specific flora of the region and treatments of raw material. Tunisian propolis ethanol extract was proven to have anticariogenic and antibiofilm effects in an in vitro experiment[19]. In all the antioxidant assay systems, aqueous extract of propolis (AEP) showed higher activity compared to the ethanolic extract of propolis (EEP). This may be due to its higher polyphenols content. The antioxidants present in propolis play a great role in its immunomodulatory properties[20, 21,22]. The flavonoids concentrated in propolis are powerful antioxidants.

The effective component of propolis could inhibit both glucosyltransferase activities and bacterial growth[23].Furthermore, a previous study investigated the ethanolic extract of a novel type of propolis and its purified hexane fraction on *mutans streptococcus* biofilms and the development of dental caries in rats. The results suggested that the cariostatic properties of propolis were due to the reduction of acid production and acid tolerance of *cariogenic streptococci*; these biological activities may be attributed to its high content of fatty acids [24]. Koru *et al*.[25] reported that the propolis samples were more effective against Gram-positive anaerobic bacteria than Gram negative ones. The author also stated that it can be effectively used in managing oral cavity diseases as it contains flavonoids such as pinobanksin, quercetin, naringenin, galangine, chrysin, and aromatic acids such as caffeic acid determined by GC-MS analysis. Ali *et al*.[26] and Giamalia*et al.*[27]demonstrated the remineralization efficacy of propolis as evidenced with an increase microhardness level of demineralized enamel block following treatment with propolis.

MAGNOLIA BARK

Magnolia (Magnolia officinalis) bark is a plant part, the extract of which (MBE) has been widely used in medicine for thousands of years, especially in traditional Chinese, Japanese, and Korean medicine [28]. The two effective components of magnolia bark extract are magnolol and honokiol which have been reported to inhibit the growth of *S. mutans, Streptococcus sobrinus, Porphyromonasgingivalis, Fusobacterium nucleatum, Aggregatibacteractinomycetemcomitans, Capnocytophagagingivalis and Veillonelladisparin vitro*, and to reduce dental caries in an animal experiment model [29,30,31]. MBE is acknowledged for several pharmacological activities, including benefits for the digestive, respiratory, cardiovascular, and central nervous systems, it also possesses antioxidant, anti-inflammatory, and antimicrobial activities [32,33,34]. Furthermore, MBE is safe for dietary consumption and does not show any toxic or mutagenic potential in animal [35,36]. Thanks to these properties, MBE has the potential of a natural feed additive with positive implications for animal health and product quality. For

example, dietary MBE increased growth performance and transcripts for antioxidant enzymes in chickens [37]. Also, MBE supplementation is reported to modify the levels of certain metabolites absorbed in the chicken intestine.

Magnolia bark extract has previously shown beneficial effects on oral health, including reduction of *salivary mutans streptococci*, plaque acidogenicity and bleeding on probing, when added into a xylitol chewing gum, when used for 30 days, in a randomized controlled intervention trial[38]. Ghorbani*et al*.[39] investigated the effect of Magnolia Grandiflora bark mouth-wash on the prevalence of *Streptococcus mutans* in dental plaque and proved that 0.3% Magnolia Grandiflora mouthwash tends to decrease the number of *S. mutans* in dental plaque significantly[40].

TEA

Tea is an aqueous extract of various dried and processed Camellia sinensis(CS) leaves[41], there are 3 main varieties of Camellia sinensis which includes; green, black and oolong[42].The main chemical difference between green and black tea is that the former contains simple catechins whereas in the later many of these have been oxidized and condensed, during a manufacturing process known as 'fermentation' to larger dark-colored molecules including theaflavins and thearubigins. Black tea contains other elements such as Al, K, Mn and Mg which may substitute Ca^{2+} of hydroxyapatite crystals thus forming other crystals [42]. They have numerous medicinal properties, mainly attributed to their bioactive chemicals, including; polyphenols, alkaloids, mineral, volatile oils that exert antibacterial and antioxidant effects [43-47].

The anticariogenic properties of tea have been researched on and off over many decades [46,48]. Tea showed several useful effects on both dental plaque[49] and caries [50,51] as a result of its high fluoride and organic constituents [52,53] that inhibit bacterial activity [54] and re-mineralize enamel. Tea, especially green tea, is rich in catechins like epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin that exhibit profound inhibitory effects on both collagenase and elastase[55]. Jose et al.[55] stated in their study, tea was increased in microhardness of enamel through these components. Taha *et al*. [56] in their study on the effect of green tea against acidic drinks showed that green tea may help stop the erosive effect of the acidic drinks and may support the remineralization. Aidaros*et al*.[57] showed that both black tea and green tea enhanced the remineralization process of demineralized enamel and dentin, which could be attributed to the action of tea on collagen network by stabilizing the collagen and maintaining it in an expanded state. This enables interfibrillar remineralization. Allah *et al*.[58] reported that black tea exhibited strong antimicrobial effect against S. mutans and lactobacillus bacteria as it contains phenolic compounds that degrade the cell wall leading to cell death. Awadalla*et al*.[59] found that oral rinsing of green tea solution without sugar for a short time could strongly inhibit salivary and plaque numbers of *S. mutans* which are important causative bacteria of caries both initially and secondarily. Lee *et al.*[60] showed that green and black tea can be used as a convenient, slow-release source of catechins and theaflavins to prevent dental caries.

HESPERIDIN

Hesperidin (C28H34O15) is a flavonoid glycoside[61] which was first isolated from citrus peel by French chemist Lebreton[62]. Its name is derived from the word “Hesperidium” present in many fruits including bergamot, banana and all citrus fruits belonging to the genus Rutaceae[63]. It is also present in the aerial part of the genus Rubiaceae and the Cruciferous plant leeks, with roots and whole grasses. Hesperidin has an aglycon (hesperetin or methyl eriodictyol) bonded to rutinose [6-O-(α-l-Rhamnopyranosyl)-D-glucopyranose] and/or [6-O-(α-l-Rhamnosyl)-D-glucose], as a disaccharide, in its structure. The major bioactive constituents present in hesperidin are polyphenolic in nature, especially flavonoids. Hesperidin possesses anti-inflammatory, anti-oxidative and anticancer activities by immunoregulatory activity mediated by nuclear factor κβ pathway[64]. Further, hesperidin is also shown to lower cholesterol levels and blood pressure due to its therapeutic action. In restorative dentistry, hesperidin, was shown to reduce the susceptibility of dentin lesions to acid-dependent demineralization with the potential to promote the remineralization process [65]. Interestingly, hesperidin was observed to reduce caries lesion depth and mineral loss even under fluoride-free conditions [66]. Further, hesperidin was able to preserve bovine dentin collagen against proteolytic degradation which may be related to its interaction with collagen and/or noncollagenous proteins, resulting in stabilization of the collagen matrix and induction of remineralization[67].

GUM ARABIC

Gum arabic is an exudate collected from the stems and branches of Acacia senegal and other related African species of Acacia. It consists mainly of high molecular weight polysaccharides and contains high concentrations of calcium, magnesium, and potassium salts, which yield arabinose, galactose, rhamnose, and

glucuronic acid following hydrolysis[69]. Gum arabic has been shown to play a crucial role in homeostasis by regulation of Ca^{2+} and phosphate (PO_4^{2-}) metabolism, as evidenced in an animal study. Remineralizing solutions composed of gum arabic exhibited excellent remineralization activity when applied on caries like enamel lesions, owing to the presence of high Ca^{2+} content [70]. Gum arabic also contains cyanogenic glycosides and several different types of enzymes, such as oxidases, peroxidases, and pectinases, that exhibit good antimicrobial as well as antiplaque properties[71,72,73]. Further, the growth of *Porphyromonasgingivalis*and*Prevotella intermedia* was reported to be inhibited by Acacia arabica gum[73]. Owing to the plaque inhibitory activity and remineralizing effects, gum arabic may serve as a potential caries preventive agent.

GRAPE SEED EXTRACT(GSE)

Grape seed extract (GSE) is rich in polyphenols, anthocyanins, proanthocyanidins (PA), procyanidins, and stilbene derivative resveratrol, which have been shown to possess potent free-radical scavenging activity. GSE is a complex matrix containing 40% fiber, 16% oil, 11% protein and 7% complex phenols such as tannins. Grape seeds are rich sources of flavonoids and contain monomers, dimers, trimers, oligomers and polymers. The monomeric and dimeric compounds include (+)-catechin (pCT), (−)-catechin (CT), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), procyanidin B2. Grape seed extract has been reported to have a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as anti-diabetic, anti-cholesterol, and anti-platelet functions. GSE is gaining more importance because its constituent PA possesses excellent anticaries, remineralizing, and antibacterial properties. Further, PA has dual functionality as collagen cross linker and matrix metalloproteinase (MMP) inhibitor. PA is also shown toupregulate the synthesis of collagen in human skin fibroblasts through TGF β1/ SMAD signaling pathway and inhibit the degradation collagen 1 by regulating MMPs/TIMPS system, thereby maintaining the stability of extracellular matrix[74.]PA-treated collagen matrices are non-toxic and inhibit the enzymatic activity of glucosyl transferase, F-ATPase and amylase. Glucosyl transferases, are enzymes produced by *S. mutans* that polymerize the glucosyl moiety from sucrose and starch carbohydrates into glucans. This constitutes the sucrose-dependent pathway for cariogenic pathogen to establish on the tooth surface resulting in plaque formation and development of caries[75]. Silva *et al.*[76] created artificial caries and compared the remineralization potential of grape seed extract derived PA and fluoride on enamel and dentin of bovine teeth.

PA is capable of inhibiting not only the production of organic acids and the formation of biofilms by cariogenic bacteria but also the progression of artificial root caries by affecting the balance of demineralization and remineralization under dynamic pH cycling conditions[77]. Xie*et al.*[78] compared sodium fluoride and GSE and inferred that GSE may serve to be a promising adjunct or alternative to fluoride in the treatment of root caries during minimally invasive therapy. Bedran-Russo *et al.*[79] reported that GSE treatment significantly increased the ultimate tensile strength of demineralized dentin, indicating the potential of GSE to induce cross-links in the dentin collagen. Moreover, the efficacy of GSE was verified in clinically relevant settings and in the presence of phosphoric acid, accentuating its great potential as a priming agent and etchant additive in bonding applications [80-82]. Vidhya *et al.*[83] evidenced that treatment with 5% PA increases bond strength significantly when compared to 10% sodium ascorbate, following immediate and delayed bonding up to two weeks post bleaching. The authors also stated that this improved therapeutic action of PA could be attributed to the following reasons: 1. The specificity of 5% PA for hydroxyl free radicals 2. The presence of multiple donor sites on 5% PA that trap superoxide radicals 3. The esterification of (-) epicatechin by gallic acid in 5% PA which enhances the free radical scavenging ability. Green et al.[84] reported that the presence of grape seed extract PA in dental adhesives inhibited the biodegradation of unprotected collagen fibrils within the hybrid layer when challenged by the collagenase solution. Zhang et al.[85] studied the synergistic effect of PA with biomimetic remineralization systems, namely carboxymethyl chitosan/amorphous calcium phosphate/collagen self-assembly on demineralized dentin and observed that PA can effectively serve as an analogue of glycosaminoglycans to preserve the porosity of collagen scaffolds. Further, biomimetic intrafibrillar mineralization was achieved along with significant antimicrobial effects against *Enterococcus faecalis*, *Porphyromonasgingivalis*, *and Fusobacterium nucleatum* thereby highlighting the dual-function strategy of therapy using PA for dental caries[86-88].

LEMON OIL:

The genus Citrus belongs to the Rutaceae family that comprises about 140 genera and 1300 species and, for instance, Citrus limon (Lemon) is among important species of genus Citrus. Citrus plants are grown in many countries all over the world, mainly in African region. Lemon oil mainly contains Limonine in addition to other constituents such as terpene compounds, phenolic compounds, and ketones. As evidenced in literature, these

essential lemon oils exhibit anticariogenic effects by potentially inhibiting the bacterial growth, adhesion, acid production and other cariogenic virulence factors including insoluble glucan [89-91]. This has led the global researchers to analyze the remineralizing effects of lemon oil. For instance, Ma *et al.*[92] reported that lemon oil can affect the progress of early dentin lesions not only by inhibiting collagen hydrolysis but also by enhancing collagen stability. However, further studies are mandatory to validate the findings of these preliminary studies pertaining to the remineralization efficacy of lemon oil.

CACAO

Cocoa / cocoa (Theobroma cacao), the main constituent of chocolate, contains some polyphenols which exhibit anti-glucosyltransferase activity. Cocoa / cocoa (Theobroma cacao) as natural materials can be potentially utilized in the field of dentistry along with the extract containing theobromine, which is expected to be an alternative to fluoride[93]. The cariostatic activity of cacao mass extract was established both *in vitro* and *in vivo* animal study models, however, its anticaries activity is not strong enough to significantly suppress the cariogenic activity of sucrose. Ooshima*et al*. also reported that the husk extract of cacao beans might be able to change a cariogenic into a non-cariogenic flora by significant reduction of *S. mutans*, without destroying the ecological balance inside the oral cavity by sparing other *oral streptococci*[93]. Osawa*et al.*[94] attributed the anticariogenic activity of cacao bean husk to the presence of high molecular weight polyphenolic compounds and unsaturated free fatty acids. Percival *et al*. [95] observed that cacao polyphenols can effectively inhibit biofilm formation and acid production by *S. mutans*. In addition, the anti-glucosyltransferase activity of cacao beans also contributes to the improved antibacterial protection.

In a comparative evaluation of the remineralizing potential of theobromine and sodium fluoride dentifrice by Amaechi *et al.*, a significantly higher mineral gain was observed with theobromine and fluoride toothpaste relative to artificial saliva[96]. Syafira G *et al.* have shown an increased enamel microhardness after treatment with theobromine on the enamel surface[97]. Meanwhile, AbdillahImronNasution*et al.* has noticed that the increase in hardness of the enamel surface by fluoride application is higher than that of theobromine[98].

COFFEE

Coffee, another popular worldwide drink, was reported to combat dental caries [99-100], mainly attributed to the presence of polyphenols. Antonio *et al.* evidenced the antibacterial action of Coffea canephora extract against *S. mutans*, as well as its inhibitory effect against enamel demineralization when tested under biofilm conditions[100,101]. C. canephora results in bacterial lysis and consequently the release of calcium into the medium rendering anticariogenic activity. The anticaries effect of coffeacanephora varies with varying concentrations with the higher concentrations (6-10%) exhibiting improved therapeutic effect when compared to lower concentrations[102].

CONCLUSION

Though numerous papers focused on how to use natural products to combat dental caries, there are still some problems to be solved. Firstly, the natural products showed weaker effects in caries prevention compared with traditional chemical agents, like antimicrobials or fluoride. Secondly, the mechanisms of the effective components are still unclear for most of the natural products. So,in future studies, more efforts should be made to find potential agents isolated from natural products, investigate their mechanisms and modify them to have stronger inhibiting effects on dental cariesagents. In addition, biologicalvalidation of these herbal therapeutics through *in vivo* animal and clinical studies is mandatory prior to commercialization and their clinical translation.

AUTHORS' CONTRIBUTIONS

SM, PK and SN provided the study design, PK prepared the first draft, SK, SM edited and completed the manuscript. All authors read and approved the final version of the manuscript for publication.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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