



ORIGINAL ARTICLE

Formulation of New Herbal *Salvadora* toothpaste with Anti-decay, Wound healing and caring tooth and Periodontal Diseases

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ABSTRACT

*Herbal toothpaste *Salvadora* with comprehensive effective materials for dental health ranging from antibacterial, detergent and whitening properties including benzyl isothiocyanate, alkaloids, and anions such as thiocyanate, sulfate, and nitrate with potential antibacterial feature against oral microbial flora, silica and chloride for oral disinfection and bleaching the tooth, fluoride to strengthen tooth enamel, and saponin with appropriate detergent, and resin which protects tooth enamel by placing on it and is aggregated in *Salvadora* has been formulated. The paste is also from other herbs extract including valerian and chamomile. Current toothpaste has antibacterial, anti-plaque, anti-tartar and whitening, and wood extract of the toothbrush strengthens the tooth and enamel, and prevents the cancellation of enamel. From the other side, resin present in toothbrush wood creates a proper covering on tooth enamel and protects it against decay and benzyl isothiocyanate and also alkaloids present in miswak wood gives *Salvadora* toothpaste considerable antibacterial and bactericidal effects. Anti-inflammatory effects of the toothpaste are for apigenin and alpha bisabolol available in chamomile extract and sesquiterpen components including valeric acid with sedating features give the paste sedating and calming effect to oral tissues.*

Key words: dental disease, herbal toothpaste, *Salvadora*, periodontal disease, effective herbal material

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INTRODUCTION

Tooth is one of the most important parts of body which is responsible for chewing, and in the case of non-health of the parts, various digestive and malnutrition problems occurs, and in addition teeth have an important role in beauty of face and speaking. The aim of modern dentistry is maintaining masticator system through whole human lifespan, and without endodontic treatment, obtaining the purpose in most patients will be difficult [1]. It can consider the oral and dental diseases including dental caries, gingival inflammation diseases, and periodontium tissue. The most important and common cause of dental caries, and inflammations and gingival diseases and periodontium tissue is lack of hygiene and accumulation of plaque and germs [2]. However, there are other factors which can increase the germ and plaque effect, which can refer to some systemic diseases (like leukemia or anemia and ...) and special systemic condition and hormonal changes (like puberty and pregnancy) or using some drugs (like some antiepileptic and some antihypertensive drugs). Biofilm dental plaque is as a milky and opaque color layer and contains more than 600 different microorganisms which can be removed largely after mechanical methods of mouth and dental hygiene such as brushing with effective tools, and effective and proper materials and toothpaste, and dental floss [3]. In the case of non-removal, microbial plaque becomes hard and changes into germ or tartar, which cannot be removed by brushing and needs a mechanical tool to remove it. Bacterial activities at the tooth surface causes irritate the gingival and gingival immune response leads to its inflammation. Gingival is a fibrous tissue covered by a mucosal membrane. The tissue becomes extremely thin near tooth, which the area (sulcus) is a critical region for sensitivity to heat, cold, and cases like periodontal diseases. Gingival, boundary between the gum and tooth, root surface, connective tissue appendix, and part of jaw bone are called periodontium. Periodontal diseases are set of problems dealing with gingival, especially boundary between gingival and tooth [4]. It can refer to gingival color change from pale pink to red, gingival bleeding during brushing, eating food, or even spontaneous bleeding and halitosis as clinical signs of gingivitis. If gingivitis left untreated, it would lead to periodontal diseases including gingival and jaw bone erosion (bone around the teeth), incidence of periodontal abscess, and pus extraction from gingival, and ultimately spontaneous falling of the tooth [5,

6]. It has been proved that gingivitis of an inflammation is from substitution of bacteria especially at the gingival and tooth margin. Development and intensification of the disease depends on the amount of dental plaque accumulation, type of microorganism and host immune system properties [7]. There are more than 350 species of microorganism in the healthy individual mouth, which only 5 percent of them could be involved in incidence of periodontal infections. Mouth bacteria are categorized in two groups which include aerobic gram positive bacteria and anaerobic gram negative bacteria [8]. Nevertheless, it can prevent the incidence of such diseases in many cases. It should understand that accumulation of dental plaques on teeth is the main cause for incidence of dental cavities and gingivitis and periodontal disease [5]. The most important present microorganisms and leading cause of plaque is streptococcus species that *Streptococcus mutans* is the major initiating species of damage and dental cavities [9]. Another microorganism participated in development of damages is lactobacilli that initially have no role but latter they have a role. *Streptococcus* clones on the teeth surface and instead of other microorganisms where their fermentation product is lactic acid, their product is propionic acid and barbituric acid; produces by fermentation of surplus food rich of lactic acid which the acid makes erosion and calcium phosphate (constituent material of tooth enamel) resolve by low pH created in the mouth (one unit lower than other organic acids)[10]. For essential role of *S.mutans* in dental damage and also regarding the statistics of world health organization (WHO) statistics that 60-90 % of school kids and about 100 % of adults have dental decay and cavities, severe periodontal diseases is observed in 15-20 % of adults with mean age of 35-44 years, many efforts have been done to produce a vaccine for the microorganism that so far has not been successful in human [2]. Recently a molecule named Keep 32 has been synthesized which is able to kill *S.mutans*. Nevertheless, if we could reduce absorption of *S.mutans* of tooth surface or reduce and or remove its physiological capacity on dental biofilms, it can decrease acidifying potential of biofilm and constituting next cavity [11]. In recent years, numerous reports have been presented about natural factors in control dental plaque and prevent periodontal consequences [12-14]. With this approach, oral hygiene care which includes brushing and rinsing with proper mouthwash and also using appropriate antibiotics and anti-microbial can help to reduce plaque and remove microbial flora of the mouth. Meanwhile, proper use of toothbrush and toothpaste is the most effective way to control plaque [11]. For this purpose, toothpaste should have certain properties including abrasive, anti-microbial, detergents, flavoring and thickener. Fluoridated toothpastes are very effective in decrease dental decay by strengthening tooth enamel structure and inhibiting effect on enolase enzyme (needed in fermentation of sucrose by microorganisms), and of course it is not recommended for under 6 years children, and its excess use causes some pigments on teeth. Whitening toothpastes have abrasive material and saponin and strong detergents. The toothpastes are proper for healthy and strong teeth but they are not suitable for teeth having numerous decays and weak enamel. In general it weakens the enamel by abrasion of tooth surface and gradually yellows the tooth and its sensitivity. Toothpastes lowering tooth sensitivity are effective in lowering tooth sensitivity particularly dental neck sensitivity. Their usage should be prolonged to give result and the toothpastes are usually potassium nitrate and or strontium and so on. Among antibacterial materials used for mouth environment sterilization, chlorhexidine (a chlorophenyl bisbiguanide with wide anti-microbial activity) is used as inhibitor of flat surfaces decay and dental plaque controller [15]. Due to its high antibacterial power, partially prolonged effect and stability, and non-toxicity it has also been introduced as channel washer [16]. However chlorhexidine has various problems such as dental pigmentation and pigmentation of mouth and tongue environment, altered sense of taste, irritation and oral dryness, scaling of gingival and negative systemic effects in ingestion. Sodium hypochlorite has also been always considered due to its high antibacterial power and solving pulp tissue. The matter has disadvantages including bad odor and erosion. Considering the bacterial nature of tooth and surrounding tissues infections, using the herb with comprehensive effective materials for tooth hygiene ranging from antibacterial, detergent and whitener including benzyl isothiocyanate, alkaloids and anions such as thiocyanate, sulfate and nitrate with inherent antibacterial feature toward mouth microbial flora, silica and chloride to sterilization of mouth and whitening the tooth, fluoride to protect tooth enamel and saponin with appropriate detergent power, and resin which protect tooth enamel by placing on it [17] named *Salvadora* which had been recommended by Islam prophet, is considered and has been formulated as paste with some other herbs to prevent and treat tooth and periodontal diseases [18], to both prevent and maintain teeth and surrounding tissues from disease, and improve available diseases including inflammations of bacterial infections and difficulties resulted from other diseases such as cancer or so on.

INVESTIGATION OF DENTAL DISEASES BACKGROUND:

Accumulation of microbial plaque on teeth is the leading cause of dental decay, gingivitis, and periodontal disease. Streptococcal species are the most important microorganisms in plaque and cause of dental cavities, which *Streptococcus mutans* is the major initiator species for incidence of damage and dental cavities [15]. Beside the microorganism, lactobacilli have also an important role in continuation of decaying process. Instead of producing acetic acid, propionic acid and barbituric acid with pH 4.8, through fermentation of the remaining of foods rich of sucrose, streptococcus mutans produces lactic acid with pH 3.8 which lead to resolving calcium phosphate (constituting material for tooth enamel). The bacteria can survive and grow in acidic environment by their acid resistant enzymes. Further, lactic acid can change calcium phosphate into calcite, and conduct the chemical equilibrium toward the mucus environment supersaturated from calcium phosphate which cannot replace it equal to the rate of its separation. Therefore microorganisms producing lactic acid remove mineral materials from tooth body and lead to dental cavities and also development of infection to lower layers of tooth. Although tooth damage and decay is a complicated multifactorial disease, the rate of patient mouth hygiene is one of the most important factors in decaying process. If mouth hygiene supplied, the will be no plaque and no bacteria cloning occurs in tooth structure [19]. Fluoride in water reduces the dental decay. Fluoride places in calcium phosphate matrix of enamel, and reduces the decaying; and also has inhibiting effect on enolase enzyme. The aim of dentistry treatment is controlling infection. Number and types of treatment are different depending on disease severity. First treatment includes cleaning tooth depth and scaling polishing the tooth. In more advanced stages, drugs including antimicrobial mouthwash having materials such chlorhexidine have been used to control bacterium in treatment of gingivitis and after gingival surgery as sterilizing chip to control bacterium and decrease the size of periodontal pockets size. By intervening in bacterium adsorption on the surface of teeth, chlorhexidine has been used as inhibitor of dental plaque and decay on flat surfaces, and controls *S.mutans* population. However, chlorhexidine has different complications such as creating dental pigments; alter in tasting sense, irritation and mouth dryness, scaling the gingiva and negative systemic effects in ingestion form. Antibiotic gels including doxycycline and minocycline antibiotic micro particles to control bacteria and reduce the periodontal pockets size and also nutritional antibiotics to resist against periodontal infections, are other drugs used to treat inflammations and gingival diseases which are always accompanied with risk of developing antibiotic resistant infections. In more advanced stages, periodontal tissue repair surgery is conducted [15]. Using toothbrush and toothpaste is the first preventive action to maintain oral and teeth hygiene. Fluoride is considered as the most important parts of toothpaste to protect tooth enamel. Triclosan and metal salts as antibacterial, peroxide and baking soda as teeth whitener, sugars and detergents are used in toothpastes. Antimicrobial and odorant mouthwashes are also effective in mouth hygiene which is not used for children under 12 years old. Herbal toothpastes were initially made for those searching for a natural cleaner and didn't like to use fluoride containing toothpastes. The pastes contain materials such as myrrh gum, herbal extracts like strawberry, oils, and special detergents. Green tea extract is the other herbal material has been used for oral and tooth hygiene in various studies, which is rich of antioxidant and naturally prevents *S.mutans* growth, kills mouth bacteria, resists with dental plaque, and inhibits collagenase activity [20]. Tea tree oil is also effective to kill some bacteria including *S.mutans* in the form of mouthwash, and removes gingivitis. Corcominoid (effective ingredient of turmeric) has demonstrated anti-biofilm activity and also has anti-acidogenic activity against *S.mutans* [21]. Also the compound extracted from liquorice root called glycerizol A has high antibacterial activity against dental cavity causing bacteria [22].

MATERIALS AND METHODS

Components of new herbal toothpaste:

Herbal toothpaste *Salvadora* is an accurate mixture of extraction and powder of miswak tree with scientific name of *Salvadora persica* [Fig 1] with extraction of chamomile (*Matricaria recutita*) [Fig 2] and valerian (*Valeriana officinalis*) [Fig 3] extraction that each applied herb has considerable properties for preventive and curative toothpaste, which has been formulated with proper formulation. Extraction of miswak as an antibacterial, sterilizer, abrasive, whitener and natural detergent, chamomile extraction as anti-inflammation and valerian extraction as relaxing for inflamed tissue were used [23]. Herbal extractions of miswak, chamomile, and valerian were mixed with less than 1 % of a pigmentation agent (like titanium oxide or zinc oxide) and 10-50 % of abrasive (like silica, calcium carbonate, and sodium hydrogen carbonate or salvadora powder); then they are mixed with 10-30 % of a humectant (like propyl glycol, glycerin or sorbitol), and ultimately 1-3 % of natural flavoring (like *Mentha*, cinnamon or orange essence) and 1-2 % of a detergent are added to the mixture.



Fig 1: Plant of *Salvadora persica*



Fig 2: Plant of *Matricaria recutita*

Formulation of toothpaste:

Herbal toothpaste *Salvadora* is formulated from a mixture of miswak extraction as anti-bacterial, sterilizing, abrasive, whitener and natural detergent, chamomile as anti-inflammation, and valerian extraction as relaxing for inflamed tissues [24]. Herbal extractions of *Salvadora*, chamomile and valerian are mixed together and less than 1 % of pigmentation agent (like titanium oxide or zinc oxide) preferably

titanium oxide and 10-50 % abrasive (like silica, calcium carbonate, sodium hydrogen carbonate or Salvadora powder) preferably Salvadora, and then are mixed with 10-30 % of a humectant (like propylene glycol, glycerin or sorbitol) preferably sorbitol; and ultimately 1-3 % of natural flavoring (like Mentha essence, cinnamon or orange) preferably mentha essence and 1-2 % of sodium lauryl sulfate is added to mixture.



Fig 3: Valeriana officinalis

Microbial tests:

In order to investigate the antimicrobial and antifungal effect of formulated toothpaste, toothpaste diluted with fluid casein digest lecithin polysorbate 20 was used. Plate count agar culture was used to growth *Streptococcus mutans* bacterium. Lauryl sulfate tryptose broth and MacConkey broth cultures were used to evaluate to overall growth of forms. Further, julietteganton broth culture and pseudomonas agar were used to identify *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth, respectively. Evaluation of candida albicans fungus growth was also studied in 25°C in Sabouraud Dextrose Agar culture. All experiments were investigated in two groups control and experiment.

RESULTS

In control group which was without toothpaste in the environment, in all plates microbial and fungal growth was clearly seen after passing the incubation period. However, in test group which was designed with three different dilutions of new formulated herbal toothpaste, after passing the special incubation period, each mentioned bacterium had no sign of growth. Also in Sabouraud dextrose agar culture which was grown with *candida albicans* fungus, there could not be seen any sign of mouth fungus after 5 days of incubation in 25 degrees.

Table 1: Bacterial Test under Different culture

microorganism	culture	Control group	Test group1 (tooth paste dilution 10^{-1})	Test group2 (tooth paste dilution 10^{-2})	Test group3 (tooth paste dilution 10^{-3})
<i>Streptococcus mutans</i>	Plate count agar	+	-	-	-
<i>Salmonella</i>	Lauryl sulfate	+	-	-	-

	tryptose broth				
	MacConkey broth	+	-	-	-
<i>E.coli</i>	Lauryl sulfate tryptose broth	+	-	-	-
	MacConkey broth	+	-	-	-
<i>Staphylococcus aureus</i>	julietteganton broth	+	-	-	-
<i>Pseudomonas aeruginosa</i>	pseudomonas agar	+	-	-	-
<i>candida albicans fungus</i>	Sabouraud Dextrose Agar	+	-	-	-

DISCUSSION

Production of new toothpaste:

Since the most important and prevalent cause of dental decay, inflammations, gingival diseases, periodontium tissue is lack of hygiene and accumulation plaque and tartar, and plaque is the site for accumulation and cloning myriad of microorganisms' and are dynamic environment inside the tooth which streptococci are the most important and harmful of them, as it was stated *S. mutans* are the most important initiator species of dental decay, which beside lactobacilli produce lactic acid from fermentation of remaining of the food enriched from sucrose, which (has one unit of pH lower than other organic acids), lead to abrade and resolve the calcium phosphate, and causes damage and dental cavity by low pH created in mouth [15]. Considering the importance of *S. mutans* role, using different antibacterial such as chlorhexidine, triclosan, and metal salts in toothpastes and mouthwashes is one of the greatest efforts to reduce dental decay and subsequently gingival diseases, which sometimes will destroy all beneficial and harmful microorganisms and lead to create dental pigments, altering taste sense, irritation and mouth dryness, scaling the gingival, negative systemic effects in ingestion type, and danger of development of antibiotic resistant infections. Chemical abrasives have also been used to remove plaque on tooth, which cause more abrasion on tooth and gingivitis regardless of the time of using time [25].

Toothbrush wood is the root and stem of *Salvadora persica*, prevailing among Muslims to clean teeth; according to conducted studies the herb is very effective to prevent oral-dental problems by selective influence on microorganisms *S.mutans*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis*, and its antibacterial, antiplaque, anti-tartar, halitosispreventive and gingival diseases improver, anti-inflammatory and gingival antiallergic and tooth whitener properties have been proved [26]. Chamomile (*Matricariarecutita*) has anti-inflammatory and antiallergic effects and it is proper to treat mouth ulcers and relief dental pains [27]. Valeria (*valerianaofficinalis*) is also of the herbs having sedative effect, and tremendous impact in wound healing and removing many diseases for its pleasant smell [28].

Herbal toothpaste Miswak has been formulated with comprehensive effective material for dental hygiene ranging from antibacterial, detergent and whitener including benzyl isothiocyanate, alkaloids and anions such as thiocyanate, sulfate and nitrate with inherent antibacterial feature to mouth microbial flora, silica and chloride to sterilize mouth and whitening tooth, fluoride to protect tooth enamel and saponin with appropriate detergent and resin which protects the tooth by placing on tooth enamel that all are integrated in miswak [24]. Further the paste has other herbs extract including Valeria and chamomile, and has antibacterial, antiplaque, anti-tartar, and whitening effects. Miswak extract leads to protect tooth and gingival (especially in sensitive and susceptible for bleeding people) and prevent cancellation of

gingival. However, resin available in miswak creates a proper cover on tooth enamel and protects it against decaying [11, 27, 28, 29]. Moreover, benzyl isothiocyanate and alkaloids present in miswak gives the *Salvadora* toothpaste considerable antibacterial and bactericidal effects. Further it can attribute the anti-inflammatory effects of the toothpaste to apigenin and alpha-bisabolol present in chamomile extract; and sesquiterpen compounds including valerenic acid give the toothpaste soothing and sedative effect for dental tissues with soothing properties.

CONCLUSION

With unique formulation and considerable effective herbal material the toothpaste has been designed for all family members and its abrasive and antibacterial materials have herbal source. Current toothpaste has been formulated with a mixture of miswak extract (*Salvadora persica*), chamomile (*Matricaria arcutita*) and valerian (*Valeriana officinalis*). In order to prepare it, herbal extracts of miswak, chamomile, and valerian are mixed together and mixed with less than 1 % of a pigmentation agent (like titanium oxide or zinc oxide) and 10-50 % of abrasive (like silica, calcium carbonate, sodium hydrogen carbonate or miswak powder); then they are mixed with 10-30 percent of a humectant (like propylene glycol, glycerin or sorbitol), and ultimately 1-3 % of natural flavoring (like mentha, cinnamon, or orange essence) and 1-2 % sodium lauryl sulfate is added to the mixture.

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