World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(REVIEW ARTICLE)

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Potential of pomegranate mouthwash in inhibiting periodontopathogens bacteria development as alternative to halitosis therapy: A review

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World Journal of Advanced Research and Reviews, 2023, 20(03), 532–539

Publication history: Received on 22 October 2023; revised on 03 December 2023; accepted on 05 December 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.3.2466

Abstract

Background: Halitosis is unpleasant odors originating from the oral cavity caused by metabolic waste of periodontopathogens bacteria in the form of volatile sulphur compounds (VSCs). According to Riskesdas 2018, the oral health prevalence in Indonesia reached 57.6%. Doctors recommend using a mouthwash made from chlorhexidine. However, it is less effective because it can cause various side effects. The alternative treatments using herbal ingredients using pomegranate. This literature review aim is to analyze the potential and mechanism of pomegranate mouthwash as an alternative to halitosis therapy against periodontopathogens bacteria.

Material and Methods: The literature review was carried out in PUBMED, ResearchGate, and ScienceDirect using with the keywords: pomegranate, mouthwash, anti-bacteria, and halitosis.

Conclusions: Pomegranate mouthwash has the potential to be an alternative halitosis therapy by inhibiting plaque formation and the growth of periodontopathogens bacteria.

Keywords: Halitosis; Periodontopathogens; Plaque; Pomegranate Mouthwash

1 Introduction

Halitosis is common complain that characterized by bad breath from the oral cavity when talking or breathing. The etiological factor of halitosis condition is formation of volatile sulphur compounds (VSCs) as a bioprocess result of anaerobic activity in oral cavity [1,2]. Based on data released by the Indonesian Ministry of Health in a basic health research report in 2018 shown that the prevalence of oral health problem in Indonesia up to 57.6% [3]. Halitosis often causes shyness in socializing, which has a psychological impact resulting in decreased self-confidence, avoidance of social interactions, and loss of self-image. The etiological factors of halitosis are multifactorial, including 10% of extra-oral origin such as kidney disease, diabetes, lung and respiratory tract infections, sinus inflammation, chronic bronchitis, and digestive tract disorders as well as risk factors such as tobacco, alcohol, food, drinks and drugs. while 90% comes from intraoral origin caused by less of oral hygiene index, food impacted in tooth, large caries cavity, using of orthodontic appliances, periodontal disease, pericoronitis, xerostomia, orofacial abscess, bone infections, and tongue coating [4,5].

Nowadays, the treatment used to eliminate halitosis is to use mouthwash. Mouthwash is on of treatment of choice that has effectiveness to reducing halitosis because it comes into direct contact with all surfaces of oral cavity that cannot be

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reached up by brushing your teeth. The use of mouthwash containing chemicals such as chlorhexidine can effectively reduce levels of Volatile sulphur compounds (VSCs) but continuous use can cause several local side effects such as mucosal irritation, burning sensation, discoloration (stain) on teeth and restorations, disruption taste, triggers calculus formation, allergic reactions, and discomfort after gargling. In certain cases, long-term using of mouthwash can give some side effects such as formation of desquamation and peeling of the oral mucosa. Apart from considering the weaknesses of previous therapy, dentist and researcher recommend the patient to using herbal medicine-based drug including herbal medicine mouthwash. Previously research shown that herbal-based medicine is one of trend in dentistry because it has quite adequate potential as anti-inflammatory and antibacterial activity such as synthetic-chemical agent. In the future this literature review can be used as a basis for further research and clinical references in developing halitosis therapy innovations based on herbal medicine resources. This encourages the development of innovation, both research and writing on the potential of natural ingredients that thrive in Indonesia, namely red pomegranate (*Punica granatum* L.) as an alternative therapy for halitosis. The problem raised in this review article is whether pomegranate mouthwash has the potential to inhibit the development of periodontopathogens bacteria as an alternative therapy for halitosis and what is the mechanism of action of pomegranate mouthwash in inhibiting the development of periodontopathogens bacteria as an alternative therapy for halitosis [2,4,6,7].

2 Materials and methods

2.1 Search strategy

A literature search in English was performed, using the PUBMED, ResearchGate, and ScienceDirect base to identify research of the potential of pomegranate mouthwash in inhibiting periodontopathogens bacteria development as alternative to halitosis therapy. Research and literature reviews from 2018 to 2023 were included. The following keywords were searched: pomegranate, mouthwash, antibacteria, and halitosis [8].

3 Results and discussion

3.1 Periodontopathogens Bacteria

Periodontopathogens bacteria are bacteria that are known to contribute significantly to periodontal disease and halitosis. Approximately 700 species of bacteria identified in the normal microflora of oral cavity and 300 species known that has contributing to forming biofilms in periodontal pockets. These normal microflora bacteria in oral cavity can promote inflammation condition of the soft tissue located under the teeth, causing gingivitis. As the gingivitis gets worse, the bacteria will penetrate further and reach deeper pockets where the bone and underlying tissue are located leading to tooth loss. Several bacteria that are classified as periodontopathogens are gram-negative bacteri such as *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum,* and *Prevotella intermedia* [9,10]. *F. nucleatum* is one of a gram-negative bacteria that is the most abundant in the oral cavity. It is involved in various periodontal diseases. *F. nucleatum* has a pathogenic role in periodontal infection. Alveolar bone loss or abscess can be caused by *F.nucleatum* alone in mouse models of periodontitis. Co-Aggregation infection by *Porphyromonas gingivalis* or *Tannerella forsythia* and By releasing LPS, *F. nucleatum* can trigger the host's immunological response and lead to alveolar bone loss [11,12].

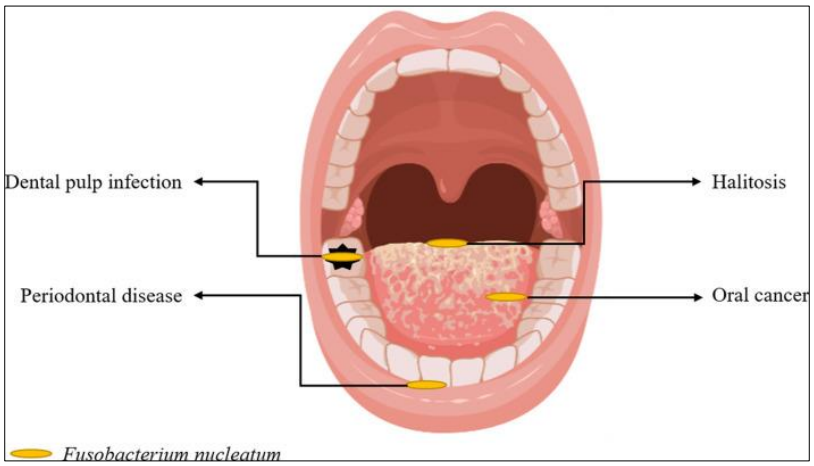

Figure 1 Predilecting site of *Fusobacterium nucleatum* in pathogenesis of several oral disease [12]

Through proteins and receptors extra membrane, *F. nucleatum* is linking bacteria with early and late phase of dental plaque development and maturation. In particular, *F. nucleatum* may cling to the surface of dental plaque where Streptococcus mutans first colonizes it by using RadD, CmpA, and Aid1. Following biofilm colonization, it subsequently combines with dental plaque that has colonized late (eg: *P. gingivalis*) through the use of FomA and Fap2 [12,13].

3.2 Halitosis

The term "halitosis" refers to an unpleasant stench that emerges from the oral cavity and is brought on by bacteria that can create volatile sulphur compounds (VSCs) on the tongue's ventral and dorsum surfaces. Anaerobic bacterial activity in the mouth produces sulphur compounds, which quickly evaporate and lead to halitosis. This process produces volatile sulphur compounds or VSCs. Gram-negative anaerobic bacteria, such as *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum, Prevotella intermedia*, and *Treponema denticola*, which produce the amino acids cysteine, methionine, and cystine, break down protein substrates into peptide chains to form VSCs [14]. To create VSCs, these amino acids will be reduced chemically, resulting in the production of dimethyl sulfide from extra-oral sources and hydrogen sulfide and methyl mercaptan from intra-oral sources. One more element that raises. Periodontal disease, dry mouth, smoking, alcohol use, certain food habits, infrequent tooth brushing, diabetes, obesity, and psychological stress are other variables that raise the risk of halitosis. The physiological state of the body, including hunger, dehydration, excessive physical activity, and advanced age, can also have an impact on halitosis. The prevalence of halitosis in the Indonesian arround 0.9% till 25.9%, although the global incidence is as high as 25% [2,5].

The survey conducted by Aninda et al through a google form distributed to the general public in Arjuna Bandung sub-district found that 23.8% claimed to have bad breath, 76.2% claimed not to have bad breath. 95.2% claimed to have encountered people who had bad breath. 52.4% rarely visit the dentist [3]. The underlying cause of halitosis is pathological due to periodontal disease. The etiology of halitosis is due to bacteria growing in the mouth, specifically on the mucosa behind the tongue. The oral microbes that are most likely to cause halitosis are gram-negative bacteria. The presence of these bacteria causes the bacteria described by the protein to produce several odor-causing compounds/vapors (Volatile Sulphur Compounds), including putricine, H_2S, cadaver, methyl mercaptan, and skatol. VSCs are the main cause of Intra-Oral Halitosis (IOH), especially H_2S and methyl mercaptan. This compound produces bacteria that will cause enzymatic reactions from amino acids that contain sulphur, such as L-cysteine and L-methionine. Some of the most active bacteria are *Peptostreptococcus anaerobius*., *Fusobacterium nucleatum*., *Prevotella intermedia*., *Treponema denticola*., etc. Halitosis can also be caused by a buildup of plaque and caries. Bad breath occurs due to the microbial breakdown of amino acids by enzymes which produce compounds that produce bad breath, including VCSs, diamines, short chain fatty acids. Volatile sulphur compounds (VSCs) are the result of the decomposition of anaerobic bacteria from normal flora or bacteria originating from the oral cavity. When the levels are normal, it will not cause anything disturbing. However, when levels increase, the activity of anaerobic bacteria will increase in the mouth. This is what causes the smell. Increased VSCs activity can occur due to a decrease in oxygen levels in the oral cavity when saliva or saliva production decreases, as well as tartar or caries in a person. In saliva, VSCs produce three main amino acids, including L cysteine produces (CH3)2S, L methionine produces CH3SH, and L cysteine produces H2S [15–17].

Halitosis often has far-reaching psychological and social impacts on sufferers. This condition will result in several social problems where sufferers will feel uncomfortable, anxious, embarrassed, shunned, and find it difficult to approach and interact directly socially with each other. As a result, social rejection can arise where sufferers will isolate themselves from a community. In certain cases, halitosis can also disturb the work productivity and psyco-sosial conditions of sufferers in a community. One of the recommended therapies to treat halitosis is to use mouthwash. Peppermint mouthwash is a safe formulation for home use and has been shown to be successful in treating halitosis over a 1-week observation period. The zinc component of mouthwash has been proven effective in reducing protein breakdown by bacteria and can be reducing the production of VSCs. In another research conducted that chlorhexidine (CHx) is on of an antiseptic agent that also can inhibit a microbes broadly, controlling plaque accumulation and thereby reducing halitosis. The mechanism of action of CHx is by penetrating intra membrane of bacteria, causing cell leakage and disruption of bacterial metabolism, thereby inhibiting bacterial growth. However, this treatment can cause side effects such as causing an alteration of taste persception in the patient and long-term use can cause reversible staining on the tooth surface and mucosal surface, mucosal irritation, burning sensation, taste alteration, allergic reactions, and discomfort after gargling [1,5].

3.3 Pomegranate as a Promising Source of Herbal Medicine

Pomegranate is a fruit that has latin name of *Punica granatum* L. that the trees can grow up to 5-8 m. Pomegranate is a dicotyledonous plant from the Punicaceae family that originates from the Middle East and is believed to have been a natural medicinal plant since 1550 BC (Ismail et al, 2012). Tree or shrub with a height of 2-5 m, woody stem, faceted branches, many branches, sometimes thorny, brown when young, and dirty green when old. Single leaf opposite or

spread out, without supporting leaves, leaf blade oval to lanceolate, sharp base, blunt tip, flat edge, pinnate spines, green color. Pansy flowers, actinomorphs, are separate. The flower axis is hollow, conical in shape. 5-7 petals, not deciduous. 5-7 crown leaves, irregular in buds. Many stamens, free pollen stalks, two anthers. Fruit ovary sunken, numerous, multi-tiered chambers, lower part with ovaries seeds in the axil, the top with the ovule on the wall. The pistil is slender, only one. Buni fruit with calyx crowns that do not fall off at the top end. Many seeds with a juicy seed coat and can be eaten, no endosperm, body with rolled body leaves. Although it is believed that this plant originated in Iran, it has been cultivated for a long time in the Mediterranean. Additionally, South China and Southeast Asia, which includes Indonesia, are home to this plant. Since the days of Ayurvedic medicine, pomegranates have been widely utilized as a natural remedy to prevent a variety of illnesses. Numerous substances found in pomegranates have the potential to be employed as natural dental products. Pomegranate peel waste mostly contains tannic acid, catechin, and epigallocatechin. Many chemicals containing punicic acid are found in pomegranate seeds. In the meanwhile, several compounds found only in pomegranates, such as punicalagin, gallic acid, ellagic acid, and ellagitannins, are present in the fruit of the pomegranate plant. Three different chemicals, including ellagitannins, gallotannins, hydroxy benzoic acid, and hydroxy cinnamic acid, are the tannins found in pomegranate peel waste [18–22].

Figure 2 Pomegranate fruit (*Punica granatum*) [23].

3.4 Mechanism of Pomegranate Mouthwash in Inhibiting Dental Plaque Formation

In its role as an antibacterial, pomegranate mouthwash works as an inhibitor of plaque formation. The pomegranate extract contained in pomegranate mouthwash works by diffusing into bacterial cells due to differences in concentration gradients. After the pomegranate extract diffuses into the bacterial cytoplasm, the pomegranate will reduce the activity of proteins and enzymes which play an important role in the bacterial cell membrane synthesis process. This will have the effect of lysis of bacterial cells. Apart from that, pomegranate extract will work by being a competitive inhibitor between bacterial glycoproteins and the glucosyltransferase enzyme which plays an important role in the process of initiating biofilm formation. Biofilm is a community of microorganisms that adhere to the tooth surface and are encased in an extracellular polymer matrix. There are five steps involved in the process of biofilm formation. The first step is reversible attachment aided by the bacteria's adhesive surface. The second step is irreversible attachment where bacterial structural adhesions are involved. Certain bacteria have flagellum, fimbriae, and special adhesins that aid this step causing an irreversible attachment between the bacteria and the surface. The third step is extracellular polymeric substances production that mediate bacteria cohesion and biofilm adhesion leading to the growth of the biofilm. The fourth step is biofilm maturation where bacteria continue to multiply and form microcolonies. The fifth step is dispersal, the terminal stage of biofilm development. In this fifth step, the bacteria cells leave the biofilm and continue to form new biofilms in other surfaces [24]. Biofilm formation begins with the attachment of glycoproteins on the surface of the *Streptococcus mutans* bacterial membrane to the glucosyltransferase enzyme to initiate attachment and formation of the biofilm. The mechanism of pomegranate here is due to the presence of tannins, which cross bacterial cell walls and precipitate proteins through complex formation, increasing bacterial lysis and inhibiting bacterial adhesion by suppressing enzymes such as glucosyltransferase which plays an important role in initiating the adhesion of *Streptococcus mutans* to the tooth surface. By inhibiting the performance of the glucosyltransferase enzyme and inhibiting the aggregation of *Streptococcus mutans* bacteria, the process of biofilm formation will also be hampered. By inhibiting biofilm formation, the aggregation of other bacteria such as *Porphyromonas gingivalis* will be hampered from continuing the formation and maturation of dental plaque. This can suppress the breakdown of proteins into peptides, especially cysteine (Cys) and methionine (Met), which make a major contribution to bad breath odor/halitosis [22,25–27].

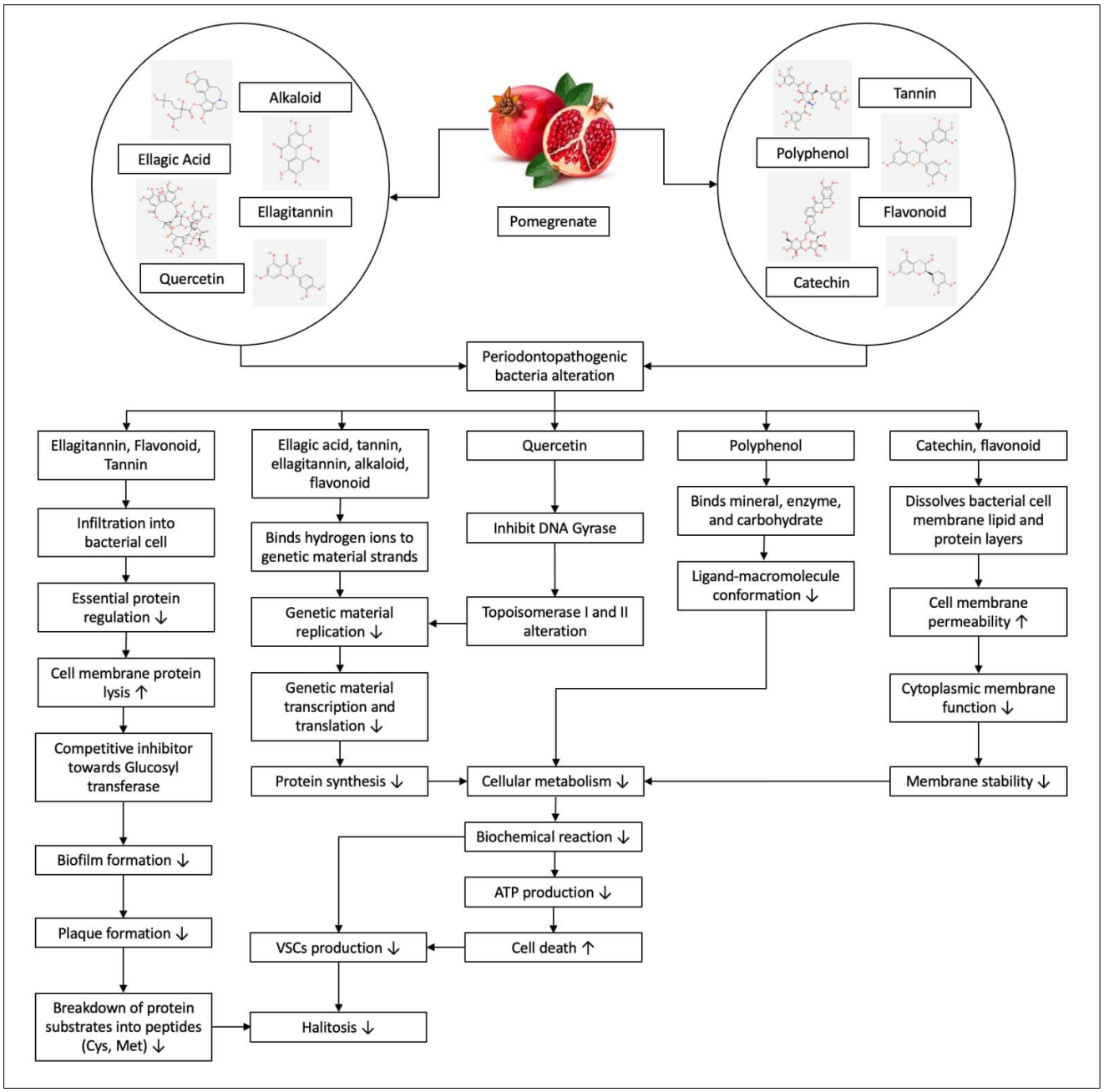

Figure *3* Mechanism of action of pomegranate mouthwash as an antibacterial agent to reduce halitosis.

3.5 Mechanism of Pomegranate Mouthwash in Inhibiting the Genetic Material Synthesis Activity of Periodontopathogens Bacteria

The pomegranate extract in pomegranate mouthwash has the ability to inhibit the synthesis process of bacterial genetic material. Inhibition is carried out through DNA and RNA found in bacteria. Pomegranate contains antibacterial substances, namely alkaloids and polyphenols in the form of flavonoids and their derivatives such as ellagic acid, tannins and ellagitannins. Flavonoids can reduce the activity of aspartate aminotransferase, increasing the activity of antioxidant, and inhibit bacterial growth. The benzene ring (ring B) of the flavonoids contained in pomegranate interacts by binding hydrogen bonds originating from RNA and DNA. The flavonoid compound in pomegranate in the form of quercetin can inhibit DNA gyrase in bacteria which causes bacterial death. This is caused by increasing the permeability of the bacterial membrane, impairing its ability to inhibit bacterial ATP production, and disrupting membrane transport and bacterial movement. Apart from that, the alkaloid compounds contained in pomegranate mouthwash have an antibacterial effect by inhibiting DNA synthesis and also reacting with amino acid compounds in DNA which functions. By disrupting DNA synthesis, bacteria will experience difficulty in multiplying or replicating, causing a decrease in the amount of genetic material. Disruption of DNA synthesis will then inhibit protein synthesis,

thereby triggering disruption of bacterial metabolism. The amount of protein synthesis will decrease and cause a decrease in bacterial cellular metabolism [2,28].

3.6 Mechanism of Pomegranate Mouthwash in Inhibiting Cellular Metabolic Activity of Periodontopathogens Bacteria

The next mechanism of pomegranate mouthwash that plays a role in overcoming halitosis is inhibiting the cellular metabolic activity of periodontopathogens bacteria, especially *Fusobacterium nucleatum*. Research conducted by Sri Hernawati and Pratiwi Soesilawati in 2021 showed that at levels of 50% and 100% concentration of pomegranate extract had the role of MIC and MBC, in *Fusobacterium nucleatum* bacterial cultures. In its role as an inhibitor of cellular metabolic activity, pomegranate extract works by binding minerals, enzymes and carbohydrates which are essential for cells to carry out their metabolic processes. This can have a direct effect because by binding minerals, enzymes and carbohydrates which are essential in metabolism, it will reduce the opportunity for the formation of ligand-macromolecule conformations with cellular metabolic enzymes. This will have a significant impact on reducing the metabolic rate of periodontopathogens bacteria. By decreasing the metabolic rate of bacteria, the number of biochemical reaction chains responsible for producing ATP in bacterial cells will also decrease, so that the bacterial ATP supply will continue to decrease until the bacteria run out of ATP energy supply which has an impact on the death of periodontopathogens bacteria. With the death of periodontopathogens bacteria, the production of VSCs metabolic waste products will also decrease. Apart from having a direct impact on cell death, decreasing the chain of biochemical reactions in bacterial cells will cause a decrease in the metabolic waste substances themselves, which in this case are VSCs. By reducing the production of waste metabolites from VSCs, it will reduce the incidence of halitosis in the patient's oral cavity [23,28–30].

3.7 Mechanism of Pomegranate Mouthwash in Disrupting the Cell Membrane Function of Periodontopathogens Bacteria

Pomegranate extract (*Punica granatum* L.), which is the main ingredient in making pomegranate mouthwash, contains several specific compounds that work by interfering with the function of the cell membrane of the *Fusobacterium nucleatum* bacteria. These compounds are flavonoids and catechins. These two compounds have the ability to form complexes with dissolved extracellular proteins which can disrupt the integrity of the bacterial cell membrane so that it will dissolve the bacterial cell membrane which consists of a lipid bilayer and amino acids. Damage to this layer can decreasing the surface tension of the bacterial cell membrane, thereby increasing the permeability of the bacterial cell membrane to important intracellular components, such as nucleic acids and nucleotides. This will cause leakage of intracellular molecules and ions as well as a decrease in the function of the cytoplasmic membrane in bacteria so that membrane stability is disrupted. As a result, the internal osmotic pressure becomes disturbed, causing the release of cell components/cell organelles such as the nucleus, mitochondria, lysosomes, ribosomes, Golgi bodies, and so on. These cell organelles function to carry out the life of bacterial cells and maintain the normal function of bacterial life. If disturbed, this situation will cause denaturation of proteins and nucleic acids in bacteria. The denaturation causes protein coagulation and disrupts the metabolism and physiological function of the bacteria. Disturbed metabolism will result in permanent cell damage due to insufficient energy needs and will ultimately cause bacterial cells to lyse. This will cause a decrease in bacterial metabolic waste substances, namely VSCs. Reducing the waste metabolic products of VSCs will reduce the incidence of halitosis in the patient's oral cavity [7,28].

4 Conclusion

Pomegranate mouthwash has the potential to inhibit the development of periodontopathogens bacteria as an alternative therapy for halitosis through several mechanisms, namely inhibiting plaque formation by being a competitive inhibitor of periodontopathogens bacteria, thus inhibiting the conversion of proteins into peptides which play an important role in the occurrence of halitosis. Another mechanism is inhibiting the development of *Fusobacterium nucleatum* bacteria by inhibiting genetic material synthesis activity, cellular metabolic activity, and disrupting cell membrane function. This causes lysis of bacterial cells which leads to bacterial death, so that the production of VSCs is also reduced. In the near future, researchers hope that this literature review can carry out further tests including in vitro, in vivo tests, as well as clinical trials of innovative halitosis therapy using pomegranate mouthwash. In the near future the researcher hope that pomegranate mouthwash can be produced and used by dentists and the public to treating or reducing halitosis with minimum side effects.

Compliance with ethical standards

Acknowledgements

All author acknowledged their equal contribution, read the manuscript, and gave their approval.

Disclosure of conflict of interest

We declare that there was no major conflict of this article.

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