

ANTIMICROBIAL POTENCY of *Stevia rebaudiana Bertoni* as HERBAL MOUTHWASHES AGAINST CARIOGENIC BACTERIAL *Streptococcus mutans*

Sinta Deviyanti, Margaretha Herawati, Adibah Ferhad
Universitas Prof. Dr. Moestopo (Beragama), Indonesia.

ABSTRACT

Background: Dental caries is a disease that is produced by the dissolution of local chemical surfaces of the teeth caused by product of microbial fermentation from dietary carbohydrates in the biofilm that attached to the teeth. *Streptococcus mutans* as the primary microbial pathogen in the aetiology of caries. They have a characteristic ability to produce voluminous amounts of sticky, extracellular polysaccharides in the presence of dietary carbohydrates, thus, help tenacious binding of the organism to enamel. Caries can be prevented by regular tooth brushing and flossing as mechanical plaque removal for cleaning the tooth. However, for many individuals, it difficult to eliminate *Streptococcus mutans* from the surfaces of the tooth by mechanical means alone over prolonged periods. Consequently, for effective caries control, these methods should be combined with the antimicrobial agent such as mouthwash would have a direct inhibitory effect on the microorganism that can be bactericidal or bacteriostatic. However, they have several undesirable side effects reported from clinical studies. The use of medicinal plants is highly considered, because of side effects of chemical substances. Recently, *Stevia rebaudiana Bertoni* leaves extracts as herbal mouthwashes are being tried and have been scientifically proven from several studies to be safe and effective to protection against dental caries because of its inhibitory effect or antimicrobial property against *Streptococcus mutans*. **Purposes:** The main aim of this literature review article is to explain the potentially antimicrobial herbal mouthwashes from *Stevia rebaudiana Bertoni* leaves extracts towards cariogenic bacterial *Streptococcus mutans*. **Conclusions:** *Stevia rebaudiana Bertoni* leaves extracts as herbal mouthwashes can be strongly recommended as a caries preventing agent.

Keywords: Caries, *Streptococcus .Mutans*, Antimicrobial , Mouthwash, *Stevia Rebaudiana Bertoni*.

1. INTRODUCTION

Dental caries is the disease on the teeth caused by demineralization of the inorganic portion and destruction of the organic substance of the tooth caused by particularly lactic acid produced from the microbial fermentation of dietary carbohydrates which often leads to cavitation (Marsh et al., 2009; Fejerskov et al., 2015). It was discovered that bacterial *Streptococcus mutans* was the main causative agent of dental caries. It can ferment most of the sugar present in food as sucrose (Samaranayake, 2018). The presence of more than 10^6 *Streptococcus mutans*/ml saliva may indicate a high caries risk or activity (Pallavi, 2011).

Studies have shown that caries can be prevented by regular tooth brushing and flossing as mechanical plaque removal for cleaning the tooth (Marsh et al., 2009; Mittal et al., 2011). However, for many individuals, it difficult to eliminate *Streptococcus mutans* from surfaces of the tooth by mechanical means alone over prolonged periods (Marsh et al., 2009). Consequently, for effective caries control, these methods should be combined with the antimicrobial agents (not including antibiotics) or chemical plaque control with chemoprophylactic agents, e.g., chemical mouthwash act by lowering the number microorganism or inhibiting dental plaque formation (Marsh et al., 2009; Mittal et al., 2011). However, they have several undesirable side effects reported from clinical studies, including tooth staining, increased supragingival calculus formation, reversible desquamation of the oral tissue, burning sensation, bitter taste and altered taste sensations (Mittal et al., 2011; Perry et al., 2014; Perry and Beemsterboer, 2007).

Herbal medicines with their “naturally occurring” active ingredients offer a gentle and enduring way for restoration of health by the most trustworthy and least harmful way (Malhotra et al., 2011). Herbal medicine is both promotive and preventive in its approach (Amruthesh, 2007). Natural Herbs like stevia (*Stevia rebaudiana Bertoni*) leaves extracts as herbal mouthwashes, have been scientifically proven to be safe and effective to protection against dental caries because of its inhibitory effect or antimicrobial property against *Streptococcus mutans* (Ajagannanavar et al., 2014; Sreekumar and Hegde, 2018; Usha et al., 2017). The major strength of these natural herbs is that their used has not been reported with any side effects till date (Malhotra et al., 2011; Amruthesh, 2007).

The main aim of this literature review article is to explain the potentially antimicrobial herbal mouthwashes from *Stevia rebaudiana Bertoni* leaves extracts towards cariogenic bacterial *Streptococcus mutans*. Thus, can serve as a good alternative mouthwashes and has the important impact in order to preventive dental caries and promotes commonly oral health.

Dental caries

Dental caries is a disease that is produced by the dissolution of local chemical surfaces of the teeth caused by metabolic changes in the biofilm attached to the teeth. Dental caries can affect the enamel, dentin and cementum, where biofilms develop and persist for long periods of time (Fejerskov et al., 2015).

Metabolic activity is influenced by changes in nutritional conditions such as increased intake of fermentable carbohydrates. When metabolic activity in the biofilm increases causing the pH to drop, it will cause demineralization of the hard surface of the teeth and caries lesions begin to occur (Fejerskov et al., 2015). The demineralization process will continue as long as there is no disturbing process. Dental caries lesions will continue to develop until the hard surface of the tooth is destroyed (Fejerskov et al., 2015).

The main etiology of dental caries is the presence of bacteria in biofilms attached to the teeth producing acid in response to the fermentation of carbohydrates consumed. There are three important causal components of caries, namely teeth, dental plaque (biofilm) and diet known as the Keyes triad. Other factors that influence caries emergence namely salivary rate, buffer capacity, sugar clearance rates and socio-behavioral factors. An important causal relationship is time which indicates a shift in the process of demineralization and remineralization of tooth surfaces (Fejerskov et al., 2015).

Streptococcus mutans as cariogenic bacterial

Streptococci comprise a diverse group of Gram-positive cocci. They are distributed widely in humans and animals, mostly forming part of their normal flora. The oral streptococci, which include the cariogenic mutans group, are important members of the genus (Samaranayake, 2018). *Streptococcus mutans* was originally isolated from carious human teeth by Clarke in 1924 (Marsh et al., 2009). The term *Streptococcus mutans* is now limited to human isolates previously belonging to serotypes c, e, f and k. This is the most commonly isolated species of *Streptococcus mutans*. Epidemiological studies have implicated *Streptococcus mutans* as the primary pathogen in the aetiology of enamel caries.. (but in the absence of predisposing factors, such as sucrose, they cannot cause caries). They have a characteristic ability to produce voluminous amounts of sticky, extracellular polysaccharides in the presence of dietary carbohydrates; these help tenacious binding of the organism to enamel and to each other (Marsh et al., 2009; Samaranayake, 2018).

Streptococcus mutans make extracellular soluble and insoluble extracellular polysaccharides (glucan, mutan and fructan) from sucrose that are associated with plaque maturation and cariogenicity. *Streptococcus mutans* can scavenge dietary sugars very efficiently, and rapidly convert them to acidic fermentation products (mainly lactate); significantly, they are also able to grow and survive under the acidic conditions they generate, by the induction of specific molecular stress responses (Marsh et al., 2009).

Mouthwash as antimicrobial agent and chemical antiplaque agent

Antimicrobial agent have a direct inhibitory effect on the microorganism that can be either lethal (bactericidal) or inhibitory but non lethal (bacteriostatic). Both type of agent can be delivered or formulated into oral care products such as mouthwashes (Marsh et al., 2009; Perry et al., 2014). To provide the most beneficial effects, mouthwash which also act as chemical antiplaque agent, should demonstrate several properties likes antiplaque action (bactericidal, or bacteriostatic), substantivity (ability to adhere to structure in the oral environment and be released slowly over time, enhancing the duration of effectiveness), low permeability (to allow its retention in the oral cavity), low toxicity and nonirritating (allows them to extend their effect on the bacterial population without damaging host tissues) (Perry et al., 2014).

Various product mouthwashes as antibacterial and chemical antiplaque agents available today in market. This agent reduces plaque and gingivitis in humans and has the active ingredients 0,12% chlorhexidine digluconate, phenolic compounds and quaternary ammonium compounds (Perry et al., 2014; Perry and Beemsterboer, 2007). However, they have several undesirable side effects reported from clinical studies. Dark brown staining of the teeth, increased supragingival calculus formation, reversible desquamation of the oral tissue and altered taste sensations can occur in the used of mouthwash containing chlorhexidine digluconat 0,12%. This mouthwash also contains 11,6% alcohol, so people who are sensitive about using products containing alcohol should be warned (Perry et al., 2014). Another mouthwash containing essential oils, thymol, eucalyptol, menthol methyl salicylate and alcohol up to 26,4%, have strong flavor and can cause staining too. The side effects of burning sensation and bitter taste have been also report in the used of mouthwash containing phenolic compounds (Perry and Beemsterboer, 2007).

Botanical description, nutritional aspects, biochemical aspects, functional component for food industry and health benefits of *stevia rebaudiana bertonii*

Stevia rebaudiana was botanically classified in 1899 by Moises Santiago Bertoni, who described it in more detail. Initially called *Eupatorium rebaudianum*, its name change to *Stevia rebaudiana Bertoni* in 1905 (Khiraoui et al., 2017). *Stevia* is a genus of about 200 species of herbs and shrubs in the sunflower family (*Asteraceae*), native to the Amambay region in the north east of Paraguay. Today its cultivation has spread to other regions of the world, including Canada and some part of Asia and Europe (Hossain et al., 2010). The leaves are sessile, 3-4 cm long, elongate lanceolate or spatulate shaped with blunt tipped lamina, serrate margin from the middle to the tip and entire below (Lemus-Mondaca et al., 2012).

Stevia rebaudiana Bertoni is a natural sweet herb native. Sweetness of 1 g of stevia in 100 ml water was equivalent to a sucrose solution containing 20 g of sucrose (sugar) (Cardello et al., 1999). The leaves of *Stevia* naturally contain a complex mixture of eight sweet diterpene glycosides, including stevioside, steviolbioside, rebaudiosides (A,B,C,D,E) and dulcoside A (Abou-Arab et al., 2010). The dry extract from the leaves of *stevia* contains flavonoids, alkaloids, water soluble chlorophylls and xanthophylls, hydroxycinnamic acids (caffeine, chlorogenic, etc), neutral water-soluble oligosaccharides, free sugars, amino acids, lipids, essential oils and trace elements (Komissarenko et al., 1994). The steviol glycosides are currently in use as a sweetener in a number of industrial foods (Goyal et al., 2010).

Like many plant glycosides, *stevia* has shown activity in cancer prevention, as well as antidiabetic, antiobesity, antineoplastic effect antifungal and antibacterial properties and also for the prevention of caries (Goyal et al., 2010; Bernal et al., 2011; Pól et al., 2007). As toxicological studies have shown that stevioside does not have mutagenic, teratogenic or carcinogenic effects and no allergic reaction has been observed when it is used as a sweetener (Pól et al., 2007). It can be safely used in herbal medicines and daily usage products such as mouthwashes and toothpastes.

Effectiveness antimicrobial potency of *stevia rebaudiana bertonii* herbal of mouthwashes toward cariogenic bacterial *streptococcus mutans*

An *in vitro* study conducted by Ajagannanavar SL et al (2014) has compared the efficacy of aqueous and alcoholic *Stevia rebaudiana* leaves extract against *Streptococcus mutans* in comparison to chlorhexidine (CHX) 0,2%. The result of this study explained that MIC value of aqueous and alcoholic *Stevia rebaudiana Bertoni* leaves extract for *Streptococcus mutans* was 25% and 12,5% respectively. Mean zone of inhibition of the aqueous and alcoholic *Stevia* leaves extract against *Streptococcus mutans* at 48 hours were 22,8 mm and 26,7 mm respectively. Mean zone of inhibition of CHX against *Streptococcus mutans* at 48 hours were 20,5 mm. It is concluded that the inhibitory effect shown by alcoholic *Stevia* leaves extract against *Streptococcus mutans* was superior when compared with that of the aqueous leaves extract and chlorhexidine.

An *in vivo* study conducted by Usha C et al (2017) has evaluated the anticariogenicity of mouthwash from microwave-assisted 0,5% leaves extract of *Stevia rebaudiana* and 0,12% CHX in high caries patients. The results of this study revealed that significant difference in pre- and post-rinse values of pH and buffer was found in both groups. However, no difference was noted between groups. Both groups decrease the bacterial count to $< 10^5$ colony-forming units (CFUs) in all the patients. It is concluded that *Stevia rebaudiana* extract 0,5% improved the pH and buffering capacity of the saliva in a high risk patient. It also reduced cariogenic organism in saliva.

Another *in vitro* study conducted by Sreekumar S and Hegde VK (2018) has compared the antibacterial action of three commercially available herbal products against *Streptococcus mutans*. They were dry powder of 100% pure extract *Alloe Barbadensis Miller (Aloe vera)* which diluted 50 mg powder with 100 ml distilled water until concentration 50%, liquid plant extract *Ocimum tenuiflorum (Thulasi)* concentration 50% and liquid plant extract *Stevia rebaudiana (Stevia)* concentration 50%. Chlorhexidine (CHX) 0,2% was taken as positive control and distilled water was taken as negative control. The mean zone inhibition after 24 hour incubation measured for *Stevia*, *Thulasi* and *Aloe vera* was 22,33 mm, 11 mm and 0 mm, respectively. The mean zone inhibition of positive control CHX was found to be 13,6 mm. The antibacterial effect shown by *Stevia* was superior when compared with CHX and *Thulasi*. Thus, *Stevia* product can be strongly recommended as a caries preventing agent after sufficient clinical trials by future research.

2. DISCUSSION

The development of a targeted antimicrobial therapy against *Streptococcus mutans* by selectively killing or inhibiting the cariogenic bacteria within a pathogenic dental plaque, a non pathologic, commensal microbial community could be established. This healthy plaque would then serve as an effective barrier to prevent the subsequent colonization of cariogenic bacteria on the tooth surface, leading to a sustained anti-caries therapeutic effect (Ajagannanavar et al., 2014).

Chlorhexidine 0,2% was considered as the positive control in several studies because it is currently the most potent antimicrobial agent against *Streptococcus mutans* and dental caries. Chlorhexidine is highly substantive,

not being cleared from the mouth for several hour. It acts by altering the bacterial cell wall and interfering with the absorption of bacteria to the teeth (Perry and Beemsterboer, 2007).

The reason why the mean inhibition rates were more with alcoholic Stevia leaves extract than the aqueous form is unknown. However, the reason may be better solubility of the Stevia compound in alcohol or the very presence of alcohol. This finding is in agreement with the findings of other studies (Debnath, 2008; Ajagannanavar et al., 2014).

Salivary pH and buffer are biological indicator for high caries risk (Guo and Shi, 2013). In study conducted by Usha C et al (2017), Stevia rebaudiana leaves extract 0,5% concentration can be used as a mouthwash for moderate to high caries risk patients because decreased the acidic pH and improved the buffer in high risk individuals. The leaves of *Stevia rebaudiana* are a natural sweetener. It has two main glycosides that are stevioside (110-270 times sweeter than sugar) and rebaudioside A (180-400 times sweeter than sugar). Both mouthwashes of Stevia and CHX in study by Usha C (2017) were effective in favorably altering the pH and the buffer. It was found that Stevia mouthwash performs as good as the CHX mouthwash in altering the salivary parameter. This is accordance to a study by Brambila et al (2014) that was found the pH dropped to acidic with sucrose solutions and not with stevia solutions, concluding that stevia is nonacidogenic. Thus, it can be inferred from the current study that stevia extract which contains stevioside is not only antibacterial but also a non fermentable sugar (not cariogenic) compare with sucrose and tends to lower the acidic pH of saliva.

The presence of numerous essential oils is attributed to be one of the reasons for the antibacterial action (Hossain et al., 2010). It also contains non glycosidic diterpenes, flavonoids and phenolic compound. Steviol glycosides have also shown to be potent antioxidants, which might retard the growth of cariogenic organism. This has high correlation to the total phenolic and flavonoid contents of the extract (Wölwer-Rieck, 2012). The antibacterial effect shown by Stevia leaves extract 50% was superior when compared with 0,2 % CHX and other herbal product in result study conducted by Sreekumar S and Hegde VK (2018), based on higher zona inhibition against *Streptococcus mutans*.

3. CONCLUSIONS

Antimicrobial potency of *Stevia rebaudiana Bertoni* leaves extract as herbal mouthwash was superior against *Streptococcus mutans*, thus, can be strongly recommended as a caries preventing agent. The presence of steviol glycosides, numerous essential oil, flavonoids and phenolic compound, as antimicrobial property of *Stevia rebaudiana Bertoni* leaves extract appears to offer protection against *Streptococcus mutans* as the main causative agent of dental caries.

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