

Comparison Of Antibacterial Effects Between Aloe Vera And Sodium Fluoride On The Amount Of Streptococcus Mutans Colony (ATCC) In Vitro

Yetty Herdiyati Nonong, Arya Arta Augusta, Mieke Hemiawati Satari, Willyanti Soewondo

Abstract: Streptococcus mutans is a bacterium that plays an important role in the process of caries. These bacteria can produce glucosyltransferase (GTF) enzymes which can change the disaccharide, particularly sucrose into glucan that serves as a medium for the initial attachment of bacteria to the tooth surface and produce acids in the metabolic process which is the beginning of dental caries. Aloe vera is natural materials that has a lot of active substances that contains antibacterial effects. Sodium fluoride is a material commonly used in the prevention of caries, serves to remineralization of the teeth and has anti-bacterial properties. The purpose of this study was to analyze differences in decline in the number of colonies of Streptococcus mutans after in vitro administration of Aloe vera and Sodium Fluoride. This research was conducted using pure experimental research on the media containing artificial saliva, enriched with sucrose and the culture of Streptococcus mutans (ATCC 25 175) was incorporated in it. Samples are given treatments of Sodium Fluoride and Aloe vera extracts to see the difference in the number of bacterial colonies. Statistical analysis uses the t test to see a decrease in the number of colonies of Streptococcus mutans between treatment Aloe vera and Sodium Fluoride. T-test equality of two mean the two populations doesn't show a significant difference between the decline in the group of Aloe vera and Sodium Fluoride group, with p-value of 0.2144. This study concludes that there is no difference in the decreasing in the number of colonies of Streptococcus mutans significantly after in vitro administration of Aloe vera and Sodium Fluoride.

Index Terms: Streptococcus mutans, Aloe vera, sodium fluoride, antibacterial effects, glucosyltransferase enzyme, caries, remineralization

1 INTRODUCTION

Dental caries is a chronic multifactorial disease which is a progressive demineralization in hard tissue of the tooth surface by acid derived from the fermentation of food waste that accumulates on the tooth surface.¹ Caries process involves many factors and one of them is bacteria. Streptococcus mutans is a bacterium that is most responsible in the process of caries and is a highly cariogenic bacterial pathogens with high asidogenik level so that it can destroy the hard tissue of the tooth.^{[1],[2]} Streptococcus mutans is a bacterium capable of breaking down sucrose into glucan and fructan that can be fermented by bacteria to acid. Fermented acid irritates the outer surface of the tooth and enamel demineralization is the beginning of dental caries.^[3] Various ways and materials to prevent caries has been widely studied. One of them was Miller in 1890 who stated that the antiseptic ingredient could kill or reduce the number and activity of bacteria. Antiseptic ingredients can be used to eliminate the accumulation of biofilm and can even damage the bacterial cell.^[4] Fluor has long been used as the gold standard in caries prevention. Fluor is safe and effective in preventing and controlling dental caries.^[5] Application of topical fluoride can reduce the incidence of caries by preventing the demineralization of enamel, enhancing remineralization of enamel lesions and inhibiting the activity of bacterial enzymes.^[6] Fluor is a halogen agent which can inhibit the use of carbohydrate by bacteria to resist the enzymes used in the process of glycolysis so that fluor acts as a bacteriostatic agent.^[7] Herbal ingredients are widely used as an alternative material in the field of health. Aloe vera is one of the herbal ingredients that has many health benefits because of its many benefits. Aloe vera contains many active substances including amino acids, anthraquinone, enzymes, minerals, vitamins, lignin, monosaccharide, polysaccharide, salicylic acid, saponins, hormones, tannins, lectin, and sterols.^{[8],[9],[10],[11]} Anthraquinone is a phenol compound contained in Aloe vera. Low concentrations of phenolic compounds can inhibit the growth of bacteria by causing lysis of bacterial cells.^{[10],[12]} It

can be applied to the prevention of caries with one of the main factors that cause caries is bacteria.

2. RESEARCH METHOD

The study was conducted at the Laboratory of Chemistry Faculty of Mathematics and Natural Sciences Universitas Padjadjaran in media containing artificial saliva, enriched with sucrose and fed cultured Streptococcus mutans (ATCC 25 175) in it . Samples are given treatments of Sodium Fluoride and Aloe vera extracts to see the difference in the number of bacterial colonies. The samples were divided into 6 groups of NaF - 1 (NaF day 0), NaF - 2 (NaF day 1), NaF - 3 (NaF day 2), AV - 1 (Aloe vera day 0), AV - 2 (Aloe vera day 1), AV - 3 (Aloe vera day 2). In this study, differences in the number of colonies of Streptococcus mutans were calculated using colony counting method by means of colony counter and expressed with a numerical scale and units of CFU / mL in dilution 10-3 to 10-6 with the Duplo replication on Muller Hinton agar medium enriched with sucrose 20 %. Examination of the number of colonies of bacteria Streptococcus mutans was performed after 1 hour incubation at group NaF - 1 and AV - 1. At group NaF - 2 and AV - 2, examination was performed after 1 day of incubation , and after 2 days of incubation in group NaF - 3 and AV - 3. Prior to this research, the Minimum Inhibitory Concentration checks have been conducted on Aloe vera extract and Sodium Fluoride. This is done to determine the most minimal concentration in material or extracts to be used to inhibit the growth of colonies of Streptococcus mutans. Minimum Inhibitory Concentration Test results on Aloe vera extract is 18.75 % and the examination results on the Minimum Inhibitory Concentration Sodium Fluoride is 1250 ppm. Aloe vera used in this study were taken from the plants cultivation area in the Chemical Laboratory of the Faculty of Mathematics and Natural Sciences Universitas Padjadjaran. This plant has been identified as Aloe barbadensis Miller or Aloe vera. The manufacture of Aloe vera extract is done in Laboratory of Chemistry Faculty of Mathematics and Natural Sciences Universitas Padjadjaran.

3 STATISTIC TEST

Results of the study will be tested statistically using t test to see equality between Aloe vera and Sodium Fluoride based on a decrease in the number of colonies of Streptococcus mutans.¹³

4 RESULTS

Table 1. Examination of Number of Streptococcus mutans colonies of group Sodium Fluoride (in CFU / mL)

Dilution Concentration	Natrium Fluorida 0 day	Natrium Fluorida 1 day	Natrium Fluorida 2 days
	The average number of colonies	The average number of colonies	The average number of colonies
10 ⁻³	3		0
	4,5	0	1
	1,5		0
	0		0

Research result on Sodium Fluoride group showed a decrease in the number of colonies ranging from day 0 to day 1. Day 2 after treatment showed stable results when compared to day 1 despite the growing colonies of Streptococcus mutans.

concentration of 10⁻⁴ also showed colonies that grow as much as 6 CFU / ml on the first agar plate first and 3 CFU / ml on the second agar plates. At a dilution concentration of 10⁻⁵, the colonies are still visible as much as 2 CFU / ml on the first agar plate and 1 CFU / ml on the second agar plates.

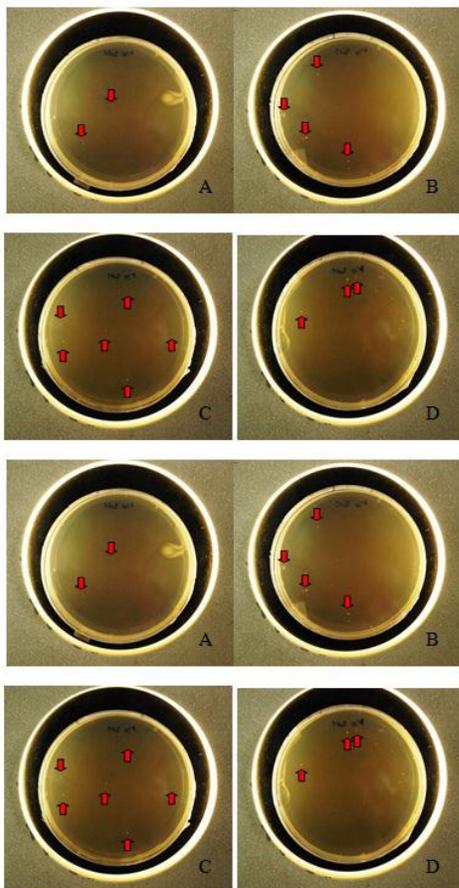


Figure 1. NaF agar plate 0 day

Day 0 indicates there are colonies that grow at dilution concentrations of 10⁻³ as much as 2 CFU / ml on the first plate and 4 CFU / ml on the second agar plates. At a dilution

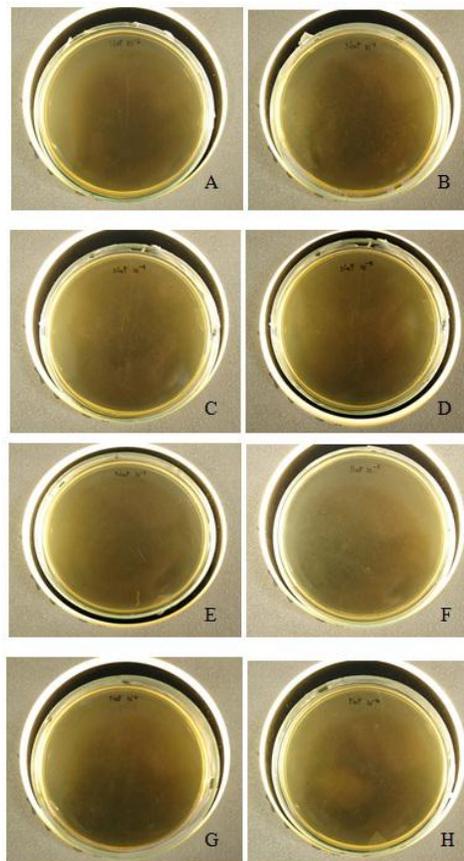
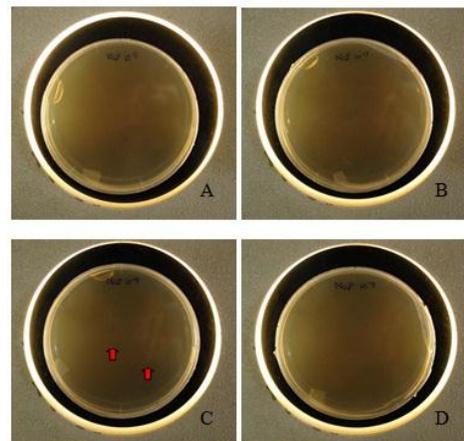


Figure 2. NaF agar plate 1 day

One day after the treatment showed that no colonies grew on agar plate for each dilution concentration.



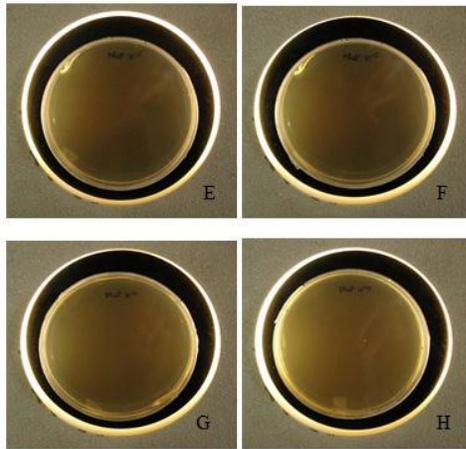


Figure 3. NaF agar plate 2 days

Day 2 overall showed good results in a decrease in the number of colonies of Streptococcus mutans. However, the dilution concentration of 10^{-4} on the first agar plate still shows Streptococcus mutans colonies that grow on as many as 2 CFU / ml.

Table 4-4 Examination of Number of Streptococcus mutans colonies of group Aloe vera (in CFU / mL)

Dilution concentration	Aloe vera 0 day	Aloe vera 1 day	Aloe vera 2 days
	The average number of colony	The average number of colony	The average number of colony
10^{-3}	5	0,5	0
10^{-4}	11,5	0	0
10^{-5}	0,5	0	0
	0	0	0

Research result on Aloe vera group showed relatively more colony growth than the Sodium Fluoride group.

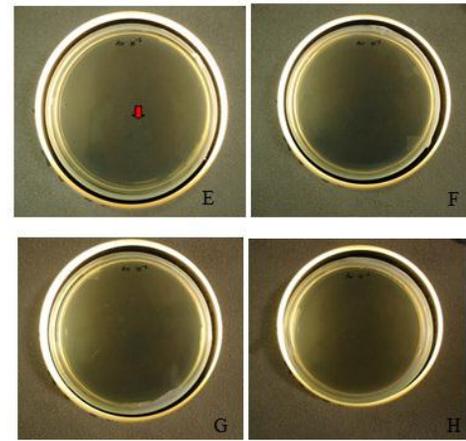
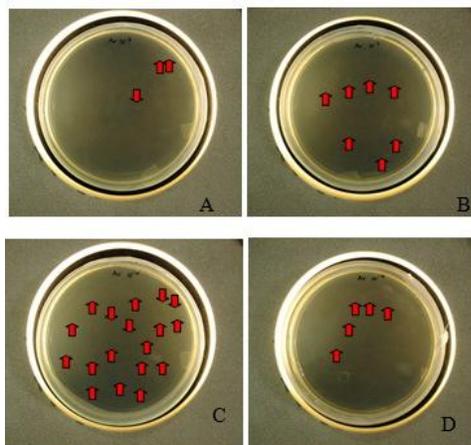


Figure 4. Aloe vera agar plate 0 day

Day 0 showed colonies that grew at dilution concentrations of 10^{-3} as much as 3 CFU / ml on the first agar plate and 7 CFU / ml on the second agar plate. At a dilution concentration of 10^{-4} the colony grew as much as 18 CFU / ml on the first agar plate and 5 CFU / ml on the second agar plate. At a dilution concentration of 10^{-5} , the colonies grew as much as 1 CFU / ml on the first agar plate.

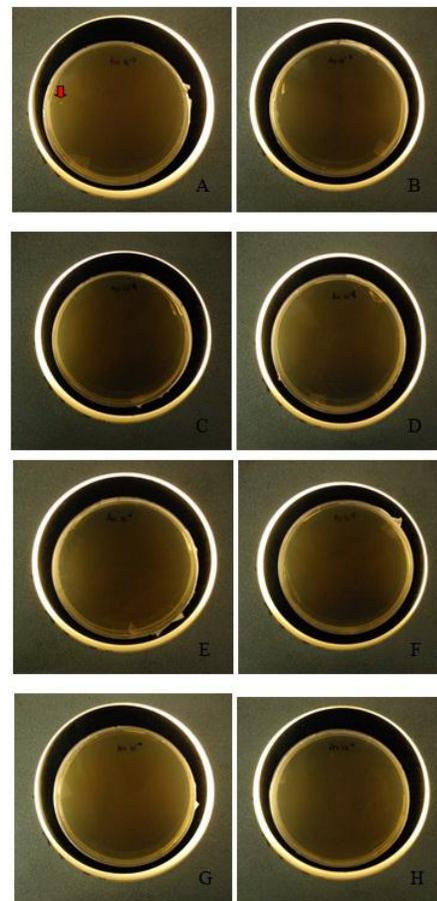


Figure 5. Aloe vera agar plate 1 day

Day 1 show good results with a decrease in all dilution concentrations. Colony growth only occurred on the first agar plate at a dilution concentration of 10^{-3} as much as 1 CFU / ml.

On the other dilution concentration, the plate looked clean and it signifies that there was no colony growth.

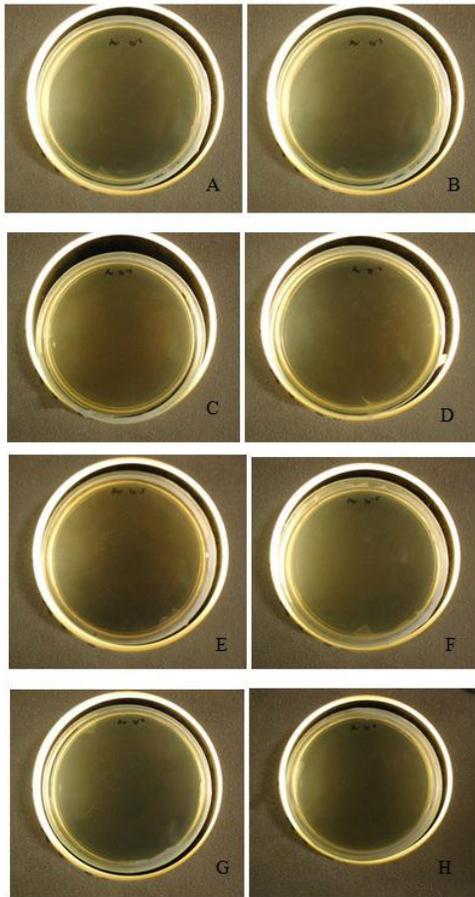


Figure 6. Aloe vera agar plate 2 days

Day 2 showed that the decrease in the number of colonies was good with no colonies growing on each dilution concentrations. It can be seen with the clean plates at each concentration dilution that were planted.

4.1 Statistic Test Results

Statistical test used t test equality of two mean of the two populations to determine the difference in decreasing the number of colonies of Streptococcus mutans after the administration of Aloe vera and Sodium Fluoride with $\alpha < 0.05$.

Table 3. T test analysis to determine the average of the number of Streptococcus mutans colony after Aloe vera and Sodium Fluoride treatment

	Pvalue			Value t _{count}	Means
	NAF0 and NAF1	NAF0 and NAF2	NAF1 and NAF2		
AV0 and AV1	0,2144			0,82	Non Significant
AV0 and AV2		0,1663		1	Non Significant
AV1 and AV2			0,3308	-0,45	Non Significant

In the statistical test with t test, a significant difference was not seen between the average number of Streptococcus mutans colony in the Sodium Fluoride group day 0 and 1 with a mean number of Streptococcus mutans colony in the Aloe vera group day 0 and 1, with p value 0.2144 and tcount 0.82. On the average number of Streptococcus mutans colony in the Sodium Fluoride group day 0 and -2 with the average number of Streptococcus mutans colony in the Aloe vera group day 0 and 2, a significant difference was not seen with p value 0.1663 and tcount 1. In the mean number of Streptococcus mutans colonies in the Sodium Fluoride group day- 1 to -2 with the average number of Streptococcus mutans colonies in the Aloe vera groups day-1 to 2, a significant difference was not seen with p value of 0,3308 and tcount -0.45. Conclusion : there is no significant differences in the decrease in the number of Streptococcus mutans colonies after treatment Aloe vera and Sodium Fluoride.

5. DISCUSSION

Streptococcus mutans is a bacteria that has an important role in the caries process. One of the cariogenic properties owned by these bacteria are the ability to produce an enzyme called extracellular glucosyltransferase (GTF). These enzymes convert the disaccharide, particularly sucrose into glucan which is a component that serves as a medium for the initial attachment of bacteria to the tooth surface, facilitating the accumulation of bacteria and are useful in the process of metabolism that produce acid.¹⁴ Streptococcus mutans is able to attach to the surface of the pellicle on the tooth enamel and synthesize polysaccharide which is an important factor in the formation of caries.^[14,15] This study was conducted to determine the decrease in the number of Streptococcus mutans colonies in vitro after treated with Sodium Fluoride and Aloe vera then incubated for 0-2 days at a temperature of 37°C with anaerobic conditions. Times of 0-2 days is used based on the preliminary research that has been done before. In the preliminary research incubation was for 3 days and the result showed no colonies growing on both materials that were applied. To know the process of reduction of the number of colonies, the examination was carried out starting from day 0 to day 2 to get more detailed data from a decrease in the number of colonies after administration of Aloe vera and Sodium Fluoride. The concentration of Sodium Fluoride and Aloe vera used are based on the results of the examination conducted by Minimum Inhibitory levels before the study began. Of the examination, Minimum Inhibitory Levels of Sodium Fluoride for 1250 ppm (1.25 %) and the minimum inhibitory concentration of Aloe vera extract of 18.75 % is obtained. Streptococcus mutans colony was calculated by the counting colony method with a colony counter equipment and expressed with a numerical scale and units of CFU / mL in dilution of 10⁻³ to 10⁻⁶. The concentration of sucrose used on agar plates and the media is 20%^[16] It is intended so the bacteria can grow well and have a fairly large colony to facilitate the colony counting process later. This is consistent with previous studies conducted by Hasslof et al, but the agar media used are different in this study. The Agar Media used in this study is Muller Hinton whereas Hasslof research used TYBS20. In the group of Sodium Fluoride, a pretty good decrease in the number of colonies happens from day 0 to day 2. It can be proved that Sodium Fluoride has antibacterial properties with a decrease in the number of colonies of Streptococcus mutans. The decrease is due to the mechanism

of fluoride that can inhibit enzymes needed by Streptococcus mutans to metabolic processes. Fluor can intervene in glucose transport into the cell through a system of phosphoenolpyruvate (PEP) –dependent phosphotransferase (PTS), because PEP is formed by 2-phosphoglycerate through the action of enolase sensitive fluor.^[17] Enolase is an enzyme used in the process of glycolysis in turn 2 Phosphoglyceric be fosfofenolpiruvat.^[18] Inhibition of this enzyme activity causes the transport of glucose 6 phosphate disrupted so that the metabolic processes of bacteria into terganggu.^[18] Disruption of this metabolic process will lead to a lack of nutrition for the bacteria so that the growth of bacteria will be disturbed and decreased the number of colonies. Fluor is also capable of inhibiting another enzyme system required by Streptococcus mutans in the process of glycolysis which is glucosyltransferase and fruktosiltransferase.^{[19],[20]} In addition, Sodium fluoride may also interfere with intracellular polysaccharide synthesis thus limiting the supply of nutrients for the Streptococcus mutans.^[19] Aloe vera group also showed good results in a decrease in the number of colonies from day 0 to day 2. This decrease in the number of colonies proves that Aloe vera also has antibacterial properties. Aloe vera is a natural ingredient of which has many benefits for human health. This material has many active substances that has anti-bacterial capabilities, such as anthraquinone and quinone. Anthraquinone and quinone contained in Aloe vera have antibacterial effects antibakteri.^[21] This antibacterial effect works by blocking the action of an enzyme in the biosynthetic process peptidoglycan and lipopolysaccharide / lipotekhoat, damaging the plasma membrane and causes disruption of membrane permeability so that bacteria growth can be inhibited.^[12] Anthraquinone also have similar properties with soaps that can lower the surface tension of the cytoplasmic membrane of the bacterial cell so that the cell membrane permeability down. Saponin-containing glycosides can dissolve lipids in the cell membrane of bacteria (lipoproteins), thus making the surface tension of lipid to be down, and cause bacterial cell function becomes abnormal, lysis and mati.^[12] The ability of anti-bacterial causes a decrease in the number of colonies of Streptococcus mutans. Other ingredients such as acids aloetik has the effect of a natural antibiotic that works synergistically with barbaloin, isobarbaloin, and aloe-emodin.^[22] Aloe-emodin has polyphenolik structure that is able to inhibit the protein synthesis of the bacterial cell, so that it works as an antibacterial and antiinflamasi.¹² This is similar to research conducted Fani and Kohanteb 2011. The research states that Aloe vera most effectively inhibit Streptococcus mutans compared with Aggretibacter actinomycetemcomintas, Porphyromonas gingivalis, Bacteroides fragilis with a minimum inhibitory concentration of 12.5%, the width of inhibition zone of 10mm.^[22] Another study conducted by George et al, suggests that Aloe vera in gel form have the same effectiveness with a toothpaste containing fluoride on bacteria growth S.mitis.^[23] Statistically, a decrease in the number of colonies on Aloe vera group has no significant difference with a decrease in the group of Sodium Fluoride or in other words a decrease in the number of colonies of Streptococcus mutans in vitro is equivalent. This shows that Aloe vera has antibacterial capabilities similar to Sodium Fluoride. The use of Aloe vera as an alternative material to reduce the rate of growth of Streptococcus mutans is an effective step in the effort to reduce the rate of occurrence of dental caries. This material is also a natural substance that has many properties

and are easily found in the environment around us. However, the use of Aloe vera can not be used as a single agent in caries prevention measures as Aloe vera does not have any effect on the remineralization of teeth. The combination of Aloe vera with Sodium Fluoride may produce a material that can provide better results in prevention of caries because it has antibacterial capability and power remineralization of teeth. Levels of use of Aloe vera should also be considered as Aloe vera has a laxative effect which can irritate the digestive system when overly consumed.^[12]

6 CONCLUSION

Based on these results it can be concluded that there was no significant difference in decreasing the number of colonies of Streptococcus mutans after administration of Aloe vera and Sodium Fluoride. In other words, Aloe vera has anti-bacterial capabilities equivalent to Sodium Fluoride in terms of decreasing the number of colonies of Streptococcus mutans.

ACKNOWLEDGMENT

The authors wish to thank Dr Nina Djustiana, drg, M.Kes as the dean of Faculty of Dentistry Universitas Padjadjaran and Dr. Eriska Riyanti, drg, Sp. KGA as the head of Pedodontic Department Faculty of Dentistry Universitas Padjadjaran.

REFERENCES

- [1] Karpinski TM, Szkaradkiewicz AK. Microbiology of dental caries. Journal of Biology and Earth Sciences. 2013;3:1.
- [2] Amin MS, Harrison RL, Benton TS. Effect of povidone-iodine on Streptococcus mutans in children with extensive dental caries. J Ped Dent 2004;26:1.
- [3] Pratiwi T, Sutadi H, Mangundjaja S, Apriati Y. Pengaruh sorbitol dalam permen terhadap populasi Streptococcus mutans dalam saliva. Majalah Kedokteran Gigi Dental Journal. 2001;34:3a.
- [4] Scheie AA, Petersen FC. Antimicrobials in caries control. Dalam Fejerskov O, Kidd E. Editor. Dental caries. The disease and its clinical management. 2nd edition. Blackwell Munksgard. 2008.266-271.
- [5] American Academy of Pediatric Dentistry. Guideline of Fluoride therapy. Clinical Guideline of AAPD. 2014.171.
- [6] Weyant RJ, Tracy SL et al. Topical fluoride for caries prevention: full report of the updated clinical recommendations and supporting systematic review. A report of the ADA Council on Scientific Affairs. 2013.2-3
- [7] Poureslami HR, Barkam F, Poureslami P, Salari Z, Salari S. Comparison of Antibacterial Effect of Fluoride and Chlorhexidine on two Cariogeni Bacteria: An in Vitro Study. Journal of Dental Biomaterials. 2014;1:1.
- [8] Shelton RM. Aloe vera Its Chemical and Therapeutics Properties. International Journal of Dermatology. 1991;30:10.

- [9] World Health Organization. Aloe vera gel. Dalam World Health Organization. WHO monograph on selected medicinal plants Vol. 1. WHO. 1999. 43-48.
- [10] Aloe vera properties: Properties of Aloe Barbadensis Miller Constituents. Diunduh dari <http://www.aloevera-usa.com/aloeprop.htm> tanggal 20 Oktober 2014.
- [11] Subramanian S, Kumar DS, Arulselvan P, Senthilkumar GP. In vitro Antibacterial and Antifungal Activities of Ethanolic Extract of Aloe vera Leaf Gel. *Journal of Plant Sciences*. 2006;1:4. 348-355.
- [12] Hamman HJ. Compositions and Applications of Aloe vera leaf gel. *Molecules*. 2008;13.1599-1616.
- [13] Warrens MJ. A Comparison of Cohen's Kappa and Agreement Coefficients by Corrado Gini. *IJRRAS*. 2013. 16 (3).
- [14] Quivey RG. Caries. Dalam: Lamont RJ et al. Editor. *Oral microbiology and immunology*. ASM Press; 2006. 234 – 237.
- [15] Selwitz RH, Ismail AI, Pitts NB. Dental caries. *Lancet* 2007; 369: 51–59.
- [16] Forsten SD, Bjorklund M, Ouwehand MC. Streptococcus mutans, Caries and Simulation Models. *Nutrients*. Finland. 2010. (2).290-298
- [17] Tafu M, Masutani T, Takemura Y, Toshima T, Chohji T. Effect of Hydroxyapatite on Reaction of Dicalcium Phosphate Dihydrate (DCPD) and Fluoride Ion. *Bioceram Dev Appl*. 2013.1-3
- [18] Barandozi FN. Antibacterial activities and antioxidant capacity of Aloe vera. *Organic and Medicinal Chemistry Letters*. 2013.3:5.
- [19] Deery C, Toumba KJ. Diagnosis and prevention of dental caries. Dalam Welbury R, Duggal MS, Hosey MT. *Paediatric Dentistry*. 4th edition. Oxford University. United Kingdom. 2012.94-95
- [20] Kumar KPS, Bhowmik D, Chiranjib, Biswajit. Aloe vera : A Potential Herb and its Medicinal Importance. *Journal of Chemical and Pharmaceutical Research*. 2010, 2(1): 21-29.
- [21] Karim B, Bhaskar DJ, Agali C, Gupta D, Gupta RK, Jain A, Kanwar A. Effect of Aloe vera Mouthwash on Periodontal Healths: Triple Blind Randomized Control Trial. *OHDM*. 2014;13:1. 14-19.
- [22] Fani M, Kohanteb J. Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. *Journal of Oral Science*. 2012;54:1. 15-21.
- [23] George D, Bhat SS, Antony B. Comparative evaluation of the antimicrobial efficacy of Aloe vera tooth gel and two popular commercial toothpastes: An in vitro study. *General Dentistry*. 2009. 238-241.