Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2016 June; 49(2): 104–109

Research Report

Antibacterial effect of 70% ethanol and water extract of cacao beans (*Theobroma cacao L.*) on *Aggregatibacter actinomycetemcomitans*

Ayu Rafania Atikah, Hendrik Setia Budi, and **Tuti Kusumaningsih**
Departement of Oral Biology
Faculty of Dental Medicine, Universitas Airlangga
Surabaya - Indonesia

ABSTRACT

Background: *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) is a Gram negative bacteria that form a subgingival plaque causing periodontitis. Nowadays, many natural resources can be used as a basic ingredient of drugs. One of the resources used as an antibacterial material is cacao bean. It contains of polyphenol flavonoids, such as catechin, epicatechin, anthocyanin, and proanthocyanidin. Chemical compounds contained in ethanol extract and water extract are different in quantity from those in cocoa beans.* ***Purpose:*** *This research aimed to find out difference in antibacterial activity between the 70% ethanol and water extract of cacao beans (Theobroma cacao L.) on A. actinomycetemcomitans.* ***Method:*** *This research was an in vitro laboratory experiment. The serial dilutions was performed on the 70% ethanol and water extract of cacao beans a concentration of 100% to 3.125%. At each concentration, the 70% ethanol and water extract of cacao beans were added with grown bacterial suspension of A. actinomycetemcomitans. After they were incubated for 24 hours, the bacteria grown on Luria Berthani media were observed. Bacteria colonies then were measured in CFU/ml.* ***Result:*** *There were significant differences in bacterial colonies grown at the concentrations of 6.25% and 3.125% between the 70% ethanol extract of cacao beans and the water extract of cacao beans as p-value = 0.000 ($p<0.05$).* ***Conclusion:*** *70% Ethanol beans and water extract of cacao beans have antibacterial activity against A. Actinomycetemcomitans. The concentrations of MIC and MBC extracts were 6.25% and 12.5% respectively.*

Keywords: *Aggregatibacter actinomycetemcomitans; Cacao beans; antibacterial effect*

Correspondence: Hendrik Setia Budi, Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga. Jln. Mayjend. Prof. Dr. Moestopo no. 47 Surabaya 60132, Indonesia. E-mail: hendriksetiabudi@gmail.com

INTRODUCTION

Periodontal disease is an infectious disease of the oral cavity that can be suffered by teens and adults. Based on Health Research (RISKESDAS) in 2013, the prevalence of oral and dental problems, including periodontal disease reached 25.9%. The most common periodontal tissue diseases are gingivitis and periodontitis.[1-3]

Periodontitis is an infectious disease resulting in inflammation of the tissues supporting teeth, gingival attachment loss progressively, and bone loss. The disease is caused by the induction of facultative anaerobic bacteria and Gram-negative bacteria. One of the Gram-negative anaerobic bacteria that plays a role in the formation of subgingival plaque causing periodontitis is *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*).[1,4]

A. actinomycetemcomitans are Gram-negative bacteria that have a small, non-motile, capnophilic, fermentative coccobacillus form. *A. actinomycetemcomitans* can be found in dental plaque, periodontal pockets, and buccal mucosa in 36% of patients in the normal population.[1] *A.actinomycetemcomitans* release virulence factors, deliver adhesion on oral surfaces, inactivate the host immune response, as well as induce inflammation and tissue

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i2.p104-109

damage. *A. actinomycetemcomitans* are also frequently detected in periodontal tissues of healthy people and then can be grouped into a part of the normal flora in oral cavity.[5]

Currently, some herbal ingredients have been developed as an alternative to medicine ingredients, one of which is cocoa beans. Indonesia ranks on the third position in the world cocoa producers. Cocoa production in Indonesia has the potential to be improved, but it depends on political and local economic factors.[6] The size of cocoa growing areas in Indonesia in 2002 has reached 776, 900 hectares scattered in all provinces, except in the capital city of Jakarta (DKI).[7]

Cocoa is composed of stems, fruits and seeds, flowers, roots, bark seeds, and leaves. Cocoa (*Theobroma cacao L.*) has polyphenolic compounds, such as catechin, epicatechin, anthocyanin, proanthocyanidin, phenolic acid, condensed tannins, flavonoids, and other small compounds.[8] Cocoa polyphenols are useful as an antioxidant, anticarcinogenic, anti-inflammatory, and antimicrobial.[9] Generally, the majority of people consume cocoa only on the seeds alone. Several researches also have shown that cocoa bean skin and cocoa beans can be used as antibacterial ingredients.[7]

Cocoa beans contain polyphenolic flavonoid of flavonol class, namely monomeric catechin and epicatechin flavonoids as well as proanthocyanidin polymerized flavonoids.[10] Flavonols are classified into a flavonoid group composed of several molecules of phenols (polyphenols).[11] Flavonols contained in cocoa stimulate peripheral blood mononuclear cells to secrete interleukin-5 (IL-5) and stimulate immunoglobulin A (IgA) production that protects the oral cavity from *Streptococcus mutans*.[8]

Cocoa beans can also inhibit the growth of *Streptococcus mutans* at an effective concentration of 12.5%.[12] The extract water of cocoa beans and the 70% ethanol extract of cocoa beans even have antibacterial activity against *Escherichia coli* and *Bacillus subtilis*. A research on cocoa beans extracted with the ethanol 70% shows a higher antimicrobial activity against *Streptococcus mutans* and *C. albicans* bacteria than with water.[13]

Cocoa, furthermore, will generate a different antibacterial activity when extracted with different polarities of its solvents.[14] Extraction method varies depending on the samples to be tested. Several different solvents used include water, 70% ethanol, 70% methanol, acetonitrile, diethyl ether, and acetone. 70% ethanol (ethyl alcohol) is a solvent which has a low boiling point and is widely used by industries. 70% ethanol has a boiling point of 70° C so that extraction temperature is used to attract all the components in the basic materials.[15] 70% ethanol is widely used as a solvent of various chemical or natural materials devoted to drugs. In in vitro research, 70% ethanol can disrupt transport ions, namely Na +, K +, and ATP. The use of 70% ethanol in antibacterial material has a risk to human health, especially in liver because of the residual concentration and ethanol exposure.[16]

Polyphenol level, furthermore, will be higher when extracted with a lower solvent polarity. Solvent water will tend to dissolve inorganic compounds and salts of acids or bases.[14] The use of water compared to the 70% ethanol as cosolvent can generate higher residual polyphenols, so the solvent water is considered to be better because it can maintain a high quantity of beneficial compounds.[17]

This study aimed to determine whether there were differences in antibacterial activity between the 70% ethanol extract of cocoa beans and the water extract of cocoa beans against *A. actinomycetemcomitan* bacteria.

MATERIALS AND METHOD

This study was an in vitro laboratory experimental research using randomized post test only control group design. This study was conducted at the Laboratory of Microbiology, Faculty of Dental Medicine, Universitas Airlangga from June to October 2015. Samples were *A. actinomycetemcomitans* bacteria taken from the microbiology laboratory, Faculty of Dental Medicine, Universitas Airlangga, and then cultured in Brain Heart Infusion Broth media (BHIB). Minimal sample size eligible to be analyzed is determined by Lemeshow formula, as many as five.

Manufacture of 70% ethanol extract of cocoa beans and water extract of cocoa beans was conducted. The 70% ethanol extract of cocoa beans and the water extract of cocoa beans were obtained from Integrated Service Unit (UPT) Materia Medika Batu, East Java. Cocoa beans used were non-fermented cocoa beans and macerated using 70% ethanol and water solvent. Having obtained the 70% ethanol extract of cocoa beans and the water extract of cocoa beans, the preparation of *A. actinomycetemcomitans* bacteria then was performed by suspending the BHIB media until turbidity standard was equivalent to 0.5 Mc Farland (1.5 108 CFU/ ml). Manufacture of the 70% ethanol extract of cocoa beans and the water extract of cocoa beans at various concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% was performed using serial dilution method.

The determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in the 70% ethanol extract of cocoa beans and the water extract of cocoa beans against *A. actinomycetemcomitan* bacteria was started from the preparation of the test tube as much as 9 tube. Six test tubes were filled with bacterial suspensions of *A. actinomycetemcomitans* as much as 0.05 ml that had been standardized to 0.5 Mc Farland and mixed with BHIB media as well as the extracts at the various concentrations (100%, 50%, 25%, 12.5%, 6.25 %, and 3.125). The test tube K + (as positive control) containing 0.05 ml bacterial suspension of *A. actinomycetemcomitans* were planted in the BHIB media, while the test tube K- (as negative control) contained only media BHIB and control extracts. Control is useful to make sure that no bacterial contamination in the media.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i2.p104-109

The reading of the results of antibacterial activity in the 70% ethanol extract of cocoa beans and the water extract of cocoa beans against the growth of *A. actinomycetemcomitans* bacteria was conducted by measuring the number of colonies grown on bacteria subculture as much as 0.1 ml of each tube as well as a positive control and a negative control on the Luria Berthani media. Planting on the Luria Berthani media was conducted using spreader, and incubated at 37 ° C for 24 hours.

The results showed 90% of *A. actinomycetemcomitans* bacterial growth was inhibited compared with the positive control used as MIC. The results also indicated 99.9% of *A. actinomycetemcomitans* bacterial death compared with the positive control was used as MBC. MIC and MBC were determined by measuring the number of colonies grown on the Luria Berthani media manually and expressed as CFU/ ml. The calculation was repeated five times by three different observers, and then the mean values were measured.

Processing data was conducted using a statistical analysis test for normality with Kolmogorov-Smirnov test to see whether the data obtained were normally distributed or not. Next, homogeneity test using Levene test was performed. A statistical test for antibacterial power difference in the 70% ethanol extract of cocoa beans and the water extract of cocoa beans at every concentration then was carried out using Independent t-test.

Table 1. The analysis results of the chemical compounds contained in 70% ethanol extract of cocoa beans and the water extract of of cocoa beans

No.	Chemical compounds	70% ethanol extract of cocoa beans	Water extract of cocoa beans
1.	Flavonoids	1.32%	2.18%
2.	Catechins	2.01%	1.22%
3.	Epicatechin	1.36%	1.08%
4.	Anthocyanins	1.74%	1.67%
5.	Proanthocyanidin	1.93%	2.51%

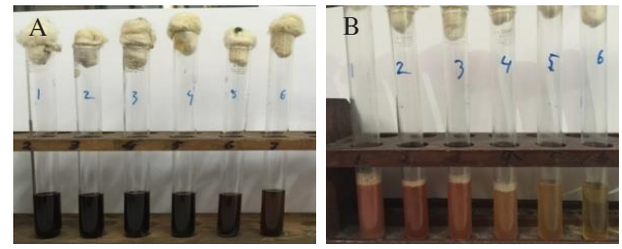

Figure 1. Serial dilution (A) the 70% ethanol extract of cocoa beans and (B) the water extract of cocoa beans against *A. actinomycetemcomitan* bacteria at a concentration of 100% in tube 1; a concentration of 50% in tube 2; a concentration of 25% in tube 3; and a concentration of 12.5% in tube 4; a concentration of 6.25% in tube 5; and a concentration of 3.125% in tube 6.

RESULTS

This research used *A. actinomycetemcomitan* bacteria incubated for 1 x 24 hours at 37° C in the BHIB media, synchronized with the *0.5* McFarland standard. Materials used were non-fermented cocoa beans from Kota Batu, Malang. Next, cocoa extracts were made in UPT Materia Medika Batu in East Java by maceration method using 70% ethanol and water.

Based on the analysis results of chemical compounds contained in the 70% ethanol extract of cocoa beans as well as in the water extract of cocoa beans, there were different dosages of flavonoids, catechins, epicatechin, anthocyanin, and proanthocyanidin contained (Table 1). Catechin, epicatechin, and anthocyanin contained in the 70% ethanol extract of cocoa beans were higher than in the water extract of cocoa beans. Meanwhile, flavonoids and proanthocyanidin contained in the water extract of cocoa beans were higher than in the 70% ethanol extract of cocoa beans.

Serial dilution method is a method usually used in a comparative research of MIC and MBC in both the 70% ethanol extract of cocoa beans and the water extract of cocoa beans against *A.actinomycetemcomitan* bacteria (Figure 1).

During the observation of MIC and MBC in the 70% ethanol extract of cocoa beans and the water extract of cocoa beans at every concentration, the results of serial dilution were planted in Luria Berthani agar media using streak technique as a cross-check to see the growth of bacterial colonies. It was necessary to do because of dark-colored extract material and turbidity occured at any concentration.

Furthermore, based on the observation results on the number of bacterial colonies grown in the Luria Berthani media expressed in colony forming units (CFU), 70% ethanol extract of cocoa beans and water extract of cocoa beans could be able to inhibit and kill *A. actinomycetemcomitan* bacteria (Table 2).

The comparative test on the MIC and MBC in the 70% ethanol extract cocoa beans and the water extract of cocoa beans was conducted using serial dilution method. The results showed that the extracts at the concentrations of 100%, 50%, 25%, and 12.5% contained no bacterial growth. Meanwhile, the mean bacterial growth in the 70% ethanol extract cocoa beans at the concentration of 6.25% was 11.4. On the other hand, the mean bacterial growth in the water extract of cocoa beans at the same concentration was 7.8 (Table 2).

Before the antibacterial power difference analysis test on the 70% ethanol extract of cocoa beans and the water extract of cocoa beans against *A. actinomycetemcomitan* bacteria was performed, the normality test using Kolmogorov-Smirnov test as well as the homogeneity test using *Levene* test were conducted in each group. The results of the

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i2.p104-109

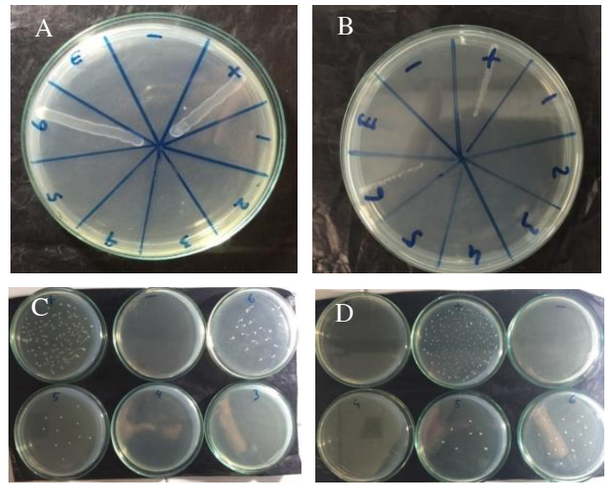

Figure 2. a) Streak results of the 70% ethanol extract of cocoa beans against *A. actinomycetemcomitan* bacteria at any concentration as well as the controls grown in the Luria Berthani media; b) streak results of the water extract of cocoa beans against *A.actinomycetemcomitan* bacteria at any concentration as well as the controls grown in the Luria Berthani media; c) replanting results of the 70% ethanol extract of cocoa beans against *A. actinomycetemcomitan* bacteria at any concentration as well as the controls grown in the Luria Berthani media; d) replanting results of the water extract of cocoa beans against *A. actinomycetemcomitan* bacteria at any concentration as well as the controls grown in the Luria Berthani media.

Table 2. The number of *A. actinomycetemcomitan* bacterial colonies grown in the Luria Berthani media at various concentrations of 70% ethanol extract of cocoa beans and water extract of cocoa beans

Treatment Group	N	The means number of the bacterial colonies (x10^8 CFU/ml)			
		70% Ethanol extract	Water extract	Positive control	Negative control
At the concentration of 100%	5	0	0	109	0
At the concentration of 50%	5	0	0		
At the concentration of 25%	5	0	0		
At the concentration of 12.5%	5	0	0		
At the concentration of 6.25%	5	11.4	7.8		
At the concentration of 3.125%	5	27.2	18		

Note: n: number of replication; 0: no growth on the negative control; 109: the number of bacterial colonies grown as the positive control

Table 3. The mean and standard deviation of the number of bacterial colonies of *A. actinomycetemcomitans*

Treatment control	Mean ± SD		Significance
	70% Ethanol extract	Water extract	
At the concentration of 6.25%	11.4 ± 0.54772 [a]	7.8 ± 0.83666 [b]	P = 0.000
At the concentration of 3.125%	27.2 ± 1.64317 [a]	18.0 ± 1.22474 [b]	P = 0.000

Note: [a b]: significant difference

normality and homogeneity tests showed that data obtained in the water extracts of cocoa beans at the concentrations of 6.25% and 3.125% were normally distributed and homogeneous (p>0.05). Whereas, data obtained in the 70% ethanol extract of cocoa beans at the concentration of 6.25% were not normally distributed (p <0.05), and at the concentration of 3.125% was not homogeneous (p<0.05). Next, Kruskal-Wallis test was carried out to test significant differences in antibacterial activities between the 70% ethanol extract of cocoa beans and the water extract of cocoa beans since there were groups which were not normally distributed and not homogeneous. The results showed value of p was less than 0.05 (p = 0.000), indicating that there was a difference in antibacterial activity between the

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i2.p104-109

70% ethanol extract of cocoa beans and the water extract of cocoa beans at the concentrations of 6.25% and 3.125% (Table 3).

DISCUSSION

This research was an in vitro research aimed to see the difference in antibacterial power beween the 70% ethanol extract of cocoa beans and the water extract of cocoa beans against *A. actinomycetemcomitan* bacteria. Antibacterial agents used in this research were non-fermented cocoa beans since non-fermented cocoa beans actually have higher antibacterial effects than fermented ones. Fermentation and drying processes, however, can reduce the active components of natural materials, such as polyphenol.[18] This research also used a serial dilution method with the concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. *A. actinomycetemcomitan* bacteria grown on the BHIB media were considered as the positive control (+), whlie the bacteria grown on the BHIB media and the control extracts were considered as as the negative control (-). Serial dilution method was used because it can give the significant calculation results of microorganism colonies at small concentrations. Thus, if the serial dilution method is not conducted, there will be a number of colonies stacked so that the calculation of the colonies will be not accurate.[19] Serial dilution method has widely been used for many years.[20]

In the antibacterial activity test, properties owned by the cell wall of bacteria may affect the ability of cocoa bean extracts to inhibit tested bacterial growth.[21] *A.actinomycetemcomitan* bacteria are gram-negative bacteria that have a cell wall in the form of peptidoglycan surrounded by a membrane structure, called as outer membrane. Outer membrane of Gram-negative bacteria has lipopolysaccharide or endotoxin components that can prevent an antibacterial agent to penetrate into the cells.[22,23] In the layers of lipopolysaccharide, Gram-negative bacteria have a system of selection against strange substances so that Gram negative bacteria are more resistant to strange substances than Gram positive bacteria.[21] The presence of polyphenols, such as flavonoids in cocoa beans can penetrate the membrane of Gram-negative bacteria to mediate the cell response.[24]

Flavonoids have antibacterial activity through the barrier function of bacterial DNA gyrase so that replication capabilities and bacterial translation can be inhibited.[25] Flavonoids may cause damage to the permeability of bacterial cell wall, microsomes, and lysosomes as a result of the interaction of flavonoids with bacterial DNA.[26] Catechins, moreover, are natural polyphenolic compounds and secondary metabolites included in the constituent group of tannins.[21] Catechins may damage cytoplasmic membrane causing the release of important metabolites that inactivate bacterial enzyme system. Proanthocyanidin, furthermore, is condensed tannins that can inhibit the formation of cell walls and lead to termination of the crosslinking peptide that combines the glycan chains of peptidoglycan in another chain, causing cell membrane damage.[28]

How to cultivate antibacterial materials, thus, is a thing that needs attention. Antibacterial material can be extracted by using various methods, one of which is by solvent extraction (maceration). Solvents widely used for polyphenol extraction method are water and 70% ethanol. Cocoa, on the other hand, will yield a different antibacterial activity when extracted with a different solvent polarity.[14]

Ethanol is a polar solvent that dissolves polar compounds, while water is an inorganic solvent that dissolves inorganic materials. In addition, cacao bean is an inorganic material because it is derived from plants. To dissolve the cocoa beans using ethanol, therefore, will take a long process because of evaporation process. The process of evaporation can affect the polyphenol content in the extract. Water, on the other hand, is a solvent that can maintain a high quantity of essential compounds contained in natural materials.[17] Water solvent will dissolve inorganic compounds and salts of acids and bases.[14] Cocoa beans diluted with water is more soluble and requires a shorter time than cocoa diluted with ethanol. The analysis results of chemical compounds in the 70% ethanol extract of cocoa beans contain flavonoids (1.32%), catechin (2.01%), epicatechin (1.36%), anthocyanin (1.74%), and proanthocyanidin (1.93%). The water extract of cocoa beans contain flavonoids (2.18%), catechin (1.22%), epicatechin (1.08%), anthocyanin (1.67%), and proanthocyanidin (2.51%). Proanthocyanidin contained in cocoa beans was 58%, catechin amounted to 37%, and anthocyanin amounted to 4%, thus indicating that the water extract of cocoa beans contain more useful chemical compounds than the ethanol extract of cocoa beans.[10]

In the 70% ethanol extract of cocoa beans and the water extract of cocoa beans at the concentration of 6.25%, moreover, there was still bacterial colony growth on the Luria Berthani media after incubated for 1x24 hours. It means that not all bacteria die, or there are barriers to the growth of colonies by 90%.[28] Nevertheless, in the 70% ethanol extract of cocoa beans and the water extract of cocoa beans at the concentration of 12.5%, there was no colony growth. It indicates that the 70% ethanol extract of cocoa beans and the water extract of cocoa beans at this concentration could kill bacteria by 99.9% of the total average bacteria that managed to grow on the positive control and were considered as bactericide.[29,30]

It can be concluded that water and 70% ethanol extract of cocoa beans have antibacterial activities against *A. actinomycetemcomitans*. Both the extracts also have the same MIC and MBC at concentrations of 6.25 and 12.5%.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i2.p104-109

REFERENCES

1. Carranza P, Klokkevold F, Newman M, Takei H. Carranza's clinical periodontology. St. Louis, MO: Saunders Elsevier; 2009. p. 187-192
2. Riset Kesehatan Dasar (Riskesdas) Nasional. Jakarta: Badan Penelitan dan Pengembangan Kesehatan, Departemen Kesehatan Republik Indonesia; 2013. p. 113.
3. Chauhan VS, Chauhan RS, Devkar N, Vibhute A, More S. Gingival and periodontitis diseases in children and adolescents. J Dent & Allie Sci 2012 ; 1(1): 26.
4. Newman M, Takei H, Klokkevold P, Carranza F. Carranza's clinical periodontology. St. Louis, MO: Saunders Elsevier; 2012. p. 185-93.
5. Umeda EJ, Priscila LL, Maria RL, Marcia PAM. Differential transcription of virulence genes in Aggregatibacter actinomycetemcomitans serotypes. Brazil: Departement of microbiology, Institute of Biomedical Sciences, University of Sao Paulo; 2013. p. 4-6.
6. Gu F, Tan L , Wu H, Fang Y , Xu F , Chu Z, Wang Q. Comparison of cocoa beans from China, Indonesia and Papua New Guinea. Foods 2013; 2(2): 183-97.
7. Misnawi. Pemanfaatan Biji Kakao sebagai Sumber Antioksidan Alami. Jember: Pusat Penelitian Kopi dan Kakao Indonesia; 2005. p. 6.
8. Arlorio M, Coisson JD, Travaglia F, Varsaldi F, Miglio G, Lombardi G, Martelli A. Antioxidant and biological activity of phenolic pigments from Theobroma cacao hulls extracted with supercritical CO2. Food Research International 2005; 38(8-9): 1009-14.
9. Hii CL, Law CL, Suzannah S, Misnawi, Cloke M. Polyphenols in cocoa (Theobroma cacao L.). As J Food Ag-Ind 2009; 2(04): 702-22.
10. Sulistyowati, Misnawi. Effects of alkali concentration and conching temperature on antioxidant activity and physical properties of chocolate. International Food Research Journal 2008; 15(3): 297-304.
11. Lee SY, Yoo SS, Lee MJ, Kwon IB, dan Pyun YR. Optimization of Nib Roasting in Cocoa bean processing with lotte-better taste and color process. Food Sci Biotechnol 2001; 10: 286-93.
12. Purnamasari DA, Munadziroh E, Yogiartono RM. Konsentrasi ekstrak biji kakao sebagai material alam dalam menghambat pertumbuhan Streptococcus mutans. Jurnal PDGI 2010; 59(1): 14-8.
13. Gianmaria F. Ferrazzano, Ivana Amato, Aniello Ingenito, Antonino De Natale, Antonino Pollio. Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). Fitoterapia 2009; 80(5): 255–262
14. John NA, Fang Z, Kebitsamang JM, Mohamed LB, Camel L. Quantification of Total Polyphenolic Content and Antimicrobial Activity of Cocoa (Theobroma cacao L.) Bean Shells. Pakistan Journal of Nutrition 2012; 11(7): 574-579
15. Manuchair Ebadi. Pharmacodynamic basic of herbal medicine. 2nd ed. New york: Taylor & Francis; 2007.p. 647.
16. Darmono. Farmasi forensik dan toksikologi. Jakarta: UI Press; 2009. p. 23.
17. Boakye S. Levels of selected pesticide residues in cocoa beans From Ashanti and Brong Ahafo Regions of Ghana. Dissertation. Ghana: Faculty of Physicial Science College of Science; 2012.
18. Smullen J, Koutsou GA, Foster HA, Zumbe A,Storey DM. The antibacterial activity of plant extract containing polyphenols against Streptococcus mutans. Caries Res 2007; 41: 342-9.
19. Mejos Jay Al, CaraignJ.W, De Pano J, Labador AV, Macapagal EM. Bacterial colony isolation using serial dilution techniques. Quezon City: Institute of Biology, College of Science, University of the Phillipines; 2010. p. 6.
20. Seeley HW, VanDemark PJ. Selected exercises from microbes in action a laboratory manual of microbiology. 3 rd ed. Sanfrancisco: Freeman and Company; 1981. p. 37-41.
21. V.S.T. Saito, T.F. dos Santos, C.G. Vinderola, C. Romano, J.R. Nicoli, L.S. Araújo, M.M. Costa, J.L. Andrioli, A.P.T. Uetanabaro. Viability and Resistance of Lactobacilli Isolated from Cocoa Fermentation to Simulated Gastrointestinal Digestive Steps in Soy Yogurt. Journal of food and science 2014; 79(2): doi. 10.1111/1750-3841.12326.
22. Kenneth T. Structure and function of bacterial cells. Online Textbook of Bacteriology2009; 5.
23. Zainal H, Made A, Kasno, Anggraini AD. Uji aktivitas antibakteri propolis lebah madu Trigona spp. Prosiding Seminar Nasional Indonesia, 2006; p. 204-14.
24. Ariza BTS, Mufida D, Fatima N. In vitro antibacterial activity of cocoa ethanolic extract against Escherichia coli. International Food Research Journal 2014; 21(3): 935-40.
25. Misnawi, Wulandari P, Suswati E, Rianul A. Antibacterial effect of ethanol extract cocoa beans (Theobroma cacao) on growth in vitro By Shigella dysentriae. Jurnal Medika Planta 2012; 1(5): 73.
26. Sabir A. Aktivitas antibakteri flavonoid propolis Trigona sp terhadap bakteri Streptococcus mutans (in vitro). Majalah Kedokteran Gigi (Dental Journal) 2005; 38(2): 135-41.
27. Chinami Hirao, Eisaku Nishimura, Masanori Kamei, Tomoko Ohshima, Nobuko Maeda. Antibacterial effects of cocoa on periodontal pathogenic bacteria. Journal of Oral Biosciences 2010; 52(3): 283-291.
28. Duskova M, Karpiskova R. Antimicrobial resistance of Lactobacilli Isolated from food. Czech J Food Sci 2013; 31(1): 27-32.
29. Surbhi Leekha, Christine L. Terrell, and Randall S. Edson. General principles of antimicrobial therapy. Mayo Clin Proc 2011;86(2): 156–167.
30. Akinyemi KO, Oluwa OK, Omomigbehin EO. Antimicrobial activity of crude extracts of three medicinal plants used in south-west Nigerian folk medicine on some food borne bacterial pathogens. African Journal of Traditional, Complementary and Alternative Medicines 2006; 3(4): 13-22.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

DOI: 10.20473/j.djmkg.v49.i2.p104-109