Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2016 June; 49(2): 99–103

Research Report

Antibacterial ability of arabica (*Coffea arabica*) and robusta (*Coffea canephora*) coffee extract on Lactobacillus acidophilus

Willy Wijaya, Rini Devijanti Ridwan, and **Hendrik Setia Budi**
Department of Oral Biology
Faculty of Dental Medicine, Universitas Airlangga
Surabaya-Indonesia

ABSTRACT

Background: *Dental caries is the most commonly dental health problem found in Indonesia. Lactobacillus acidophilus (L. acidophilus) is bacteria playing a role in the development and continuation of caries. Some researches in Dentistry Faculty show that many plants are efficacious for oral health. One of them is coffee bean. Coffee bean containing caffeine, phenolic, trigonelline, and chlorogenic acid is reported to have antimicrobial activity.* ***Purpose:*** *This research aimed to determine the differences in the inhibition of Arabica and Robusta coffee extract to L. acidophilus.* ***Method:*** *This research was an laboratory experimental research. The method used was well diffusion method using seven samples for each treatment group. BHI-A and inoculated L.acidophilus bacteria was poured into each petri dish, and then 8 pitted holes were made with a diameter of 5mm and a depth of 3mm using a ring. Next, Arabica or Robusta coffee extracts at a concentration of 100%, 75%, 50%, 12.5%, 6.25%, and 3.125% were put into each of the pitted hole until it was full, and a negative control was also prepared. They then were put in an incubator at a temperature of 37 °C for 24 hours. Afterwards, measurements and observations were conducted on inhibition zone area.* ***Result:*** *Robusta coffee extract at the concentrations of 100% and 75% had greater inhibitory than Arabica coffee extract ($p<0.05$). Meanwhile, Arabica and Robusta coffee extracts at the concentrations of 50% and 25% had no significant inhibitory difference ($p>0.05$).* ***Conclusion:*** *In conclusion, Robusta and Arabica coffee extracts have inhibitory effects on L.acidophilus. Robusta coffee bean extract, nevertheless, has better inhibitory effects than Arabica coffee bean extract.*

Keywords: *arabica extract coffee; Robusta extract coffee; Lactobacillus acidophilus; antibacterial*

Correspondence: Rini Devijanti Ridwan, Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjend. Prof. Dr. Moestopo no. 47 Surabaya 60132, Indonesia. E-mail: devi.rini@yahoo.co.id

INTRODUCTION

The teeth are part of masticatory apparatus of the digestive system in the human body. A dental health problem mostly found in Indonesia is dental caries. Based on the results of Basic Health Research in 2013 conducted by the Agency for Health Research and Development, the national prevalence of oral health problems was amounted to 25.9%, with the highest proportion of 30.5% in the productive age of 35-44 years and 31.9 % in the age of 44-45 years, thus indicating an increase in the national prevalence of oral health problems compared to in 2007, about 23.5%.[1]

Caries is a demineralization process of dental hard tissue due to metabolic activity of bacteria. This involve of vulnerable hosts, bacteria causing caries, and substrate for bacteria. The bacteria causing caries include *Streptococci, Lactobacilli,* and *Actinomycetes*.[2] *Streptococcus* mutants play a role in the initiation of dental caries, while Lactobacillus play a role in the development and continuation process of caries. The most dominant *Lactobacillus* species causing dental caries are *Lactobacillus acidophilus (L. acidophilus)*.[3] The amount of *Lactobacillus* in dental plaque ranges 104-105 cells/ mg in patients with active caries. The amount of *L. acidophilus* identified in the saliva of the subjects exposed to caries is as

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.p99-103

much as 3-24%. *L. acidophilus* can ferment carbohydrates and produce acid so that pH of plaque will decrease. Decrease in pH repeated within the specified time results in demineralization of vulnerable tooth surface and even caries process is started.[4]

Many researches on plants are useful as an effective herbal treatment with minimal side effects have been conducted. Coffee plant is an export commodity that has relatively high economic value in the world market, in addition to the one commodity developed in Indonesia. Coffee is favored because it has a special taste and aroma. [5] Coffee beans naturally contain various types of volatile compounds, such as aldehydes, furfural, ketones, alcohols, esters, formic acid, and acetate acid. [6] In addition to volatile compounds responsible for the aroma of coffee, the coffee also contains caffeine, phenolic compounds, trigonelline, and chlorogenic acid which reportedly have antimicrobial activity.[7] Chemical composition of the beans may vary depending on types of coffee and geographic conditions in which the coffee is planted.[8]

Coffee, moreover, is a beverage that has been consumed since the days of our ancestors, and now coffee is one of the world's favorite beverage consumption level of 6.7 million tons per year.[9] According to statistics from the International Coffee Organization in 2000-2010, world coffee consumption continues to rise by 3-4% annually. Generally, there are two types of coffee most often consumed namely Arabica and Robusta coffee. Arabica coffee contains caffeine from 0.4 to 2.4% of the total dry weight, while Robusta coffee contains 1-2% of caffeine and 10.4% of organic acid.[8]

Furthermore, a research conducted by Daglia *et al*.,[10] shows that coffee can help to prevent caries. Other researches also show that coffee made from roasted coffee beans has antibacterial ability against certain microorganisms, both Gram positive and Gram negative bacteria, including *S. mutans* as the main cause of dental caries. A research conducted by Aroma states that the smallest concentration of Robusta coffee bean extract even still has inhibitory effects on *S. mutans* growth of 12.5%. Thus, the researchers want to conduct this research on the effects of the antibacterial ability of Robusta and Arabica coffee extracts on *L. acidophilus* bacteria with the objective of verifying and comparing inhibition of Arabica and Robusta coffee extract to *L. acidophilus*.

MATERIALS AND METHOD

This research was a laboratory experimental research using post-test only control group design. Materials used were Arabica and Robusta coffee beans, *L. acidophilus* bacterial culture, sterile distilled water, 96% ethanol, BHI-B media, BHI-A media, standard comparator of caffeine, trigonelline, caffeic acid, polyphenols, chlorogenic acid, diterpene ester, caffeic acid, and coffee oil compounds. Manufacture of Arabica and Robusta coffee extract was performed in UPT Materia Medica, Malang. This research was conducted in the laboratory of Microbiology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya.

In addition, Arabica and Robusta coffee extracts were made in several stages. Arabica and Robusta coffee powder that had been weighed was macerated with 1000 ml of 96% ethanol solution, and then shaken with digital shaker at a speed of 50 rpm for 24 hours. The coffee extract was filtered and accommodated in erlemeyer. The liquid extract obtained by the evaporation process was carried out using a rotary evaporator for 1 hour. The results obtained were re-evaporated above water bath for 2 hours to obtain concentrated extracts of Coffee Arabica or Robusta with 100% concentration.

Moreover, to make the extracts with a concentration of 75%, 0.75 ml of 100% extracts was mixed with 0.25 ml of sterile distilled water. To make the extracts with a concentration of 50%, 1 ml of 100% extract was mixed with 1 ml of sterile distilled water. To make the extracts with a concentration of 25%, 1 ml of 50% extract was mixed with 1 ml of sterile distilled water. To make the extracts with a concentration of 12.5%, 1 ml of 25% extract was mixed with 1 ml of sterile distilled water. To make the extracts with a concentration of 6.25%, 1 ml of 12.5% extract was mixed with 1 ml of sterile distilled water. And, to make the extracts with a concentration of 3.125%, 1 ml of 6.25% extract was mixed with 1 ml of sterile distilled water.

After Arabica and Robusta coffee extracts with various concentrations were completely made, the extracts then were added with sodium carboxyl to change the extracts into the form of a gel. Sodium carboxyl was put little by little as 1-3 grams to achieve the desired consistency.

Furthermore, antibacterial inhibition test was performed using two methods, namely dilution tube method and well diffusion method. Dilution tube method was used to determine the minimum inhibitory concentration and the minimum bactericidal concentration (MIC and MBC) required by Arabica and Robusta coffee extracts in inhibiting the growth of *L. acidophilus*. Meanwhile, well diffusion method was used to determine the inhibitory power generated large extracts of Arabica and Robusta coffee to *L. acidophilus*.

Furthermore, dilution tube method was conducted by serial dilution. Arabica or Robusta coffee extract with the concentration of 100% on the first tube as much as 5ml was poured into the second tube containing 5ml of BHI-B media. The solution in the second tube was taken about 5 ml, then added to the third tube, and so on to obtain the extracts with the concentrations of 100%, 50%, 25%, 12.5%, 6.25% and 3.125%. For Arabica and Robusta coffee extracts with a concentration of 75% was prepared by mixing the Arabica coffee extract or Robuta coffee extract with the concentration of 100% as much as 7.5 ml into tubes that had contained 2.5 ml of BHI-B media. After the serial dilution, 0.1 ml of *L. acidophilus* bacteria equivalent to 0.5 Mc Farland standard was put into each tube with various concentrations. Next, the BHI-B 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.p99-103

were incubated for 1x24 hours with a temperature of 37° C. After the incubation, each tube was planted in the BHI-A media, and then the media were incubated for 1 x 24 hours with a temperature of 37° C. Observation then was performed on the results to know whether there was bacterial growth or not. MIC value was obtained from the lowest concentration, indicating there was no bacterial growth.[11] Meanwhile, to determine MBC, the number of *L. acidophilus* bacterial colony growth on the BHI-A media emerged was observed.[12]

On the other hand, the initial steps in well diffusion method was to make a well hole on each petri dish using a ring as much as 8 wells with a diameter of 5 mm and a depth of 3 mm in the BHI-A media which had been inoculated by *L. acidophilus* bacteria. Next, Robusta or Arabica coffee bean extract with the concentration of 100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125% was put into each well that had been tagged previously until the pitting holes were filled. One hole then was used for a Negative control. After that, they were incubated for 24 hours at 37° C. The amount of inhibition zone was measured using calipers, and then data analysis was conducted.

Finally, normality test was performed using Kolmogorv-Smirnov test, and then homogeneity test was carried out using Levene's test. If the results of both tests show that the data were normal and homogeneous (p>0.05), then T test was conducted to determine whether there were significant differences between the effects of Arabica coffee extract and the effects of Robusta coffee extract on *L. acidophilus*.

RESULTS

The results of dilution method in this research showed that MIC valueobtained in Arabica and Robusta coffee extract to inhibit the growth of *L. acidophilus* was 25%, while MBC was 50% (Figure 1). The mean diameter of the inhibition zone in Arabica and Robusta coffee extracts can be seen in Table 1.

Figure 1 shows the new *L. acidophilus* bacterial growth was emerged at the concentration of 12.5%, while at the concentrations of 100% -25% there was no *L. acidophilus* bacterial growth. Based on these results, it can be concluded that a concentration of Arabica and Robusta coffee extracts, which still can inhibit the growth of L. *acidophilus* is 25%. And, for determining MBC required, the number of *L. acidophilus* colonies was measured.

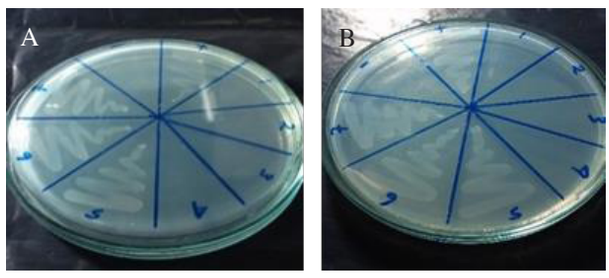

Figure 1. Planting coffee extract dilution culture: (a) Arabica and (b) Robusta on BHI-A media at concentrations of 100-3.125%.

Table 1 shows the number of bacterial colonies growing on the media BHI-A at each concentration of the Arabica and Robusta coffee extracts. It also shows that there was no growth of *L. acidophilus* bacterial colonies at the concentrations of 100%, 75%, and 50%. The growth of new *L. acidophilus* bacterial colonies was also emerged at the concentration of 25%. The number of *L. acidophilus* bacterial colonies increased at the concentration of 12.5%. Based on these results, it can be concluded the MBC value of Arabica and Robusta coffee extracts is equal at the concentration of 50%.

Table 2 shows that the largest diameter mean of the inhibition zones found on Robusta coffee extract at the concentrations of 100% and 75% were 13.83 mm and 12.62 mm. Meanwhile, the largest diameter mean of the inhibition zones found on Arabica coffee extract at the concentrations of 50% and 25% were 9.31 mm and 8.14 mm.

The normality of data obtained was tested using Kolmogorv-Smirnov test. The results of the normality test showed that the data obtained was normally distributed (p> 0.05). After that, homogeneity test was conducted using Levene's test. The results of Levene's test showed the value of p was less than 0.05 in the inhibition zone of Arabica and Robusta coffee extracts at the concentration of 100%, indicating the data were not homogeneous. Meanwhile, the value of p was more than 0.05 in the

Table 1. The number of *L. acidophilus* bacterial colonies given with Arabica and Robusta coffee extracts

	100%	75%	50%	25%	12.5%
Arabica	0	0	0	38	62
Robusta	0	0	0	51	77
C^+			109		
C^-			0		

Note: G+: Positive control group; G-: Negative control group

Table 2. The calculation results of the mean inhibition zone diameter of Arabica and Robusta coffee extract

Concentration	Mean(mm) ± SD	
	Arabica	Robusta
100%	12.53 ± 0.307^a	13.83 ± 0.71^b
75%	10.66 ± 0.373^a	12.62 ± 0.42^b
50%	9.31 ± 1.003^a	9.23 ± 1.07^a
25%	8.14 ± 0.821^a	8.03 ± 0.89^a

Note: Mean: Average diameter of inhibition zone; SD: Standard deviation; N: number of samples; ab: indicating the presence of significant value difference; aa: indicating the absence of significant value difference.

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.p99-103

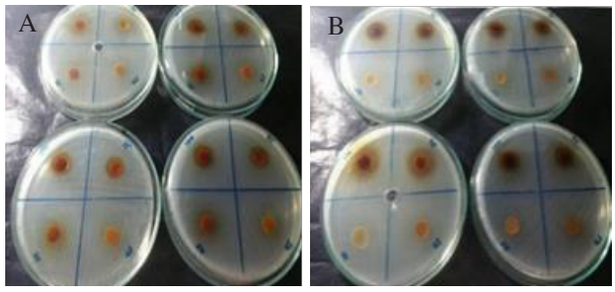

Figure 2. The results of inhibition zone diameter of Arabica coffee extract (a) and Robusta coffee extract; (b) at concentrations of 100-25%.

inhibition zone of Arabica and Robusta coffee extracts at the concentrations of 75%, 50%, and 25%, indicating the data were homogeneous.

After the homogeneity test was conducted, T test then was performed then to know the differences in inhibiting the growth of *L. acidophilus* bacteria between Arabica coffee extract and Robusta coffee extract. The results of T test showed that the value of p was less than 0.05 at the concentrations of 100% and 75%. It means that there was a significant difference in inhibiting the growth of *L. acidophilus* bacteria between Arabica coffee extract and Robusta coffee extract at the concentrations of 100% and 75%. On the other hand, the value of p was more than 0.05 at the concentrations of 50% and 25%. It indicates that there was no significant difference in inhibiting the growth of *L. acidophilus* bacteria between Arabica coffee extract and Robusta coffee extract at the concentrations of 50% and 25%.

DISCUSSION

In this research, the antibacterial inhibitory effects of Arabica and Robusta coffee extracts at various concentrations on the growth *L. acidophilus* were observed using well diffusion method in the BHI-A media. The antibacterial inhibitory effects of Arabica and Robusta coffee extracts were indicated with the existence of a clear zone around the well hole. In other words, the larger the diameter of the clear zone is formed, the greater the inhibitory effects are.[13] Before doing the research on the amount of inhibition zone on Arabica and Robusta coffee extracts, however, the values of MIC and MBC of Arabica and Robusta coffee extracts to inhibit and kill the growth of *L. acidophilus* were measured using dilution tube method on the BHI-B media.

The results showed that the MIC values of Arabica and Robusta coffee extracts to inhibit the growth of *L. acidophilus* were obtained at the concentration of 25%, while the MBC value was obtained at the concentration of 50%. On the other hand, the minimum concentration of Robusta coffee extract in inhibiting *S. mutans* bacteria was at a concentration of 12.5%.[7]

The results of this research, furthermore, showed that the inhibitory zone of Robusta coffee extract was greater than the inhibitory zone of Arabica coffee extract at the concentrations of 100% and 75%. On the other hand, there was no difference in the inhibitory zone of Arabica and Robusta coffee extracts at the concentrations of 50% and 25%. The differences in the diameter of the inhibition zone at each concentration may be due to a large difference in the active substances contained in Arabica and Robusta coffee extracts that are antibacterial, such as caffeine, trigonelline, caffeic acid, and chlorogenic acid. It means that the greater the concentration is, the greater the components of the active substances contained are, as a result, the inhibition zone formed is also different in each concentration.[14] In addition, according to Butler *et al*., an increase and a decrease in inhibition zone are caused by the component substances contained in medicinal plants that can mutually weaken, strengthen, improve, or change completely the effects of the medicinal plants. The quality and quantity of the substances contained in the medicinal plants are determined by environmental factors, such as growing climate, soil, sunlight, and growing conditions until harvesting date.[15]

Based on examination results conducted by in the Laboratory of Research, and Industrial Consultation Agency (Balai Penelitian dan Konsultasi Industri) in Surabaya, East Java, the greatest components contained in Arabica coffee bean extract were *caffeine* (0.18%), trigonelline (0.17%), diterpene ester (0.05%), caffeic acid (0.15%), chlorogenic acid (0.07%), polyphenols (0.54%), and coffee oil (0.09%). On the other hand, the greatest components contained in Robusta coffee bean extract were caffeine (0.21%), trigonelline (0.12%), diterpene ester (0.08%), caffeic acid (0.11%), chlorogenic acid (0.09%), polyphenols (0.72%), and coffee oil (0.08%).

Caffeine and trigonelline are ones of the largest components of the alkaloid compounds found in coffee beans serving as antibacterial.[10] This statement is supported by a research conducted by Nuhu *et al*.,[16] that *trigonelline* contained in Robusta coffee bean extract is positively correlated to a decrease in *S. mutans* biofilm formation through its bacteriostatic action. According to a research conducted by Almeida *et al*.,[17] trigonelline, caffeine, and chlorogenic acid contained do not differ in their antimicrobial activity. Caffeic acid and trigonelline are known to have the same inhibitory effect on the growth of microorganisms. Caffeine and chlorogenic acid are also known to have a very strong antibacterial effect in inhibiting the growth of Serratia marcescens and Enterobacter cloacae. It can be concluded that Robusta and Arabica coffee bean extracts have inhibitory effects on *L. acidophilus.* Nevertheless, Robusta coffee bean extract has a greater inhibition ability than Arabica coffee extract.

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.p99-103

REFERENCES

1. Worotitjan I. Pengalaman karies gigi serta pola makan dan minum pada anak sekolah dasar di desa Kiawa Kecamatan Kawangkoan Utara. Jurnal e-gigi 2013; 1(1): 59-68.
2. Quivey RG, Koo H, Seils J, Abranches J, Robert A, William H. Influence of Apigenin on gtf gene expression in Streptococcus mutans UA159. Antimicrobial agents and chemotherapy. J Microbiology 2006; 50(2): 542-6.
3. Sanders ME, Klaenhammert TR. The scientific basis of Lactobacillus acidophilus NCFM functionality as a probiotic. 2012. p. 319-31.
4. Fitrianti D, Noorhamdani, Setyawati S. Efektifitas ekstrak daun ceplukan sebagai antimikroba terhadap methicillin-resistant staphylococcus aureus in vitro. Jurnal Kedokteran Brawijaya, 2011; 26(4): 3.
5. Wang Y, Ho CT. Polyphenolic chemistry of tea and coffee: a century of progress. Journal of Agricultural and Food Chemistry 2009; 57: 8109-14.
6. Widyotomo S, Sri M. Ekstraksi kafein dari dalam biji kopi. Warta Pusat Penelitian Kopi dan Kakao Indonesia 2007; 22(3): 13-41.
7. Suhad AMS, Ekbal AK, Ali AS. Anti microbial investigation of Suaeda baccata (chenopodiaceae). J Plant Science 2004; 2(1): 49-51.
8. Farida A, Ristanti E, Kumoro AC. Penurunan kadar kafein dan asam total pada biji kopi robusta menggunakan teknologi fermentasi anaerob fakultatif dengan mikroba nopkor MZ-15. Jurnal Teknologi Kimia dan Industri 2013; 2(3): 70-5.
9. Baylin A, Hernandez-Diaz S, Kabagambe EK, SIles X, Campos H. Transient exposure to coffee as a trigger of a first nonfatal myocardial infarction. J Epidemiology 2006; 17(5): 506-11.
10. Namboodiripad A, Kori S. Can coffee prevent caries?. J Conserv Dent 2009; 12(1): 17-21.
11. Mardiyaningsih A, Aini R. Pengembangan potensi ekstrak daun pandan (Pandanus amaryllifolius Roxb) sebagai agen antibakteri. J Pharmaciana 2014; 4(2): 188.
12. Ayini U, Hanina S, Dewi TC. Efek Antibakteri ekstrak daun mimba (Azadirachta indica A. Juss) terhadap bakteri vibrio alganoliticus secara in vitro. Journal of Biology & Biology Education 2014; 6(1): 4-6.
13. Taguri T, Kouno TA. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food borne disease. Biol Pharm Bull 2004; 27: 1965-9.
14. Quivey RG. Influence of apigenin on gtf gene expression in Streptococcus mutans UA159. Antimicrobial agents and chemotherapy 2006; 50(2): 542-6.
15. Mendez CR, Badet C, Yanez A, Domingue ML, Giono S, Richard B, Nancy J, Dorignac G. Identification of oral strains of Lactobacillus species isolated from Mexican and french children. Journal of Dentistry and Oral Hygiene 2009; 1(1): 9-16.
16. Nuhu A. Bioactive Micronutrient in coffee: recent analytical approaches for characterization and quantification. Nigeria: Hindawi Publishing Corporation ISRN Nutrition; 2013. p. 1-13.
17. Almeida AA, Farah A, Daniela AM, Elziria, Beatriz M. Antibacterial activity of coffee extracts and selected cofee chemichal compounds against Enterobacteria. J Agric Food Chem 2006; 54 (23): 8738-43.

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.p99-103