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

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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

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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%. 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. 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). 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...
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 beverages. 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.\(^9\)

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 5 ml 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 Robusta 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...
RESULTS

The results of dilution method in this research showed that MIC value obtained 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.

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 Kolmogorov-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

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Arabica</th>
<th>Robusta</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>75%</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>50%</td>
<td>0</td>
<td>109</td>
</tr>
<tr>
<td>25%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Arabica (Mean±SD)</th>
<th>Robusta (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>12.53 ± 0.307a</td>
<td>13.83 ± 0.71b</td>
</tr>
<tr>
<td>75%</td>
<td>10.66 ± 0.373a</td>
<td>12.62 ± 0.42b</td>
</tr>
<tr>
<td>50%</td>
<td>9.31 ± 1.003a</td>
<td>9.23 ± 1.07a</td>
</tr>
<tr>
<td>25%</td>
<td>8.14 ± 0.821a</td>
<td>8.03 ± 0.89a</td>
</tr>
</tbody>
</table>

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.
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. 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%.

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. 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.

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. This statement is supported by a research conducted by Nuhu et al., 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., 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.
REFERENCES


