

# Effect of ethanol extract of robusta coffee leaves (*Coffea canephora var. robusta*) against *Streptococcus mutans* growth

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**ABSTRACT** The study to determine the inhibition capability of robusta coffee's (*Coffea canephora var. robusta*) leaf extract against *S. mutans* growth. Robusta coffee's (*Coffea canephora var. robusta*) leaf extract was made by maceration method using 96% ethanol. The antibacterial examination using well diffusion on Mueller Hinton Agar (MHA) media. The concentration of robusta coffee's (*Coffea canephora var. robusta*) leaf extract used in this study was 25%, 50%, and 75%. The study was analyzed using the One Way Analysis of Variance (ANOVA) test and continued with the Least Significant Difference (LSD) test. The results showed a value of  $p < 0.05$ , which proves that robusta coffee's (*Coffea canephora var. robusta*) leaf extract has an antibacterial effect on the growth of *S. mutans* with low inhibition potency.

**KEYWORDS:** *Streptococcus mutans*, dental caries, robusta coffee leaves (*Coffea canephora var. robusta*)

## INTRODUCTION

Based on the 2013 Baseline Health Research (Riskesdas), the percentage of oral and dental problems in the Indonesian population in 2007 and 2013 increased from 23.2% to 25.9%. Cavities or dental caries are one of the problems that many children and adults complain about.<sup>1</sup> Dental caries is an infectious disease that damages tooth structures such as the surface of the enamel, dentin, or cementum. This infection may develop into pulp necrosis.<sup>2</sup>

Fitriyanti et al. (2014) reported the prevalence of dental caries in Indonesia is 90.05%, indicating the prevalence of dental caries is still high in Indonesia.<sup>3</sup> Dental caries occurs when the causative factors such as host, agent or microorganisms, substrate or diet, and time are mutually supporting and related to one another.<sup>2, 4</sup> The agent or microorganism factor that plays a role in the process of caries is bacteria. These caries-causing bacteria usually originate from the continuous accumulation of plaque on the teeth.

Bacteria involved in dental caries are; *Streptococcus mutans*, *Streptococcus*

*sanguinis*, *Lactobacillus sp.*, *Actinomyces sp.*, *Veillonella sp.*, *Bifidobacterium sp.*, *Propionibacterium sp.* *Streptococcus mutans* is reported as the primary agent of dental caries. It has properties of aciduric and acidogenic. When these bacteria population increases in the oral cavity it converts these bacteria into pathogens to cause dental caries. Therefore, one of the prevention measures is to control the growth of the cariogenic bacteria *S. mutans* as the leading cause of dental caries.<sup>6</sup>

Treatment options for preventing dental caries are controlling the growth of bacteria in plaque or plaque control. Plaque control can be done mechanically, such as brushing teeth and chemically using antibacterials such as mouthwash. However, the side effects of long-term use of chemicals in mouthwashes such as chlorhexidine can cause discoloration and restoration, burning sensations, and harming the mouth's soft tissues. It is necessary to have alternative treatments such as treatments from herbal plants that have antibacterial effects.<sup>7</sup>

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One of the most common herbal plants in Aceh is the coffee plant. As part of the robusta plant (*Coffea canephora* var. *Robusta*), Robusta seeds also have an antibacterial effect. Tilaar and Kaseke (2016) reported that robusta coffee bean extract (*Coffea canephora* var. *Robusta*) can inhibit the growth of *Enterococcus faecalis*.

The average diameter of the inhibition zone that has been reported is 13.8mm.<sup>8</sup> In general, Indonesians only use coffee beans for drinking, but in West Sumatra, people have also used the leaves (*Coffea canephora* var. *Robusta*). This type of coffee drink from its leaves is actually beneficial for health, warms the body, lowers high blood pressure and increases stamina. Robusta coffee leaves (*Coffea*

*canephora* var. *Robusta*) contain antimicrobial compounds such as alkaloids, saponins, flavonoids, and polyphenols.<sup>9</sup>

Based on Putri's (2018) research, disc diffusion method of robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) has ability to inhibit *S. mutans* growth at a concentration of 25%. In another study by Anggraini (2017) that using ethanol solvent, it was stated that robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) can inhibit the growth of *Lactobacillus acidophilus* bacteria. Based on this, researchers are interested in conducting research to determine the inhibitory ability of robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) againsts *Streptococcus mutans* growth.

## MATERIALS AND METHODS

The *S. mutans* isolates were obtained from the Microbiology Laboratory of the Faculty of Veterinary Syiah Kuala University, Banda Aceh. Samples of robusta coffee leaves (*Coffea canephora* var. *Robusta*) were obtained from Sumatra Barat (West Sumatra Province). The type of research is an experimental laboratory design with a post-test only control group.

Robusta coffee leaves (*Coffea canephora* var. *Robusta*) are obtained from 3rd and 4th stalks of each tree branch. Leaves are washed under running water, drained, and sliced into small pieces (about 2cm in size). Then, let the leaves re-dry for three days at room temperature. Simplicia Robusta coffee leaves (*Coffea canephora* var. *Robusta*) are blended until the coffee leaves are smooth. The fine powder is put into a sterile Erlenmeyer flask by adding 96% ethanol solvent until the powder was immersed completely. The Erlenmeyer flask is tightly closed and left for two days. After the maceration process is complete. The entire filtrate obtained is filtered and concentrated using a rotary evaporator.<sup>10</sup>

A phytochemical test was conducted to determine Robusta coffee leaves (*Coffea canephora* var. *Robusta*). In this study, a phytochemical test was conducted to determine alkaloids, flavonoids, tannins, terpenoids, and saponins from the extract of the Robusta coffee leaves (*Coffea canephora* var. *Robusta*). The pure extract (100%) that has been obtained is diluted with distilled water to get a concentration of 100%, 75%, 50%, and 25%, then homogenized using a vortex. The dilution formula adopted to Permatasari et al.<sup>11</sup>

*Streptococcus mutans* was cultured in Trypticase Soy with Sucrose and Bacitracin (TYS20B) with T streak technique. Petri dishes inoculated with the bacteria, placed in an anaerobic jar and incubated at 37°C for 1x24 hours in an incubator.<sup>12</sup> The *S. mutans* confirmation test carried out using Gram stain, which demonstrates a purple color for Gram-positive bacteria.<sup>16</sup> Using rose, *Streptococcus mutans* put into a tube containing 5ml of 0.85% NaCl solution, then homogenized with a vortex. The turbidity of the solution equalized using Mc densitometer. Farland 0.5 ( $1.5 \times 10^8$  CFU/ml).<sup>15</sup> Analyzing of inhibition of Robusta coffee leaves (*Coffea canephora* var. *Robusta*) extract against *S. mutans* was carried out using the well diffusion method using Mueller Hinton Agar (MHA) media. Then three Petri dish MHAs were made into six wells with a diameter of 6mm and a thickness of 4cm. Then *S. mutans* was inoculated using a hole punch device. Then filled the well with 50 µl of 100%, 75%, 50%, and 25% nutmeg pulp extract, then incubated at 37°C for 24 hours in an incubator. The positive control used chlorhexidine 0.2%, and for negative control using distilled water. The formation of the zone of inhibition around the well is measured using a caliper in millimeters using the overall diameter minus the well diameter.<sup>10</sup> The results were assessed based on Davis dan Stout<sup>15</sup> Data analysis was performed using SPSS software. Data analysis used One Way Analysis of Variance (ANOVA) with the significant difference is  $p < 0.05$ .

## RESULTS

Phytochemical test results showed alkaloids, flavonoids, tannins, terpenoids, and saponins in the

Robusta coffee leaves (*Coffea canephora* var. *robusta*). The culture results of *Streptococcus mutans* on

Trypticase Soy with Sucrose and Bacitracin (TYS20B) media with the T streak technique and incubated for 1 x24 hour at 37°C showed a yellowish-white colony of *S. mutans* with a convex surface. The results of Gram stain show that purple Gram-positive bacteria are cocci and chains form. Antibacterial test results of robusta coffee leaf extract (*Coffea canephora* var.

*Robusta*) on *S. mutans* growth carried out in triplo, showed an inhibitory power as evidenced by the presence of a clear zone around the well containing the extract. Antibacterial robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) against the growth of *S. mutans* is demonstrated in Table 1 below:

Table 1. Antibacterial of robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) against *S. mutans* growth

| Concentration | Inhibition Zone (mm) |      |      | Mean (mm) | Davis and Stout Category |
|---------------|----------------------|------|------|-----------|--------------------------|
|               | I                    | II   | III  |           |                          |
| 25 %          | 0,5                  | 0,7  | 0    | 0,4*      | Weak                     |
| 50 %          | 1,1                  | 0,8  | 1    | 0,63*     | Weak                     |
| 75 %          | 2,33                 | 3,1  | 3    | 2,81*     | Weak                     |
| CHX           | 21,7                 | 22,5 | 20,7 | 21,63*    | Poweful                  |
| Aquades       | 0                    | 0    | 0    | 0*        | None                     |

\*p = 0,000

## DISCUSSION

Robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) positively contains alkaloids, saponins, flavonoids, and polyphenols. All of these compounds are active substances that can inhibit the growth of *S. mutans* bacteria. Alkaloids are known to work by disrupting the peptidoglycan component in bacterial cells. The cell wall layer is unformed entirely and ultimately leads to cell death—the mechanism of inhibition of saponin compounds by reacting with transmembrane proteins of the bacterial cell wall. Sehen forms a strong polymer bond that damages porins and reduces the permeability of bacterial cell membranes. The reduced permeability of the bacterial cell membrane results in the cell being deficient in nutrients, which causes slow growth of bacteria and even causes death. Flavonoids act by inhibiting bacterial growth forming extracellular protein complexes needed for the integrity of bacterial cell membranes.

Polyphenol can denature proteins cause cell components is damage. Some of these components, such as cell membranes, microsomes, and lysosomes, and this effect is irreparable. The mechanism of inhibiting the growth of the microorganisms from polyphenols is by inhibiting the enzyme's performance in the cell wall. Polyphenols also interfere with microorganism interactions to cause adhesions with other microorganisms, known as co-aggregation.<sup>21,22</sup> Besides, *S. mutans* with Gram stain observed under light microscopy is described as coccus-shaped (oval) colonies, colored purple and, arranged in

chains. This display proves that *S. mutans* is a Gram-positive bacterium. Gram-positive bacteria have a thicker peptidoglycan layer whose layer retains a crystal violet dye even when alcohol is applied.<sup>23</sup>

The test results showed that robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) affected *S. mutans*. Based on the classification of inhibition according to Davis and Stout (Table 1.) shows that robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) at a concentration of 25%, 50%, and 75% has a weak inhibitory. One of the factors that contribute is environmental factors. The environmental changes such as rainfall, day and night temperatures, duration and intensity of sunlight, and season may influence leaves' compounds. The age of the leaves also affects the content of active compounds found in leaves. The research of Ramadhan, Aziz and Ghulamahdi (2015) reported that the levels of flavonoids in mature leaves were higher than medium and young leaves.<sup>26</sup> The effect of differences in extract concentrations also affected the ability to inhibit bacterial growth. The greater the concentration, the greater the inhibitory ability of the extract.<sup>58</sup>

The length of time between the freshly picked leaves and the extraction process also affects the leaves' chemical compounds. In this study, the leaves went through a shipping process until the drying process was over 24 hours. This gap causes the leaf wilting process to overtime. It is known, the length of withering time affects the decrease in water content, total phenol, tannin content, and

decreased antioxidant activity in leaves. The study result is in line with Putri's (2018) research on the inhibition of robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) on *S. mutans* growth. This study also used the maceration method and 96% ethanol solvent for extraction with variable concentrations of 25%, 50%, 75%, and 100%. Leaves taken from different areas and using the disc paper diffusion method showed that the robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) could inhibit the growth of *S. mutans* at a concentration of 25%. However, Davis and Stout's classification of inhibition showed a weak category.

Murtafiah (2012) stated that the inhibition of robusta coffee bean extract (*Coffea canephora* var. *Robusta*) on the growth of *S. mutans* using maceration method and 97% ethanol solvent for the extraction process with variable concentrations respectively: 12.5%, 25%, 50%, and 100%. This study also used the well diffusion method to test for

bacterial inhibition. The results of this study indicate that the average diameter of the inhibition zone is greater than the robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) in this study in each concentration. This result proves that beans as a part of the robusta coffee plant have a better antibacterial effect than the leaves.<sup>29</sup>

Several factors affect the results of the extract's contents, one of which is selecting the solvent. The choice of a particular solvent is known to be influenced by the following factors: selectivity, solubility, density, reactivity, and boiling point. Besides, the extraction method can also affect the content of the extract constituent compounds. Each extract certainly shows advantages and disadvantages facts in the extraction process.<sup>20,30</sup> Thus, the selection of a different solvent and extraction method can produce different extract compound content.

## CONCLUSION

Robusta coffee leaf extract (*Coffea canephora* var. *Robusta*) has an inhibitory power against the growth of *S. mutans*. The concentration of robusta

coffee leaf extract (*Coffea canephora* var. *Robusta*) of 25%, 50%, and 75% can inhibit the weak category.

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