

# EFFECTS OF ANTIBACTERIAL SUBSTANCE OF BALI ROBUSTA COFFEE (*Coffea canephora*) SEED TOWARD *Salmonella typhi* GROWTH

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**Abstract:** Typhoid fever is a disease caused by infection with *Salmonella typhi* bacteria which is transmitted through consumption of contaminated food or water. WHO announces typhoid fever patients around the world to reach 17 million every year. Infection is one of the biggest causes of death in Indonesia. From these data, the government needs to take action to improve public health. However, a healthy world launches interventions in the implementation of curative therapy for typhoid fever, one of which is *Salmonella typhi* (MDRST). One alternative is to use active substances contained in medicinal plants. The general objective of this study was to study the inhibitory test results of extract of Bali robusta coffee beans (*Coffea canephora*) against *Salmonella typhi* in vitro. This type of research is experimental with post-test only. The Group Control design used the diffusion disk method (Kirby-bauer) with a sample size of 30 samples. The conservation group was given Robusta coffee bean extract with a concentration of 25%, 50%, 75%. Then the positive control was given ceftriaxone, and 96% ethanol in the disk is blank as a negative control. The results of the study were formed by inhibition zones at a concentration of 25% by 6.33 mm, a concentration of 50% by 8.5 mm, and a concentration of 75% by 10.5 mm. This study could show that Bali Robusta coffee extract could inhibit the growth of *S. typhi* in vitro with the results of the analysis associated with differences ( $p < 0.05$ ) between Bali Robusta coffee extract at concentrations of 25%, 50%, and 75% *Salmonella typhi* in vitro. The concentration of 75% becomes the most effective concentration in inhibiting the growth of *Salmonella typhi*.

**Keywords:** Bali Robusta Coffee extract, inhibitory test, *Salmonella typhi*.

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## I. INTRODUCTION

Infection of *Salmonella typhi* bacteria that is transmitted through consumption of contaminated food or water can be referred to as typhoid fever.(1) This is caused by poor hygiene and sanitation. The number of typhoid fever cases in 2010 is estimated at 26.9 million.(2) Worldwide, there are 17 million people who suffer from typhoid fever according to the WHO each year. Every year, as many as 600,000 of them die. WHO estimates that 70% of deaths occur in Asia. One of the endemic areas of typhoid fever is Indonesia, with the majority occurring in the age group 3-19 years with a percentage of 91%

Infectious disease is one of the biggest causes of death in Indonesia. Typhoid or typhoid fever is one of these infectious diseases. Typhoid fever is an acute contagious disease that attacks the human digestive tract. This disease causes the sufferer to experience digestive disorders and awareness and a fever that lasts more than 7 days.(3)

From these data, the government needs to take action to improve public health. However, the world of health has problems in implementing curative typhoid fever, one of which is Multi Drug Resistant *Salmonella typhi* (MDRST). Research into antibacterial properties needs to be done to find new antibiotics at affordable prices that have the potential

to inhibit or kill antibiotic-resistant bacteria. Utilizing active substances from bacterial killers found in medicinal plants can be an alternative. One plant that is empirically used as an antibacterial drug is coffee. (4)

Coffee contains chemical compounds that are almost the same as tea, including caffeine and tannin. The content of tannin and polyphenol compounds in coffee also have a similar effect on tea which gives a sense of sepet and brownish. Some health benefits of polyphenols are as antioxidants, cardiovascular disease, cancer, reduce the risk of diabetes mellitus, and reduce blood uric acid levels. (5)

Antibacterial inhibition of the growth of *Porphyromonas gingivalis* at concentrations of 25%, 50%, and 100% is owned by robusta coffee bean extract. In a study conducted by Tanauma and Citraningtyas in 2016 stated that the administration of Robusta coffee extract (*Coffea canephora*) on the growth of *Escherichia coli* will inhibit the growth of these bacteria. Based on this background, research should be conducted on the antibacterial inhibition test of Robusta coffee beans (*Salmonella canephora*) extract against *Salmonella typhi* bacteria.

## II. MATERIAL AND METHOD

### A. Coffee extract and experimental procedure

This research was approved by the Chair of the Unud Biomedical Laboratory Unit of Unud and the knowledge of the Dean of the Unud FK in a letter number 344 / UN.14.2 / LBT / Permit / VI / 2018 in Denpasar on June 7, 2018.

This type of research is a true experimental posttest only study to determine the effect of Bali Robusta coffee (*Coffea canephora*) extract on the growth of *Salmonella typhi* which then the treatment results were assessed based on the diameter of the inhibition zone produced. The samples were divided into two groups, namely the control group (K) and the treatment group (P). The control group was negative control ie acetyl acetate 100% (K1) and positive control namely meropenem (K2). The treatment group was divided into 3 groups based on the concentration of the extract of Bali Robusta coffee (*Coffea canephora*) which was tested, namely the concentration of 25mg / ml (P1), 50mg / ml (P2), and 75 mg / ml (P3). The bacterial samples used in the study were *S. typhi* bacterial specimens. This bacterium is obtained from the SMF Laboratory Clinical Microbiology Installation Sanglah Hospital, Faculty of Medicine, Udayana University.

Samples of Bali robusta coffee extract (*Coffea canephora*) used were obtained from coffee bean collectors who were in the coffee garden in Pupuan, Tabanan, Bali. the independent variable is a solution of Bali robusta coffee extract with a concentration of 25%, 50%, 75%. and the dependent variable in this study is the diameter of the inhibitory zone of Bali robusta coffee with a concentration of 25%, 50%, 75% on the growth of *Salmonella typhi* bacteria.

### B. Statistical Analysis

Statistical tests were carried out in a comparative analysis that was processed with SPSS (Statistic Program for Social Science) computer peripheral tools.

## III. RESULT AND DISCUSSION

### A. Result

After the Bali Robusta Coffee Extract (*Coffea canephora*) is ready to be used, the next step is the inhibitory test stage against *Salmonella typhi*. The first step is to make *Salmonella typhi* bacterial suspense, then the bacterium is districting evenly on SDA media, then place a blank disc containing extract with concentrations of 25%, 50%, 75%, ceftriaxone positive control and negative control 96% ethanol . Next, incubated for 24 hours. The inhibition zone formed by each blank disc is measured using a caliper. The inhibition zone of the inhibitory results of extract of Bali Robusta coffee beans (*Coffea canephora*) against *S. typhi* in vitro is shown in Figure 1.

Viewed from table 1, extracts of Bali Robusta coffee beans (*Coffea canephora*) have a inhibitory effect on *Salmonella typhi* bacteria. From the results of observations carried out for 24 hours, the highest inhibition results were obtained to inhibit the growth of *Salomnella typhi* at a concentration of 75%. The average diameter of the inhibitory zone formed was 6.33 mm for a concentration of 25%, then 8.5 mm for the average concentration value of 50%, and 10.5 mm for a mean concentration value of 75%. For ceftriaxone positive control the inhibition zone average was 27.67 mm and negative control was 0 mm. From the table above it can be seen that the higher the extract concentration the greater the inhibition zone of Bali Robusta coffee extract (*Coffea canephora*) in inhibiting growth from *Salmonella typhi* bacteria.

Test of Statistic Effect of Bali Robusta Coffee Extract (*Coffea canephora*) on the growth of *Salmonella typhi* Statistical tests were carried out in a comparative analysis that was processed with SPSS (Statistic Program for Social Science) computer peripheral tools. The stages are carried out by normality test using Saphiro Wilk. The analysis was continued by homogeneity test with Kruskal-wallis test and obtained data that was normally distributed and homogeneous. The data were then analyzed by Mann-Whitney test.

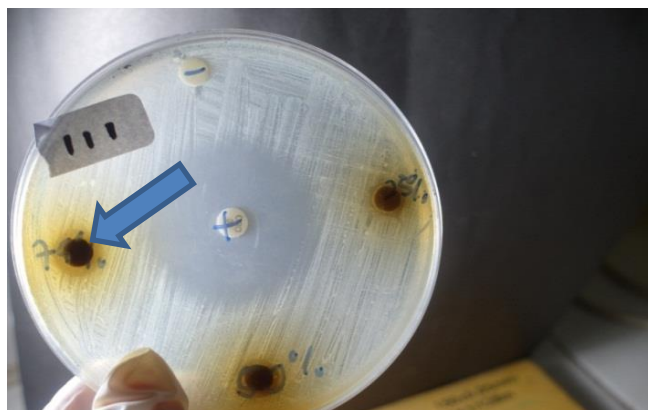


Figure 1: The inhibition zone formed on the SDA media was indicated by the arrow

Table 1: Diameter of the inhibitory zone formed

N	K 25%	K 50%	K 75%	K +	K -
1	6 mm	8 mm	9 mm	28 mm	0 mm
2	7 mm	8 mm	10 mm	29 mm	0 mm
3	6 mm	9 mm	11 mm	26 mm	0 mm
4	7 mm	9 mm	14 mm	26 mm	0 mm
5	6 mm	9 mm	10 mm	28 mm	0 mm
6	6 mm	8 mm	9 mm	29 mm	0 mm
	6.33 mm	8.5 mm	10.5 mm	27.67 mm	0 mm

### B. Discussion

From the research, the inhibitory test results of extract of Bali Robusta coffee beans (*Coffea canephora*) have the ability to inhibit the growth of *Salmonella typhi* bacteria in vitro. Bali Robusta coffee extract (*Coffea canephora*) has the ability to inhibit the growth of *Salmonella typhi* bacteria because there are bioactive compounds contained in extract of Bali Robusta coffee (*Coffea canephora*). Based on the inhibitory test of extract of Bali Robusta coffee (*Coffea canephora*) against *Salmonella typhi*, from observations made for 24 hours, the average diameter of the inhibitory zone formed was 6.33 mm for a concentration of 25%, then 8.33 mm for the average concentration value is 50%, and 10.5 mm for the average concentration value of 75%. Positive ceftriaxone control has a mean inhibition zone of 27.67 mm and negative control of 0 mm. The highest inhibition power to inhibit the growth of *Salmonella typhi* is at a concentration of 75%. The average results of the inhibitory zone formed indicate that the higher the concentration of the extract, the greater the diameter of the inhibitory zone formed. The average diameter of the inhibition zone above 20 mm means that the inhibition is very strong, the diameter of the inhibition zone is 11-20 mm means that the inhibition is strong, the diameter of the inhibition zone is 5–10 mm means that the inhibition is moderate and the inhibition is weak for 0–4 mm. Robusta coffee bean extract has antibacterial effect on the growth of *Porphyromonas gingivalis* at concentrations of 100%, 50%, and 25%, so this study is relevant to research on robusta coffee beans in *S. typhi*. In another study conducted by Tanauma and Citraningtyas in 2016 stated the growth of *Escherichia coli* will be inhibited after administration of Robusta coffee extract (*Coffea canephora*), *Escherichia coli* and *Salmonella typhi* are gram negative bacteria. 5 From other studies with the same bacteria namely *Salmonella typhi* and using different antibacterial substances, fvc coffee, we concluded that kombucha coffee can inhibit the growth of *Salmonella typhi* bacteria. Statistically there was no significant difference in antibacterial power between fermented kombucha coffee 6, 12 and 18 days (5)

Polyphenol compounds contained in coffee are 200-550 mg per cup. (6) Coffee is included in the phytochemical group or commonly called plant phenols (flavonoid polyphenolics). Chemical compounds containing antioxidants, namely

stilbenes, coumarins, lignans, lignins cinnamic acid, benzoic acid, flavonoids, proanthocyanidins, and chlorogenic acid and also from plants are called Plant phenols. Kadnungan phenol has biological activity as an antioxidant that can fight free radicals so that it can protect DNA, lipids and proteins. Rather than that the risk of a chronic disease can be prevented from phenol compounds. Secondary metabolite compounds produced from the process of adapting plants to environmental conditions, pathogenic aggression, stress, or radiation from UV light can be called polyphenol compounds. The acid phenol content of coffee and the tea is 97mg / 100g and 30-36mg / 100 g.(7) In addition to caffeine contained in Robusta coffee beans there are also antibacterial substances such as chlorogenic acid, phenol compounds, and trigonelline. (8) flavonoids contained in robusta coffee beans is one of the phenol compounds. The activity carried out by flavonoids is to damage the walls of bacterial cells, which are obtained from different polarity between alcohol groups and lipids from the constituent DNA in flavonoid compounds so that the cell wall will be broken or damaged which can then enter the nucleus of the bacterial cell. From the research that has been done, robusta coffee beans have been shown to reduce the growth of *S. typhi*.(9)

From the results of the statistical tests performed, the comparison of the results of the inhibitory test between the five concentrations statistically and experimentally proved to be significant. With such results, it can be interpreted that the concentration of 75% is the most effective concentration.

Based on the results of this study it was found that extract of Bali Robusta coffee (*Coffea canephora*) can inhibit the growth of *Salmonella typhi* bacteria. The higher the concentration of the solution given, the higher the inhibition zone. The concentration with the largest inhibition zone area is in the 75% group of 10.5 mm, then followed by the second concentration sample, namely 50% group at 8.5 mm, and the group with the smallest inhibition zone of 25%, which is 6.33 mm .

Biological activity of the compounds contained in Robusta coffee beans greatly affects the ability of alkaloid compounds, because there is nitrogen contained in the base group. Contact base groups with bacteria will react with amino acid compounds that make up the cell wall and bacterial DNA which is the main constituent of the cell nucleus. The cell nucleus is the center of regulating all cell activity. Based on chemistry an acid compound will have a reaction with compounds that are alkaline in nature. Reactions that occur cause changes in the composition and structure of amino acids, this occurs because the majority of amino acids have reacted with alkaline groups found in alkaloid compounds. Changes from the base group of these amino acids will affect by changing the arrangement of the DNA chain in the nucleus of the original cell having a pair of alkaline and acidic groups that are in pairs. Changes from the level of genetic balance so that bacterial DNA undergoes an improper change caused by changes in the sequence of amino acid chains in DNA. The cell nucleus will be damaged because there is a change in the DNA sequence because it does not match the initial sequence. This is because DNA is the primary substance that composes the cell nucleus. Damage to DNA will cause damage to the cell nucleus from bacteria which will later make lysis in the nucleus of bacterial cells. Cell damage in bacteria in the long run will result in bacterial cells unable to carry out metabolism so that lysis cannot be avoided and will occur. Therefore, the bacteria will become inactive and over time will be destroyed

#### IV. CONCLUSION

Based on the results of this study, it can be concluded that extract of Bali Robusta coffee (*Coffea canephora*) can inhibit the growth of *Salmonella typhi* in vitro both at concentrations of 25%, 50%, and 75%. Then the antibacterial inhibition of extract of Bali Robusta coffee beans (*Coffea canephora*) is the most effective against the growth of *Salmonella typhi* in vitro at a concentration of 75%. Proven effective results and statistics.

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