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***Jelita* Tea: Herbal Drink Anti-Typhoid Fever From Pomelo's Peel (*Citrus maxima*) and Stevia Leaf (*Stevia rebaudiana*)**

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ABSTRACT

Background: Typhoid fever is an acute systemic infection caused by *Salmonella typhi* with the prevalence of this disease in Indonesia reaches 1.6%. The commonly used treatment for typhoid fever is antibiotic chloramphenicol, but it causes aplastic anemia and inhibits the formation of blood cells. Alternative treatment is needed with the use nature ingredients such as of pomelo's peel and stevia leaves. Pomelo has been known with the active ingredients content such as alkaloid, tannin, and vitamin C. The most active ingredients found in its peel, but has not been utilized enough. Stevia leaves are a natural sweetener that did not increase the blood sugar that can increase the bacterial virulence. The purpose of this study was to determine the effectiveness of *Jelita* (Jeruk Bali and Stevia) Tea as an anti-typhoid. **Methods:** The experimental study was conducted in vivo method with posttest-only-control group design using 3 treatment groups and 2 groups of control. **Results:** In vitro test results obtained a signification of 0.037 ($p < 0.05$) showing a significant difference between inhibitory zone produced by the test solution and negative control. In vivo tests result a decrease of widal antibody titer against antigen O and H of *Salmonella typhi* showed by antibody titer 1/640 in control group and 1/80 in treatment group. So, the conclusion is *Jelita* tea has potential as an anti-typhoid fever.

Keywords: Typhoid Fever, Pamelos Peel, Stevia Leaf, Alternative Herbal Drink



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INTRODUCTION

Typhoid fever is an acute infectious disease of a systemic nature caused by gram-negative bacteria *Salmonella typhi* (S. typhi). The prevalence of typhoid fever in Indonesia is 1.6% and ranks 5th in infectious diseases that occur in all ages with the number of cases of 350-810 per 100,000 population (Khairunnisa et al., 2020). According to the World Health Organization (WHO) the number of cases of typhoid fever sufferers in Indonesia reaches 81% per 10,000 population (DEPKES RI, 2013). Typhoid fever can attack humans by entering through food or drinks contaminated with *Salmonella typhi* bacteria. The pathophysiology of typhoid fever is a complex process through several stages. Typhoid fever has an incubation period of 10 – 14 days, but can vary up to 5 – 40 days in children (Imara, 2020). Complications in the incidence of typhoid fever often occur in patients who do not get treatment properly such as visceral abscesses (Naveed & Ahmed, 2016).

The high morbidity rate of typhoid fever and the consequences caused to patients who are not treated immediately will be very dangerous for humans. The initial therapy commonly given to patients with typhoid fever is antibiotics. However, improper administration of antibiotic therapy causes resistance problems and side effects in patient (Abdurrachman, 2018). One antibiotic that is often used for typhoid fever sufferers is chloramphenicol. However, chloramphenicol antibiotics causes several side effects such as aplastic anemia or commonly known as bone marrow depression and inhibit the formation of blood cells that arise within 5 days after the start of therapy (Rahmasari, n.d.). Therefore, there is a need for

alternative treatments that are effective and safer for people with typhoid fever using the natural materials namely stevia leaves and pomelo's peel.

Stevia leaves are natural sweeteners with a sweetness level of 100-200 times the sweetness of sucrose and do not have carcinogenic effects (Aina et al., 2019). The main components in stevia leaves are steviosides and rebaudiosides which cause sweetness (Siagian et al., 2020). Research (Winarsih et al., 2017) showed that the higher the glucose level, the higher the expression of AdhO36 protein which indicates an increase in bacterial virulence factors. In addition, *Salmonella typhi* is a bacterium that produces biofilms. Bacterial biofilm will increase along with increased glucose levels and increase bacterial virulence (Waldrop et al., 2014). Stevia leaves as a natural sweetener do not cause an increase in blood sugar levels (Limanto, 2017). Stevia has been authorized for use as a food additive by the Food and Drug Administration (FDA) in 2018 and is classified in the Generally Recognize as Safe (GRAS) category with an Acceptable Daily Intake (ADI) consumption limit of 4 mg/kg/BB/day (Siagian et al., 2020). In Indonesia, the use of stevia extract received BPOM approval in 2004 (circular letter of the head of BPOM number HK.00.055.2.3877).

Pomelo's peels are often thrown away as waste. In fact, most of the antioxidant and vitamin C content of pomelo is in the skin. The content of antioxidants and vitamin C in pomelo serves to maintain and increase endurance (imunostimulator). The function of body cells that are not optimal due to free radicals that enter the body can reduce the immune system.

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Free radicals that enter will bind electrons owned by molecules around them, including compounds from cells in the body. Vitamin C contained in the body will donate electrons to free radicals so as not to interfere with the function of body cells. Vitamin C also stimulating the production of interferon. Interferon is one of the cytokines that has a regulatory effect so that it can act as a mediator of inflammation and tissue repair processes to increase the immunity (Suharyanto et al., 2022). Besides to increasing immunity, pomelo is also antibacterial. The mechanism of pectin compounds as antibacterial by increasing permeability and changing the structure of bacterial cell membranes so that the bacterial metabolic process will be disrupted (Wana & Pagarra, 2018). Based on the analysis of these ingredients and some previous studies that say that pomelo's peel is effective as an antibacterial *Salmonella typhi*.

Based on the problems and the potential of pomelo's peel and stevia leaves, in this study was created *Jelita* tea (Jeruk Bali and Stevia), which is tea from pomelo's peel and stevia leaves which can be the alternative herbal drinks to boost the immune system that can affect the body's antibodies in fighting bacteria *Salmonella typhi* causes typhoid fever.

METHOD

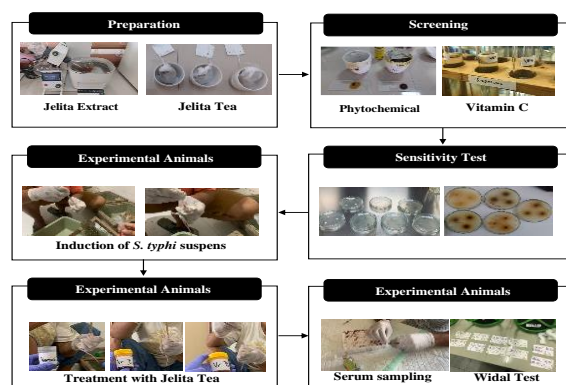
INSTRUMENTS

The tools used in this study were laboratory glassware, oven (ELOS), blender (STATMESIN ks 10000), tea bag, evaporator (Rotavapor BUCHI R.300), petri dish, autoclave (TOMY SX-500), biosafety cabinet (ESCO AC2-458), paper disc (OXOID), incubator (ESCO), and centrifuge (GEMMYCO PLC-025).

The ingredients used in this study were pomelo's peel taken from fruit traders in Denpasar City and Badung Regency, stevia powder purchased from supermarkets, *Salmonella typhi* bacteria, HCl 2N, dragendorff reagent, mayer reagent, wagner reagent, Mg powder, concentrated HCl, FeCl₃, 70% ethanol, iodine, MHA media, chloramphenicol, and widal test KIT.

RESEARCH METHOD

Pre experimental study was conducted in vitro method by testing the effectiveness of *Jelita* extract to inhibit the *Salmonella typhi*. The experimental study was conducted in vivo method with posttest-only-control group design. This research was conducted in May 2023 in Denpasar City. The research flow are shown in picture 1.



PROCEDURE

The Making of Jelita Extract

Pomelo's peel dried in oven at 50°C and then mashed. Each 150 grams of pomelo's peel powder and stevia leaf powder were macerated separately with 70% ethanol and concentrated with a rotavapor until a thick extract was obtained. The extract is used for phytochemical screening, vitamin C presence test, and



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inhibitory ability test against *Salmonella typhi* bacteria.

The Making of Jelita Tea

Dry pomelo's peel powder put in a tea bag together with stevia powder. Tea formulations are made with three types of formulations, namely variation 1 (VR1) which is made with a combination of 1.5 grams of stevia powder and 0.5 grams of pomelo's peel powder; variation 2 (VR2) made with a combination of 1.0 grams of stevia powder and 1.0 grams of pomelo's peel powder; and variation 3 (VR3) made with a combination of 0.5 grams of stevia powder and 1.5 grams of pomelo's peel powder.

Phytochemical Screening and Presence of Vitamin C

Phytochemical screening is carried out on the content of alkaloids, flavonoids, tannins, saponins, and qualitative testing for the presence of vitamin C. The alkaloid test is performed by reacting the test solution with Drendorff reagent, Mayer reagent, and Wagner reagent. The flavonoid test is performed by

37°C for 1 x 24 hours. Observations were made on the formation of an inhibitory zone around the paper disc. Blank discs are used as negative controls and chloramphenicol as positive controls.

Organoleptis Test

The organoleptical test consists of taste, color, and aroma. Conducted by giving tea samples to 20 respondents then filling out a test questionnaire.

Experimental Animals and Research Design

Experimental animals in the form of 15 female mice bulb / c strain were divided into 5 test groups, where K1 was the normal group, K2 was the control group, and K3 – K5 as the treatment group. The maintenance and treatment of mice was carried out at the Pemeliharaan dan Pembiakan Hewan Percobaan (P2HP) Bio Mice and Rat Pulau Moyo, South Denpasar. Group 1 was untreated mice, groups 2, 3, 4, and 5 were mice induced by a 300 microliters suspension of *Salmonella typhi* 0.5 Mc. Farland with the difference that group 2 was not given product consumption, group 3 was given product VR1, group 4 was given product VR2, and group 5 was given product VR3. The product is given twice a day as much as 300 microliters at 10 am and 5 pm for 5 days. On the 5th day, serum sampling was carried out for antibody titer measurement using the widal test by collecting the blood from the mice's eyes, and the serum was separated using a centrifuge.

DATA COLLECTION AND ANALYSIS TECHNIQUES

Primary data in the form of tests on tea samples is carried out by testing in the laboratory of the Department of Medical Laboratory Technology Poltekkes Denpasar. Phytochemical tests of alkaloids, flavonoids,

Picture 1. Research flow

reacting the test solution with concentrated Mg and HCl powder. The tannin test is performed by reacting the test solution with 5.5% FeCl. The saponin test is performed with the addition of hot water. Vitamin C test is done by adding a test solution to iodine.

Sensitivity Test of Salmonella typhi Bacteria

The 0.5 Mc Farland *Salmonella typhi* suspension implanted on 7 MHA media. Each variety of extract is soaked on 3 paper discs for 30 minutes. The disc paper is placed on MHA media that has been inoculated with a suspension of *Salmonella typhi* bacteria. Incubation is carried out at a temperature of



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saponins, and tannins as well as tests for the presence of vitamin C are carried out directly in the Applied Chemistry laboratory, and sensitivity tests of *Salmonella typhi* bacteria are carried out directly in the microbiology laboratory. Serum samples from experimental animals were measured antibody titers by the widal test method at the Immunology Laboratory. Organoleptical tests are carried out in the class of Medical Laboratory Technology. In the organoleptis test, the color, taste, and aroma of *Jelita* tea were tested on 20 respondents using questionnaire.

The research was carried out in three stages, namely the preparation stage, the implementation stage, and the data analysis

stage. The qualitative data such as phytochemical screening results, vitamin C presence tests, organoleptic tests, and antibody titer of experimental animals were processed manually and analyzed descriptively in the form of tables and narratives with relevant literature reviews, while quantitative data such as inhibition zone diameter and antibody titer of experimental animals was processed statistically.

Feasibility of Research Ethics

Feasibility of research ethics from the Health Research Ethics Commission of the Ministry of Health Denpasar Health Poltekkes number: LB.02.03 / EA / KEPK / 0561 / 2023.

RESULTS

PHYTOCHEMICAL AND VITAMIN C SCREENING

Table 1. Phytochemical and Vitamin C Screening Result

| Sample | Alkaloid | Flavonoid | Tanin | Saponin | Vitamin C |
|---------------|----------|-----------|-------|---------|-----------|
| Pomelo's Peel | + | - | + | - | + |
| Stevia Leaf | + | + | + | + | - |
| VR1 | + | + | + | + | + |
| VR2 | + | + | + | + | + |
| VR3 | + | + | + | + | + |

Based on table 1, the pomelo's peel sample showed positive results containing alkaloids, tannins and vitamin C while the stevia leaf samples showed positive results containing alkaloids, flavonoids, tannins and saponins. In *Jelita* tea formulation variations 1, 2 and 3 showed positive results containing alkaloids, flavonoids, tannins and saponins. Pomelo's peel samples did not show positive results for flavonoids and saponins. However, in

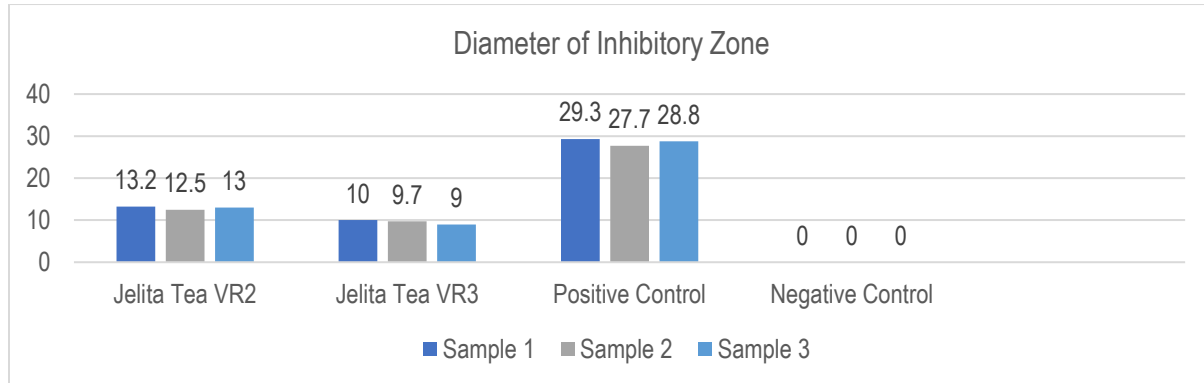
phytochemical screening tests, stevia leaf samples showed positive results containing flavonoids and saponins but did not contain vitamin C.



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SENSITIVITY TEST OF *Salmonella typhi* BACTERIA

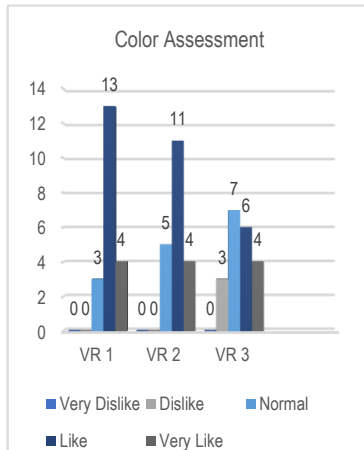


Picture 2. Diameter of Inhibitory Zone

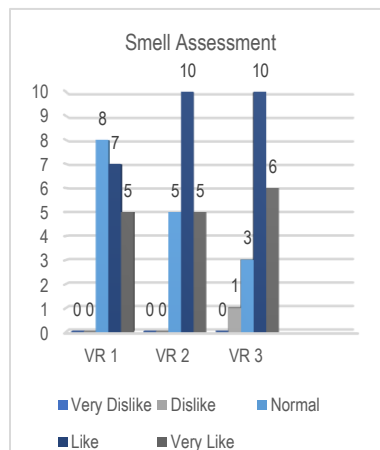
Picture 2 showed the results of an inhibitory power test with the disc diffusion method carried out on various variations of pomelo's peel extract and stevia leaves which are ingredients for making *Jelita* tea. The positive control used is chloramphenicol. Based on the

test results, it was found that the three variations of *Jelita* tea ingredients have the potential to be antibacterial, with an average diameter of VR1 inhibitory zone of 13.1 mm, VR2 of 12.9 mm, and VR3 of 9.6 mm.

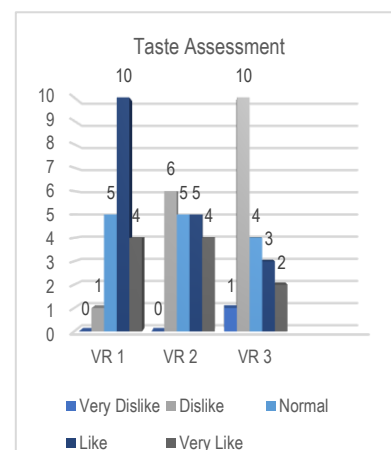
ORGANOLEPTIC TEST



Picture 3. Color Assessment



Picture 4. Smell Assessment



Picture 5. Taste Assessment

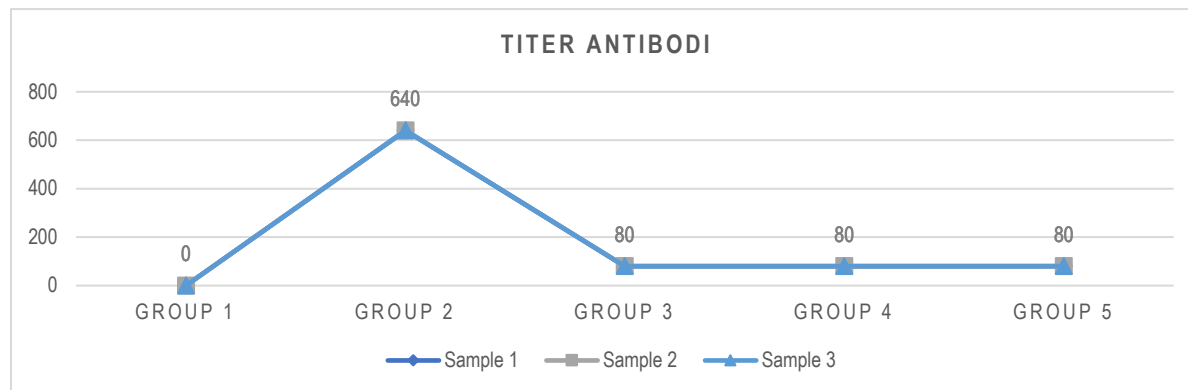
The results of the organoleptical test showed that in terms of color and taste, respondents successively preferred variation 1, variation 2 and then variation 3. Meanwhile, in terms of smell, respondents successively prefer variation 3, variation 2 and variation 1.



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EXPERIMENTAL ANIMAL TEST



Picture 6. Antibody Titer

Picture 6 showed the difference between the antibody titers of the normal, control, and the entire test group. Group 1 was not given a suspension of *Salmonella typhi* bacteria or *Jelita* tea so that *Salmonella typhi* antibodies were not formed. In group 2 – group 5 *Salmonella typhi* antibodies are formed due to bacterial suspension on the first day. In picture 6, it can be seen that group 2 has the highest antibody titer and decreases in group 3 – group 5.

DISCUSSION

In this study, phytochemical and vitamin C screening was carried out on samples by measuring qualitatively using appropriate reagents. Based on table 1, there are differences active ingredients content between pomelo's peel and stevia leaves. However, the *Jelita* tea formulation variations 1, 2 and 3 showed positive results in all active ingredients. This means that the combination of both ingredients, pomelo's peel and stevia leaves can provide complex ingredients in *Jelita* tea formulations shown by positive phytochemical screening results containing alkaloids, flavonoids, tannins, saponins, and vitamin C. The content of

alkaloids, flavonoids, tannins and saponins in *Jelita* tea showed its potential as an herbal drink that acts as an anti-typhoid. Vitamin C itself is a micronutrient that plays an important role for the human body. Vitamin C is also known as ascorbic acid which is a powerful antioxidant that can improve the body's immune system and is useful in overcoming infections (Kumari et al., 2020).

Picture 2 showed the results of the sensitivity of *Salmonella typhi* test with the disc diffusion method carried out on various variations of *Jelita* extrac which are ingredients for making *Jelita* tea. Based on the test results, it was found that the three variations of *Jelita* tea ingredients have the potential to be antibacterial, with an average diameter of VR1 inhibitory zone of 13.1 mm, VR2 of 12.9 mm, and VR3 of 9.6 mm. Thus, VR1 and VR2 have strong antibacterial potential, and VR3 has medium antibacterial potential. This is in accordance with the provisions according to Davis and Stout (1971) in (Rastina et al., 2015) regarding the criteria for antibacterial power strength as follows: the diameter of the inhibitory zone of 5



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mm or less is categorized as weak, the diameter of 5 – 10 mm is categorized as medium, the diameter of 10 – 20 mm is categorized as strong, and the diameter of 20 mm or more is categorized as very strong.

The difference in the diameter of the inhibition zone of the three *Jelita* tea variations with the control was then analyzed statistically. The normality test showed a significance value of 0.009 ($p < 0.05$) means the data is abnormally distributed. Followed by the homogeneity test, a significance of 0.074 ($p > 0.05$) showed there was no difference in variance in results (homogeneous data). Then the Kruskal Wallis test was carried out with a 95% confidence interval or 5% error level and obtained a significance value of 0.011 ($p < 0.05$) means that there is a significant difference in the diameter of the inhibitory zone between *Jelita* tea variations and controls. The Mann-Whitney tests were conducted to determine the difference in inhibitory ability between controls with each variation of *Jelita* tea. A significance value of 0.037 ($p < 0.05$) was obtained in the negative control test with all variations means there was a significant difference between the diameter of the negative control inhibitory zone and VR1, VR2, and VR3. Thus, it can be concluded that the *Jelita* tea formulation has the ability to inhibit the growth of *Salmonella typhi* bacteria and can be used as an antibacterial alternative.

The ability to inhibit bacterial growth is due to the presence of phytochemicals in the test formulation. Alkaloids can be antimicrobial by damaging cell membranes by lipophilic compounds. The flavonoid content contained in *Jelita* tea can damage the permeability of bacterial cell walls, microsomes, and lysosomes due to the interaction between flavonoids and

bacterial DNA. In addition, the lipophilic nature of flavonoids can damage bacterial cell membranes (Rastina et al., 2015). Tannin can disrupt the pH balance of bacteria by binding to H^+ ions and inhibiting the process of RNA reverse transcriptase enzymes and DNA topoisomerase so as to inhibit the process of bacterial replication and result in inhibition of bacterial growth. Saponins can cause bacterial death because saponins can increase permeability and change the structure of bacterial cell membranes, thereby disrupting bacterial metabolic processes and bacterial cell wall surface tension (Wana & Pagarra, 2018).

Picture 3, 4, and 5 showed the results of the organoleptic test showed that in terms of color and taste, respondents successively preferred variation 1, variation 2 and then variation 3. Meanwhile, in terms of aroma, respondents successively prefer variation 3, variation 2 and variation 1. The aroma is more popping and comfortable so much liked by respondents because the sweet aroma found in stevia leaves is balanced with the fresh aroma of pomelo's peel. The preferred color in variation 2 looks more attractive to respondents because it is not too dense. In taste, respondents showed that they preferred variation 1 which had more stevia content compared to variations 2 and 3.

The experimental animals used in this study were female mice that had received treatment for 5 days. Blood samples were taken from all mice from all groups and prepared to obtain serum samples. The serum sample was then carried out a widal test to determine the titer of *Salmonella typhi* antibodies.

Picture 6 showed the difference between the antibody titers of the normal, control, and the entire test group. In picture 6 it can be seen



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that group 2 has the highest antibody titer and decreases in group 3 – group 5. Group 2 was not given treatment by *Jelita* tea while group 3 received *Jelita* VR1 tea, group 4 received *Jelita* VR2 tea, and group 5 received *Jelita* VR3 tea. Mice that induced by *Salmonella typhi* bacteria but not consuming *Jelita* tea had an antibody titer of 1/640, while mice induced by *Salmonella typhi* bacteria and consuming *Jelita* tea had an antibody titer of 1/80. Widal test used to detect the antibody against the antigen O and antigen H of *Salmonella typhi* bacteria. This method conducted a dilution step until there is no agglutination which indicated a negative result. The last dilution used as the titer (Rahayu, 2022). The higher of antibody titer means more antigen detected in the serum sample and increase the risk of bacterial infection (Widari, 2022). In the research result showed that consumption of *Jelita* tea can reduce the risk of *Salmonella typhi* bacterial infection indicated by lower antibody titers. Antibody titers were tested statistically to determine if there was a significant difference between the antibody titers of the control group and the test group. The Mann-Whitney test obtained a significance of 0.025 ($p > 0.05$) showing a significant difference in antibody titers between the control group and each test group.

The decrease of antibody titer in the *Jelita* tea treatment group was caused by the phytochemical content in *Jelita* tea. *Salmonella typhi* are included in pathogens that can invade the body. Antigens that enter the body through pathogens such as bacteria will generally trigger the immune system. It started from the destruction of pathogens (bacteria) by non-specific body defenses and continues to more complex specific body defenses through the

production of antibodies (Pancawari Ariami et al., 2021). Flavonoids are the largest group of phenol compounds and have the ability to improve the host response that activates monocytes. Monocytes are a type of white blood cell with the functions to phagocytosis pathogens (Patimah et al., 2015). This showed that *Jelita* tea can increase non-specific body defenses in the face of exposure to *Salmonella typhi* and reduce bacterial pathogenicity indicated by a decrease in antibody titer. The decrease of antibody titer showed that the number of bacteria that successfully pass through the body's non-specific defense system has been reduced due to the phagocytosis process against bacteria, so that the antigens that trigger the formation of antibodies in specific body defenses have decreased.

CONCLUSION(S)

From the research that has been done, it can be concluded that *Jelita* tea has the ability as an anti-typhoid fever as evidenced by the results of in vitro tests using the disc diffusion method showing the formation of an inhibition zone against the growth of *Salmonella typhi* bacteria on discs soaked in *Jelita* tea formulation. The ability as an anti-typhoid fever *Jelita* tea is also proven by in vivo tests with antibody titer measurement parameters against *Salmonella typhi* showing mice with tea consumption treatment have lower antibody titers.

Conflict of Interest

The author(s) declare that they have no conflict of interest.

Acknowledgment

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