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Antibacterial Test of Keji Beling Leaf Starch Extract (*Strobilanthes crispus*) against Inhibition Zone *Salmonella thypi*

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Abstract

Salmonella typhi is a gram-negative rod-shaped bacterium, which can cause typhoid fever. Keji Beling (*Strobilanthes crispus*) is a medicinal plant that contains metabolic substances such as flavonoids that function as antibacterial. The aim of this research was to determine the antibacterial activity of the starch from the leaves of the Keji Beling (*Strobilanthes crispus*) against the inhibition zone of Salmonella thypi. This type of research is quantitative research, the methods used are laboratory experimental methods and Completely Randomized Design (CRD) with 5 treatments (Thiamphenicol as positive control using concentrations of 55%, 70%, 85%, 100%) and 5 repetitions and perform statistical tests using *One Away Annova*. *One Way Anova* statistical test results were not significant. The conclusion in this study was that the concentration of starch from the leaves of Keji Beling (*Strobilanthes crispus*) had no effect on the inhibition zone of *Salmonella thypi*.

Keywords: Antibacterial; Keji Beling (*Strobilanthes crispus*); *Salmonella thypi*

INTRODUCTION

Typhoid fever is a typhoid disease caused by a bacterial infection in the small intestine and sometimes in the bloodstream caused by bacteria. The disease can be transmitted through food and drink contaminated by *Salmonella thypi* (Crump, 2019). The World Health Organization (WHO) estimates that the number of cases of typhoid fever in the world reaches 16-33 million with 500-600 thousand deaths each year. It is estimated that there are about 22 million people with typhoid with a death rate of 200,000 each year worldwide. The prognosis of typhoid fever depends on the age and endurance of the patient (Nugroho, 2011). Cases of children suffering from typhoid fever increased, the figure reached 1,079 people. In detail, at the DR Sobirin Hospital in 2018, there were 380 children suffering from typhoid fever. Then in 2019, it jumped to 411 pediatric patients with bacterial infections in the small intestine. While at Siti Aisyah Hospital during 2019, there were 288 patients (Prime, 2020).

Disease can be caused by various factors, namely a weak immune system, genetic factors, and unhealthy diet and nutritional intake. In addition, environmental factors are very influential on a person's health. The environment that is not clean can cause the growth of pathogenic bacteria. There are many pathogenic bacteria in the human body, one of which is *Salmonella typhi* which causes typhoid fever. These bacteria enter the mouth and then into the digestive tract.

Bacteria in the small intestine can stimulate fever and bowel disorders. This can stimulate white blood cells to produce interleukins and cause symptoms of fever, bowel obstruction, and decreased appetite and other symptoms. Incubation time depends on the quantity of bacteria and *host factors*. In addition, the incubation time depends on the characteristics of the infecting bacterial strain. Generally, the incubation period for typhus is approximately 1-3 weeks (Maier, *et al.*, 2000).

Treatment of typhoid can be done medically and traditionally. Medical treatment can use chemical-based drugs such as Amoxilin, Thiampenicol, Azithromycin and other drugs (Juwita, *et al*., 2013). Thiampenicol is a broad-spectrum antibiotic that works by inhibiting the growth of bacterial cells that cause infection. Thiampenicol is effective against aerobic and anaerobic gram-positive and negative bacteria. This drug works by inhibiting bacterial protein synthesis (ho, 2000). Meanwhile, traditional medicine uses natural ingredients. Traditional medicine has been known since ancient times which is generally inherited and spread by word of mouth. Each region has its own characteristics in traditional medicine. This is influenced by natural conditions and the availability of plants in each area (Peneng and Sumatra, 2007).

Traditional medicine is processed traditionally and from generation to generation which has medicinal properties. Efficacy as a drug is known from the results of clinical scientific studies that have proven beneficial for health based on recipes from ancestors, customs, beliefs, local customs and traditional knowledge. Dewoto (2007) says medicinal plants are ingredients or ingredients derived from plants, animals, minerals that have been used for generations as treatment based on experience. Traditional medicine commonly used by the community to treat typhus is a decoction of the leaves of Keji Beling (*Strobilanthes crispus*).

The general public believes that the vile shard plant (*Strobilanthes crispus*) is known to cure several diseases including diabetes, typhoid, kidney stones, and can also inhibit the growth of cancer cells (Dalimartha, 2006). The vile shard plant (*Strobilanthes crispus*) contains saponins, flavonoids, terpenoids, polyphenols and potassium. The content of polyphenols and flavonoids in keji shard (*Strobilanthes crispus*) has effective antibacterial benefits against bacterial growth. This plant produces various kinds of metabolic substances such as flavonoids which function as antibiotics (Amalia, *et al*, 2015). Keji shard (*Strobilanthes crispus*) contains flavonoids which are antibacterial. The mechanism of action is by denaturing bacterial cell proteins and damaging the cytoplasmic membrane which can cause leakage of important metabolites and inactivate enzyme systems in bacteria. This damage can allow nucleotides and amino acids to seep out and prevent the entry of active ingredients into cells so that this situation can cause bacterial death (Agoes, 2010).

The results of a preliminary test that was carried out at the Biology Laboratory of PGRI Lubuklinggau University on January 6, 2021, showed that the leaf starch of Keji Beling (*Strobilanthes crispus*) with a concentration of 80% inhibited bacterial growth, which was visible in the presence of a clear zone. Therefore, the purpose of this study was to determine the antibacterial power of the starch from the leaves of keji shard (*Strobilanthes crispus*) against the inhibition zone of *Salmonella thypi*.

METHOD

This study uses a laboratory experimental method, this type of research is a quantitative study with a completely randomized design (CRD) and performs statistical tests using *One Away Annova* and Thiamphenicol as positive controls using concentrations of 55%, 70%, 85%, 100%.

A0 : Thiampenicol (positive control)
A1 : *Strobilanthes crispus* 55 %
A2 : *Strobilanthes crispus* 70%
A3 : *Strobilanthes crispus* 85%
A4 : *Strobilanthes crispus* 100%

The repetition was carried out 5 times, the determination of this repetition was based on the formula repetition calculation.

$$t\,(r-1) \geq 21$$

The parameter measured in this study was the microbiological antibacterial activity against *Salmonella thypi* which was measured using a caliper (modification from Rahmati, *et al*., 2017). This research was carried out at the Biology Laboratory of PGRI Lubuklinggau University in March 2021. The tools used in this research were petri dishes, bunsen, mortal, tweezers, oven, *hot plate*, measuring cup, *erlenmeyer*, *magnetic stirrer*, electric scales, aluminum bucket , lighter, *perforator* and calipers with an accuracy of 0.01 mm. While the materials used in this study were leaves of vile shard (*Strobilanthes crispus*) cultured *Salmonella thypi* which were purchased online at the Gajah Mada University Laboratory, 70% alcohol, aquades, Nutrien Agar (Na), *aluminium foil*,m asks, gloves, *tissue*, *buds*, gauze, and paper discs with a diameter of 5.5 mm.

This research procedure was carried out in several stages, namely as follows:

a. Sterilization of tools and materials

   Sterilization in this study was carried out using two methods, namely boiling and dry heat sterilization with oven and bunsen heating (modification from Hidayat, 2013)

b. Making Na (Nutrient Agar)
   1) Prepare 2 grams of Na using an electric scale on an *aluminum foil* that has been sterilized with 70% alcohol and heated by passing a Bunsen fire.
   2) Heating *hot plate* by pouring 30 ml of distilled water into a sterilized measuring cup, then pouring the distilled water into the *Erlemeyer* with *aluminum foil* then setting the *hot plate* to 100°C, waiting for it to boil.
   3) Lower the temperature to 0°C and turn off the *magnetic stirrer*. After the temperature drops, then enter the Na into the *Erlemeyer* and turn on *magnetic stirrer* temperature *hot plate* to 120°C, wait until the Na is well mixed and dissolved completely (not too thick).
   4) Cool the Na for 15 minutes then put the Na into the oven at 50°C for 7 minutes. Remove the Na from the oven then wait for the Na to not get too cold.
   5) Pour Na into a sterilized petri dish in the oven with a thickness of 1-2 cm. Cover the petri dish with sterile tissue until tightly.
   6) Spray the 70% alcohol back into the oven. Cover again and heat the Na at 50°C for 7 minutes.
   7) Incubating Na for 1x24 hours in the oven (Winato, *et al*., 2019).

c. Manufacture of vile shard leaf starch (*Strobilanthes crispus*)
   1) Prepare vile shard leaves (*Strobilanthes crispus*) that have matured and are not polluted by pests, then wash them thoroughly under running water and dry by airing.
   2) Cut the vile shard leaves (*Strobilanthes crispus*) into small pieces then grind them by grinding using a mortar and measure the starch essence of the *Strobilanthes crispus* using measuring cups as much as 55 ml, 70 ml, 85 ml, and 100 ml.

3) Mixing 55 ml of starch from the leaves of Keji shard (*Strobilantes crispus*) with 10 ml of distilled water until homogeneous, then filtered using gauze to a concentration of 55%.
4) Mixing 70 ml of starch from the leaves of Keji shard (*Strobilanthes crispus*) with 10 ml of distilled water until homogeneous, then filtered using gauze for a concentration of 70%.
5) Mixing 85 ml of starch from the leaves of keji shard (*Strobilanthes crispus*) with 10 ml of distilled water until homogeneous, then filtered using gauze to a concentration of 85%.
6) Mixing 100 ml of starch from the leaves of Keji shard (*Strobilanthes crispus*) with 10 ml of distilled water until homogeneous, then filtered using gauze for a concentration of 100%.
7) Positive control using one tablet of thiampenicol dissolved in 10 ml of distilled water (Permatasari, *et al*., 2013)

d. Antibacterial activity test
1) Scraping *Salmonella typhi* on a petri dish containing Na which is not contaminated by bacteria or fungi.
2) Divide the petri dish into 4 quadrants then heat the lips of the petri dish over a Bunsen fire for 30 seconds.
3) Take *Salmonella thypi* using *cotton bud* and then press it on the tube wall until the *cotton bud* is not too wet then apply it on the Na surface until smooth.
4) Enter the paper discs that have been shaped using *perforator* with a diameter of 5.5 mm as many as 5 pieces for each petri dish with 4 concentrations of leaf starch essence vile shard (*Strobilanthes crispus*).
5) Put a positive control paper disc placed in the center of the petri dish.
6) Incubate 1x24 hours in a cold oven that has been sprayed with 70% alcohol.
7) Calculating the clear zone formed using a caliper (Noviyanti, *et al*., 2014)

Data were obtained by observing and measuring the diameter of the inhibition zone formed in mm units. The measurement was carried out using a caliper with an accuracy of 0.01 mm. The clear zone formed around the paper disc is an indication of the sensitivity of the bacteria to the test material which is indicated by the presence of an inhibition zone. The results of the entire inhibition zone formed were recorded on the observation sheet. Data analysis technique is a data processing technique or data calculation from research results. The analytical technique used in this study is quantitative data analysis, the data obtained by measuring the inhibition zone around the paper disc. The data obtained will be tabulated and analyzed descriptively (Lingga, *et al*., 2015). The data obtained were tested with One-way Analysis of Variance (*ANOVA*) if the data were normal and homogeneous and would be calculated manually using the inhibition zone calculation formula in Figure 1.

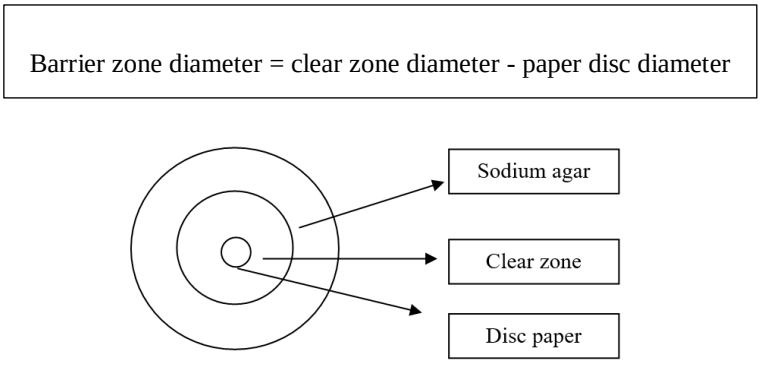

Figure 1. Measuring the Diameter of the Infectious Zone (Source: Hidayat, 2013)

The clear zone formed from the results of the study can be seen in Figure 2.

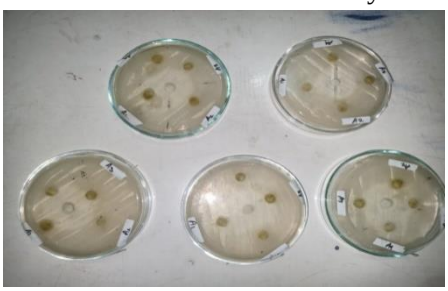

Figure 2. Measurement of Inhibitory Zone Diameter (Source: Personal Data, 2021)

The calculated data are presented in the form of Table 1 and the decision returns with the general standard of inhibition as follows:

Table 1. Response Criteria for Bacterial Growth Inhibitory Zone

No	Barrier Zone	Inhibition
1	>20 mm	Very Strong
2	10-20 mm	Strong
3	5-10 mm	Medium
4	<5 mm	Weak
5	No barrier zone	

(Source: Lauma, *et al*., 2015)

RESULT AND DISCUSSION

The results of the research on the inhibition zone of the starch of the vile shard leaf (*Strobilanthes crispus*) against *Salmonella thypi* can be seen in Table 2.

Table 2. Results of Inhibitory Zones for Sari Starch Leaves Keji Beling (*Strobilanthes crispus*) Against *Salmonella thypi*

Concentration	Barrier Zona (mm)					$\bar{X}$±SD	Reaponse of inhibitory zone
	P1	P2	P3	P4	P5		
A0 Thiampenicol	9,6	9,9	10,2	9,8	9,5	9,8±0,27	Medium
A1: 55 %	0	0,1	0,2	0,1	0,3	0,14±0,11	Weak
A2 : 70%	0,3	0,4	02	0,3	0,8	0,4±0,67	Weak

A3 : 85%	0,4	0,9	0,6	0,5	2,3	0,94±0,54	Weak
A4 : 100%	1,4	1,1	4,2	1,3	3,7	2,38±6,63	Medium

Description:
P : Repeating
X : Mean
SD : Standard Deviation

Inhibition zone response criteria:
Very Strong : > 20 mm
Strong : 10-20 mm
Medium : 5-10 mm
Weak : < 5 mm

The variables observed in this study were the inhibition of *Salmonella thypi* on Na media given different concentrations of starch from the leaves of Keji Beling (*Strobilanthes crispus*) and positive control thiampenicol. The calculated data is presented in the form of a bar chart to see the level of comparison of the average inhibition zones formed. The diagram of the average inhibition zone of the starch of the keji shard leaf (*Strobilanthes crispus*) against the activity of *Salmonella thypi* can be seen in Figure 3.

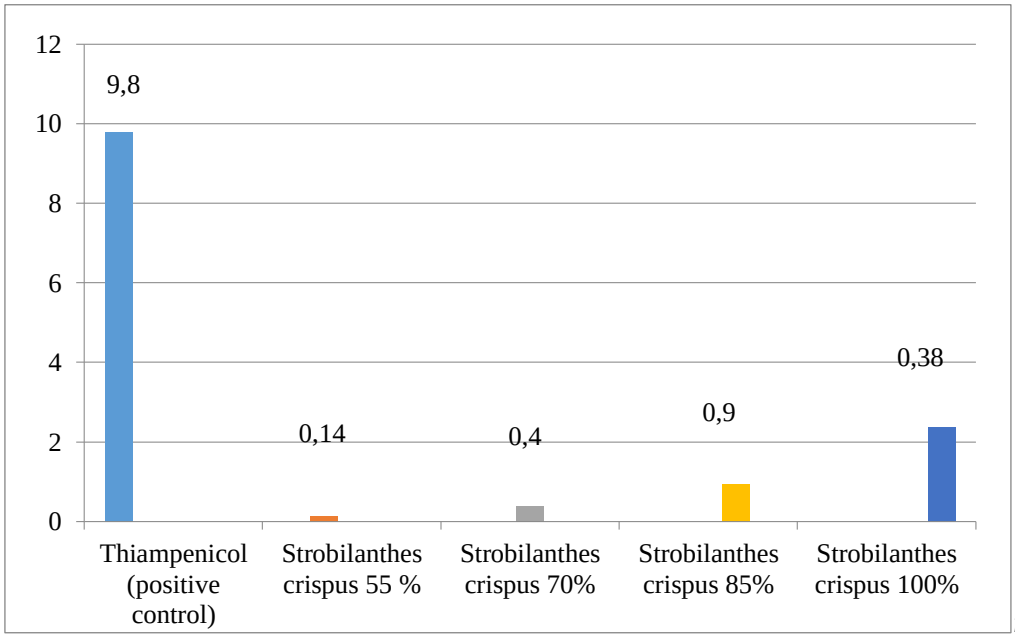

Figure 3. Average diameter of inhibition zone of *S. crispus* against *S. thypi*

Normality testing is done to determine whether a data distribution is normal or not. The results of the normality test used the calculation of the *One Sample Kolmogorov-Smirnov Test* on SPSS version 21 with the test criteria if sig. > then H_0 is accepted and the data is normally distributed. The results of the normality test are shown in Table 3.

Table 3. Normality Test Results

α	Sig.
0.05	0.12

Based on the data obtained, the results obtained were 0.12 > 0.05 so it can be said that the data obtained were normally distributed. Homogeneity test is used to test the similarity of variance of each data group. The homogeneity test used the Barlett test because the data tested were more than two groups of data with a level of 0.05. If sig. > then H_0 is accepted and the data obtained is homogeneous. The results of the homogeneity test are shown in Table 4.

Table 4. Homogeneity Test Results

α	Sig.
0.05	0.99

Based on the data obtained, the results were 0.99 > 0.05 so that the data obtained were homogeneous.calculation *ANOVA* was carried out to determine whether there was a significant effect on the starch of Keji shard leaves (*Strobilanthes crispus*) on the inhibition zone of *Salmonella thypi*. With the test criteria if sig. < then the data is significant which means that the treatment has an effect on the variables analyzed. From the results of the *ANOVA* the results are shown in Table 5.

Table 5. *One Way Annova*

Inhibition

	Sum of squares	Df	Average	F	Sig.
Between Groups	3,970	4	0,992	0,059	0,993
In Group	335,256	20	16,763		
Total	339,226	24			

Based on the results of calculations obtained the results that sig. > i.e. 0.993 > 0.05 which means the data is not significant, this indicates that the treatment with starch leaves of vile shard (*Strobilanthes crispus*) has no effect on the inhibition zone of *Salmonella thypi*.

This study found that the leaf extract of the vile shard (*Strobilanthes crispus*) has antibacterial properties that affect the growth of *Salmonella thypi*. This is evidenced by the formation of an inhibitory zone around the paper discs in the treated medium. The Vile Beling plant (*Strobilantes* crispus) has compounds such as potassium, sodium, calcium, silicic acid, alkaloids, saponins, flavonoids and polyphenols (Nurraihana, 2013). Compounds such as flavonoids and alkaloids are

compounds that have the potential as antioxidants and can inhibit bacterial growth (Andriani, 2016).

Inhibition of the growth of bacterial colonies can occur due to damage to the structural components of the cell membrane in bacteria. Damage to the bacterial cell membrane can interfere with the nutrient transport process. So that the cells will experience a lack of nutrients needed in the process of bacterial growth (Foster *et al.*, 2019). The mechanism of action of flavonoids is to denature bacterial cell proteins and damage the cytoplasmic membrane which causes leakage of important metabolites and inactivates enzyme systems in bacteria. The damage that occurs allows nucleotides and amino acids to seep out and prevent the entry of active ingredients into cells and can cause bacterial death (Agoes, 2010).

The diameter of the clear zone formed around the paper disc indicates the size of the concentration given to inhibit bacterial growth. Based on the results of the measurement of the diameter of the inhibition zone of the starch of the leaves of keji shard (*Strobilanthes crispus*) at a concentration of 55% the average diameter was 0.14 mm with a weak category. At a concentration of 70% the average diameter is 0.4 mm in the weak category, at 85% concentration the average diameter is 0.9 mm in the weak category, at 100% concentration the average diameter is 2.38 mm in the medium category. The positive control treatment used thiampenicol which had an average diameter of 9.8 mm. In this study, 100% starch concentration had the largest average diameter of the inhibition zone. This is in accordance with research conducted by Ajizah (2004) that the more concentrated the concentration, the more secondary metabolites contained therein, so that it can affect the clear zone formed. Based on the calculation of the standard deviation of the leaf extract of vile shard (*Strobilanthes crispus*) with concentrations of 55%, 70%, 85%, and 100% had an effect on the growth of *Salmonella thypi* seen by the formation of a clear zone on Na media. The average clear zone in the positive control was greater than the treatment group. Thiampenicol is a broad-spectrum antibiotic that is effective against gram-positive and gram-negative bacteria. It works by inhibiting bacterial protein synthesis so that the resulting inhibition zone is wider (Ho, 2000).

The difference in the diameter of the inhibition zone formed could be caused by the incubation temperature, the time of insertion of the disc and the distance of the antimicrobial disc. The bacteria used can also affect the inhibition zone that is formed, when viewed from the measurement of the standard deviation of the average diameter of the inhibition zone at each concentration of the leaf extract of Keji shard (*Strobilanthes* crispus) it can restrain the growth rate of *Salmonella thypi*. This is because the bacterium *Salmonella typhi* is a gram-negative bacterium that has a large amount of lipoprotein, liposaccharide and fat, and the presence of a cell wall layer that can affect the work activity of antibacterial substances (Bachtiar, *et al*., 2012). The solubility properties of each of the active ingredients contained in the keji shard leaves also affect the differences in the inhibition zones formed (Tuna, *et al*., 2015).

Before being calculated with the *one way annova* , the data that has been obtained are tested for normality and homogeneity first. Normality test using *One Sample Kolmogorov-Smirnov Test.* The results obtained show 0.12 > 0.05 which means the data is normally distributed. Then the homogeneity test was carried out and the results were 0.99 > 0.05 which showed the data obtained were homogeneous and could be used for further statistical tests using the *one way annova* and the results obtained were sig. > ie 0.99 > 0.05which means that the results are not significant and there is no difference in the effect of inhibition of the starch extract of Keji Beling (*Strobilanthes crispus*) leaves at different concentrations on the growth of *Salmonella typhi*. In the calculation of Kruskall Wallis obtained the results of sig. > ie 0.90 > 0.05. This shows that the concentration of starch from the leaves of Keji Beling (*Strobilanthes crispus*) has no effect on the inhibition zone of *Salmonella thypi*.

There were several factors that caused the results to be insignificant, including differences in the content of secondary metabolites contained in the leaf extract of Keji Beling (*Strobilanthes crispus*). The concentration given to the starch from the leaves of Keji shard (*Strobilantes crispus*)

greatly affected the results obtained, namely the size of the inhibition zone could be influenced by the size of the concentration given. The results of Ambarwati's research (2007) that the cause of insignificant results could occur due to differences in the rate of diffusion of antibacterial compounds on agar media as well as different types and concentrations of bacterial compounds.

CONCLUSION

Based on the results of the research conducted, it can be concluded that the concentration of starch from the leaves of the vile shard (*Strobilanthes crispus*) has no effect on the inhibition zone of *Salmonella thypi.*

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REFERENCES

Agoes, A. (2010). *Indonesian Medicinal Plants*. South Jakarta: Salemba Medika.

Ajizah, A. (2004). Sensitivity *Salmonella thypimurium* to Guava Leaf Extract (*Psidium guajava* L.) *Bioscientie.* 1(1), 31-8.

Amalia, SN, Syafnir, L., Purwanti, L. (2015). *Effect of Leaf Location on Total Catechin Levels in Keji Beling (Strobilanthes Crispus) Leaves*, Proceedings of the Academic Civitas Research (Health and Pharmacy), 1(1), 24–32.

Ambarwati. (2007). The Effectiveness of Neem Seed Antibacterial Substance (*Azadirachta indica*) to Inhibit the Growth of *Salmonella* and *Sthapylococcus aureus*. *Biodiversity*. 8(3), 320-325.

Andriani, Y., et al. (2016). Biological Activities of Isolated Compounds from Three Edible Malaysian Red Seaweeds, *Gracilaria changi* snd *Gracilaria sp*. *Natural Product Communications*. 11(8), 1117-1120

Bachtiar SY, Tjahjjaningsih W., & Sianita N. (2012). Effect of Brown Algae Extract (*Sargassum sp.*Bacteria Growth *Escherichia coli*. *Journal of Marine and Coastal Science*, 1(1), 53-60.

Crump, John A. (2019). Progress in Typhoid Fever Epidemiology. Clinical Infectious Diases. 68 (1). Doi : https://doi.org/10.1093/cid/ciy846

Dalimartha, S. (2006). *Atlas of Indonesian Medicinal Plants Volume 4*. Jakarta: Puspa Swarna.

Dewoto. (2007). Development *of Indonesian Traditional Medicines To Become Phytopharmaceuticals.* Department of Pharmacology. Medical School. University of Indonesia, Jakarta. 57(7), 1-205.

Foster, Kevin R., Elisa T. Granato. & Thomas A. M. L. (2019). The Evolution and Ecology of Bacterial Warfare. *Current Biology*. 29 (11).

Hidayat, MN Hifiza, A. & Asmar, I. (2013). Inhibitory Test of Herbal Ingredients (Garlic, Betel Leaf, and Cinnamon) Against the Growth of *Basillus subtilis* and *Eschiricia coli*. *Animal Husbandry Industry Science*, 1(1), 13-23.

ho, Shupeng & HSU, Tain-Yao & Che, M & WANG, Way-Shyan. (2000). Antibacterial Effect of Chloramphenicol, Thiamphenicol and Florfenicol against Aquatic Animal Bacteria. The *Journal of veterinary medical science / the Japanese Society of Veterinary Science*. 62. 479-85. 10.1292/jvms.62.479.

Juwita, S., Hartoyo, E., & Budiarti, LY (2013). In Vitro Sensitivity Pattern of Salmonella Typhi To Antibiotics Chloramphenicol, Amoxicillin, And Co-trimoxazole: In Children's Section of Ulin Hospital Banjarmasin Period May-September 2012. *Periodical Medicine*, *9*(1), 25-34.

Lauma, MM, Pangemanan, DHC, & Hutagulung, BSP (2015). Test the effectiveness of lime juice (citrus aurantifolia s) on the growth of staphylococcus bacteria.

Linga, RA Pato, U., & Rossi, E. (2015). Antibacterial test of kecombrang (Niculaia spesiosa horan) stem extract against staphylococcus aureus and eschericia coli. JOM Faperta, 3(1) 1-7.

Mairer, RM, Pehaler, IL., Gerba. (2000). *Environmental Microbiology,* Fourth edition, Williams and Wilkins, Ballimore, Maryland, United States of America.

Noviyanti, Y., Pasaribu, SP, & Tarigan, D. (2014). Phytochemical test of toxicity and antibacterial activity of ethanol extract of rambusa leaf (Passiflora foetida I) against Staphylococcus aureus and Eschericia coli bacteria. Mulawarman Chemistry, 12(1), 31-36.

Nugroho, S. (2011). *Typhoid Fever Treatment*. Yogyakarta: Nuha Medika.

Nurraihana, H., Norfarizan-Hanoon, N, A. (2013). Phytochemistry, pharmacology and Toxicological properties of *Strobilanthes crispus*. *International Food Research Journal.* 20(5), 2045-2056.

Peneng, INM, & Sumatra IW (2007). *Investment in Plants with Traditional Wound Medicines in Jatiluwih Village, Penebel District, Tabanan Regency, Bali,* Proceedings of the Bali Usada Plant Conservation Seminar and Its Role in Supporting Ecotourism, UNUD, LIPID, UNHI, 118-123.

Prime, A. (2020). Thousands of Typhoid Fever Children. Accessed on December 23, 2020, https://linggaupos.sumeks.co/ribuan-anak-demam-tipoid/

Permatasari, GAAA, Besung IN K & Mahatmi H. (2013). Inhibitory Power of Soursop Leaf Juice Against *Escerichia coli*. *Indonesia Medicus Veterinus*. 2(2), 162-169.

Rahmawati, F., Bintang, M., & Artika, M. (2017). Antibacterial activity and phytochemical analysis of geranium homeanum turez leaves. *Current Biochemistry*. 4(3), 13-22.

Tuna MR, Billy JK Michael AL (2015). Inhibitory Test of Soursop Leaf Extract (*Annona muricata L.*) Against the Growth of *Staphylococcus aureus* In vitro. *Scientific Journal of Pharmacy I,* UNSRAT, 4(4), 2302 – 2493.

Winato, BM Sanjaya, E. & Siregar L. (2019). Test of Antibacterial Activity of Ethanol Extract of Bay Leaf (*Syzigium poliantha*) against *Streptococcus mutans* and *Kliebsiella pneumoniae* and their formulation in the form of lozenges. *Pharmaceutical proceedings*. 2(2), 358-365.