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Comparison of The Effectiveness Antibacterial Test of Natural Ingredients and Antibiotic in *Salmonella typhi* Isolated from Hospital Waste and Household Waste

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Abstract: *Salmonella typhi* is a bacterium that causes salmonellosis which is a serious disease in Indonesia and is still endemic. Until now, the treatment and handling of cases of bacterial infection are still using antibiotics. The comparison of antibacterial effectiveness using turmeric extract, a ginger extract which is a natural ingredient with the antibiotics Ampicillin and Ciprofloxacin on *Salmonella typhi* isolates from hospital waste and household waste has been conducted. The purpose of this research is to compare the antibacterial effectiveness of *Curcuma longa* (turmeric) extract, *Zingiber officinale* (Ginger) extract, Ampicillin, and Ciprofloxacin antibiotics in inhibiting the growth of *Salmonella typhi* which are isolated from hospital waste and those isolated from household waste. This research is experimental research (experimental), using a control group and the research is carried out in a laboratory. The data collection technique was carried out by measuring the inhibition zone. Size of the inhibition zone of hospital isolates after treated by turmeric and ginger extract is smaller than household isolates. This result indicates that excessive usage of antibiotics will be able to increase the resistance or immunity of *Salmonella typhi*. Substances which are contained in turmeric and ginger extracts have ability to inhibit the growth of *Salmonella typhi,* with strong category against hospital isolates and very strong category against household isolates. It is concluded that turmeric and ginger extracts are able to substitute synthetic antibiotics such as Ampicillin.

Keywords: Antibiotics; Ginger; *Salmonella typhi;* Turmeric

Introduction

Bacterial resistance to antibiotics has been widely reported (Depkes RI, 2013). The results of the antimicrobial resistance research in Indonesia (AMRIN-Study) proved that of 2,494 individuals spread throughout Indonesia, 43 percent of *Escherichia coli* resistant to various types of antibiotics (Walewangko et al., 2015), but for data on the level of resistence of the bacteria *Salmonella typhi* that causes *Salmonellosis* is still very rare in Indonesia (Monica et al., 2013).

Salmonella typhi is a bacterium that causes *salmonellosis* which is a serious disease in Indonesia with a *case fatality rate* of 10% (Nainggolan, 2011). This occurs because the incidence is quite high (0.36-0.81% per year) and various obstacles in the picture group. clinical setting, diagnosis, and treatment (Yuliana, 2015). This disease is considered serious because it can be accompanied by various diseases and also has a fairly high mortality rate (Yuliana, 2015). *Salmonellosis* can occur at all ages, mostly at the age of 3-19 years, about 77% with the highest peak at the age of 10-15 years (Darmawati & Dewi, 2008).

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Handling cases of infections caused by bacteria are still using antibiotics. Its use during the last five decades has increased tremendously (Utami, 2011). Due to unwise use, various problems of resistance to antibiotics arise which cause the treatment of infectious diseases with antibiotics to be inefficient, relatively more expensive, and even a serious problem is when there are no longer antibiotics that can be used and are able to eradicate the bacteria that cause infection. which can be life-threatening for the patient.

Infection by bacteria that have become resistant results in ineffective treatment so that the infection continues and increases the risk of spreading the infection to others (WHO 2016). Resistance genes can be inherited or can be obtained from cellular genetic elements such as plasmids that can occur between bacteria (Read & Woods, 2014). Low-dose antibiotic concentrations (*subtherapeutic*) can increase the development of antibiotic resistance by triggering genetic changes (Ventola, 2015). Because the use of antibiotics is considered to have too great a risk, so many people turn to traditional medicine. Treatment of typhoid fever or *salmonellosis* can be done medically and traditionally, the appeal of traditional medicine mainly comes from its natural nature so that it is considered safer and better tolerated than modern medicine.

The types of plants that are considered capable of being used as drugs for bacterial infection are turmeric and ginger, these two types of plants have compounds that are able to inhibit bacterial growth and act as anti-cancer drugs (Sulistyarsi et al., 2019). Turmeric contains chemicals such as curcumin, essential oils, starch, and ash. The active compound of turmeric, namely curcumin, acts as an antitumor, antibacterial and antioxidant. This compound has the ability to inhibit growth and kill bacteria. Compounds that act to inhibit the growth of *Salmonella typhi* is curcumin indicated by a inhibition zone. Turmeric can also play a role in inhibiting the growth of *Salmonella typhi* causes typhus by denaturing and damaging cell membranes so that cell metabolism processes will be disrupted and damaged.

Based on the above analysis, we conducted a comparative study of antibacterial effectiveness using turmeric extract, a ginger extract which is a natural ingredient with the antibiotics ampicillin, and ciprofloxacin on *Salmonella typhi* from the hospital and household waste. The purpose of this practicum is to compare the antibacterial effectiveness of *Curcuma longa* (turmeric) extract, *Zingiber officinale* (Ginger), ampicillin, and ciprofloxacin antibiotics in inhibiting the growth of *Salmonella typhi* isolated from hospital waste and those isolated from household waste.

Method

This research is experimental. Experimental research is research that is used to find the effect of certain treatments on others under a controlled condition. A controlled conditions means that the results of the research are converted into numbers (Sugiyono, 2011). Based on the inhibition zone formed, the antibacterial activity can be classified into several groups as shown in the following table.

Table 1. Category of Antibacterial Activity Strength

Category	Zone Diameter
weak	<5 mm
currently	5 – 10 mm
strong	10 – 20 mm
very strong	>20 mm

Source: Handayani et al., (2015)

The tools used in this research are: laminar airflow, mask, petri dish, cutter, sonication, porcelain dish, Bunsen, spatula, round loop, test tube, 20 mL vial, autoclave, oven, tweezers, label paper, matches, aluminum foil, 10 mL measuring pipette, dropper, object-glass, dish blank, bunsen, stove, glass stirrer, tweezers, incubator, measuring cup, test tube, Erlenmeyer, centrifuge, analytical balance, razor, ruler, grate, filter, and other practical tools.

The materials used in this study were: a) Extraction and Antibiotics: The materials used in this experiment were *Curcuma longa* extract (turmeric extract), *Zingiber Officinale* (Ginger extract), ampicillin and ciprofloxacin; b) Bacterial Test: Extract of turmeric rhizome (*Curcumae longa*), Ginger extract (*Zingiber officinale*), equates, cotton swabs, aluminum foil, MHA media (*Mueller Hinton agar*), ampicillin and ciprofloxacin; c) Bacteria Test Bacteria: The bacteria used in this practicum are not isolated by the practitioner, but the bacteria obtained at the Laboratory of the Faculty of Medicine, University of Mataram *Salmonella typhi* obtained from the hospital environment, for *Salmonella typhi* originating from the household environment obtained from the Laboratory Mataram Health and Calibration.

Research Procedures

Making Turmeric and Ginger Extracts. a) Prepare tools and materials used; b) Samples of turmeric rhizome were sorted wet, washed, and then sliced thinly; c) dried or aerated to dry; d) After drying the turmeric rhizome samples were mashed using a blender; e) Maceration is carried out in separate containers by soaking each Simplism as much as 50 grams in 200 ml of acetone for 3 days; f) Stirring every day 3 times during the maceration period; g) Filtering the maceration results using filter paper; h) The maceration result is evaporated using an evaporator until the acetone is

separated and leaves a pure turmeric extract; i) Perform steps b to h on ginger; j) Extracts of turmeric and ginger are ready to use.

Making MHA (*Muller Hinton Agar*) media. a) The media for the test bacteria using Mueller Hinton Agar (MHA) was weighed as much as 19 grams and dissolved in 600 ml of distilled water; b) The solution is then heated on the stove and stirred with a glass stirrer until homogeneous, let cool, then cover with aluminum foil; c) MHA media (*Muller Hinton Agar*) was sterilized in an autoclave at 121^{o}C pressure 15 atm for 15 minutes; d) Reheat the MHA (Muller Hinton Agar) media on the stove; e) Sterilize the lab table using 96% alcohol; f) Pour MHA (*Muller Hinton Agar*) media into Petri dishes, each petri dish contains 15-20 ml and is allowed to solidify; g) Divide the solidified MHA (*Muller Hinton Agar*) media into 2 parts, 12 media for *Salmonella typhi* from hospitals, 12 media for *Salmonella typhi* from households, then label them.

Sterilization of Tools and Materials. All equipment and materials to be used are wrapped in paper or plastic, then sterilized using an autoclave at 121 ^{0}C pressure 15 atm for 15 minutes. Effectiveness Test. Testing the effectiveness of turmeric extract, ginger extract, ampicillin antibiotics, and ciprofloxacin against *Salmonella typhi* was carried out using the disc method. Bacterial resistance test was carried out by sterilizing the lab table using 96% alcohol and then preparing MHA (*Muller Hinton Agar*) media, cotton swabs, and bacterial suspension. These steps are followed by dipping a cotton swab in the bacterial suspension and then spread it on the prepared MHA (*Muller Hinton Agar*) media. This last step is repeated 3 times on each bacterium with a different cotton swab, then let it standing for 15 minutes. Disc paper or disc are dipped into turmeric extract or ginger extract with a concentration of 25%, 50%, 75% and 100% for 1 hour, remove and let it drain for a moment, then place the paper disc or disc on the surface of the MHA media (*Muller Hinton Agar*) which has been implanted with bacteria. The disc paper is pressed using tweezers so that it sticks perfectly on to the agar surface. Place ampicillin and ciprofloxacin antibiotic disc using tweezers and press it so that it sticks perfectly to the agar surface and then incubating for 24 hours at 37^{o} C. The diameter of the inhibition zone of bacteria can be measured using a millimeter block or ruler.

Result and Discussion

Picture of Inhibition Zone Observation on Effectiveness Test

Samples of *Salmonella typhi* were isolated from hospital waste and household waste and grown on LB media which is commonly used for inoculation of various microorganisms, both aerobic and anaerobic. After growing well, *Salmonella typhi* was re-cultured on MHA (*Muller Hinton Agar*) media for sensitivity testing. Isolate *Salmonella Typhi* taken from hospital waste and household waste that has gone through the rejuvenation stage.

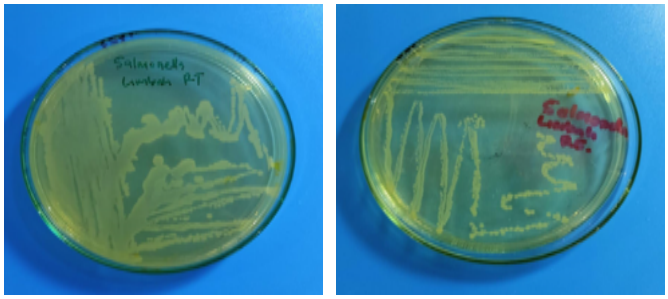

Figure 1. Isolates of *Salmonella typhi* from Hospital Waste and Household Waste that have undergone a rejuvenation

After having rejuvenation, the effectiveness of antibiotics ampicillin, ciprofloxacin, turmeric extract, and the ginger extract was tested against *Salmonella typhi* taken from the hospital and household waste.

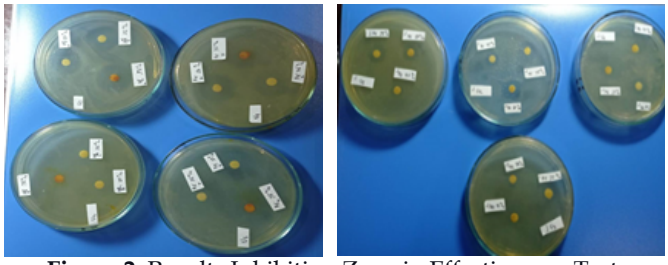

Figure 2. Results Inhibition Zone in Effectiveness Test, Turmeric Extract and Ginger Extract against *Salmonella typhi* from hospital waste

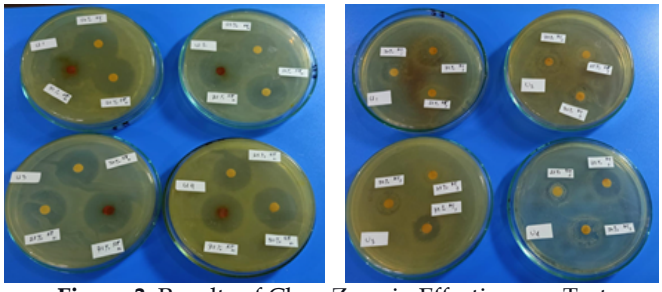

Figure 3. Results of Clear Zone in Effectiveness Test, Turmeric Extract, and Ginger Extract against *Salmonella typhi* from household waste

Inhibition Zone Observation Results Table

Based on the results of testing the antibacterial effectiveness of ampicillin, ciprofloxacin, turmeric extract, and ginger extract against *Salmonella typhi* from the hospital and household waste, the following data were obtained:

The zone formed on average from 4 replications, ciprofloxacin has the highest value of 26.25 mm in the very strong category, followed by 75% turmeric extract with a inhibition zone formed 19.17 mm in the strong

category. Furthermore, for ginger extract at a concentration of 75%, the inhibition zone area was 11.75, which was larger than ampicillin with a zone area of 11.25 in the strong category. If we look at the concentration of turmeric and ginger with a concentration of 75% has been able to form a zone with a strong category, it is possible that the higher the concentration, the larger the zone formed. The zone formed shows the ability of antibiotics as well as the content of natural ingredients extracts of turmeric and ginger in inhibiting the growth of *Salmonella typhi* from hospital waste.

Table 2. Measurement Results of *Salmonella typhi* Inhibition Zone Hospital Waste (mm)

S	A	C	Turmeric Extr			Ginger Extract		
			75%	50%	25%	75%	50%	25%
RS1	12	25	24	20.67	19.67	9.67	8	6.67
RS2	11	27	14.67	8.67	7.67	18	14.33	9.67
RS3	12	27	14	12	8	10.33	7	6.33
RS4	10	26	24	20.33	19.33	9	7	6.67
Total	45	105	76.67	61.67	54.67	47	36.33	29.33
Average	11.25	26.25	19.17	15.42	13.67	11.75	9.08	7.33
Description	S	VS	S	S	S	S	C	C

S = Sample; A = Ampicillin; C = Ciprofloxasin
RS = Rumah Sakit (hospital isolates)
S = Strong; VS = Very Strong; C = Currently

Table 3. Inhibition Zone *Salmonella typhi* from Household Waste (mm)

S	A	C	Turmeric Extr			Ginger Extract		
			75%	50%	25%	75%	50%	25%
RT1	17	40	27	25.66	22.66	18.66	13.66	8
RT2	18	42	27.66	25.33	24.33	15	12.33	8
RT3	18	41	27.33	24.33	22.33	17.33	13.66	8.33
RT4	17	40	26.33	25	22.33	19	15.33	11.33
Total	70	163	108.32	100.32	91.65	69.99	54.98	35.66
Average	17.65	40.75	27.08	25.08	22.92	17.50	13.75	8.92
Description	S	VS	VS	VS	VS	S	S	C

S = Sample; A = Ampicillin; C = Ciprofloxasin
RT = Rumah Tangga (household isolates)
S = Strong; VS = Very Strong; C = Currently

Furthermore, the effectiveness test was carried out on *Salmonella typhi* from household waste, based on the results of the calculation of the inhibition zone formed from the table above, can be seen the comparison of the anti-bacterial effectiveness of ampicillin, ciprofloxacin, Turmeric Extract, and Ginger Extract against *Salmonella typhi*. The zone formed by an average of 4 replications, ciprofloxacin has the highest value of 40.75 mm in the very strong category, followed by 75% turmeric extract with a inhibition zone of 25.08 mm in the very strong category. Furthermore, for ginger extract at a concentration of 75%, an inhibition zone area of 17.50 was obtained, the same as ampicillin with a zone area of 17.50 in the strong category.

If we look at the concentration of turmeric and ginger with a concentration of 75% has been able to form a zone with a very strong and strong category, it is possible that the higher the concentration, the larger the zone formed. The zone formed shows the ability of antibiotics as well as the content of natural ingredients extracts of turmeric and ginger in inhibiting the growth of *Salmonella typhi* from household waste.

Comparison of Antibacterial Effectiveness Test Results for Turmeric Extract, Ginger Extract, Ampicillin, and Ciprofloxacin

Based on the results of the calculation of the inhibition zone of each antibiotic, turmeric extract and ginger extract from a concentration of 75% were tested on *Salmonella typhi* isolated from hospital waste and household waste, based on the values obtained with four repetitions, the following data were obtained as table 4.

Table 4. Average Measurement Results *Salmonella typhi* from Hospital and Household Waste (mm)

Average Sample	Sample Name	Amphi cillin	Ciproflo xacin	TE 75%	GE 75%
Inhibition zone	RS	11.25	26.25	19.17	11.75
	RT	17.50	40.75	27.08	17.50

TE = Turmeric Extract
GE = Ginger Extract
RS = Rumah Sakit (hospital isolates)
RT = Rumah Tangga (household isolates)

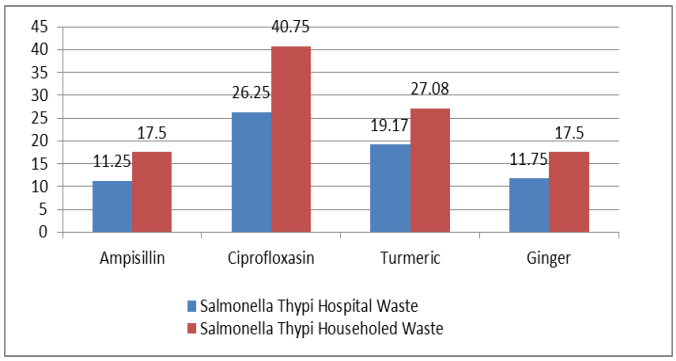

Figure 4. Measurement Result of *Salmonella typhi* Inhibition Zone *from* Hospital and Household Environmental Waste (mm)

From the results of testing for antibiotics, turmeric extract and ginger extract against *Salmonella typhi* derived from different isolates, namely hospital waste and household waste, which are shown by tables and graphs, showing different data, the area of the inhibition zone formed indicates the level of strength of a substance or antibiotic, the wider the zone formed. the materials used can be said to be good materials to inhibit the growth of *Salmonella typhi*.

The ampicillin antibiotic for sensitivity test on *Salmonella typhi* taken from hospital waste has a zone of 11.25 mm while that from household waste shows a zone size of 17.50 mm. For testing using ciprofloxacin data obtained 26.25 mm on isolates from hospital waste while that of household waste is 40.75 mm.

Tests using 75% turmeric extract on isolates originating from hospital waste, the zone formed was 19.17 mm while those from household waste were 27.08, then the ginger extract was formed zones of 11.75 mm and 17 respectively. ,50 mm zone area.

If we look at the area of the zone formed, the zone from household waste has a larger zone, namely Ciprofloxacin 40.75, which shows a much lower level of bacterial strength from household waste when compared to bacteria from hospital waste. As stated by Ventola (2015) that low-dose (*subtherapeutic*) antibiotic concentrations can increase the development of antibiotic resistance by triggering genetic changes. So it should be suspected that *Salmonella typhi* originating from hospital waste can be said to have begun to experience a level of immunity and even at times can become resistant to antibiotics.

Until now, the treatment and handling of cases of bacterial infection is still using antibiotics. Its use over the last five decades has increased tremendously. As a result of unwise use, various problems of resistance to antibiotics arise which cause the treatment of infectious diseases with antibiotics to be inefficient (Utami, 2011). Thus the difference in the zones formed indicates the level of strength of *Salmonella typhi* from hospital waste and *Salmonella typhi* from household waste.

Excessive use of antibiotics will be very worrying, so it is necessary to strive for antibiotics from natural ingredients that can replace synthetic antibiotics so that they do not have too big a risk in their use and are easily obtained in nature. Based on the tests carried out on *Curcuma longa* extract (turmeric extract) and *Zingiber Officinale* (ginger extract) they can be used as alternative antibiotics to replace synthetic antibiotics that have been used in hospitals.

The use of natural ingredients such as turmeric can be used as an alternative to natural antibiotics, because of the content it contains. Turmeric contains various compounds such as alkaliods, flavonois, curcumin, essential oils, saponin, tannins, and terpenoids. *Curcumin* and essential oils have been shown to have anti-inflammatory properties. In addition, the *curcuminoid* has properties that have antibacterial, anti-seizure, analgesic, anti-iarrheal, antipyretic, and anti-tumor properties. Curcumin compounds are the same as other chemical componends such as antibiotics, *alkaloid*, *steroid*, *essential oils*, *resins*, and *phenols* which are included in the secondary metabolites of a plant (Afidatul et al., 2019).

When viewed from the results of the effectiveness test, turmeric and ginger extract with a concentration of 75% obtained a zone of 19.17 mm against *Salmonella typhi* from hospital waste and 27.08 mm showed a strong and very strong level of effectiveness while using ginger extract showed a zone with a strong category. where the zones formed are 11.25 mm and 17.50 mm, respectively, meaning that if we look at the size of the zone formed, the content of turmeric and ginger extracts has been able to exceed the ability of the antibiotic ampicillin to inhibit the growth of *Salmonella typhi*. So that in the future it is recommended that the use of synthetic antibiotics can be reduced and switch to antibiotics derived from natural ingredients such as turmeric and ginger or even from other types of natural ingredients.

Conclusion

Size of the inhibition zone of hospital isolates after treated by turmeric and ginger extract is smaller than household isolates. This result indicates that excessive usage of antibiotics will be able to increase the resistance or immunity of *Salmonella typhi* Substances are contained in turmeric and ginger extracts have ability to inhibit the growth of *Salmonella typhi,* strong category against hospital isolates and very strong category against household isolates. It is concluded that turmeric and ginger extracts are able to substitute synthetic antibiotics such as Ampicillin.

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