EFFECT OF EARTHWORMS (*Lumbricus Sp*.) EXTRACT ANTIBACTERIAL ACTIVITY AGAINST THE BACTERIA *Salmonella Typhii*

Imannita Septianda[1], Kartuti Debora[2], Maftuchah Rochmanti[3]
[1]Medical Doctor Study Program, [2]Department of Medical Microbiology,
[3]Department of Medical Pharmacology, Faculty of Medicine,
Airlangga University, Surabaya

ABSTRAK

*Demam tifoid masih menjadi salah satu penyakit endemik di Indonesia. Penyakit infeksi sistemik ini disebabkan oleh infeksi bakteri Salmonella typhii. Cacing tanah merupakan hewan yang banyak dan mudah ditemukan. Cacing tanah merupakan salah satu alternatif obat berbagai penyakit, salah satunya penyakit demam tifoid. Penelitian ini bertujuan untuk menunjukkan keberadaan aktivitas antibakteri ekstrak cacing tanah Lumbricus sp. terhadap bakteri Salmonella typhii secara in vitro. Penelitian ini merupakan jenis penelitian eksperimental laboratorik dengan isolat Salmonella typhii 0,5 McFarland dengan ekstrak cacing tanah Lumbricus sp. dalam konsentrasi 50 mg/mL, 25 mg/mL, 12,5 mg/mL, 6,25 mg/mL, yang diuji dengan menggunakan metode dilusi agar. Pengamatan pertumbuhan bakteri dilakukan pada Mueller Hinton agar plate setelah diinkubasi selama 24 jam pada suhu 37^{o}C. Hasil penelitian menunjukkan ekstrak cacing tanah Lumbricus sp. sampai dengan konsentrasi 3200 mg/mL tidak mempunyai efek aktivitas antibakteri terhadap bakteri Salmonella typhii. Dapat disimpulkan bahwa ekstrak cacing tanah Lumbricus sp. sampai dengan konsentrasi 3200 mg/mL tidak mempunyai efek aktivitas antibakteri terhadap bakteri Salmonella typhii, sehingga diusulkan untuk dilakukan penelitian lebih lanjut untuk menguji aktivitas antimikroba ekstrak cacing tanah Lumbricus sp.. **(FMI 2012;48:102-108)***

Kata Kunci: *ekstrak cacing tanah Lumbricus sp., Salmonella typhii*

ABSTRACT

*Typhoid fever is still one of endemic disease in Indonesia. This systemic infectious disease caused by infection of bacteria Salmonella typhii. Earthworms are animal that are alot and easily found and has a cheap price. Earthworms are used for an alternative medicine for many diseases, including typhoid fever This study aims to show whether or not the effect of antibacterial activity from the extract of earthworms (Lumbricus sp.) against bacteri Salmonella typhii in vitro. This study was one of experimental laboratory research with 0,5 mcFarland isolates of bacteri Salmonella typhii that use concentration of the extract of earthworms Salmonella typhii are 50 mg/mL, 25 mg/mL, 12,5 mg/mL, 6,25 mg/mL, were tested with use the method of dilution plate in order. Observations are made by the growth of bacteria Salmonella typhii in Mueller Hinton plates after incubation for 24 hours at 370 celcius. The result showed that the extract of earthworms (Lumbricus sp.) until the concentration is 3200 mg/mL did not have the effect of antibacterial activity against bacteri Salmnonella typhii. In conclusion, based on the result of this study can be concluded that the extract of earthworm (Lumbricus sp.) until the concentration is 3200 mg/mL did not have the effect of antibacterial activity against bacteri Salmnonella typhii, so it is proposed to do further research to test microbacterial activity of the extract of earthworm (Lumbricus sp.). **(FMI 2012;48:102-108)***

Keywords: *the extract of earthworm (Lumbricus sp.), Salmonella typhii*

Correspondence: Imannita Septianda, Medical Doctor Program, Faculty of Medicine, Airlangga Univesity, Jalan Prof dr Moestopo 47, Surabaya 60131, Indonesia. Phone: 62-31-5020251.

INTRODUCTION

Typhoid fever is still endemic disease in Indonesia. Data from the World Health Organization (2003) estimates that there are approximately seventeen million cases of typhoid fever worldwide with an incidence of 600,000 deaths annually. In Indonesia, the disease incidence between 600,000 to 1.5 million cases per year. Approximately 95% of cases of typhoid fever is an outpatient cases, so the actual incidence is 15-25 times greater than the reported inpatient hospital (PB PAPDI 2006). The disease is a systemic infection caused by *Salmonella typhii* bacteria infection. *Salmonella typhii* is a genus of bacteria enterobacteria gram-negative rod-shaped (Ryan & Ray, 2003). *Salmonella typhii* is Salmonella enterica serotype of the species. It grows best between 35 and 37°C but can grow to between 7 and 45°C. Grow through a variety of pH 3.8 to 9.5, have a minimum water activity level of 0.94, grown either the presence or absence of oxygen and under nitrogen growth is only slightly less than that under oxygen (King & Metzger, 1968).

The bacteria are spread through the faecal-oral way, so it is less easy to spread from one individual to another individual (Safitri 2010). These bacteria thrive in water a lot, especially dirty water. Therefore, many of Indonesia's population is afflicted by this disease, especially populations living in environments with poor sanitation such as in riverbank (Anonymous 2009). Earthworm is known as a lot of animals help farmers. In Indonesia earthworms have only utilized as bulking land animals, whereas the number of earthworms very much, but can not be fully utilized (Anonymous 2009).

Unlike the case in developed countries, the worm has been more widely used for example in China, Korea, Vietnam, and many other places in Southeast Asia, especially from the ground to the worms *Lumbricus sp*p types, can be used as medicine for thousands of years ago. Earthworms have been included in "*Ben Cao Gang Mu*", a book ingredient standards (pharmacopoeia) in traditional Chinese medicine. In China, the familiar earthworm called 'dragon lands'. Name of dried earthworm market among traders of traditional Chinese medicine is *ti kam lung* (Anonymous 2009). Even in some countries of Asia and Africa, earthworms that have been cleaned and then dried in the sun to dry cut, commonly used as medicinal food (healing foods). Usually dry roasted or fried, eaten as chips worms. Allegedly this worm eating habits can help reduce the number of deaths due to diarrhea in poor countries of Asia and Africa (Anonymous 2009). Whether in the form of fresh or dried, the dragon land in Korea is processed into refreshing soup is commonly eaten at bedtime, so the next day those who ate it can work energetically. After cleaning the dirt through a special processing technique, earthworms are sold as a traditional medicine in Korea (Anonymous 2009).

Typus disease if treated properly actually is not fatal, but if the disease typhus allowed to drag on without proper treatment or no treatment at all can lead to death (Anonymous 2009). Krištufek et al (1999) found different types of Streptomyces on earthworms. Antibacterial compounds produced from the fermentation of Streptomyces sp. isolated from the stomach of earthworms *Allolobophora rosea* was only inhibits gram-positive bacteria (Hardijulendra 2002). In vitro studies by Chong (as cited in Hardijulendra 2002) to the biological material earthworm flour also managed to find a positive antibacterial activity against a broad spectrum of bacteria. Cho et al (as cited in Hardijulendra 2002), there is mention of the worm lumbricin compounds that showed broad-spectrum antimicrobial activity. With the administration of some antibiotics such as doxycycline, tetracycline, chloramphenicol typhoid disease can be cured (Baumslag 2005), but the price of these drugs is not cheap, therefore this study was conducted in order to find an alternative medicine typhoid diseases are easily available and the price within reach of circles. The purpose of this study was to determine whether the extract of earthworm (*Lumbricus sp*.) can be used as an anti-microbial *Salmonella typhii* bacteria.

MATERIALS AND METHODS

This study is a preliminary type of true-experimental, to test that the *Salmonella typhii* dilution on Mueller Hinton agar medium plate which will then be given the extract of the earthworm *Lumbricus sp*. concentrations were determined on a pre-study. This study used a research design Separate Sample Post Test Control Group Design (Campbell et al 1966). The population in this study is the bacterium *Salmonella typhii*. The sample used in this study were *Salmonella typhii* 0.5 McFarland of Central Laboratory Diagnostic Center Dr. Soetomo Hospital in Surabaya. Samples were cultured or grown in Mueller Hinton agar plate.

Earthworms are made in the form of extracts to take active ingredient. Extraction using ethanol, it is based on Singh et al (2002) who have observed that ethanol is the best solvent for the taking, the active ingredient in *Lumbricus sp*. The results of the extraction are given in cultured *Salmonella typhii* which has grown 24 hours on Mueller Hinton To Plate. The evaluation was conducted to determine whether this extracts meaningful use as an anti-bacterial in *Salmonella typhii* to see whether or not the growth of *Salmonella typhii* bacteria on Mueller Hinton To Plate. To prove the existence of the work of the antimicrobial compound earthworm extract against *Salmonella typhii* bacteria, so in this study using the broth dilution test in order to determine the minimum inhibitory concentration (MIC).

Treatment to be given to the *Salmonella typhii* samples numbered four, namely providing the type of concentration earthworm *Lumbricus sp*. 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, so that the corresponding formula Federer, repetitions performed on the first and second kind of sample numbering at least four times in each treatment. Samples were taken by random sampling. Data collection was performed 24 hours after administration of the treatment to see whether or not the growth of bacteria, *Salmonella typhii* on Mueller Hinton agar plates. Antimicrobial activity test used the dilution method. With this method it can be seen minimal inhibitory concentration (MIC) is the smallest concentration that was able to inhibit the growth of germs.

Extract earthworm *Lumbricus sp*. prepared at a concentration of 62.5 mg/mL, 12.5 mg/mL, 25 mg/mL, and 50.0 mg/mL, and DMSO which will be used to thin gradually. Then we made a suspension of bacteria. Available stock first cultured to obtain healthy growth (thrives and the logarithmic growth phase or not mutated or phase lag or dead). Suspension initial test was made equivalent to 0.5 Mc Farland turbidity (cloudiness mixture of barium sulfate and HCl) or proportional to the number of bacteria 1. 108 CFU/ml (CFU: Colony Forming Unit) or 250-300 colonies on solid media. Taken a few colonies of bacteria then thinned or diluted with isotonic solution (PBS or PZ) so that the concentration in accordance with the concentration of 0.5 McFarland.

In the dilution test is provided by 4 test tubes and 2 cup partitionade petri dish experiments with labeled T1-T4 and C1-C2. T-1 tube filled with sterile medium of Mueller Hinton agar were mixed with extracts of the earthworm *Lumbricus sp*. concentration of 50 mg/mL with a volume of 1: 1. Between the medium and the extract earthworms mixed. Then take half of the mixture of molten nutrient agar medium and extracts of this tube to be inserted into the tube T-2. Mixture taken from the T-tube into the tube 1dimasukkan T 2 is then added liquid medium with a sterile nutrient agar mixture volume ratio of the tube T - 1 with sterile nutrient agar liquid medium at 1: 1. Mix well so that the concentration of the extract obtained earthworm *Lumbricus sp*. on the tube T - 2 of 25.0 mg/mL. Then take half to put the tube T - 3 so that the concentration in the tube T-3 to 12, 5 mg/mL. The above step is continued until the tube row T - 4, after being mixed and concentrations obtained earthworm *Lumbricus sp*. on the tube T 4 by 6, 25 mg/mL. Once all mixed, it was poured into the cup partitionade petridish.

Then we planted the germ suspension into each dish and incubate all tubes for 24 hours in an incubator at a temperature of 37ºC. After 24 hours, look at each individual cup or not the growth of *Salmonella typhii* bacteria. Then we determine the minimum inhibitory concentration (MIC) is to look at the cup which is not overgrown with bacteria. The extract earthworms *Lumbricus sp*. The smallest in the cup can not be overgrown with bacteria, the minimum inhibitory concentration (MIC). These observations were done visually. The analysis was performed by observing the presence or growth of *Salmonella typhii* bacteria in culture medium after 24 hours of treatment. Data from the treatment group were processed and tested descriptive statistics. The study was conducted in the laboratory of the Department of Microbiology, Faculty of Medicine, University of Airlangga.

RESULTS

Table 1. Results of the dilution test that the antibacterial activity of extracts of the earthworm *Lumbricus sp*. against *Salmonella typhii* bacteria in various concentrations on the first replication

Tube Concentration	Bacterial Growth
50 mg/ml	+
25 mg/ml	+
12.5 mg/ml	+
6.25 mg/ml	+

Notes : + : growth, - : no growth

Table 2. Results of the dilution test that the antibacterial activity of extracts of the earthworm *Lumbricus sp*. against *Salmonella typhii* bacteria in various concentrations on the second replication

Tube Concentration	Bacterial Growth
200 mg/ml	+
100 mg/ml	+
50 mg/ml	+
25 mg/ml	+
12.5 mg/ml	+
6.25 mg/ml	+

Notes : + : growth, - : no growth

Figure 1. The result of mixing the extract earthworms *Lumbricus sp*. and Mueller Hinton agar medium

Figure 2. Results of the agar plate dilution test replication of both

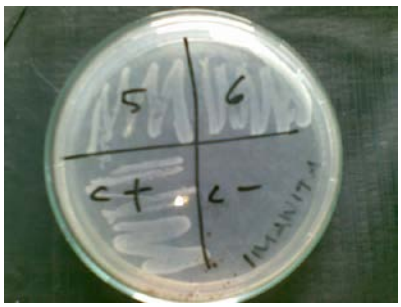

Figure 3. Results of the agar plate dilution test replication of both

Table 3. Results of the dilution test that the antibacterial activity of extracts of the earthworm *Lumbricus sp*. against *Salmonella typhii* bacteria in various concentrations on the third replication

Tube Concentration	Bacterial Growth
3200 mg/ml	-
1600 mg/ml	+
800 mg/ml	-
400 mg/ml	+
200 mg/ml	+
100 mg/ml	+

Table 4. Results of the dilution test that the antibacterial activity of extracts of the earthworm *Lumbricus sp*. against *Salmonella typhii* bacteria in various concentrations on the fourth replication

Tube Concentration	Bacterial Growth
3200 mg/ml	+
1600 mg/ml	+
800 mg/ml	+
400 mg/ml	+
200 mg/ml	+
100 mg/ml	+

Table 5. Results of the dilution test that the antibacterial activity of extracts of the earthworm *Lumbricus sp*. against *Salmonella typhii* bacteria in various concentrations on the fifth replication

Tube Concentration	Bacterial Growth
3200 mg/ml	+
1600 mg/ml	+
800 mg/ml	+
400 mg/ml	+
200 mg/ml	+
100 mg/ml	+

Figure 4. The result of mixing the extract earthworms *Lumbricus sp* and Mueller Hinton agar medium

Figure 5. Results of the agar plate dilution test replication fifth

At that dilution experiments of replication to-1 to -5, to determine whether or not the growth of germs planting mixing results *Salmonella typhii* bacteria, earthworms *Lumbricus sp* extract, and liquid nutrient medium on nutrient agar. In the first experiment, it was showed that the extract of the earthworm *Lumbricus sp*. does not have antimicrobial activity up to levels of 50 mg/ml against *Salmonella typhii* bacteria. This can be seen from the results of the agar diffusion test in which they obtained the growth of bacteria on the order of the first

tube (T1) which has a concentration of the extract at 50 mg/ml to tube-4 (T4) which has a concentration of 25 mg extract/ml, 12,5 mg/ml, 6.25 mg/ml.

Figure 6. Results of the agar plate dilution test replication fifth

In the second replication also obtained the same result, even after the concentration is increased up to 200 mg/ml, extract earthworms *Lumbricus sp*. still not obtained any antimicrobial activity against the bacteria *Salmonella typhii*. In the third through fifth replication, extract concentration increased up to 3200 mg/ml, 1600 mg/ml, 800 mg/ml, 400 mg/ml, 200 mg/ml. In the third replication, no growth of *Salmonella typhii* bacteria obtained at a concentration of 800 mg/ml and 3200 mg/ml, but at a concentration of 1600 mg/ml, 400 mg/ml, 200 mg/ml of the growth of *Salmonella typhii* bacteria obtained.

In the fourth replication, the growth of *Salmonella typhii* bacteria obtained even at a concentration of 3200 mg/ml, *Salmonella typhii* bacteria that grow approximately 7 points on Mueller Hinton agar medium, whereas at a concentration of 1600 mg/ml, 800 mg/ml, 400 mg/ml, 200 mg/ml was found to be the growth of the bacteria *Salmonella typhii*. To be sure, made the fifth replication. In the fifth replication, the growth of *Salmonella typhii* bacteria obtained even at a concentration of 3200 mg/ml, 1600 mg/ml, 800 mg/ml, 400 mg/ml, 200 mg/ml was found to be the growth of the bacteria *Salmonella typhii*. Based on the results of the agar diffusion test third replication, showed the presence of inhibition at a concentration of 3200 mg/ml, but the fourth and fifth replication was found to be the growth of *Salmonella typhii* bacteria at these concentrations.

DISCUSSION

Typhoid fever remains one of the diseases endemic in Indonesia, based on data from the World Health Organization (2003). The disease is a systemic infection caused by *Salmonella typhii* bacteria infection. Salmonella is a genus of gram-negative bacteria enterobacteria sticks (Ryan & Ray, 2003). Earthworms are animals are more easily found in Indonesia, as well as cheap price. Animals that normally help these farmers have many properties, but its use in Indonesia has not been much. Whereas in developed countries used earthworm is one alternative to cure various diseases, one of which typhoid fever (Anonymous 2009).

Antimicrobial compounds contained in extracts of the earthworm *Lumbricus sp*., Which allegedly lumbricin lumbricin 1 and 2, the two types of factors that have antibacterial activity of lysozyme with a case of hemolytic activity and pattern recognition protein named coelomic cytolitic factor (CCF) has been identified in Eisenia foetida earthworm. While the mechanism of antimicrobial peptides owned by earthworms is alleged to create pores in the cell wall of bacteria. This causes the cytoplasm of bacterial cells become exposed to the external environment that can interfere with the activity in the bacterial cells and can cause bacterial death. By using this method, the bacteria will become resistant because it is more difficult for Damaging the structure of the bacterium itself (Jeyakumar et al, 2011). It is also noted in the study of Cho et al (as cited in Hardijulendra 2002) that the flour contained earthworms lumbricin compounds which showed broad-spectrum antimicrobial activity of the compound. In previous studies, research and Muliasari Affandi (as cited in Waluyo et al 2007) have proved the existence of antibacterial power of protein extraction results earthworms Lumbricus rubellus and Pheretima sp can inhibit the growth of Gram-negative bacteria *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Salmonella typhii*. Research extracts for antibacterial activity against *Salmonella typhii* earthworms is expected to provide information on the use of extracts of the earthworm (*Lumbricus sp*.) Against *Salmonella typhii* bacteria, cheap, minimal side effects, and easily obtained.

The method used in this study is that the dilution method. Based on research Waluyo et al (2007), Proteins contained in the earthworm has a concentration minimum barrier against Gram-negative bacteria: Salmonella typhi, *Shigella dysenteriae*, *Escherichia coli*, respectively 40.0 mg/mL, 40.0 mg/mL , 20.0 mg/mL. So that the concentrations used for 3200 mg/mL. 1600 mg/mL, 800 mg/mL, 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml. Researchers used a maximum concentration of 3200 mg/mL based on the assumption that the researcher 3200 mg/mL is the feasible maximum concentration for widespread consumption

society. Extract solution earthworm *Lumbricus sp*. mixed with different concentrations of the nutrient agar media is still warm and then inserted into partitionade petridish. After that planted the germ and incubated for 24 hours at 37 degrees Celsius. After that, it can be seen whether or not the growth of bacteria on agar plate media nutrition.

The results of this study indicate that extracts of the earthworm *Lumbricus sp*. with concentrations up to 3200 mg/mL can not provide an antibacterial effect against *Salmonella typhii*. Based on research conducted by Budiastuti et al (1991) found that 844 mg of earthworm activity equivalent to 0,125 mg of dried chloramphenicol against *Salmonella typhii* and 0.149 mg of chloramphenicol against *Escherichia coli*. For cloramphenicol therapeutic dose for typhoid fever about 2-3 g, 35 patients received therapy chloramphenicol 2-3 g orally four times daily for 14 days. Thirty patients with chloramphenicol therapy showed improvement or healing (Butler et al 1999).

If obtained 844 mg of extract equivalent to earthworm activity cloramphenicol (comparator antibiotics) 0.125 mg, whereas therapeutic doses used for the treatment of typhoid fever at 2 -3 g then when converted to extract earthworms takes about 1.688 million or 1.688 mg g for the equivalent dose therapy with chloramphenicol. Judging from the above sources, the concentration of the extract earthworms *Lumbricus sp*. the researchers used is still very poor to be able to inhibit the growth of *Salmonella typhii*.

This study tried to find whether or not the antibacterial effects of extracts of the earthworm *Lumbricus sp*. *Salmonella typhii* bacteria against accordance with the above conversion, but according to the researchers use the extract earthworms *Lumbricus sp*. earthworms weighing 1688 g with a concentration of more than 3200 mg/mL less effective and efficient, because if the earthworm *Lumbricus sp*. the number being made up in the form of a capsule or other dosage forms, capsules would be very large and difficult to be consumed by the community at large.

The results of this study showed that the extract of the earthworm *Lumbricus sp*. to a concentration of 3200 mg/mL did not have an antibacterial effect against *Salmonella typhii* bacteria, likely to be caused by several other things. Among them is in terms of extraction of earthworms *Lumbricus sp*. which can cause the contents of active antibacterial compounds in extracts of earthworms, such as the enzyme lysozyme, agglutinin, and lytic factor isolated lumbricin be properly or is damaged or becomes non-active. Or other possibilities in terms of the extraction techniques used by researchers. Researchers used the extract earthworms *Lumbricus sp*. derived from the whole body of the earthworm.

Then the other possibilities in the choice of solvents, which can lead to non-antibacterial compounds active can also affect the results of this study. The authors used the solvent DMSO (dimethyl sulfoxide) which has the properties of a weak acid. DMSO is aprotic polar solvents and less toxic compared to other members of the class. DMSO is often used as a solvent and extracted in biochemistry and cell biology (Day & Stacey 2007). Mechanism of action of DMSO is the solvent DMSO weakly acidic (pKa = 35) due to the stabilizing carbanion produced by sulfur (O) R and deprotonisasi process with a strong base. The sodium salt of DMSO produced in this way (also known as "sodium dimsyl") is a substance that reacts on dissolution (Bordwell 1988).

In various previous studies that have been done there is a difference in the minimum inhibitory concentration generated, this happens because of different methods used. This causes a bias for researchers in determining the minimum inhibitory concentration used for research. One example of previous studies which have shown that earthworms have an antibacterial effect against *Salmonella typhii* bacteria, research Waluyo et al (2007) note that the protein fractionation javanica or *Lumbricus pheretima sp*. in MOPS solvent by chromatography on DEAE group of proteins produced three peaks, where the peak third group of proteins has antibacterial activity. Active protein fractionated further using filtration and chromatography yielded two peaks of protein groups. The test results stated that the second peak of activity has antibacterial activity. The highlight of the second protein is then purified by Native-PAGE and produced seven kinds of protein bands. The test results of the activity of each band showed that the protein bands no.6 has antibacterial activity.

In research Waluyo et al (2007) carried out the fractionation of proteins on earthworms showed that isolated protein produced has antibacterial activity against *Salmonella typhii* bacteria. Based on the research results Waluyo et al (2007) for the antibacterial activity of earthworms *Lumbricus sp*. against *Salmonella typhii* required effective and efficient extraction technique further so as to isolate the most active antibacterial compounds against *Salmonella typhii* bacteria. Researchers conclude that the earthworm (*Lumbricus sp*.) With the whole extraction techniques extracted up to a concentration of 3200 mg/mL did not have antibacterial activity against *Salmonella typhii* bacteria, so that when the earthworm (*Lumbricus sp*.) Used in the form of whole and raw as

an antibacterial against Salmonella baktreri typhii can not deliver the intended results.

In this study, the control variables are kept to the same at all concentrations used. Further research is needed to determine the antibacterial activity of extracts of the earthworm *Lumbricus sp*. against *Salmonella typhii* bacteria regarding the extraction of earthworms *Lumbricus sp*., pengekstrasiannya techniques, as well as purification of the active compounds contained in the earthworm *Lumbricus sp*.

CONCLUSION

Extract earthworm *Lumbricus sp*. up to a concentration of 3200 mg/mL had no effect typhii antibacterial activity against Salmonella.

REFERENCES

Anonymous (2009). Cacing Tanah Sebagai Obat Bukan Cuma Mitos. Available from http://74.125.153.132/search?q=cache:5PR5v0RUG7EJ:sman1ampekangkek.sch.id/index.php%3Foption%3Dcom_content%26view%3Darticle%26id%3D177%26joscclean%3D1%26comment_id%3D19+penggunaan+obat+cacing+untuk+obat+tifus&cd=19&hl=id&ct=clnk&gl=id. Accessed April 25, 2011

Baumslag N (2005). Murderous Medicine: Nazi Doctors, Human Experimentation, and Typhus, Connecticut, Praeger Publisher

Bordwell FG (1988). Equilibrium acidities in dimethyl sulfoxide solution. Acc Chem Res 21, 456-463

Budiastuti J, Yulinah ES, Andreanus AS (1991). Uji Air Rebusan Cacing Tanah Sebagai Antipiretik dan Uji terhadap Beberapa Bakteri Penyebab Diare. Available from http://bahan-alam.fa.itb.ac.id/detail.php?id=214. Accessed April 27, 2011

Butler T, Sridhar CB, Daga MK, Pathak K, Pandit RB, Khakhria R, Potkar CN, Zelasky MT, Johnson RB (1999). Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicentre trial in India. J Antimicrob Chemother 44, 243-250

Campbell DT, Stanley JC, Gage NL (1966). Experimental and Quasi-Experimental Designs for Research, 18th ed, Michigan, Rand McNelly

Day JG and Stacey GN (2007). Cryopreservation and Freeze-Drying Protocols, 2nd ed, Totowa, Humana Press Inc

Hardijulendra (2002). Uji Aktifitas Antibakteri dari Cacing Tanah (Lumbricus terestris) Sebagai Bahan Pakan Ayam Terhadap Pergrowthan Bakteri Salmonella pullorum Secara Invitro.

Jeyakumar E, Lawrence R, Pal T (2011). Comparative evaluation in the efficacy of peppermint (Mentha piperita) oil with standards antibiotics against selected bacterial pathogens. Asian Pacific Journal of Tropical Biomedicine 2011, S253-S257

King S and Metzger WI (1968). A new plating medium for the isolation of enteric pathogens. I. hektoen enteric agar. Appl Microbiol 16, 577-578

Krištufek V, Fischer S, Bührmann J, Zeltins A, Schrempf H (1999). In situ monitoring of chitin degradation by Streptomyces lividans pCHIO12 within Enchytraeus crypticus (Oligochaeta) feeding on Aspergillus proliferans. FEMS Microbiology Ecology 28, 41-48

PB PAPDI (2006). Panduan Pelayanan Medik, Jakarta, Departemen Ilmu Penyakit Dalam FKUI

Prevention of Typhoid Fever, Geneva, World Health Organization

Ryan KJ and Ray CG (2003). Sherris Medical Microbiology An Introduction to Infectious Diseases, 4th ed, New York, McGraw-Hill Medical

Safitri IR (2010). Analisis penggunaan antibiotik pada pasien demam tifoid di instalasi rawat inap rumah sakit PKUMuhammadiyah Surakarta tahun 2009. Bachelor Thesis. Universitas Muhammadiyah Surakarta, Surakarta

Singh RP, Chidambara Murthy KN, Jayaprakasha GK (2002). Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. J Agric Food Chem 50, 81-86

Waluyo J, Sugiharto B, Zaini NC (2007). Purifikasi dan karakterisasi protein antibakteri dari Pheretima javanica. Jurnal Ilmu Dasar 8, 37-44

World Health Organization (2003). Background Document: The Diagnosis, Treatment and Prevention of Typhoid Fever, Geneva, World Health Organization