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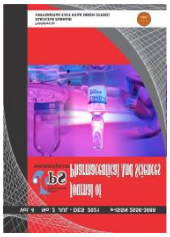

POTENTIAL ANTI-BACTERIAL POWDER BOOTING WATER, n-HEXANA EXTRACT AND EARTHWORM CHLOROFORM EXTRACT (*Peryonix sp.*) AGAINST *Salmonella typhosa* BACTERIA

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ABSTRACT
Earthworms are animals that have many benefits and are easy to find—results of information from people consume boiled water of earthworms three times a day for 7 days is used as a typhus medicine, other information is obtained from electronic media, many people also consume earthworms as a typhoid medicine in powder form which is filled into capsules Which can be found in Chinese drug stores, but until now scientific research on earthworms (Perionyx sp.) as an anti-bacterial has not been done. Extraction is done by maceration. Multilevel maceration using chloroform and n-hexane as solvent. The class of chemical compounds was determined on the ethyl acetate extract and the n-hexane extract of the earthworm Perionyx sp.), including alkaloids, glycosides, steroids/triterpenoids, flavonoids, saponins, and tannins. Testing of antibacterial activity of chloroform extract and n-hexane extract against *Salmonella typhosa* was carried out *in vitro* by agar diffusion method using metal buffer at a concentration of 500 mg/ml; 400 mg/ml; 300 mg/ml; 200 mg/ml; 100 mg/ml and 50 mg/ml. The results of determining the class of chemical compounds from the chloroform extract of earthworms (Perionyx sp.) showed the presence of chemical compounds belonging to the alkaloid group, while the n-hexane extract did not indicate the presence of chemical compounds detected. The n-hexane extract of tar h (Perionyx sp.) had an inhibitory effect on the growth of *Salmonella typhosa* at a minimum inhibitory concentration of 400 mg/ml of 10.9mm, while the chloroform extract had a minimum inhibitory concentration of 300 mg/ml with an inhibitory diameter of 12.87 mm.

Keywords : Anti-bacterial activity, Earthworm (*Perionyx* sp.), *Salmonella typhosa*

INTRODUCTION

Today, the potential of earthworm resources is being researched and developed. They are taming until finally commercial cultivation technology is found. Currently, Earthworms in the data have been identified—more than 1,800 species (species). Regarding the number of potential resources (species) of earthworms, only nine species have attracted the attention of agricultural experts, earthworm cultivators, and those interested in their field of expertise, for example, from traditional medicine experts. The nine types or species of earthworms are L. *rubellus*, *Eisenia foetida*, *Allolobophora caliginosa*, A. *Chlorotica*, *Diplocardia verrucosa*, and *Eudrilus eugeniae* (Rukmana).

In China, earthworms are familiarly called "earth dragons." The market name for dried earthworms traders of traditional Chinese medicines is Ti lung Kam. Meanwhile, earthworms are sold as traditional medicine in Korea after their feces are cleaned through special processing. Earthworms are also listed in Ben Cao Gang Mu, a book on traditional Chinese medicine. Meanwhile, in Korea, earthworms are fresh or dried to become a refreshing soup that is commonly eaten; eating the next day can work full of enthusiasm (Dimas, 2011).

Earthworms used as samples in this study were earthworms (*Perionyx* sp.) or what is often

referred to in the community as necklace worms. Easy to find in trash cans and is a local Indonesian worm. If we visit Central Java, we will find stalls selling herbal medicine specifically for typhoid. The fungus's essential ingredient is earthworms and has long been used as traditional herbal medicine for the local community. In electronic media, many people have also consumed earthworms to treat typhoid by consuming boiled water for earthworms and earthworm powder put into capsules (Hasanudin, 2010). Typhoid is caused by *Salmonella typhosa*, which is often transmitted to humans by animal manure. Symptoms of typhoid can vary greatly, namely fever with a gradual increase in temperature in the first three days, severe headache, abdominal bloating and pain, anorexia, nausea, and constipation. Then often followed by diarrhea, bronchitis, nose bleeding, and apathy (Tjay, 2002).

Based on research conducted by Khairani (2010), it was stated that earthworms had protein levels obtained from earthworms of the type *Drawida* sp., i.e., 40.32% ± 1.31% in *Pontoscolex corethrurus*, namely 50.90% ±1.37% and in *Megascolex* sp. worms. Namely 63.82%±1.85%. The results of Rasifitria's research (2011) showed that the highest protein content was found in earthworms (*Perionyx* sp.) (49.96%), and the protein content of *Pheretima peguana* (40.62%) was almost the same as *Pheretima Posthuma* (40. 65) %. The results of research by Manik (2011) on earthworm Megascolex sp. There are groups of alkaloid compounds, glucose, and saponins; the results of the anti-bacterial activity of boiled water on earthworm *Megascolex* sp. has no inhibition against the growth of *Salmonella typhosa*, while the ethanolic extract of earthworms has an effective inhibition area on *Salmonella typhosa*, where the effective inhibition area on *Salmonella typhosa* at a concentration of 100 mg/ml with an inhibitory diameter of 14.35 mm.

The author also obtained information that earthworms are used by the community in Paluh Nibong Paya Pasir, Kec. Medan Marelan, Deli Serdang, North Sumatra. Treat typhus by drinking boiled water of earthworm powder with a mixture of other ingredients (not told to the researcher) three times a day for approximately seven days. Based on the research hypothesis above, the purpose of this study was to determine the anti-bacterial activity of boiled water, n-hexane extract, and chloroform extract of earthworm powder (*Perionyx* sp.) against *Salmonella typhosa* and to determine the class content of chemical compounds contained in boiled water, n-n extract. -hexane and chloroform extract of earthworm powder (*Perionyx* sp.) have anti-bacterial properties.

MATERIALS AND METHODS

This research was conducted at the Biology Laboratory (Faculty of Mathematics and Natural Sciences USU), Phytochemical Laboratory (Faculty of Pharmacy UTND), Chemical Laboratory (Faculty of Mathematics and Natural Sciences USU), Laboratory of Microbiology (Faculty of Pharmacy UTND) Medan, and the Research Laboratory (Faculty of Pharmacy UTND) Medan. from January to March 2012.

Research Implementation

Preparation of n-hexane extract and chloroform extract

A total of 300 g of earthworm powder (*Perionyx* sp.) was macerated with n-hexane solvent in a tightly closed container for 3 days protected from sunlight while stirring frequently, then filtered, so that the maserate is obtained, the maceration work is repeated as before, so that the final maserate is clear. The maserates were combined into one and then concentrated with a *rotary evaporator* at low pressure at a temperature of not more than 50°C until a thick extract was obtained, then the extract was dried with a *freeze dryer* at a temperature of -30°C, pressure of 2 atm for 24 hours to obtain a dry extract. and weighed. The resulting dregs were dried at room temperature and then macerated again with chloroform solvent in the same way as above (Depkes RI, 1995).

inoculum preparation

The rejuvenating bacteria were taken using a sterile ose needle and then suspended into 10 ml sterile NB, then incubated for approximately 2-3 hours. Then test spectrophotometrically at a wavelength of 580 nm to obtain a transmittance of 25-30%, which means that the concentration of bacteria has reached 106 CFU/ml and Nutrient Broth (NB) is used as a blank (Ministry of Health, 1995).

Method Testing the anti-bacterial effect in vitro

. Anti-bacterial activity test of boiled water powder.

Pipette 0.1 ml of *Salmonella typhosa* with a concentration of 10^6 CFU/ml, put into a sterile petri dish. Then, 20 ml of liquid MHA medium was poured at room temperature, then homogenized and allowed to stand until the media solidified. After the solid media was put on top of the metal backing, then 0.1 ml was added to the powdered boiled water test solution with various concentrations of 500 mg/ml; 400 mg/ml; 300 mg/ml; 200 mg/ml; 100 mg/ml and 50 mg/ml. As a negative control, the metal stockpile contained sterile distilled water. The metal backing was pressed using tweezers on the surface of the slab to a depth of half the height of the medium, then incubated at 35±2°C for 24 hours in an incubator. Then the clear area around the metal backing was observed and measurements were made using a caliper, the measurements were taken twice perpendicularly. The test was carried out three times. The inhibition area is considered effective if it has an inhibitory diameter of approximately 14 mm to 18 mm (Depkes RI, 1995).

Anti-bacterial activity test of n-hexane extract and chloroform extract

. 0.1 ml of *Salmonella typhosa* with a concentration of 10^6 CFU/ml, put into sterile petri dishes. Then, 20 ml of liquid MHA medium was poured at room temperature, then homogenized and allowed to stand until the media solidified. After the media is solid, then metal backing is on it, then drop 0.1 ml of each test solution of n-hexane and various concentrations of 500 mg/ml; 400 mg/ml; 300 mg/ml; 200 mg/rpl; 100 mg/ml and 80 mg/ml, as a negative control metal storage contained DMSO and ethanol in an amount according to the ratio used to dissolve the extract at each concentration. The metal backing was pressed using tweezers on the surface of the plate to a depth of half the height of the medium, then incubated at 35±2°C for 24 hours in an incubator. Then the clear area around the metal backing was observed and measurements were made using a caliper, the measurements were taken twice perpendicularly. The test was carried out three times. The limit of the inhibition area is considered effective if it has a diameter of inhibition of approximately 14 mm to 16 mm (Ministry of Health RI, 1995).

RESULTS AND DISCUSSION

Earthworms Identification Results Earthworm

identification conducted at the Tjut Nyak Dhien University Laboratory stated that the earthworms used in this study were earthworms (*Perionyx* sp.), family *Megascolecidae.*

Earthworm Extraction

Results The maceration results of 300 g of earthworm simplicia using 5 liters of n-hexane solvent, then evaporated with *a rotary evaporator*, then dried with a *freeze dryer* and weighed, obtained a dry n-hexane extract of 35.47 g, while the results of maceration with 5 liters of chloroform solvent, then evaporated with *a rotary evaporator*, then dried with a *freeze dryer* and weighed, obtained 15.78 g of dry chloroform extract, the dry extract obtained was tested with various concentrations against *Salmonella typhosa*.

Chemical Compound Screening Results Screening

results of earthworm powder decoction showed the presence of alkaloids, glycosides and saponins, while the chloroform extract contained alkaloids. The results of screening for chemical compounds can be seen in table 1 below:

Table 1. Results of screening for chemical compounds in powdered boiled water, n-hexane extract and earthworm chloroform extract

No.	Group of compounds used Checked	Screening Results Water Powder	Extract Screened *n* - hexane	Extract Chloroform
1	Alkaloids	+	-	+
2	Glycosides	+	-	-
3	Saponins	+	-	-
4	Flavonoids	-	-	-
5	Triterpenoid Steroids	-	-	-
6	Tannins	-	-	-

Description: + = Contains the compound examined

- = Does not contain the compound examined

Activity Test Results Antibacterial Water Decoction of Earthworm Powder Against the Growth of Bacteria *Salmonella typhosa*

water did not indicate the presence of a slave area: on the growth of *Salmonella typhosa*, the data can be seen in graph 4.1 below

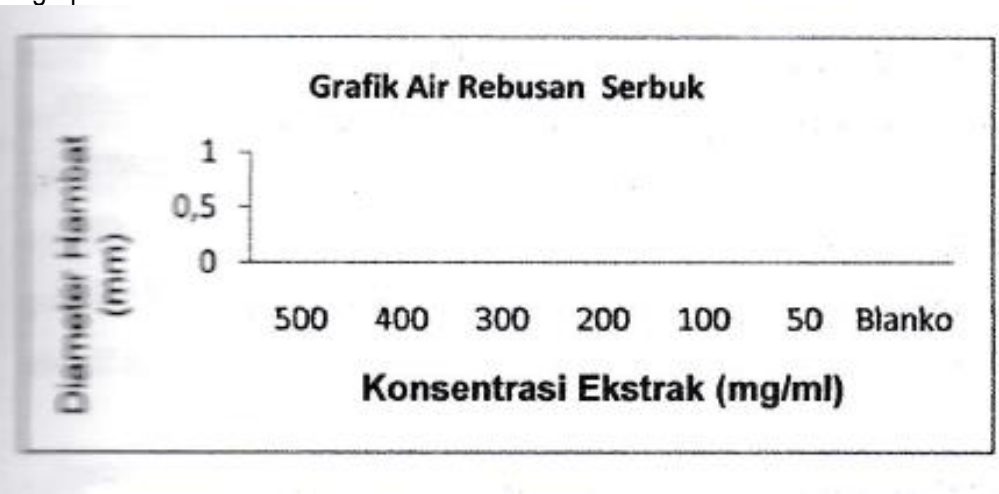
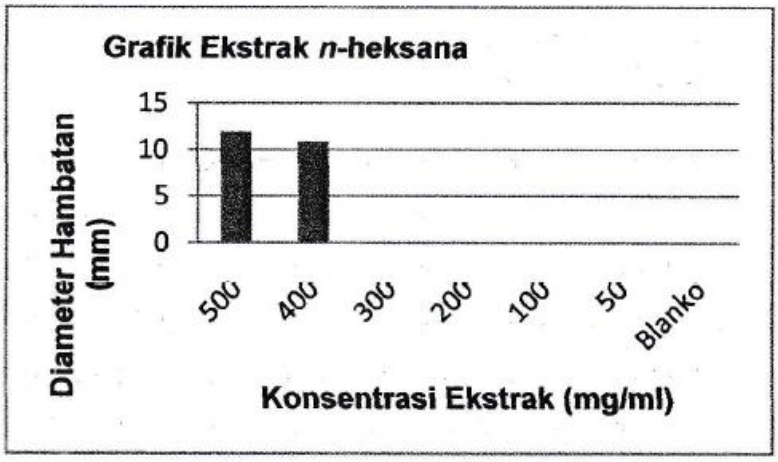


Figure 4.1 The results of measuring the diameter of the air resistance area of boiled earthworm powder against *Salmonella typhosa* bacteria

From the study results, it was not proven that boiled water of earthworm powder inhibited the growth of *Salmonella typhosa*, so the information for the treatment of typhus was not appropriate. This is because the substances or chemical compounds found in earthworms cannot be extracted with water solvents, and enzymes, which are anti-bacterial from earthworms, have been damaged by heating at a temperature of ±50°C (Sofyan, 2010). It may also be due to the addition of other ingredients besides earthworms added to the stew; besides that, information is obtained that the efficacy of boiled water for earthworm powder does not heal immediately but must be drunk continuously for three days or until results are obtained.

Anti-bacterial Activity Test Results of n-hexane Extract of Earthworms Against *Salmonella typhosa*

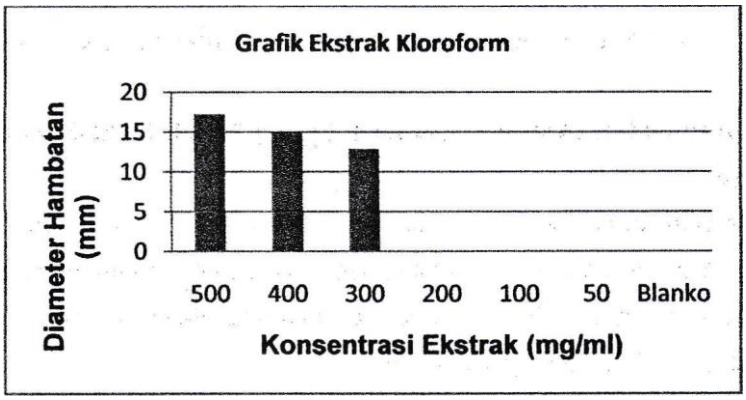
anti-bacterial activity test results showed that n-hexane extract could inhibit the growth of *Salmonella typhosa* at a concentration of 500 mg/ml with an inhibitory diameter of 10.92 mm and at a concentration of 400 mg. /ml with an inhibitory diameter of 10.9 mm. The test results of the n-hexane extract can be seen in graph 4.2 below:



Measurement of the diameter of the inhibition area of the n-hexane extract of earthworms on the growth of *Salmonella typhosa*. According to Sumardi (1998), the anti-bacterial compound of earthworm extract contains protein, carbohydrates, glycopeptides and does not contain cholesterol. Antibacterial found in earthworms (*Perionyx* sp.) contains glycopeptides which have a mechanism of action by killing bacteria by inhibiting the synthesis of their cell walls. Formation cell wall, a transpeptidase reaction is catalyzed by the enzyme transpeptidase and produces cross-links between two peptide-glucan. Enzyme transpeptidase membrane cytoplasm can also cause this enzyme to be unable to catalyze the transpeptidase even though the cell wall continues to be formed. cell wall does not have cross-links and peptidoglycan imperfectly formed

Anti-bacterial Activity Test Results of Earthworm Extract Chloroform Against *Salmonella typhosa*

anti-bacterial activity test results showed that chloroform extract could inhibit the growth of *Salmonella typhosa* at a concentration of 500 mg/ml with an inhibitory diameter of 17.20 mm, at a concentration of 400 mg/ml the power diameter inhibition of 15.06 mm, and at a concentration of 300 mg/ml with an inhibitory diameter of 12.87 mm. The results of the anti-bacterial activity test showed that the chloroform extract of earthworm powder could inhibit the growth of *Salmonella typhosa*. The higher the concentration of the extract, the larger the diameter of the inhibition area. The test results from the chloroform extract can be seen in graph 4.3 below:



Graph 4.3 The results of measuring the diameter of the inhibition area of the earthworm chloroform extract on the growth of *Salmonella typhosa*

The results of the anti-bacterial activity test of the chloroform extract were obtained at a concentration of 500 mg/ml with an inhibitory diameter of 17.20 mm. , a concentration of 400 mg/ml with an inhibitory diameter of 15.06 mm and at a concentration of 300 mg/ml obtained an inhibitory diameter of 12.87 mm, in order to obtain an effective inhibitory power at a concentration of 400 mg/ml with an inhibitory diameter of 15.06 mm. while the n-hexane extract obtained the greatest inhibitory power at a concentration of 500 mg/ml with an inhibitory diameter of 11.92 mm and at a concentration of 400 mg/ml with an inhibitory diameter of 10.9 mm. Thus the chloroform extract of earthworms was stronger than the n-hexane extract in inhibiting the growth of *Salmonella typhosa* and the possibility of alkaloid compounds being attracted by the organic solvent of chloroform more than using distilled water. Salmonella *typhosa* is a gram negative bacterium. Based on the mechanism of action, antibiotics are divided into five, namely antibiotics with the mechanism of inhibiting cell wall synthesis, destroying plasma membranes, inhibiting protein synthesis, inhibiting nucleic acid synthesis and inhibiting essential metabolite synthesis. The plasma membrane is semipermeable and controls the transport of various metabolites into and out. The disruption or damage to the structure of the plasma membrane can inhibit the ability of the plasma membrane as an osmosis barrier and disrupt a number of biosynthetic processes needed in the membrane (Pratiwi, 2008). The limit of the inhibition area is considered effective if it has an inhibitory diameter of approximately 14 mm to 16 mm (Depkes RI, 1995).

The ability of Alkaloid compounds as anti-bacterial *Salmonella typhosa* is strongly influenced by the biological activity of these compounds. The biological activity of these alkaloids is due to the presence of a nitrogen-containing base group. The presence of this base group when in contact with *Salmonella typhosa* will react with amino acid compounds that make up the bacterial cell wall and also bacterial DNA which is the main constituent of the cell nucleus which is the center for regulating all cell activities. This reaction occurs because chemically a compound that is alkaline will react with an acidic compound in this case is an amino acid. This reaction results in changes in the structure and composition of amino acids because most of the amino acids have reacted with the basic groups of alkaloid compounds. This change in the arrangement of amino acids will clearly change the arrangement of the DNA chain in the cell nucleus which originally had an acid and base arrangement that was paired with each other. Changes in the arrangement of amino acid chains in DNA will cause changes in the genetic balance of DNA acids so that the DNA of *Salmonella typhosa* will be damaged. With the damage to the DNA, the intl of *Salmonella typhosa* will be damaged. This is because DNA is the main component of the cell nucleus. Damage to DNA in the nucleus of this bacterial cell will also encourage lysis of the nucleus of the bacterial cell of *Salmonella typhosa*. The lysis of the cell nucleus of the *Salmonella typhosa* will also cause cell damage to the *Salmonella typhosa* because the cell nucleus is the center of cell activity. Cell damage in these bacteria will eventually make the cells of the *Salmonella typhosa* unable to metabolize so that they will also undergo lysis. Thus the bacteria *Salmonella typhosa* will become inactive and destroyed (Gunawan, 2009).

CONCLUSION

The anti-bacterial activity test results of chloroform extract were obtained at a 500 mg/ml concentration with an inhibitory diameter of 17.20 mm and a concentration of 400 mg/ml with an inhibitory diameter of 15.06 mm. At a concentration of 300 mg/ml, the diameter of inhibition was 12.87 mm. Then the effective inhibition was obtained at a concentration of 400 mg/ml with an inhibitory diameter of 15.06 mm; the n-hexane extract obtained the most excellent inhibitory power at a concentration of 500 mg/ml with an inhibitory diameter of 11.92 mm and a concentration of 400 mg/ml with a diameter of 10.9 mm inhibition, while in boiled water, the powder has no inhibitory power. There are glycosides and saponin alkaloid compounds in the boiled water of earthworm powder. At the same time, in the n-hexane extract, there are no chemical compounds examined, and the chloroform extract contains a group of alkaloid compounds.

REFERENCES

Anonymous. 2008. *Salmonella typhosa*. http://id. Wikipedia.org/wiki/Salmonella.

Cahyo, Dimas. 2011. *Rich from Cultivating Earthworms and Silkworms*. Arta Pustaka Publisher.

Ciptanto, Sapto. 2011. *Gaining Black Gold Through Cultivation of Earthworms*. Yogyakarta. Publisher Lily Publisher.

Indonesian Ministry of Health. 1980. *Materia Medika Indonesia*. Volume IV. Jakarta.

Indonesian Ministry of Health. 1989. *Materia Medika Indonesia*. Volume V. Jakarta.

Indonesian Ministry of Health. 1995. *Materia Medika Indonesia*. Volume Vi. Jakarta.

Directorate General of POM. 1979. *Indonesian Pharmacopeia*. ill edition. Indonesian Ministry of Health. Jakarta.

Directorate General of POM. 1995. *Indonesian Pharmacopeia*. Edition IV. Indonesian Ministry of Health. Jakarta.

Directorate General of POM. 2000. *General Standard Parameters of Medicinal Plant Extracts*. Print I. Ministry of Health of the Republic of Indonesia. Jakarta.

Dwidjoseputro. (1998). *Microbiology Fundamentals*. Jakarta: Publisher DJBanbatan. Page 38.

Gunawan, IA (2009). *Potential of Pare Fruit as Antibacterial Salmonella typhimurium*. Faculty of Educational Sciences Teacher Training. Mahasaraswati University, Denpasar.

Harborne, JB 1987. *Phytochemical Methods*. Translators: Kosasih Padmawinata and Iwang Soediro. Bandung: ITB Publisher.

Hoan, Tan Tjay. & Rahardja, Kirana. 2002. *Important Drugs Efficacy, Use, and Side Effects*, 2nd edition. Jakarta: Publisher PT. Elex Media Komputindo, Gramedia Group.

Irianto, K. 2006. *Microbiology Reveals the World of Microorganisms*. Volume I. Bandung:

Yrama Widya Publisher.
Jawetz, E. 2001. *Medical Microbiology*. Translator: Mudihardi, E., et al. Surabaya: Salemba Medika Publisher.
Khairuman, et al. 2010. *Making Profits From Raising Worms*. Jakarta: PT Agro Media Pustaka.
Khairani, ummah 2010, *Examination of Protein Levels in Earthworms Contained in Market Organic Waste by Kjeldahl*, Tjut Nyak Dhien University. Medan. Page 49.
Lay., BW (1996), *Microbial Analysis in the Laboratory*, Jakarta: Raja Grafindo Persada Publisher.
Lee, J. 1983. *Microbiology*. First Edition. USA: The Barnes and Noble Outline Series.
bead. EW 2011. *Anti-bacterial Activity Test of Boiled Water and Ethanol Extract of Earthworm (Megascolex sp.) Against Salmonella typhosa, Escherichia coli, Shigella dysenteriae bacteria*. Medan. USU.
Palczar. MJ, et al, (2008), *Fundamentals of Microbiology, First Volume*, Jakarta: UI Press. Pratiwi, ST 2008. Pharmaceutical Microbiology. Jakarta: Erllangga
Rasifirtia Publisher, 2011. *Determination of Protein Content in Pheretima and Perionyx Seca~ Kjeldahl Earthworms*, Medan: UTND.
Robinson, T. 1995. *High Organic Content of Plants*. Translator: Padma Winata, K. Bandung.ITB.
Rukmana, Rahmat. 1999. *Cultivation of Earthworms*. Yogyakarta: Kanisius.
Shofyan. M, 2010. *Factors Affecting Enzyme Activity*.
Suin., NM, 2006. *Soil Animal Ecology*, Jakarta: Publisher Earth Literacy. Case. 94.
Sumardi, 1998. *Detection and Characterization of Antibiotic Compounds from Extracts and Isolates of Microbes in the Body of the Earthworm Allolobophora rosea*. Bogor. Bogor Agricultural Institute.
Yuli Priyanto, H. 2010. *Soil Biology and Management Strategy*. Yogyakarta: Graha science.