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Antibacterial Activity of Salmonella Typhi in Combination of Earth-Worms Extract (*Lumbricus rubellus*) and Turmeric Rhizoma Extract (*Curcuma Longa L.*) In Vitro

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ABSTRACT

Salmonella typhi s a gram-negative bacteria in the form of coccus, is motile and pathogenic, which can cause a salmonellosis (typhoid fever). Treatment of thiophid fever generally uses paracetamol and chloramphenicol. The development of traditional medicine, such as earthworms and turmeric, which is believed to cure typhoid fever. This study aims to determine the activity of a combination of extracts of earthworm and turmeric on the growth of Salmonella typhi. This study applied an experimental laboratory method. The treatment group consisted of a combination of earthworm extract and turmeric rhizome extract at various concentrations (40%: 60%; 50%: 50%; 60%: 40%), solely earthworm extract, solely turmeric rhizome extract and chloramphenicol 30 µg as a control positive. Earthworm extract and turmeric rhizome extract were prepared using maceration method with 96% ethanol solvent. Antibacterial activity test was performed in vitro using disk diffusion method against Salmonella typhi for 24 hours incubated at 37˚C and then the inhibitory zone (mm) was measured. The inhibitory zone was further analyzed using One Way ANOVA statistical analysis. The results showed that turmeric rhizome single extract better combination of earthworm extract and turmeric rhizome extract could inhibit Salmonella typhi growth optimally at a concentration of 50%: 50% with inhibitory value of 11.1 mm which was closer to the value of inhibition positive control (21.93 mm) compared with the other groups (p = 0.000).

Keywords: antibacterial activity; earthworm extract; turmeric rhizome extract; concentration; *Salmonella typhi*; inhibitory power

INTRODUCTION

The development of the use of traditional herbal medicine is increasingly advanced and growing rapidly which is widely used by the community as one form of treatment options, one of which is earthworms and turmeric. Earthworms are found and are well known in the community, especially rural communities who almost every day find them in rice fields, fields or gardens. Not only earthworms, plants can also be used. One of them turmeric *(Curcuma longa Linn. syn. Curcuma domestica Val.)*, is one of the biopharmaca plants of the *Zingiberaceae* family.

Earthworms *(Lumbicus rubellus) Lumbricin* contains bioactive compounds. *Lumbricin* is an alkaloid peptide group compound so that alkaloid compounds are suspected as antimicrobials. Turmeric contains chemicals such as curcumin, essential oils, starch, and ash. Turmeric active compound namely curcumin acts as an antitumor, antibacterial, and antioxidant. This compound has the ability to inhibit growth and kill bacteria, one of which is *Salmonella typhi*. The compound that plays a role in inhibiting the growth of *Salmonella typhi* is curcumin. Turmeric can also play a role in inhibiting the growth of *Salmonella typhi* bacteria that cause typhus by denaturing and damaging cell membranes so that the metabolic process of cells will be disrupted and become damaged, both of these compounds have antibacterial activity of *salmonellosis*.(1)

Salmonellosis is an infectious condition caused by *Salmonella typhi* and causes typhoid fever. Symptoms of typhoid fever can vary greatly, namely fever with a gradual rise in temperature within the first three days, severe headache, flatulence and pain and followed by diarrhea and nasal bleeding. Transmission can occur through the mouth, enter the body through contaminated food or drinks, enter the stomach, into the small intestinal lymphoid glands, then enter the blood circulation.(2)

Background

Based on the background description of the problem above, the problem is:

1. What is the antibacterial activity of salmonella typhi in a combination of earth-worm extract (Lumbricus rubellus) and turmeric (*Curcuma Longa L.*) ?
2. At what concentration ratio does the combination of earth-worm extract and turmeric have the best antibacterial activity against *Salmonella typhi* in vitro?

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Goal

This research aims to:

1. The combination of earthworm extract and turmeric rhizome extract has the antibacterial activity of *Salmonella typhi* which is shown in the inhibitory area (mm) in vitro.
2. There is a difference in the antibacterial activity of Salmonella typhi in extracts of earthworm (*Lumbricus rubellus*) and turmeric rhizome extract (*Curcumae longa L.*) with a concentration ratio of 40%: 60%, 50%: 50%, 60%: 40% shown in the area of power inhibition (mm) in vitro.

Hypothesis

1. The combination of earthworm extract and turmeric rhizome extract has the antibacterial activity of *Salmonella typhi* which is shown in the inhibitory area (mm) in vitro.
2. There is a difference in the antibacterial activity of *Salmonella typhi* in extracts of earthworm (*Lumbricus rubellus*) and turmeric rhizome extract (*Curcumae longa L.*) with a concentration ratio of 40%: 60%, 50%: 50%, 60%: 40% shown in the area of power inhibition (mm) in vitro.

METHODS

Research Design

This research was experimental laboratory research. Antibacterial activity test was carried out in vitro by using disc diffusion method to determine the combined activity of earthworm extracts and turmeric rhizomes as antibacterial *Salmonella typhi*. Antibacterial activity test was carried out using 6 treatments namely positive control I treatment (chloramphenicol 30 µg), treatment II (single earthworm extract), treatment III (single turmeric extract), IV treatment (40:60 extract combination), V treatment (combination V extract 50:50) and VI treatment (60:40 extract combination).

Research Locations

The extraction of earthworms and turmeric was carried out in the pharmaceutical technology laboratory, followed by the observation of bacterial growth carried out in the pharmaceutical microbiology laboratory of STIKes Bhakti Husada Mulia Madiun.

Research Instruments

1. Research Tools
   The tools used in this study include a set of maceration tools, paper discs, stirring rods, waterbaths (faithful), plates, beaker cups (pyrex), funnels, spatulas, mortars, stamper, petri dishes, measuring cups, ose needles, sticks, sticks, bunsen, aluminum foil tweezers, analytical scales and autoclaves (GEA).
2. Research Materials
   Earthworm extract (*Lumbricus rubellus*.), Turmeric rhizome extract (*Curcumae longa L.*), *Salmonella typhi bacteria*, 96% ethanol, filter paper, sterile aquadest, NA media, 10% DMSO, standard chloramphenicol 30 µg.

Research Framework

1. Extraction
   Extraction was done by maceration method using 96% ethanol solvent. Separately, earthworms and turmeric rhizomes were soaked with 96% ethanol solvent until simplicia was completely submerged, for 5 days while repeatedly stirring. After 5 days, the sample was filtered using filter paper. Each extract produced was evaporated with an evaporator until a thick extract was obtained.
2. Ethanol Free Test
   Ethanol free test in extracts is carried out using the following procedure. The extract was added with H2SO4 3 drops then added with CH3COOH 3 drops then heated. The test results are negative if there is no characteristic ester odor.[3]
3. Identification of Alkaloid and Curcumin Compounds
   Alkaloid test is carried out by means of 3 drops of sample added with 2 drops of Mayer reagent solution, positive reaction of alkaloids is characterized by the formation of white or yellow lumpy deposits.[4]

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4. Identification Taken 100 mg of a thick extract dissolved in 1 ml of ethanol and then bottled on silica gel as a stationary phase then developed in the mobile phase of chloroform: ethanol: acetic acid (95: 4: 1). Boric acid spray reagents were given to see the results in the form of stains indicating the presence of curcumin compounds.(5)
5. Making Comparison of Extract Concentrations
   Making a single extract both 40% worm extract and 40% turmeric rhizome is made with a concentration of 10% DMSO each concentration of a single extract is made by adding 10% DMSO to several grams of each earthworm extract and turmeric rhizome to 1 ml volume.
6. Making a comparison of extracts of earthworms and turmeric rhizome each concentration was made using 10% DMSO. Each concentration ratio was made by adding 10% DMSO to several grams of each earthworm extract and turmeric rhizome in 1 ml volume. Concentrations are made in 3 kinds of comparisons. Comparison of concentrations of extracts of earthworm extracts and turmeric rhizomes are 40:60, 50:50, and 60:40 in 1 ml of 10% DMSO solvent solution. Each treatment was replicated 2 times.
7. Testing of Salmonella typhi Antibacterial Activity
   This test uses the Disk Disc Diffusion Method. NA test media were sterilized at 121ºC and then put in 20 ml petri dishes and allowed to condense. Test bacteria were transferred into NA media by being scratched. Comparison of the extract concentration was added to the petri dish by dipping the disc paper into the extract and placing it on the media, labeled and incubated for 24 hours. Repeated 2 times and then taken the average value. For positive control, paper discs containing 30 µg chloramphenicol were placed on NA media and incubated for 24 hours.

RESULTS

Alkaloid Test on Earthworm Extract

Phytochemical testing on earthworm extracts showed positive results for the group of alkaloid compounds. Alkaloid compounds play a role in antibacterial activity by disrupting the constituent components of peptidoglycan in bacterial cells, which results in cells not forming fully and then undergoing lysis.(6) Another study shows that earthworm extract can provide an antibacterial effect on S.typhi.(7)

Curcumin Test on Turmeric Rhizome Extract

Curcumin is a compound that is soluble in lipophilic solutions such as ethanol and methanol. Another study shows that turmeric rhizome extract can provide an antibacterial effect on Salmonella typhi. In the results of the curcumin test in turmeric rhizomes produce positive values containing curcumin which is characterized by the presence of yellow spots on silica gel that have been developed in the mobile phase and given spray reagents.(7)

Inhibitory Extract Against *Salmonella typhi*

Salmonella typhi is a gram-negative, rod-shaped, non-sporical bacteria, usually motion with flagella. Gram negative bacteria do not retain the violet crystal dye used in the gram staining method due to the fact that they have thin cell walls. *Salmonella typhi* can survive for several months to a year if it is attached to feces, butter, milk, cheese and frozen water. Antibacterial activity test was carried out in vitro by using disk disc diffusion method to determine the combined activity of earthworm extract and turmeric rhizome as an antibacterial against *Salmonella typhi* as indicated by the presence of inhibitory power (mm). This test was conducted on 6 treatments namely positive control I treatment (chloramphenicol 30 µg), treatment II (single earthworm extract), treatment III (single turmeric extract), IV treatment (40:60 extract combination), V treatment (50 extract combination : 50) and VI treatment (60:40 extract combination).

Table 1. Inhibition of each treatment to the conversion of salmonella typhi bacteria

Treatment group	Average inhibitory power (mm)	Inhibitory Response: > 20 mm (Very strong); 10-20 mm (strong); 5-10 mm (medium); 0-5 mm (weak)	p-value
I	21.93	Very strong	0.000
II	17.03	Strong	
III	21.53	Very strong	
IV	10.07	Strong	
V	11.1	Strong	
VI	9.9	Medium	

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In the above results show the inhibition of the combination of extracts of earthworm extract and turmeric rhizome to the growth of *Salmonella typhi* bacteria that were stored for 24 hours, showed a positive control group including very strong category and 2 single extract treatments showed a single extract of turmeric rhizome has a very strong inhibitory response (21.53 mm) compared with a single earthworm extract (17.03 mm), while for the combination of the concentration of earthworm extract and turmeric rhizome extract (40%: 60%, 50%: 50%, 60%: 40%) showed the 50:50 extract combination has a better inhibitory response (11.1 mm) compared to other extract combinations.

Separate tests provide stronger inhibition compared to combinations. A previous research proving that turmeric rhizome extract can inhibit the growth of *Salmonella typhi* bacteria at a concentration of 40% with inhibition of 37.87 mm and ethanol extract of earthworms can inhibit the growth of *Salmonella typhi* bacteria at a concentration of 40% with inhibitory power 18 .5 mm.[7]

DISCUSSION

Phytochemical testing on earth-worm extracts showed positive results for the group of alkaloid compounds. Factors of the use of solvents can also affect the results obtained, such as whether or not the solute is dissolved due to the compound content that is present in the simplicia. A previous research shows that earth-worm extract can provide an antibacterial effect on Salmonella typhi.[7] The results of alkaloid tests on earth-worms produce positive values containing alkaloids which are characterized by the formation of white or yellow lumpy deposits.

Phytochemical testing on turmeric extract showed positive results for the presence of curcumin compounds. The solvent use factor can also affect the results obtained. Curcumin is a compound that is soluble in lipophilic solutions such as ethanol and methanol. Curcumin is a polyphenol compound with a chemical structure similar to ferulic acid which is widely used as a flavor enhancer in the food industry. This phenol derivative will interact with the bacterial cell wall, then absorbed and penetrated into the bacterial cell, thus causing precipitation and denaturation of proteins, consequently it will lyse the bacterial cell membrane.[8] Turmeric rhizome extract can provide an antibacterial effect on Salmonella typhi. In the results of the curcumin test in turmeric rhizomes produce positive values containing curcumin which is characterized by the presence of yellow spots on silica gel that have been developed in the mobile phase and given spray reagents.[7]

The difference in inhibition of each concentration ratio is due to the comparison of the same concentration of compound content will produce a synergistic effect so that the inhibition in inhibiting bacteria will be stronger and produce a wider inhibition. Whereas in the comparison of extracts with different concentrations, the area of inhibition produced is small. This is because the unequal comparison will reduce or eliminate the inhibitory function of the compound of one extract, so it is not effective to use the concentration of the extract combination which is not the same. However, the difference in research that has been done shows that it is better to separate test results than combination testing. Separate tests provide stronger inhibition compared to combinations. This happens maybe because of several factors from one of the extract compounds and further research can be done to find out.

CONCLUSION

Based on the results of this study, we conclude that there are alkaloid compounds in earthworm extracts and there are curcumin compounds in turmeric rhizome extract. A single extract of 40% turmeric rhizome has a very strong slow response (21.53 mm) close to the inhibitory response in the positive control (21.93 mm). In the combination of earthworm extract and turmeric rhizome has antibacterial activity against *Salmonella typhi* which is shown by inhibition at a concentration ratio of 40%: 60% has a inhibition of 10.07 mm, a concentration of 50%: 50% has an inhibition of 11.1 mm, and concentration of 60%: 40% has a inhibition of 9.9 mm. In the concentration ratio (50%: 50%) has the greatest inhibitory value compared to other treatments.

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