ETHANOL EXTRACT FROM *Petiveria Alliacea* (*SINGOLAWANG*) INHIBITS THE GROWTH OF *Mycobacterium tuberculosis* IN VITRO

Arifa Mustika, Roostantia Indrawati, Nurmawati Fatimah
Department of Pharmacology,
Faculty of Medicine, Airlangga University

ABSTRAK

*Permasalahan Multidrug-Resistant Tuberculosis TB (MDR-TB) hingga saat ini masih tercatat pada level tertinggi. Fakta tersebut mengacu pada laporan terbaru badan kesehatan dunia (WHO). Mengingat tingginya angka kesakitan dan angka kematian penyakit tuberculosis karena resistensi M.tuberkulosi sterhadap antibiotika, maka perlu penelitian untuk menemukan obat baru yang berkhasiat sebagai anti M. tuberculosis. Petiveria alliaceae adalah tanaman dalam family Phytolaccaceae dan sudah sejak lama, secara turun temurun digunakan sebagai obat tradisional untuk penyakit tuberculosis di daaerah Bogor. Tujuan dari penelitian ini adalah untuk untuk mengetahui daya hambat ekstrak etanol daun Petiveria alliacea dan untuk menentukan dosis efektif ekstrak etanol daun Petiveria alliacea dalam menghambat pertumbuhan Mycobacterium tuberculosis HRV secara in vitro. Daun Peteveria alliacea diekstraksi dengan menggunakan metode maserasi dengan menggunakan pelarut etanol 90%. Pada kelompok perlakuan media biakan Lowenstein-Jennsen dicampur dengan ekstrak etanol daun Peteveria alliacea pada dosis 15 mg/ml, 22,5 mg/ml, 27,5 mg/ml, 32,5 mg/ml, 50 mg/ml. kelompok kontrol negatif adalah kelompok dimana media biakan tanpa ekstrak sedangkan kelompok kontrol positif adalah kelompok dimana media biakan ditambahkan rifampisin dengan dosis 40 mg/ml. Koloni kuman yang ditanam adalah 10/ml, ditanam pada media LJ sebanyak 100 ul. Selanjutnya diinkubasi selama 8 minggu pada inkubator CO dengan suhu 37 derajat C. Hasil penelitian menunjukkan bahwa pada Media LJ yang ditambahkan ekstrak daun Peteveria alliacea baik pada dosis 15 mg/ml, 22,5 mg/ml, 27,5 mg/ml, 32,5 mg/ml, 50 mg/ml tidak ditemukan adanya pertumbuhan koloni bakteri Mycobacterium tuberculosis. Pada kontrol negatif nampak pertumbuhan kuman sedangkan pada kontrol positif yang diberi antibiotika rifampisin juga tidak ditemukan adanya pertumbuhan kuman. Kesimpulannya adalah pada dosis 15 mg/ml sediaan ekstrak etanol daun Peteveria alliacea mampu menghambat pertumbuhan bakteri HRV.**(FMI 2013;49:51-54)***

Kata kunci: *Peteveria alliacea, Mycobacterium tuberculosis, Media Lowenstein-Jennsen, daya hambat*

ABSTRACT

*Multidrug - Resistant Tuberculosis Problems TB (MDR - TB) is still recorded at the highest level. This fact refers to the latest report World Health Organization (WHO). Given the high morbidity and mortality due to tuberculosis antibiotic resistance sterhadap M.tuberkulosi, it is necessary to research to find new drugs that are efficacious as anti M. tuberculosis. Petiveria is a plant in the family Alliaceae Phytolaccaceae and have long been, for generations used as traditional medicine for tuberculosis in daaerah Bogor. The purpose of this study was to determine the inhibition of ethanol extract of leaves Petiveria alliacea and to determine the effective dose of ethanol extract of leaves Petiveria alliacea in inhibiting the growth of Mycobacterium tuberculosis in vitro HRV. Alliacea Peteveria leaves extracted by maceration method using 90 % ethanol. In the treatment group Lowenstein - Jennsen culture media mixed with ethanol extract of leaves Peteveria alliacea at doses of 15 mg/ml, 22.5 mg/ml, 27.5 mg/ml, 32.5 mg/ml, 50 mg/ml. negative control group is a group in which the culture medium without extracts whereas the positive control group is a group in which rifampin was added to the culture medium dose of 40 mg/ml. Colonies of bacteria are 10/ml planted, grown on LJ medium as 100 ul. Subsequently incubated for 8 weeks at a CO incubator with a temperature of 37 degrees C. The results showed that the LJ media were added Peteveria alliacea leaf extract at doses of 15 mg/ml, 22.5 mg/ml, 27.5 mg/ml, 32.5 mg/ml, 50 mg/ml there were no colony growth Mycobacterium tuberculosis. In the negative control the growth of bacteria, while the apparent positive controls were given antibiotics rifampin also did not reveal any bacteria growth. The conclusion was that the dose of 15 mg/ml of ethanol extract of leaves Peteveria preparations were able to inhibit the growth of bacteria alliacea HRV. **(FMI 2013;49:51-54)***

Keywords:*Peteveria alliacea, Mycobacterium tuberculosis, Lowenstein-Jennsen media, inhibitory effect*

Correspondence: Arifa Mustika, Department of Pharmacology, Faculty of Medicine, Airlangga University,
Jl Prof. Dr Moestopo 47 Surabaya 60131, email: mustikaarifa@gmail.com

INTRODUCTION

Tuberculosis is an infectious disease directly caused by the bacterium *Mycobacterium tuberculosis.* Most of the attacks is in the lungs, but can also in other organs (Kritski & de Melo 2007). In 2011, the number of tuberculosis sufferers in the world was 8.7 million. The number of new cases was 3.7% and 20% suffered multiple resistance to antibiotics. Patients were most numerous in Asia 59%, Africa 26%, the Middle East

7.7%, Europe 4.3% and America 3%. The mortality rate was 1.4 million per year and 0.5 million were women so tuberculosis ranks first as the cause of death in women. So, almost all over the world is not free to tuberculosis infection. Indonesia ranks fourth (0.4-0.5 million cases) after India (2-2.5 million cases), China (0.9 to 1.1 million cases), South Africa (0.4 to 0.6 million cases) and Pakistan is ranked fifth (0.3-0.5 cases) (WHO 2012).

The cause of death in patients with tuberculosis, among others, is due to the resistance of *Mycobacterium tuberculosis* to the drugs administered. Resistance occuring in a variety of combinations of antibiotics is called Multi Drug Resistant (MDR) tuberculosis, or drug-resistant tuberculosis. The incidence of TB in the world is as much as 2.1 to 5.2% of new cases and 13-26% in patients who had received antibiotic therapy. XDR-TB cases were 6.7 to 11.2% in the world. In Indonesia, the complete drug resistance data is not known for sure. In a study in Donggala, Central Sulawesi, of isolates collected from patients, 67.1% were resistant to at least one anti-tuberculosis drug. The highest percentage of drug resistant is Rifampicin and the lowest is Isoniazid (Nurhayati 2004). Based on data released by WHO, TB cases in Indonesia is around 9.9%. TB is more difficult to treat, the recovery rate is only 50% (Aditama 2006, WHO 2012). Given the high morbidity and mortality due to tuberculosis *M. tuberculosis* resistance to antibiotics, it is necessary to conduct research to find an efficacious drug as an anti *M. tuberculosis*.

Petiveria is a plant in the family *Alliaceae phytolaccaceae* and has for generations long been used as traditional medicine for tuberculosis in Bogor. The plant is reported to have anti-fungal activity as an anti-bacterial and *S. aereus*. These data raised hopes that the plant is also able to inhibit the growth of specific bacteria, such as *M. tuberculosis* (Kim et al 2006). Therefore, we conducted a preliminary study on the inhibition of the ethanol extract of the leaves *Petiveria alliacea M. tuberculosis* HRV strains.

MATERIALS AND METHODS

This was the research laboratory experimental research. The study design is a post-test only control group design. Materials used in this study were *Petiveria alliacea* leaves, ethanol 90%, *M. tuberculosis* HRV strains derived from Labkesda Surabaya, Media Lowenstein-Jennsen. Ethanol extract of leaves *Petiveria alliacea* (Singawalang) was the extract of *Petiveria alliacea* leaves using 90% ethanol. *Alliacea petiveria* leaves was derived from Balai Materia Medika Batu . Extracting process was done by maceration. The dose used was 15 mg/ml, 22.5 mg/ml, 27.5 mg/ml, 32.5 mg/ml, 50 mg/ml. Inhibition against *M. tuberculosis* was determined by the presence or absence of bacteria growth. Petiveria ethanol extract of leaves was mixed into the culture medium alliacea Lowenstein-Jennsen, in accordance with the above doses. Negative control group was LJ medium without *Petiveria alliacea* leaf extract and as a positive control was LJ medium with the addition of rifampicin. Rifampin dose used was 40 mg/ml. The number of colonies of bacteria that is 10/ml planted, grown on LJ medium as 100 ul. Subsequently they were incubated for 8 weeks at a CO incubator with a temperature of 37 °C.

RESULTS

The results showed that the LJ media were added *Petiveria alliacea* leaf extract at doses l15 mg/ml, 22.5 mg/ml, 27.5 mg/ml, 32.5 mg/ml, 50 mg/ml there were no colony growth *Mycobacterium tuberculosis*. In the negative control the growth of bacteria, while the apparent positive controls were given antibiotics rifampin also did not reveal any bacteria growth. So at a dose of 15 mg/ml of ethanol extract of leaves *Petiveria alliacea* preparations were able to inhibit the growth of bacteria HRV.

DISCUSSION

The results of the inhibition test *Petiveria alliacea* leaf ethanol extract against bacterial *M. tuberculosis* which have shown that these plants have properties that inhibit bacterial growth. It can not be known from the growth of bacteria that have grown on media which has given the ethanol extract of leaves *Petiveria alliacea*. Extract starting from the lowest dose of 15 mg/ml to 50 mg/ml showed no bacterial growth media of *M. tuberculosis*. Bacteria growth inhibition was also seen in the media with the addition of rifampicin 40 mg/ml media, while in media without antibiotics and ethanol extracts of leaves *Petiveria alliacea*, the bacteria were grown in week four and multiply in week six. This suggests that the barriers to the growth of germs on the media by the ethanol extract of leaves *Petiveria alliacea* is caused by the active ingredient contained in, instead of caused by faulty procedure or non-virulent germs.

These results corroborate previous studies that showed that the ethanol extract of leaves *Petiveria alliacea* is able to inhibit the growth of various pathogenic bacteria such as *E. coli*, staphylococcus, pseudomonas, and shigela in vitro. In research conducted by Kim et al (2006) that both water and ethanol extracts of leaves

.hand roots *Petiveria alliacea* better able to inhibit a wide range of gram-positive and gram-negative bacteria. In these studies each bacteria required different concentrations of extracts for the inhibition of certain bacteria. The bacteria used in these studies were *Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Mycobacterium smegmatis, Streptococcus agalactiae, Escherichia coli, Pseudo-monas aeruginosa*, and *Stenotrophomonas maltophila*, and *Kleibsiella pneumoniase*. In addition to a variety of such bacteria, studies also prove that these plants are also able to inhibit the growth of fungi in vitro (Kim et al 2006). The results of the different studies presented by other researchers that the ethanol extract of leaves *Petiveria alliacea* not efficacious as an anti-microbial. These differences may occur because of differences in extraction and sample preparation. In the study conducted by Kim et al (2006), ethanol extract of leaves *Peteveria alliacea*was extracted by maceration without heating. Extraction performed after simplicia heated in water or alcohol. The differences can cause differences in the extracting of active ingredients. A number of active ingredients will suffer damage or structural changes due to heating so that the research results will be different. The difference results of the study may also be due to differences in the area where the plants grow. Nutrient content can influence the amount of active ingredients in the plant that are efficacious active ingredients become less dominant.

The data collected in this study, bringing the hope found a new type of antibiotic drugs, especially anti-tuberculosis. The discovery of antibiotics for *M. tuberculosis* is very important, given the incidence of resistance to antibiotics *M. tuberculosis* high enough. Resistance is not only on one kind of antibiotics but also on some antibiotics once called Multi Drug Resistance (MDR). Globally, the UN health agency estimates that 425,000 cases of resistance/year. Based on the survey of the world health body in November 2004 to November 2005, the prevalence of multi-drugs resistant (MDR) 20%, 20.4% and Southeast Asia an area of research in Donggala, Central Sulawesi, Indonesia one or more drug resistance was 67.1% (Aditama 2006,Nurhayati 2004). TB cure rate of 50% (Aditama 2006). The new threat that arises now is the emergence of anti-tuber-culosis drug resistance in the first-line and second-line (extensively drug resistant) so that the number of deaths from tuberculosis increased. Prevalence of extensively drug resistant (XDR) world is 2% (Aditama 2006). Therefore, the discovery of antituberculosis drugs is a priority, especially for overcoming tuberculosis that are resistant to existing antibiotics.

Various studies have also shown that in addition to being antibacterial, *Petiveria alliacea*e have a variety of other properties such as anticancer, cytotoxic, immunostimulant and anti-inflammatory. This is possible because plants have a variety of content *singowalang* compounds. Compounds that have been identified, namely: benzilhidrioxietitrisulfid compounds, esteroide (b-sitisterol), triterpenoids, saponins, polyphenols, tannins, cumarin, difeniltri-sulphur, Acido lignoceric, triterpenes types isoarbinol, acetate, cinnamate isoarbinol, and coumarin. Roots and stems containing sulphur invented, trithiolaniacine, benzenic, bensaldehyd, benzouc acid.

One of the active ingredients believed to have strong antibacterial properties as a group is a sulphur - containing compounds such as benzyl thiosulfinat, benzyl and benzyl trisulfid sulfinic acid (Kim et al 2006). Generally, plants also contain a variety of organic compounds containing sulphur. Many of these volatile compounds in the form of a sharp taste or bad smell so the presence of these compounds readily known during the extraction and isolation. An important class of compounds is the class of glucosinolates from Cruciferae, both organic disulfide of allium and third groups are acetylenic thiophene. Glucosinolates is divided into multiple classes of compounds, known to function as an antibacterial herb. The extracts *Petiveria alliacea* does contain many compounds from the class of organosulphur. The problem the amount of the compound is not known with certainty.

Another compound suspected efficacious as antibacterial is the phenol. In some plant phenolic compounds and their derivatives have antibacterial function by lowering the surface tension of the cell and protein denaturation. However, the efficacy of other phenolic compounds in plants can be of various kinds, such as antioxidants (Manna et al 1997). *Alliacea petiveria* leaves contain a variety of compounds from the class of polyphenols that are also in the class of phenolic compounds. The type and amount of these compounds in ethanol extracts performance is not known with certainty as well as the functionality and usefulness.

Alliacea petiveria leaves also contain triterpenoid compound class. In some plants this class of compounds acts as an antibacterial to soften cell membrane, resulting in the destruction of bacteria cell walls such as the triterpene asiaticoside. However, the class of triterpenoid compounds may also have various properties such as *Phylanthus niruri* is one group of plants containing compounds as terpenoids and efficacious antibacterial. This class of compounds also found in plants Eunicea pleaded efficacious anticancer and as anti-microbial (Shiet al 2002). Efficacy of anticancer compounds terpenoids group is also

identified in plants *Emilia soncheifolia* (Shyleshet al 2005). Special triterpenoid compound type, quantity and efficacy still need further research.

In view of many chemical content of plants *Petiveria alliacea*, the inhibition of ethanol extract of leaves *Petiveria alliacea* on the tuberculosis bacterium in this study is thought to be the work of a combination of various compounds contained in the leaves. As happened in the current tuberculosis treatment. Management of tuberculosis therapy today does not use monotherapy but is a combination of a variety of drugs with different mechanisms of action. The purpose of this combination is to shorten the duration of therapy, increasing the potential, decrease side effects and prevent resistance. Antituberculosis used, including isoniazid, has an action by inhibiting micolic acid synthesis so bacterial cell wall formation disturbed, by inhibiting gene transcription rifampicin, pyrazinamide causes damage to the plasma membrane and ethambutol, inhibits cell wall formation. In this study a comparison was used as an antibiotic rifampicin at a dose of 40 mg/ml media. So, it is suspected that the ethanol extract of leaves *Petiveria alliacea* containing more than one kind of antibacterials with different mechanisms of action. We should not rule out the possibility that only a single antibacterial compounds whereas other compounds are material to enhance the antibacterial activity of the compounds. To prove this, it is necessary to conduct further research.

Doses used in this study refer to previous research on herbs that also has inhibitory against *M. tuberculosis*. At the lowest dose study of ethanol extract of leaves used *Petiveria alliacea* 15 mg/ml media has been able to inhibit the growth of bacteria, while the herbal *Centella asiatica* such doses have not been able to inhibit the growth of germs. So in this study can not be determined the minimum dose that has been able to inhibit the growth of germs. Therefore, further research needs to be done to obtain the minimum dose that was able to inhibit the growth of bacteria with various concentrations of germs.

CONCLUSION

Ethanol extract of leaves Peteveria alliacea could inhibit the growth of tuberculosis bacteria in media LJ at a dose of 15 mg/ml.

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