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# Comparative antimycobacterial activity of some Indonesian medicinal plants against multi-drug resistant *Mycobacterium tuberculosis*

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**ABSTRACT**

This study has been carried out to compare antimycobacterial activity of five selected Indonesian endogenous medicinal plants of *Andrographis paniculata, Annona muricata, Centella asiatica, Pluchea indica,* and *Rhoeo spathacea* against clinical isolate of multi-drug resistant (MDR) *Mycobacterium tuberculosis*. The aqueous extracts of leaves of *Andrographis paniculata, Annona muricata, Centella asiatica, Pluchea indica,* and *Rhoeo spathacea* were obtained by maceration, and the phytochemical constituents of each extract were screened. Antimycobacterial activity of aqueous plant extracts were determined by proportion methods using Lowenstein Jensen (L-J) medium. Our study exhibited that all extracts of five selected plants showed inhibited activity against *Mycobacterium tuberculosis* H37Rv strain and multi drug resistant (MDR) strain. The proportion inhibition of aqueous extract of *Andrographis paniculata, Annona muricata, Centella asiatica, Pluchea indica,* and *Rhoeo spathacea,* against *Mycobacterium tuberculosis* H37Rv strain were 100%, 82.1%, 78.5%, 100%, and 100% respectively, whereas against MDR strain were 93.7%, 50.0%, 50.0%, 100%, and 100% respectively. The phytochemical analysis showed that the extracts were predominantly contains flavonoids, alkaloids, saponins, tannins and glycosides. *Pluchea indica,* and *Rhoeo spathacea* showed good antimycobacterial activity against MDR strains and could be useful as complementary alternative therapy in combating the emergence of MDR strains of *Mycobacterium tuberculosis*.

## INTRODUCTION

Tuberculosis (TB) is still one of the most serious public health problems in the world, including Indonesia. In 2012, WHO estimated that 8.6 million incident cases of tuberculosis worldwide. Most of the TB cases occur in Asia (58%) and African region (27%). Smaller proportions of cases occur in the Eastern Mediterranean region (8%), European region (4%) and the America regions (3%) (WHO, 2013).

Indonesia is the fourth largest country in incident cases of TB after India, China, and South Africa. The incidence of TB cases in Indonesia is estimated at about 0.5 million in 2012. According to reports from the World Health Organization (WHO) in 2012, the prevalence of tuberculosis in Indonesia is estimated at 297 per 100,000 populations, the incidence of 185 per 100,000 populations and the mortality rate 27 per 100,000 populations (WHO, 2013).

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Tuberculosis generally is asymptomatic disease and effort to control tuberculosis is still faced with many problems, especially with the increasing spread of multi drug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis. According to a WHO report in 2012 of TB morbidity caused by MDR-TB is about 450.000 people, with a high mortality rate of around 170.000 deaths from MDR-TB (WHO, 2013).

The rapid increasing of the incidence of MDR and XDR *M. tuberculosis* strains in the world, and no anti-tuberculosis agent has been introduced in the past 30 years (Gautam *et al*., 2007), necessitated the urgent need to search for new anti-tuberculosis. Medicinal plants offer a great hope to overcome these needs. Herbal medicines have been used to treat several diseases by traditional practitioner over the world.

In the past few years, a large number of plant species have been studied for their activity against *Mycobacterium tuberculosis* (Newton *et al*., 2002;; Copp, 2003; Okunade *et al*., 2004; Pauli *et al*., 2005; Billo *et al*., 2005; Soejarto *et al*., 2005; Mmushi *et al*., 2010; Luo *et al*., 2011).

More recently, several studies have also demonstrated significant activity of several compounds derived from plants against MDR strains of *Mycobacterium tuberculosis* (Camacho-Corona *et al*., 2008; Gordien *et al*., 2009; Gupta *et al*., 2010; Hannan *et al*., 2011; Antony *et al*., 2012; Shukla *et al*., 2013; Singh *et al*., 2013; Muthuswamy *et al*., 2013).

In Indonesia, there are several indigenous plants that are reportedly used traditionally to treat tuberculosis and respiratory infections. Therefore, the purpose of this study was to compare the in vitro activity of antimycobactrial activity of *Andrographis paniculata, Annona muricata, Centella asiatica, Pluchea indica,* and *Rhoeo spathacea* against multi-drug resistant (MDR) of *Mycobacterium tuberculosis*.

## MATERIALS AND METHODS

### Plant collection

Five medicinal plants were selected in this study. The selection of plants was based on their traditional use for the treatment of tuberculosis and asthma by the local traditional practitioners. The parts of selected plants of *Andrographis paniculata, Annona muricata, Centella asiatica, Pluchea indica,* and *Rhoeo spathacea* were obtained from Research Institute for Medicinal and Aromatic Plants, Bogor, and identified at Research Center for Biology, Indonesian Institute of Sciences, Bogor.

The dried leaves of *Annona muricata, Pluchea indica, Rhoeo spathacea,* and herbs of *Andrographis paniculata,* and *Centella asiatica* were cut to very small pieces and crushed into a fine powder using an electric grinder and stored in airtight containers in a dark place to prevent oxidation until the extraction stage.

### Extraction

The aqueous extracts of each plant materials from each species were obtained by maceration. Each finely powder of each plant materials were soaked in 5 L distilled water for 24 hours. The maceration was repeated 3 times to exhaustively extract the plant material. Extracts were filtered and concentrated using a rotary evaporator (Buchi, Switzerland) in a water bath set at 70°C. The dried extracts obtained from each plant were packed in glass bottles with proper labeling and the amount of extracts obtained was quantified. The extracts were stored in a refrigerator at $4^{o}$ until use.

### Preliminary analysis of phytochemicals

Each of plant extracts were screened for the presence of alkaloids, saponins, tannins, glycosides, and flavonoids using the standard procedures as described previously (Harborne, 1987).

### Mycobacterial strains

The Standard strain of *Mycobacterium tuberculosis* H37Rv and isolated multi drug resistant (MDR) strain were provided by Department of Clinical Microbiology, Faculty of Medicine University of Indonesia. MDR strain of *M. tuberculosis* was resistant to rifampicin and isoniazid.

### Antimycobacterial activity assay

The assessment of the antimycobacterial activity of the extracts was performed using Lowenstein–Jensen (L-J) proportion methods (Gupta *et al*., 2010), with slight modification. The inoculum of *M. tuberculosis* standard strain and MDR strain were prepared from Lowenstein-Jensen (L-J) slants. The inoculums were prepared by 10-fold dilution steps of the mycobacterial suspension of a 1.0 McFarland standard, to produce the dilution $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$. The $10^{-5}$ of mycobacterial dilution was used for inoculation.

A quantity of 2.5 g of dried extract was dissolved in minimum amount of DMSO and diluted using a 50.0 ml volumetric flask with distilled water to make a 50 mg/ml stock solution, and sterilized by filtration through a 0.2 µm membrane filter (Whatman, USA). Three different concentrations of 1.25 mg/ml, 2.5 mg/ml, and 5.0 mg/ml of each extracts were prepared. To make 1.25 mg/ml extract solution, 1.25 ml of stock solution (50 mg/ml) was added aseptically to 48.75 ml of sterile LJ medium, and shaken gently until mix thoroughly. Then 7 ml of the medium was dispensed into Mc.Cartney sterile bottles and then inspissated to coagulate at 85°C for 45 minutes.

Similarly, to make 2.5 mg/ml extract solution, and 5.0 mg/ml extract solution, 2.5 ml and 5.0 ml of stock solution (50 mg/ml) was added aseptically to 47.5 ml and 45 ml of sterile Lowenstein Jensen (L-J) medium, respectively. Then 7 ml of each medium was dispensed into Mc.Cartney sterile bottles and then inspissated to coagulate at 85°C for 45 minutes. The L-J slants were left at room temperature for 24 h, the caps of Mc.Cartney bottles being loosely closed in order to evaporate a part of the humidity. The control medium without extract and control drug (rifampicin, 40 µg/ml) were prepared at the same time as the extract containing media. Then all the L-J slants were inoculated with 0.1 ml of mycobacterial suspension of the chosen dilutions of $10^{-5}$ and incubated at 37°C for 4 weeks. Reading was taken weekly. For comparison, extract free control L-J slants were used.

## RESULTS

The *in vitro* antimycobacterial activity of 5 selected plants against *M. tuberculosis* H37Rv and MDR were assayed by Lowenstein–Jensen (L-J) proportion method. All extracts of five selected plants showed inhibited activity against *Mycobacterium tuberculosis* H37Rv strain and multi drug resistant (MDR) strain. The proportion inhibition of 5 mg/ml of aqueous extract of *Andrographis paniculata, Annona muricata, Centella asiatica, Pluchea indica,* and *Rhoeo spathacea,* against *Mycobacterium tuberculosis* H37Rv strain were 100%, 82.1%, 78.5%, 100%, and 100% respectively, whereas against MDR strain were 93.7%, 50.0%, 50.0%, 100%, and 100% respectively, as shown in **Table 1**.

**Table 1:** Antimycobacterial activity of aqueous plant extracts against *Mycobacterium tuberculosis* H37Rv and MDR strains.

| Plant | Part | Mycobacterial Strain | L-J proportion methods | | | |
|---|---|---|---|---|---|---|
| | | | Inhibition rate (%) | | | |
| | | | Plant extracts (mg/ml) | | | Rifampicin µg/ml |
| | | | 1.25 | 2.50 | 5.0 | 40 |
| *Andrographis paniculata* (Burm. F) Ness. | Herbs | H37Rv | 3.5 | 35.7 | 100.0 | 100.0 |
| | | MDR | 6.2 | 56.2 | 93.7 | 0 |
| *Annona muricata* Linn. | Leaves | H37Rv | 7.1 | 64.3 | 82.1 | 100.0 |
| | | MDR | 0 | 6.2 | 50.0 | 0 |
| *Centella asiatica* (L.) Urb. | Herbs | H37Rv | 3.5 | 57.1 | 78.5 | 100.0 |
| | | MDR | 6.2 | 12.5 | 50.0 | 0 |
| *Pluchea indica* (L.) Less. | Leaves | H37Rv | 3.5 | 50.0 | 100.0 | 100.0 |
| | | MDR | 6.2 | 59.3 | 100.0 | 0 |
| *Rhoeo spathacea* (L. Her) Hance. | Leaves | H37Rv | 32.1 | 100.0 | 100.0 | 100.0 |
| | | MDR | 28.6 | 100.0 | 100.0 | 0 |

**Table 2:** Phytochemicals analysis of plant extracts.

| Plant | Alkaloids | Flavonoids | Saponins | Tannins | Glycosides |
|---|---|---|---|---|---|
| *Pluchea indica* (L.) Less. | + | + | + | + | + |
| *Annona muricata* Linn. | + | + | + | + | - |
| *Rhoeo spathacea* (L. Her) Hance. | + | - | + | + | + |
| *Andrographis paniculata* (Burm. F) Ness. | + | + | + | + | + |
| *Centella asiatica* (L.) Urb. | + | + | + | + | + |

The aqueous extract of *Rhoeo spathacea* exhibited potent antimycobacterial activity. The result of phytochemical analysis of extracts showed that extracts predominantly contains flavonoids, alkaloids, saponins, tannins and glycosides as shown in **Table 2**. Alkaloids, saponins and tannins were present in all plant extracts.

## DISCUSSION

The emergence of multi drug resistant (MDR), extreme drug resistant (XDR) and recently reported total drug resistant (TDR) strains of *Mycobacterium tuberculosis* (Pandya *et al*., 2012), highlighted an urgent need to find a new compounds derived from herbal medicines. Present study demonstrated that aqueous extracts of *Andrographis paniculata, Pluchea indica,* and *Rhoeo spathacea,* showed to have antimycobacterial activity against *M. tuberculosis* H37Rv and MDR strains. In Indonesia, stems, leaves and flowers of *Andrographis paniculata* were used traditionally for the treatment of respiratory tract infection including tuberculosis and the aqueous extracts of *Andrographis paniculata* has been used as alternative medicine for tuberculosis in primary health care in Surabaya, East Java (Sulistiawati *et al*., 2010). In our study we found that aqueous extract of *Andrographis paniculata* showed good activity against *M. tuberculosis* H37Rv strain. In the previous study indicated that the administration of aqueous *Andrographis paniculata* extracts could minimize the hepatotoxic effect of rifampicin in rats (Muthulingam, 2012). These results support the use of *Andrographis paniculata* plants in traditional medicine and complementary medicine. The aqueous extracts of *Pluchea indica* and *Rhoeo spathacea* demonstrated bactericidal effect against *M. tuberculosis* H37Rv and MDR strain. Our result consistent with other study that extract of *Pluchea indica* showed antimycobacterial activity against *M. tuberculosis* H37Rv standard strain (Mohamad *et al*., 2010). In this study we found that *Rhoeo spathacea* was the potent bactericidal. The proportion of inhibition of aqueous extract (2.5 mg/ml) of *Rhoeo spathacea* was 100% against *M. tuberculosis* H37Rv and MDR strain. To our knowledge the study of antimycobacterial activity of *Rhoeo spathacea* and *Pluchea indica* extracts against MDR strain of *M. tuberculosis* are reported for the first time. Regarding to the bioactive constituents of plant extracts, we found that the plant extracts predominantly contains flavonoids, alkaloids, saponins, tannins and glycosides. Alkaloids, saponins and tannins were present in all plant extracts. It has been proposed that the antimycobacterial activity of plant extracts may be due to the main bioactive components in the extracts including flavonoids, alkaloids, tannins, saponins, and polyphenols (Arya, 2011; McCarthy and Mahony, 2011). Previous study of some flavonoids isolated from *Dorstenia barteri* showed antimycobacterial activities. Isobachalcone, kanzanol C, 4-hydroxylonchocarpin, stipulin, and amentoflavone exhibited potential activities at MIC < 10 µg/ml against *M. tuberculosis* (Kuete *et al*., 2010). Plant terpenoids have been reported to have antimycobacterial activity. Sesquiterpene, longifolene, totarol and transcommunic acid, obtained from *Juniperus communis* showed the highest activity against *Mycobacterium tuberculosis* H37Rv, whereas longifolene and totarol also showed activity against rifampicin-resistant variants (Gordien *et al*., 2009). Moreover, alkaloids extracted from several plants have also been reported to exhibit antimycobacterial activity (Copp, 2003). Those various findings indicate that the presence of active compounds such as alkaloids, polyphenols, flavonoids, and terpenoids in medicinal plants were considerably responsible for their activity against *Mycobacterium tuberculosis*.

## CONCLUSION

Our findings showed that *Pluchea indica,* and *Rhoeo spathacea* demonstrated promising antimycobacterial activity

against MDR strain of *Mycobacterium tuberculosis* and could be useful as complementary alternative therapy in combating the emergence of MDR strains of *Mycobacterium tuberculosis*. Further studies are needed on isolating and purifying active constituents of antimycobacterial agents from *Rhoeo spathacea* and *Pluchea indica.*

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