

Antituberculosis Activity of Parang Romang (*Boehmeria virgata* (Forst.) Guill) Stem Extract

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ABSTRACT

Parang Romang (*Boehmeria virgata* (Forst.) Guill) is one of the plants empirically used by the people of South Sulawesi. This study aimed to test the activity of Parang Romang Extract against *Mycobacterium tuberculosis*. The 96% ethanol extract obtained was fractionated by the liquid-solid extraction method: obtained n-Hexan soluble extract and n-Hexan insoluble extract. Samples of 96% ethanol extract, soluble extract of n-Hexan and insoluble extract of n-Hexan were made in series of concentrations of about 25, 50, 75, and 100 µg/ml. The inhibition test of *Mycobacterium tuberculosis* used the MODS method on the *Mycobacterium tuberculosis* strain H37RV. Color reagents then identify extracts that provide inhibition. The results of the minimum inhibition concentration of *Mycobacterium tuberculosis* showed that a 100 µg/ml of n-Hexan soluble extract was able to inhibit the growth of *Mycobacterium tuberculosis* strain H37RV.

KEYWORDS: Antituberculosis, *Boehmeria virgata* (Forst.) Guill.; MODS; *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis is a contagious infectious disease that is a problem in the world. Tuberculosis is the 9th leading cause of death worldwide and is the leading cause of infectious agents. In 2016 it was estimated that 10.4 million people had tuberculosis, 90% of adults, 65% of men, and 10% of those with HIV. The ten countries that reported experiencing the most significant tuberculosis cases were India, Indonesia, Nigeria, Philippines, South Africa, Pakistan, Bangladesh, the Democratic Republic of the Congo, China, and Tanzania. Indonesia is in second place with a 16% tuberculosis burden after India 25% (WHO, 2017).

One of Indonesian traditional medicine is parang romang (*Boehmeria virgata* (Forst.) Guill). This plant grows in mountainous areas such as Sinjai, Gowa, Malino, Maros, and Enrekang (Rusdi, 2014). This plant is known to have many medicinal benefits, including treating boils, fractures, dysentery, hematemesis, and can also be used as a mixture for massaging the skin (Chambie & Ash, 1994).

Parang Romang is a plant that belongs to the Urticaceae tribe and is a member of the *Boehmeria* genus. The genus *Boehmeria* is a genus group that has relatively large members. The number of species in this

genus reaches 65 (Jiarui, Friis, & Wilmot-Dear, 2003).

Several studies have been conducted on *Boehmeria virgata* (Forst.) Guill. The test results of parang romang root extract showed an inhibition of the growth of *Mycobacterium tuberculosis* strain H37RV at a concentration of 250 ppm (Rusdi, Hasan, Ardillah, & Evianti, 2018).

Previous research by Rusdi (2014) showed that the phytochemical screening results of parang romang root extract (*Boehmeria virgata* (Forst.) Guill) contained alkaloids, terpenoids, phenolics, and flavonoids. Research conducted by Kumar et al. (2010) in his review stated that several secondary metabolites that have potential as antimycobacterial are alkaloids, terpenoids, steroids and saponins (Kumar, Banik, & Sharma, 2010).

Research conducted by Semwal et al. (2009) stated that *Boehmeria rugulosa* contains the compound quercetin, inhibiting *Mycobacterium tuberculosis* (Semwal, et al., 2009). In addition, there is also a chalcone compound in *Boehmeria rugulosa* (Jash & Brahmachari, 2013).

Based on the description above, this is what underlies the need for research to test the antituberculosis activity of Parang romang (*Boehmeria virgata* (Forst.) Guill) stem extract against *Mycobacterium tuberculosis*.

METHODOLOGY

Tools and materials

The tools used are Autoclave (Hirayama), maceration vessel, chamber (Lamag), incubator (Memmert), Laminar Air Flow (LAF) (ESCO), UV lamp 254 nm and 366 nm, refrigerator (Modena), microscope, vortex mixer, oven (Memmert), micropipette (Socorex), plate 24 well, rotary evaporator (Heidolph), analytical balance (Kern), and vial.

The materials used are parang romang roots, distilled water (Aqua destillata), Iron (III) Chloride, dragendorf reagent, aluminium chloride (Merck), *Mycobacterium tuberculosis* culture were collected in Hasanuddin Hospital Laboratory, DMSO, Ethanol, n-Hexan, Ethyl acetate, Middlebrook 7H9 (Sigma Aldrich), OADC Nutrients (oxalic acid, albumin, dextrose, and catalase), PANTA Nutrients, TLC Plate.

Extraction and partition

Parang Romang stems obtained from Gowa, South Sulawesi, are cleaned, dried and powdered. Extraction was carried out by reflux method with 96% ethanol as solvent 2 times. The extract was concentrated, evaporated with a rotary evaporator until a thick extract was obtained. Parang Romang stem extract was partitioned its chemical components by the Liquid-Solid Extraction method using n-Hexan as a solvent to obtain a soluble fraction of n-Hexan and an insoluble fraction of n-Hexan

Sample preparation

As much as 50 mg extract was dissolved in 50 ml of distilled water. A series of concentrations of 25, 50, 75, and 100 µg/ml was made to test the growth inhibition activity of *Mycobacterium tuberculosis* strain H37RV.

MODS (Microscopically Observed Drug Susceptibility) Testing Method

A 24 well-plate was prepared for the H37RV strain. 50 l DMSO was pipetted and then added to the H37RV plate (in duplicate) as a negative control. 50 l of Isoniazid was pipetted and then added to the H37RV plate (triple each) as a positive control. Furthermore, 50 l of each concentration of the test extract was pipetted into each well of H37RV (each triple) except for the control. After that, 950 l of bacterial suspension was added to all wells on the plate and then homogenized. Then it was incubated for 7 days at 30 °C and observed under a microscope. As observation of cord formations, growth in drug-free control wells but not in drug-containing wells indicates susceptibility. Growth observed in both the drug-free wells and the drug-containing wells on the same day is interpreted as resistance (ECDC, 2018).

Phytochemical screening

Identification of chemical components was carried out qualitatively by TLC using the dragendorf, Lieberman Bouchard,

Aluminium chloride, and ferric chloride reagent.

RESULTS AND DISCUSSION

Parang romang steam was extracted by the reflux method using ethanol as a solvent. The extract obtained was 17.25 grams (Table 1). Furthermore, the ethanol extract was partitioned by a solid-liquid extraction method with n-Hexan as solvent. Two partition results were obtained in the partition stage, namely 0.6 g of soluble hexane extract and 2.6 g of insoluble n-Hexan extract (Table 2). The separation of this extract is based on the difference in the dielectric constant. Compounds with low polarity will be attracted to the n-Hexan solvent.

The results of the n-Hexan partition were then carried out for phytochemical screening. Screening results show the presence of terpenoid and alkaloid compounds

These three extracts were tested: ethanolic extract, soluble extract of n-Hexan, and insoluble extract of n-Hexan. The method used in the antituberculosis test is the Microscopically Observed Drug Susceptibility (MODS). This concentration difference was made to determine the level of inhibition of bacterial growth. Aquadest is a solvent that does not inhibit bacterial growth, so it does not interfere with the results of observations. The positive control used was Isoniazid because *Mycobacterium tuberculosis* strain H37RV is a bacterium

Table 1. Extraction Results of Parang Romang (*Boehmeria virgata* (Forst.) Guil) stems.

Sample	Solvent	Weight Extract	% Yield
Parang Romang Stem	Ethanol 96%	17,25 grams	1,91

Table 2. Partition of Parang romang extract

Sample	Solvent	Soluble	Insoluble
Parang romang extract	n-Hexan	0.6 g	2.5 g

Table 3. The results of the sensitive *Mycobacterium tuberculosis* H₃₇RV growth inhibition test.

Solvent	Concentration (ppm)						Isoniazid + kuman
	K-	K+	250	500	750	1000	
Ethanol extract	++	-	++	+	++	++	-
n-Hexane Soluble Extract	++	-	++	++	++	-	-
Insoluble Extract n- Hexane	++	-	++	++	++	+++	-
-	: No growth			+	: there is growth (slightly) < 10 cords		
++	: there is growth (a lot) > 10 cords			+++	: there is growth (very much)		

sensitive to Isoniazid as a standard drug and first-line drug in the treatment of tuberculosis.

The results of the activity test of Parang Romang stem extract against *Mycobacterium tuberculosis* showed that in the negative control (-) with the addition of 950 l of bacterial suspension into all wells on 50 l aqua dest plate, the result was bacterial growth. For positive control, 50 l of Isoniazid was added to the *Mycobacterium tuberculosis* H37RV plate, and the three extracts did not show any bacterial growth. Then in testing with concentrations of 25 µg/ml, 75 µg/ml, 100 µg/ml, it was seen that there was a growth of *Mycobacterium tuberculosis* more than 10 cords in the ethanol extract. Then at a concentration of 500 µg/ml there was a growth of *Mycobacterium tuberculosis* but only slightly, which was less than 10 cords/field of view. In testing with n-Hexan soluble extract at concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, it was seen that there was a growth of *Mycobacterium tuberculosis*

more than 10 cords. Then at a concentration of 100 µg/ml, there was no growth of *Mycobacterium tuberculosis*. In testing with n-Hexan soluble extract at concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, it was seen that there was a growth of *Mycobacterium tuberculosis* more than 10 cords. Then at a concentration of 100 µg/ml, there was no growth of *Mycobacterium tuberculosis*. In testing with n-Hexan insoluble extract at concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, it was seen that there was a growth of *Mycobacterium tuberculosis* more than 10 cords. Then at a concentration of 100 µg/ml, there was a lot of *Mycobacterium tuberculosis* growth. This shows that n-Hexan soluble extract has better antituberculosis activity than other extracts.

Furthermore, the soluble n-Hexan extract was identified by spraying reagents to determine the class of chemical compounds contained in the extract. These results show

that the soluble extract of n-Hexan contains alkaloids and terpenoids

CONCLUSION

The n-Hexan soluble extract of Parang Romang steams has an antibacterial effect on *Mycobacterium tuberculosis* with MIC is 100 µg/ml 2.

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