International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-3, Issue-7, July 2017
ISSN: 2395-3470
www.ijseas.com

Antituberculosis Activity Test Of Kitolod Leaf Ethanol Extract (*Laurentia longiflora* (L.) Peterm.)

Fauzia Nurul Aqila*, Sri Agung Fitri Kusuma, Yopi Iskandar

Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363

Abstract

Kitolod is one of the plants that is known to have high flavonoid content. Flavonoids are reported to play a role in inhibiting the growth of Mycobacterium tuberculosis. Therefore, this research study was aimed to evaluate the antituberculosis of kitolod leaf ethanol extract against *M. tuberculosis* H37Rv in vitro. The extraction of Kitolod leaf was conducted using a maceration method, followed by phytochemical screening of its secondary metabolites using standard methods. Antituberculosis testing was performed using the proportion method with the following antibiotic controls: rifampicin, isoniazid, streptomycin and ethambutol. The results of the phytochemical analysis revealed that the extract contained alkaloids, flavonoids, polyphenols, monoterpenoids, sesquiterpenoids, Quinones and saponins. The ethanol extract of Kitolod leaf showed good antituberculosis activity against *M. tuberculosis* H37Rv, with an extract concentration ranging from 10-50%w/v.

Keywords: antituberculosis, Kitolod, proportion, *M. tuberculosis* H37Rv

Introduction

Tuberculosis is one of the leading causes of death in the world [1]. Tuberculosis is a disease caused by *Mycobacterium tuberculosis* [2]. According to World Health Organization (WHO), in 2008 there were approximately 9.4 million cases worldwide, with 1.8 million deaths, and 4,500 reported deaths per day [3]. Indonesia is the 3rd largest country in the world after India and China, which has the largest TB patients, with about 10% of the total number of tuberculosis patients in the world. The incidence of tuberculosis cases is about 110 per 100,000 population [4, 5].

The current increases in tuberculosis cases are in line with an increase in cases of antibiotic-resistant tuberculosis especially in developing countries including Indonesia. Researchers estimate approximately 50 million people infected with M. tuberculosis strains that are resistant to at least one type of Anti Tuberculosis Drug. Resistance to antibiotics such as INH / isoniazid, streptomycin, ethambutol, rifampicin, can be caused by several

things, such as irregular treatment, medication noncompliance and single drug use in tuberculosis patients. The onset of resistance to Anti Tuberculosis Drugs in M. tuberculosis is due to a random mutation of the bacterial chromosome. The mutation process occurs spontaneously in other strains, even before contact with the drug. The nature of this resistance is also caused by the mutation of genes that are expressed into certain proteins or enzymes in the bacteria, such as the occurrence of resistance to rifampicin because mutations of expressed rpoB genes into RNA polymerase [6]. The possibility of a more drugs resistant strain to occur in the future is very great [7]. Therefore, alternative therapeutic research programs are needed to find a new antimicrobial agent. An in vitro study of the antimicrobial activity of medicinal plant needs to be done [8].

The development of research on herbs that can inhibit the growth of *M. tuberculosis* had now been widely practiced. Some natural products and their derivatives are reported to exhibit extraordinary growth inhibitory activity against Mycobacterium tuberculosis and some have even been selected as prototype molecules for the development of new anti-tuberculosis agents [9] [10]. Reportedly flavonoids have anti-tuberculosis activity against Mycobacterium tuberculosis [11].

Traditionally Kitolod plant (Laurentia longiflora (L) Peterm) has been widely used in Indonesia as an herbal remedy that has antibacterial activity [12]. Flavonoid can be found many of Kitolod's leaves [13]. The flavonoid contained in the Kitolod leaf underlies the investigation of anti-tuberculosis activity of Kitolod leaf extract against *M. tuberculosis*.

Materials and Methods

Material

Materials used include Kitolod's leaves simplicia (Laurentia longiflora (L.) Peterm) (from Manoko Plantation, Lembang), ethanol 70%, distillate water (sterile), filter paper, Lowenstein-Jensen (Merck), glycerol (Merck), duck eggs, Rifampicin (Sigma), Isoniazid (Sigma), Ethambutol (Sigma), Streptomycin (Sigma), Mycobacterium tuberculosis strain H37Rv (from Central Java Health Development Laboratory Center), toluene. For phytochemical screening include; $MgSO_4$, $CuSO_4$, KI, $HgCl_2$, Bismuth sub nitrate, $FeCl_3$ 1 %, chloroform, hydrochloric acid 10 %, acetic acid anhydrous, sodium acetate, magnesium powder, amyl alcohol, ammonia 25 % v/v, sodium hydroxide 1 N, sodium sulfate, sodium hydroxide 30% and sulfuric acid.

Methods

Extraction

Kitolod's dried leaves are chopped into small pieces using scissors, then macerated for 3 x 24 h with ethanol 70%, in the first 24 h, the solvent-soaked simplisia are occasionally stirred. After that the macerate is filtered with a filter paper, the dregs are re-macerated, then left for another 24 hours. After 24 hours, second macerate is then filtered, and for the final 24 hours the dregs are again re-macerated, after 24 hours then the third macerate filtered [14]. To thicken the macerate evaporator and water bath is used until the desired thick consistency of ethanol extract of leaf Kitolod (Laurentia longiflora (L.) Peterm) is obtained [15]. Then from obtaining extract, the yield is calculated.

Phytochemical Screening of Secondary Metabolites

Phytochemical screening of secondary metabolites in the extract was using a standard method to determine the contains alkaloids, flavonoids, tannins, quinones, saponins, steroids, and triterpenoids.

Bacterial Preparation

The Bacteria used in this study were drug-susceptible *Mycobacterium tuberculosis* strain H37Rv, taken from the Central Java Health Development Laboratory Center, incubated at 37 ° C. *M. tuberculosis* were bred in Löwenstein-Jensen medium and allowed to grow for 3-4 w in 37^0C. The inoculum then used for the proportion method prepared by diluting bacterial cultures with sterile distillated water to concentrations of 10^{-3} and 10^{-5} for subsequent testing.

Antituberculosis Activity Test

Extracts with various concentrations (50%, 40%, 30%, 20%, and 10% (w / v)) were tested for their antibacterial activity against *M. tuberculosis* strain H37Rv. A certain amount of extract is weighed, then dissolved in DMSO. A total of 0.05 mL of the extract solution was added to a tube containing a liquid L-J medium to a total volume of 5 mL, then homogenized. Medium containing extract or OAT compacted in a dry oven at 85^0C for 1 h in a sloping position.

The bacterial suspension was inoculated on medium mixed with extract, medium mixed with anti-tuberculosis drugs, medium mixed with DMSO, and medium LJ only, as follows: 20 bottles of LJ medium containing ethanol extract of Kitolod leaf with 5 concentration variations-50%, 40%, 30%, 20%, and 10% (w / v) (each concentration consisted of 2 bottles with 10^{-3} and 2 bottles with 10^{-5} bacterial suspension) and 8 bottles of LJ medium containing anti-tuberculosis drugs (respectively consisting of 1 bottle with 10^{-3} and 1 bottle with 10^{-5} bacteria suspension) was also prepared 24 bottles of only LJ medium, and 4 bottles of LJ medium containing DMSO (each

consisted of 2 bottles with 10^{-3} and 2 bottles with 10^{-5} bacterial suspension). A volume of 100 µL bacterial suspensions was inserted into each bottle.

Results and Discussion

Extraction Results

The extraction of secondary metabolite from simplisia was performed by the maceration method for three days with solvent substitution every 1 x 24 h. Solvent substitution is done so that more secondary metabolites are extracted and obtain sufficient amount of macerate for the study. Solvent selection is based on the principle of like dissolve like where polar compounds will be extracted by polar solvents and non-polar compounds will be extracted by non-polar solvents. Ethanol is a semi-polar solvent, ethanol has a good ability in extracting polar and non-polar compounds, and in addition ethanol is edible. Each liquid extract obtained from the results of maceration, mixed into one and condense to have a thick consistency. The yield of the extract after condensing was 30.8% from 500 g simplisia.

Phytochemical Screening Result

Phytochemical screening was performed to determine the secondary metabolite groups contained in the simplisia and extracts. The secondary metabolites tested qualitatively and classified. Phytochemical screening is also useful in determining whether the target secondary metabolite compound in the study, is contained in the extract. The results of the phytochemical analysis are described as varied constituents of these extracts, as follows: alkaloids, flavonoids, polyphenols, monoterpenoids, sesquiterpenoids, quinine and saponins. The result of phytochemical screening can be seen in Table 1.

Table 1: Phytochemical screening

Secondary metabolites	Results	
	Simplisia	Extract
Alkaloids	+	+
Quinones	+	+
polyphenols	+	+
monoterpenoids	+	+
sesquiterpenoids	+	+
Tannins	-	-
Flavonoids	+	+
Steroid & Triterpenoid	+	+
Saponins	+	+

Note: (+) presence; (-) absence

According to research that has been done by Xiao et al. flavonoid has antibacterial activity against some bacteria, furthermore, research conducted by Brown et al. also showed the presence antibacterial activity of flavonoid against some Mycobacterium sp. Flavonoids can release their antibacterial activity through several mechanisms, i.e.

destroying cytoplasmic membranes, inhibiting nucleic acid synthesis, inhibiting energy metabolism, inhibiting cell wall synthesis, and inhibiting cell membrane synthesis [16]. Polyphenols have antibacterial activity by denaturing proteins and interfering with cell membrane function, thereby it became lysis [17]. Tannins have antibacterial activity by damaging components of cell membranes, cell walls, enzymes, genetic material, and other protein components [18]. Tannins can also protect the intestinal mucosa thus suppressing bowel peristalsis [19]. Lipophilic terpenoid has antibacterial activity by destroying the bacterial cell membrane, this compound will react with the active side of the membrane, dissolving the lipid constituent and increasing its permeability [20]. Saponin can increase the permeability of bacterial cell membranes so as to alter membrane structure and function, causing membrane protein denaturation so it will be damaged and lysis [21].

Results of Antituberculosis Activity Test

Bacterial susceptibility is indicated by the presence or absence of the bacterial colony growth in the media compared with the positive control. The bacterial susceptibility test towards extract was carried out at concentration variations of 50%, 40%, 30%, 20%, 10% (w / v), with bacterial growth observed in the first week to sixth week. Dilution of the extract was done by dissolving 200% extract stock (diluted with DMSO) into a liquid growth medium in accordance with the calculation. Test results Bacterial sensitivity to extracts can be seen in Table 2.

Tabel 2: Suseptibility Test Result *M. tuberculosis* strain H37Rv against Ethanol Extract Leaf Kitolod

Substance	Concentration (% v/v)	Colony Growth (Weeks)						IUATLD Scale (Bacterial cons.)	
		1	2	3	4	5	6	10^{-3}	10^{-5}
Extracts	50	-	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
Anti-tuberculosis Drug	Rifampicin	-	-	-	-	-	-	-	-
	Isoniazid	-	-	-	-	-	-	-	-
	Streptomysin	-	-	-	-	-	-	-	-
	Ethambutol	-	-	-	-	-	-	-	-

Positive Control	-	-	+	+	+	+	1+	10
Negative control	-	-	-	-	-	-	-	-

Notes: (-) : No growth; (+) : Colony growth present; (1+) : IUALTD scale

Table 3: IUATLD Scale (Kemenkes RI, 2012)

Reading	Written
> 500 Colony	4 +
200 – 500 Colony	3 +
100 – 200 Colony	2 +
20 – 100 Colony	1 +
1 – 19 Colony	Colony number
No growth	negative

Based on the data in table 3, it is known that *Mycobacterium tuberculosis* strain H37Rv sensitive to the variation of extract concentration. It is characterized by the absence of bacterial colony growth at each concentration of the extract.

Conclusion

The ethanol extract of Kitolod leaf may be a promising candidate for antituberculosis in the future.

References

1. Todar K, "*Mycobacterium tuberculosis* and Tuberculosis, In: *Todar's Online Textbook of Bacteriology"*. Madison: Wisconsin, 2012, pp. 1.
2. Romero B, Rodríguez S, Bezos J, Díaz R, Copano MF, "Humans as source of *Mycobacterium tuberculosis* infection in cattle", Spain. *Emerg Infect Dis.,* 17, 2011, pp. 2393–2395.
3. World Health Organization, "Planning the development of human resources for health for implementation of the Stop TB Strategy", Geneva: World Health Organization, 2009
4. Retno AW, "Patofisiologi, Diagnosis dan Klasifikasi Tuberkulosis", Departemen Ilmu Kedokteran Komunitas, Okupasi dan Keluarga", Jakarta: Fakultas Kedokteran Universitas Indonesia, 2011.
5. Murray CJL, Styblo K, Rouillon A, "Tuberculosis in developing countries: burden, intervention and cost", Bulletin of the International Union against Tuberculosis and Lung Disease, Dis. 65, 1990, pp. 6–26.
6. Morris S, Bal H, Suffys G, Portillo-Gamez P, Fairchok LM, D, "Molecular Mechanisms of Multiple Drug Resistance in Clinical Isolates of Mycobacterium tuberculosis", *J. Infect, Dis*. 177, 1995, pp.954-960.
7. Damma J, Hallberg K, Hellman B, "Genotoxicity of plumbagin and its effects on catechol and NQNO-induced DNA damage in mouse lymphoma cells", Toxicol. In Vitro 23, 2009, pp.266–271.

8. Salie F, Eagles PFK, Leng HMJ, "Preliminary antimicrobial screening of four South African Asteraceae species", *J. Ethnopharmacol*, 52, 1996, pp. 27–33.
9. Tripathi RP, Tewari N, Dwivedi, N. & Tiwari, V. K., "Fighting tuberculosis: An old disease with new challenges", In: Medicinal Research Reviews, 25 (1), 2005, pp. 93–131.
10. Gwendolyn AM, Amit N, Eugene Uh, Sharon YW, Tathagata M, Laura EV, et al, "The Medicinal Chemistry of Tuberculosis Chemotherapy", Tuberculosis Research Section, National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD, USA, 2011.
11. Brown AK, Papaemmanouil A, Bhowruth V, Bhatt A, Dover LG, Besra GS, "Microbiology", Oct, 153(10), 2007, pp.3314-3322.
12. Siregar RM, "Aktivitas Antibakteri Ekstrak Daun dan Bunga Kitilod (*Laurentia longiflora* (L.) Paterm) Terhadap Beberapa Bakteri Penyebab Konjungtivitis", Bogor: IPB, 2012.
13. Ismailova M, "Uji Aktivitas Antibakteri Ekstrak Air Daun Kitolod *(*Laurentia longiflora *(L.) Paterm)* Terhadap Bakteri yang Diisolasi dari Pasien Penderita Konjungtivitis", Bandung: ITB, 2008.
14. SAF Kusuma, Irma E, Novianti,"Comparative Study on Antibacterial Activity of Jatropha curcas Linn. Leaves Extract and Neomycin Sulfate Against Staphylococcus aureus ATCC 25923", IJSEAS, 3(4), 2017, pp.114-119.
15. Departemen Kesehatan Republik Indonesia, "Parameter Standar Umum Ekstrak Tumbuhan Obat", Jakarta: Depkes RI, 2000, pp.1-11.
16. Cushnie TP, Lamb AJ, "Recent advances in understanding the antibacterial properties of flavonoids", Int J Antimicrob Agents, 38, 2011, pp. 99- 107.
17. Brooks GF, Carroll KC, Butel JS, Morse SA, "Jawetz, Melnick, & Adelberg's Medical Microbiology", United States of America: The McGraw-Hill Companies, Inc, 24, 2007.
18. Sabir A, "Aktivitas Antibakteri Flavonoid Propolis Trigona sp terhadap Bakteri Streptococcus mutans (in vitro)", Majalah Kedokteran Gigi, Jakarta, 2005.
19. Clinton C, "Plant Tannins A novel Approach to the Treatment of Ulcerative Colitis", USA, N Med J, 2, 2009, pp. 1-3.
20. Mayanti T, Julaeha E, Putri Y, "Isolasi dan Karakterisasi Senyawa Antibakteri dari Fraksi Etil Asetat Kulit Batang *Lansium Domesticum* Corr.', Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Padjadjaran, Bandung, 2011.
21. Juliantina F, Citra DW, Nirwani B, Nurmasitoh T, Bowo T, "Manfaat Sirih Merah (piper crocatum) Sebagai Agen Anti Bakterial terhadap Bakteri Gram Positif dan Gram Negatif", JKKI, Jakarta, 2009.