Antituberculosis Activity of Active Compound of Ethyl Acetate Extract for Patikan Kebo (*Euphorbia hirta* L.)

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ABSTRACT

Infectious diseases caused by bacteria are a concern in the world of health. The microbe *Mycobacterium tuberculosis* which causes tuberculosis (TB) is one of the main disease problems in the world, as evidenced by the existence of 10.4 million sufferers and 1.8 million deaths in the world in 2015. During 5 years (2015 to 2020), the World Health Organization was done some comprehensive programs it can reduce mortality up to 13%. However, since early 2020 were increased the mortality rate again as in 2015. In addition, the increasing incidence of bacterial resistance to antibiotics has triggered various studies to find alternative antibacterial agents. This study is related to the anti-tuberculosis activity (against *M. tuberculosis* strain $H_{37}Rv$ with the liquid dilution method with Middlebrook 7H9 (MB 7H9) and solid dilution with Lowenstein-Jensen (LJ)) in ethyl acetate patikan kebo (*Euphorbia hirta* L.) extract. The investigation of antimycobacterial tuberculosis, it was found that a concentration of 800 µg/mL had anti-tuberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$. The active compound as a result of isolation is the triterpenoid (taraxasterol) group.

Keywords: Middlebrook 7H9 method, Lowenstein-Jensen method, antituberculosis, *Euphorbia hirta* L.

INTRODUCTION

Infectious diseases caused by bacteria are a concern in the world of health. Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), an intracellular obligate and aerobic bacillus that multiplies within macrophage.[1-2] In 2015, there were 10,4 million cases of tuberculosis with a mortality rate of 1,8 million patients.[3] The World Health Organization with its programs can reduce the mortality of TB patients by up to 13% from 2015 to before Covid-19. However, with this pandemic (since early 2020) the mortality rate has increased again as in 2015.[4]

The main drugs used for tuberculosis are Rifampicin, Isoniazid, Ethambutol and Pyrazinamide.[5] The drug must be taken regularly and consumed in the long term. Tuberculosis patients are often resistant to *Mycobacterium tuberculosis* because they take drugs irregularly. With resistance, the use of traditional medicine is highly recommended because it has the same efficacy as chemicals and is more economical.[6] Several types of plants that can be used as traditional anti-TB drugs are n-hexane extract of *Maerua edulis*, n-hexane extract of *Securidaca long pedunculate*, ethyl acetate extract of *Tabernaemontana elegans*, and dichloromethane extract of *Zanthoxylum capense* which are known to have anti-TB activity based on research from Luo et al.[7]

Indonesia is one of the countries in the world that has a lot of biodiversity, especially plants. More than 40,000 species of plants in Indonesia, 940 of which are known to have medicinal properties.[8] Medicinal plants play an important role as therapeutic agents.[9] One of the plants that

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have the potential as an antibacterial against *Mycobacterium tuberculosis* and can be used as an antituberculosis drug is *Euphorbia hirta* (*E. Hirta*). L.

E. Hirta is usually erect, slender-stemmed, spreading up to 80 cm tall, though sometimes it can be seen lying down. The plant is an annual broad-leaved herb that has a hairy stem with many branches from the base to the top. The leaves are opposite, elliptical, oblong, or oblong-lanceolate, with a faintly toothed margin and darker on the upper surface. The flowers are small, numerous, and crowded together in dense cymes (dense clusters in upper axils) about 1 cm in diameter.[10-11]

According to Mothana et al.[12] and Sudhakar et al.[13] that *E. Hirta* has biological activities such as anthelmintic, antipyretic, anti-inflammatory, antioxidant, antibacterial, antifungal and anticancer. Compounds that have a role in the antibacterial activity of *E. Hirta* are reducing sugars, terpenoids, alkaloids, steroids, tannins, flavonoids and phenolic compounds.[14-15]

The ethanol extract of *E. Hirta* leaves is known to have antibacterial activity with MIC values of 4.22 mg/mL against *Shigella dysenteriae*, 15.95 mg/mL against *Eschericia coli*, and 31 µg/mL against *S. thypii*,[16-17] while the methanol extract of *E. Hirta* is known to have an MBC value of 0.125 mg/mL against *Pseudomonas aeruginosa*, 0.25 mg/mL against *Bacillus cereus*, and >1 mg/mL against *Proteus vulgaris*.[18] The methanol extract of *E. Hirta* also acts as an antibacterial against *E. coli* and *Klebsiella pneumonia* with a MIC value of 0.250 mg/mL.[19] The ethyl acetate extract of *E. Hirta* had a MIC value of 0.5 mg/mL against *Proteus mirabilis*, *S. thypii*, and *Bacillus subtilis*.

In this study, the active compound was isolated from the ethyl acetate extract of *E. Hirta*. The isolation process used preparative Thin Layer Chromatography, then characterized by infrared and mass spectroscopy, and ^{1}H NMR. Previously, the antituberculosis activity was tested against *M. tuberculosis* strain $H_{37}Rv$ by liquid dilution method with Middlebrook 7H9 (MB 7H9) and solid dilution with Lowenstein-Jensen (LJ).

MATERIALS AND METHODS

Research Material

The research materials consist of main ingredients, chemicals and supporting materials. The main ingredient is the *E. Hirta* herb which was harvested on August, 3th 2020 from Mlati Regency, Sleman, Yogyakarta, Indonesia. *Mycobacterium tuberculosis* strain $H_{37}Rv$ ATCC 25618 which is still sensitive to first-line OAT, was obtained from the laboratory of the Faculty of Medicine, Gadjah Mada University.

The chemicals used for the extraction, isolation and identification of compounds were aquadest, alcohol, n-hexane, ethyl acetate, various eluents (developer for mobile phase TLC), detection reagents, silica gel plate F254, Nutrient Broth media, Nutrient Agar, and spiritus. While the materials for the antituberculosis test were Middlebrook 7H9 Broth (Merck) liquid media, the composition of the media was LJ (KH_2PO_4, Magnesium citrate, $MgSO_4$. $7H_2O$, L-aspargine, sterile distilled water, duck eggs, potato flour, 70% ethanol, 2% Malachite green, glycerol), DMSO 5%, McFarland no. 1, technical ethyl acetate, silica gel plate 60 F254 (Merck), ethyl acetate p.a. (Merck), toluene p.a. (Merck), chloroform p.a. (Merck), methanol p.a. (Merck), quercetin p.a. (Sigma-Aldrich), thymol p.a (Sigma-Aldrich), quinine p.a. (Sigma-Aldrich), detection reagents (Dragendorff, Ce(SO4)2, ethanolic H2SO4, ammonia, vanillin sulfuric acid, sulfuric acid anisaldehyde, KMnO4, citronic acid, FeCl3) are readily available in the laboratory with p.a. solvents, and spiritus. The supporting materials used are aluminum foil, cotton, gauze, umbrella paper, filter paper, and plastic wrap.

Research Procedure

Euphorbia hirta L. plant was identified in the Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University.

Mycobacterial tuberculosis Antibacterial Test on The Ethyl Acetate Extract of *E. Hirta*

Microbial preparation was carried out aseptically in the LAF (*Laminar Air Flow*). One ose of microbial stock on

sloping agar media was taken to be suspended in liquid media and then incubated for 18-24 hours in an incubator at a temperature of 36-39°C. The next day, the suspension was diluted with liquid medium and compared its turbidity with a standard 0.5 McFarland in order to have the same concentration of 1 x 108 CFU/mL.

The antituberculosis test method of the *E. Hirta* herb was carried out by testing the antibacterial activity of the thick extract of the *E. Hirta* herb against *M. tuberculosis* strain H_{37}Rv. Liquid dilution with Middlebrook 7H9 broth (MB 7H9) and solid dilution with Lowenstein-Jensen (LJ) to test the antimycobacterial activity by knowing the minimum level to kill bacteria (MBC). The level for testing is 200 µg/mL; 400 µg/mL; 800 µg/mL.

The antimycobacterial activity test was started with liquid dilution in MB 7H9 medium for 10 days to grow *M. tuberculosis*, then observed macroscopically and compared the color and turbidity. After that, it was followed by solid dilution in Lowenstein-Jensen (LJ) medium to visualize the results of the liquid dilution of the growth of *M. tuberculosis* colonies. Lowenstein-Jensen (LJ) media will change from bluish green to yellow if it is proven to be contaminated with gram-negative bacteria. The presence of mycobacterial growth in LJ is characterized by a cream color, dryness, a rough surface and not easily emulsified.

Active Compounds Analysis by Thin Layer Chromatography (TLC)

Active Compounds analysis was carried out by Thin Layer Chromatography (TLC). Three samples of ethanol extract, the n-hexane fraction, and the ethyl acetate fraction of *E. hirta* leaves in a viscous form previously dissolved with 70% ethanol. The mobile phase used is acetic acid 15% and toluene: ethyl acetate (9:1) is put into the expansion vessel and given filter paper.

Samples were spotted on silica gel F_{254} as much as 10 spots for each sample or until it was visible in UV light. The plate was eluted for 8 cm and then dried and observed under UV_{254} nm and UV_{366} nm lamps. A qualitative examination was carried out on the chemical content of *E. hirta* leaves. Detection of these groups of compounds using spotting reagents of alkaloids, flavonoids and terpenoids.

***Euphorbia hirta* L. Leaves Fractionation**

Euphorbia hirta L. leaves were washed with water until clean. The leaves of *Euphorbia hirta* L. were dried and then made simplicia in powder form. Extraction was carried out by maceration method using 70% ethanol as solvent. A total of 100 grams of simplicia powder was put into a vessel then poured with 150 mL of 70% ethanol, closed and left for 24 hours protected from light while repeatedly stirring and then filtered.

Fractionation of the thick ethanol extract of *E. Hirta* leaves was started by dissolving in hot water. Furthermore, the extract was partitioned using an n-hexane filter with a ratio of extract volume: n-hexane is 1:1. The soluble fraction was n-hexane and the upper phase was the n-hexane fraction. The soluble n-hexane fraction was collected, the solvent was evaporated and weighed, this fraction was called the n-hexane fraction. The lower or insoluble n-hexane phase was extracted using ethyl acetate solvent with a volume of 1:1 with the same method as partitioning with n-hexane solvent. The partition was repeated until the ethyl acetate phase became colorless. The result of this fractionation is called the ethyl acetate fraction.

Active Compound Isolation

Tracing the target compound by using thin layer chromatography (TLC). The eluted compound was sprayed with a Serium Sulfate spot viewer. Spots with brown color at UV_{254} and fluorescence at UV_{366} belong to certain groups of compounds. So that certain groups of compounds will be isolated and tested for their potential activity as antituberculosis.

Isolation of the compound from the ethyl acetate fraction of the *E. Hirta* herb with preparative TLC and n-hexane as mobile phase: ethyl acetate (4:1 v/v), while the stationary phase is silica gel 60 PF254 specifically for

preparative TLC. The resulting chromatogram was detected using visible light, UV_{245} nm and UV_{366} nm light and scraped and then collected. Furthermore, it was dissolved in methanol with the help of a magnetic stirrer for ± 15 minutes then filtered and the filtrate was dried. Compounds resulting from TLC were tested for purity using TLC. Pure compounds were identified by UV-Vis spectrophotometry, FTIR, GC/MS, ^{1}H-NMR.

Data Analysis

The results of the antibacterial potency test of *E. hirta* leaves using the liquid dilution method were visually analyzed for the level of turbidity. The data obtained were in the form of Minimum Inhibitory Concentration (MIC) in µg/mL. The results of the liquid dilution test were scratched onto solid media and visually analyzed for bacterial growth as Minimum Killing Concentration (MBC) in µg/mL.

Chromatogram profiles were analyzed qualitatively with comparisons of alkaloids, flavonoids and terpenoids. Observations with visible light and UV_{254} or UV_{366} before and after spraying with spotting reagent for detection of alkaloids, flavonoids and terpenoids. While the results of the isolation using preparative TLC were tested for purity using TLC and the target compound was elucidated its structure using FTIR, GC-MS, and ^{1}H-NMR spectrometry.

RESULTS AND DISCUSSION

Ethanol extract of *Euphorbia hirta* leaves is a thick greenish brown extract, has a distinctive aroma, is sticky, and weighs 43.81 g with a yield of 15.11% w/w. Fractionation with n-hexane is used to separate non-polar compounds such as chlorophyll. Meanwhile, ethyl acetate was used to separate semi-polar compounds from crude extracts.[20] Thus, the n-hexane fraction has non-polar properties and the ethyl acetate fraction has semi-polar properties.[21] The yield of the fractionated ethanol extract of *E. Hirta* was 4.78% w/w n-hexane fraction and 6.68% w/w ethyl acetate fraction.

Anti-*Mycobacterium tuberculosis* (*M. tuberculosis*) Activity Test

Anti-*M. tuberculosis* activity test by liquid dilution method in Middlebrook 7H9 Broth (MB 7H9) media which is an Agar-based medium. The advantage of this media is that it can see growth early, because the color of the medium is transparent, besides that it can determine the morphology of the colony when viewed with a microscope.[22] Then proceed with the solid dilution method using Löwenstein Jensen media (LJ media). The use of two media will provide good homogeneity between media, materials, and bacteria.[23] The parameter of anti-*M. tuberculosis* ethyl acetate extract of *E. Hirta* was the MBC.

The anti-*M. tuberculosis* activity test used three concentrations of the test compound 200, 400, and 800 µg/mL. Anti-*M. tuberculosis* activity test was started by dilution of liquid in MB 7H9 medium for 10 days, then observed and compared the color and turbidity (Figure 1). On MB 7H9 media, the growth of *Mycobacterium tuberculosis* was indicated by the presence of turbidity in the lower layer of the media. The difference in color between the extract and the control causes difficulties in observing the turbidity level of the media, so it is necessary to visualize using the solid dilution method with LJ media.

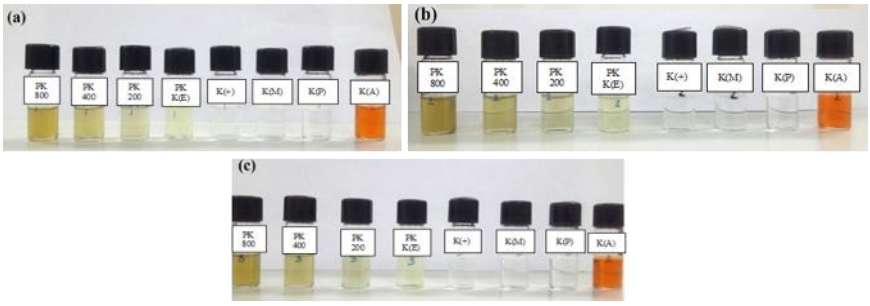

Figure 1. Anti-*Mycobacterium tuberculosis* Activity Test Result from Ethyl Acetate Extract of *E. Hirta* with Liquid Dilution on MB 7H9 Media

Description: Test 1 (a); Test 2 (b); Test 3(c); PK (800, 400, 200) = Ethyl acetate extract of *E. Hirta* with concentration series 200, 400, and 800 µg/mL; PK K(E) = Control of ethyl acetate extract of *E. Hirta* K (+) = Control of growth of *M. tuberculosis* K(M) = Control of media; K(P) = 5% DMSO solvent control; K(A) = Rifampicin antituberculosis control 40 µg/mL

Visualization of liquid dilution using solid dilution method in LJ media was carried out to make it easier to observe more clearly the growing *M. tuberculosis* colonies. The color of LJ media without mycobacteria is bluish green, if LJ media is blue it indicates the presence of bacterial contamination. The color of LJ media will change to yellow and then pink with the growth of Gram positive bacteria.[24] The growth of *M. tuberculosis* in LJ is characterized by the formation of cream-colored, dry granules, the presence of a rough surface and is not easily emulsified, while the growth of non-tuberculosis mycobacteria on LJ media is characterized by colonies that are smooth, flat, white, easily emulsified.[25]

Observations showed that the three results of repeated tests on LJ media (Figure 2), it can be seen that LJ media is a suitable medium for the growth of *M. tuberculosis* because the growth control of LJ media changes color and the surface of LJ media becomes rough indicating the growth of *M. tuberculosis*. LJ media was not contaminated as evidenced by the controlled media remained bluish green.

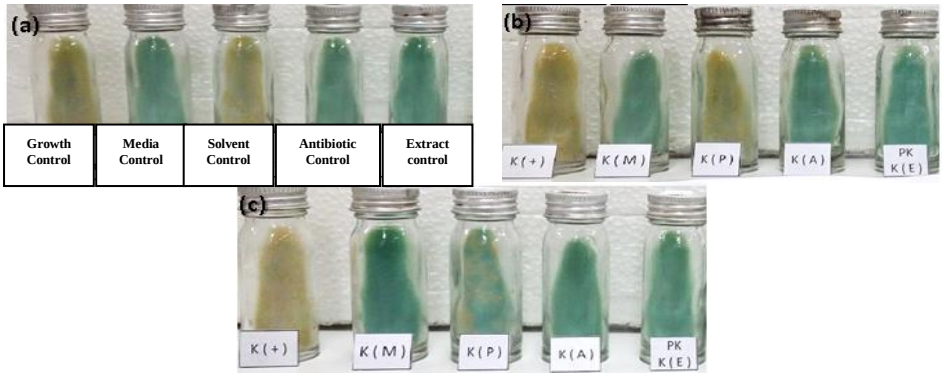

Figure 2. Control for Anti-*Mycobacterium tuberculosis* Activity Test Using Solid Dilution Method on LJ Media

Description: Test 1 (a); Test 2 (b); Test 3(c)

From the results of three tests of antibacterial activity on LJ media (Figure 3), it is known that the ethyl acetate extract of *E. Hirta* with concentrations of 200 and 400 µg/mL did not show antimycobacterial activity against *M. tuberculosis* because at these concentrations the growth of *M. tuberculosis* was still found, which is characterized by a change in the color of the LJ medium to beige with a rough surface. At a concentration of 800 µg/mL, tests 1, 2 and 3 showed anti-*M. tuberculosis* activity because there were no physical changes in LJ media and remained clean bluish green without spots (no growth of *M. tuberculosis* on the media). The extract with a concentration of 800 µg/mL was the MBC of the ethyl acetate extract of *E. Hirta* against *M. tuberculosis*, and that the ethyl acetate extract of *E. Hirta* had antimycobacterial activity against *M. tuberculosis*.

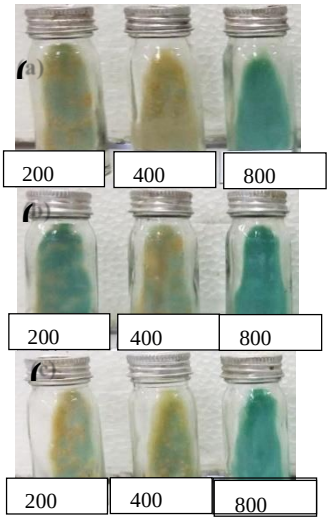

Figure 3. Anti-*Mycobacterium tuberculosis* Activity Test from Ethyl Acetate Extract of *E. Hirta* (200, 400 and 800 µg/mL) Using Solid Dilution Method on LJ Media

Description: Test 1 (a); Test 2 (b); Test 3(c)

Characterization of Active Compounds

Table 1. TLC System for Identification Groups of Compounds

	Alkaloids	**Terpenoids**	**Flavonoids**
Stationary Phase	Silica gel F_{254}		
Mobile Phase	Chloroform: ethyl acetate: methanol (3:2:1)	Toluene: ethyl acetate (92:8)	Chloroform: ethyl acetate: methanol (4:2:1)
Comparison	Quinine	Thymol	Quercetin
Spraying	• Dragendorff's reagent • $NaNO_2$ • Ethanolic H_2SO_4	• Vanillin sulfuric acid • Sulfuric acid anisaldehyde • $KMnO_4$	• Sitroborate • Ammonia vapor • $FeCl_3$

Identification of the ethyl acetate extract group in the *E. Hirta* herb was carried out using the Thin Layer Chromatography (TLC) method. TLC is used to separate various compounds in a mixture depending on their solubility in the solvent system.[26] Samples with a concentration of 20 mg/mL identified three groups of alkaloids, flavonoids and terpenoids with three eluent compositions and detected with three spraying reagents for each group of compounds (Table 1).

Before being eluted, the TLC vessel was saturated with the mobile phase. The spot color and hRf were observed after the elution process and after spraying under visible light, UV light at 254 nm and 366 nm. The identification results of the three groups of compounds showed that the ethyl acetate extract of the *E. Hirta* herb contained alkaloids, flavonoids and terpenoids.

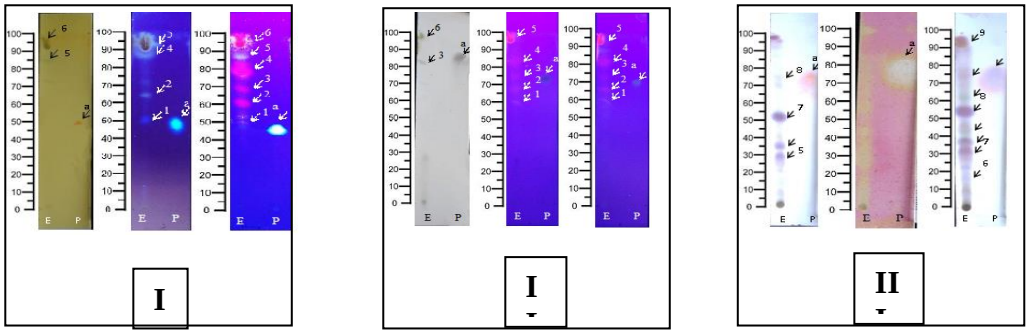

Figure 4. Results of TLC Identification of Alkaloids (I), Flavonoids (II) and Terpenoids (III)

There are three target compounds after optimization with preparative TLC (Figure 5) and isolation of the ethyl acetate fraction of *E. Hirta* with n-hexane: ethyl acetate (4:1 v/v) as mobile phase and silica gel 60 PF254 as stationary phase specifically for preparative TLC (Figure 5). The isolation results of the three target compounds were named Isolate A, Isolate B, and Isolate C. After the isolation was tested by TLC, isolates A and C were not detected so that the identification of the target compound was only carried out on isolate B. Isolate B from TLC was tested for purity using TLC.

Isolate B (weight 11.6 mg) is a colorless, crystalline isolate, and is soluble in both methanol and a mixture of n-hexane: ethyl acetate. This isolate was purified by TLC and with three different eluent systems (Figure 6) showing that a single spot on each chromatogram after eluting. The three mobile phase systems are Chloroform-Ethyl acetate (4:1 v/v), Chloroform-n-hexane (1:3 v/v) and n-hexane-ethyl acetate (2:1 v/v).

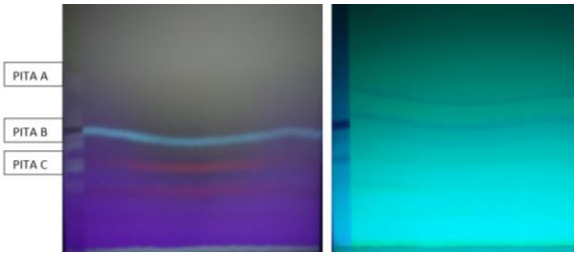

Figure 5. Preparative TLC Chromatogram

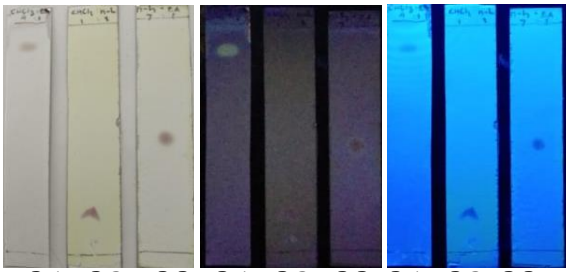

Figure 6. Purity Test for Isolate B by TLC Method Using 3 Different Eluents

1. Chloroform-Ethyl acetate (4:1 v/v); 2. Chloroform-n-hexane (1:3 v/v) and 3. n-hexane-ethyl acetate (2:1 v/v))

The infrared spectrum of isolate B (Figure 7) shows the presence of an OH group in the structure of this isolate compound. This is indicated by the absorption band of O-H stretching at a wave number of 3421 cm^{-1}. The presence of a band in this region in the infrared spectrum of a compound is a strong indication that a molecule contains the O-H functional group. Alcohol in a concentrated phase (in KBr pellets) holds strong hydrogen bonds and provides absorption until a wide absorption occurs. C-H stretching on the molecule is indicated by the absorption band at 2361 cm^{-1}. The presence of a C=C group (trans alkene) on the isolate B molecule was indicated by an absorption band at 1577 cm^{-1}.

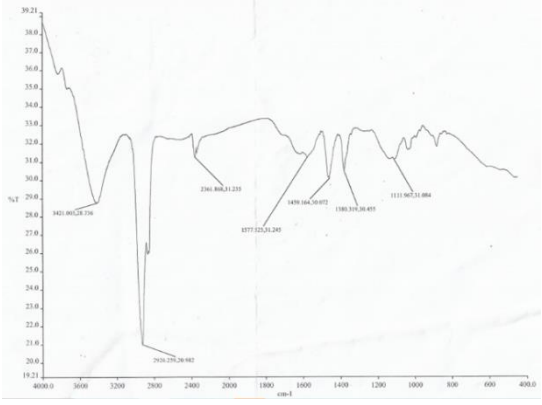

Figure 7. Spektrum of Fourtier Transform Infrared (FTIR) Isolate B

The ^{1}H-NMR spectrum of isolate B (Figure 8) showed that this molecule did not have an aromatic group, due to the presence of a peak in the chemical shift area of 0.757-1.707 ppm which is the H of the aliphatic compound. Meanwhile, the mass spectrum of isolate B (Figure 9) shows a peak at 426 m/z and a base peak at 43 m/z. Some of the fragmentation peaks were 257, 247, 229, 218, 207, 189, 175, 161, 147, 135, 121, 107, 91, 81, 68, 43, 41 and 28 m/z, and compared with the MS spectra of taraxasterol there were 12 same fragments.[27]

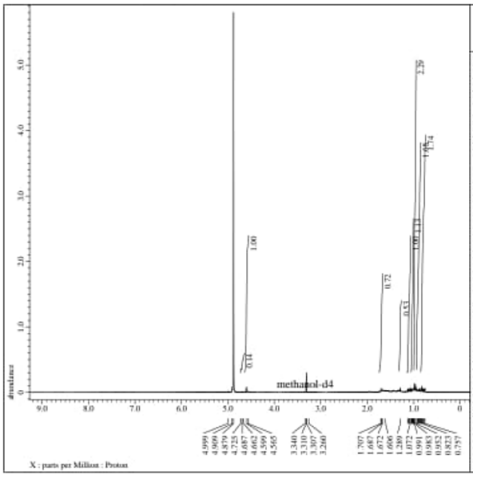

Figure 8. Spectra of Mass Spectrometry (MS) Isolate B

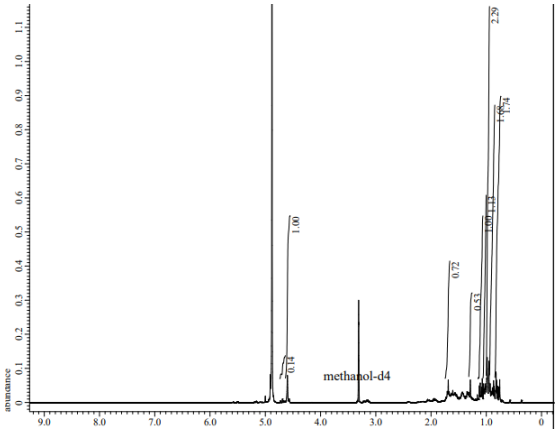

Figure 9. Spectra of Hydrogen-1 Nuclear Magnetic Resonance (^{1}H-NMR) Isolate B

Based on data from IR, ^{1}H NMR and MS, the active compound of anti-*Mycobacterium tuberculosis* strain $H_{37}Rv$ was suspected to be taraxasterol (see figure 10), which is a triterpenoid. Triterpenoid and phenolic compounds have been isolated from various plants in euphorbiacea, such as beta amyrin, qurcitrin, myricitrin, β-sitosterol, gallic acid, ellagic acid, α-Amerin, and β-amyrin.[28] While Pirmansyah et al.[29] reported that *Euphorbia milii* has potential as an antibacterial and Afrida and Sanova[30] stated that *Euphorbia thymifolia* Linn. was active as an antibacterial in their research. Isolation and Endophytic Fungi *Euphorbia antiquorum* L. identified and produced active compounds as antibacterial by TLC-Bioautography method.[31]

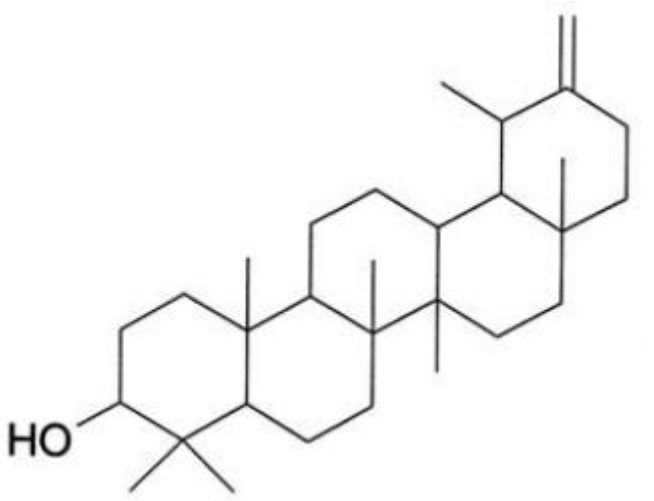

Figure 10. Chemical Structure of Taraxasterol[32]

The antituberculosis activity of *E. Hirta* has previously been studied. Nirmal et al.[33] studied anti-tuberculosis in *Lantana camara* L., *Euphorbia hirta* L., *Mukia maderaspatana* (L.) M. *Roem*, and *Abutilon indicum* (L.) in crude methanol extract of plants against multi-drug resistant (MDR) clinical isolates of *Mycobacterium tuberculosis* (Mtb) and Mtb $H_{37}Rv$ using the Luciferase Reporter Phage (LRP) assay. The MIC (Mimimum Inhibitory Concentration) of the selected plant fraction against the Mtb strain was found in the range of 400-1600 g/mL where as *Mukia maderaspatana* (L.) M. *Roem* showed the lowest MIC of 400 g/mL and this was validated by two different methods. The four medicinal plants studied are known to be useful as potential sources for anti-TB drug formulations. The difference between this study and Nirmal et al. lies in the method. This study used the Lowenstein-Jensen (LJ) method. Lowenstein-Jensen (LJ) culture is the gold standard method of identification of *Mycobacterium tuberculosis* with sensitivity and specificity of 99% and 100%, respectively.[34-35]

CONCLUSION

The antimicrobial activity of the ethyl acetate extract of *E. Hirta* was investigated as MBC value of 800 µg/mL. Based on the FTIR, MS and ^{1}H-NMR spectra, the active compound in the ethyl acetate fraction was presumed to be taraxasterol as a triterpenoid compound.

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نشاط مضادات الجراثيم للمركب النشط من خلاصة خلات الإيثيل لباتيكان كيبو(*Euphorbia hirta* L.)

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ملخص

الأمراض المعدية التي تسببها البكتيريا هي مصدر قلق في عالم الصحة. تعتبر البكتيريا المتفطرة السلية المسببة لمرض السل (TB) واحدة من المشاكل المرضية الرئيسية في العالم، كما يتضح من وجود 10.4 مليون مصاب و 1.8 مليون حالة وفاة في العالم في عام 2015. خلال 5 سنوات (2015 إلى 2020)، قامت منظمة الصحة العالمية بعمل بعض البرامج الشاملة ويمكنها خفض معدل الوفيات بنسبة تصل إلى 13٪. ومع ذلك، منذ أوائل عام 2020، تم زيادة معدل الوفيات مرة أخرى كما في عام 2015. بالإضافة إلى ذلك، أدى تزايد حدوث المقاومة البكتيرية للمضادات الحيوية إلى إجراء دراسات مختلفة لإيجاد عوامل بديلة مضادة للبكتيريا. ترتبط هذه الدراسة بالنشاط المضاد للسل ضد سلالة *M. tuberculosis* strain $H_{37}Rv$ باستخدام طريقة التخفيف السائل باستخدام Middlebrook 7H9 (MB 7H9) والتخفيف الصلب باستخدام Lowenstein-Jensen (LJ)) في ethyl acetate patikan kebo (*Euphorbia hirta* L.) استخراج. في دراسة مرض السل المضاد للبكتيريا وجد أن تركيز 800 ميكروغرام / مل له نشاط مضاد للسل ضد المتفطرة السلية .$H_{37}Rv$ المركب النشط نتيجة العزلة هو مجموعة ترايتيربينويد (تراكساستيرول).

الكلمات الدالة: مضاد السل، Lowenstein-Jensen طريقة Middlebrook 7H9 طريقة *Euphorbia hirta* L.

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