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THE ANTIMYCOBACTERIAL POTENTIAL OF SAMBILOTO (*Andrographis paniculata* Nees) EXTRACT AGAINST *Mycobacterium tuberculosis* H37Rv WITH *Microscopic-Observation* AND *Drug-Susceptibility* (MODS) METHODE

Potensi Antimikobakteri Ekstrak Sambiloto (*Andrographis paniculata* Nees) terhadap *Mycobacterium tuberculosis* H37Rv dengan Metode *Microscopic-Observation and Drug-Susceptibility* (MODS)

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ABSTRACT

Andrographis paniculata, commonly known as sambiloto, is empirically used for various treatments, including its ability to inhibit the growth of *Mycobacterium tuberculosis* (*M. tuberculosis*). This study aims to evaluate the growth inhibition activity of *M. tuberculosis* strain H37Rv using sambiloto herb extract (*Andrographis paniculata*). The sambiloto extract was prepared using the maceration method. *M. tuberculosis* was grown on MODS medium, and the toxicity of sambiloto (*A. paniculata*) was analyzed using pharmacokinetic prediction studies (pkCMS). The results showed that at concentrations of 0.255 mg/ml, 1.275 mg/ml, and 2.55 mg/ml of sambiloto extract, *M. tuberculosis* growth occurred on days 7 to 14. Further observations were made until day 28, and it was found that starting at a concentration of 6.375 mg/ml, sambiloto extract did not show *M. tuberculosis* growth in MODS medium. The positive control, isoniazid, did not show bacterial growth, while the negative control showed extensive bacterial growth on day 12. The determination of *M. tuberculosis* growth was based on microscopic observations of the bacterial colonies, focusing on cord formation. In conclusion, this study, the use of an extract with a concentration of 6.375 mg/ml in the MODS method showed no growth of *Mycobacterium tuberculosis*, indicating that the extract is effective in inhibiting bacterial growth at this concentration. The online pkCSM test conducted in this study showed that the extract used is not cytotoxic, meaning that the extract is safe for body cells and does not cause cellular damage. Therefore, it has the potential to be an adjunct therapy in the treatment of tuberculosis.

Keywords: *Andrographis paniculata, Antimycobacterial activity, MODS, Pharmacokinetic prediction*

ABSTRAK

Andrographis paniculata, yang biasa dikenal sebagai sambiloto, secara empiris digunakan untuk berbagai pengobatan, termasuk kemampuannya untuk menghambat pertumbuhan bakteri *Mycobacterium tuberculosis (M. tuberculosis)*. Penelitian ini bertujuan untuk mengevaluasi aktivitas penghambatan pertumbuhan *M.tuberculosis* strain H37Rv menggunakan ekstrak herba sambiloto (*Andrographis paniculata*). Ekstrak sambiloto dibuat dengan cara metode maserasi.

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Bakteri *M. tuberculosis* ditumbuhkan pada medium MODS dan toksisitas sambiloto (*A. paniculata*) dianalisis menggunakan studi prediksi sifat farmakokinetik (pkCMS). Hasil penelitian, pada konsentrasi ekstrak sambiloto sebesar 0,255 mg/ml, 1,275 mg/ml, 2,55 mg/ml pada hari ke 7 – 14 ditumbuhi bakteri *M. tuberculosis*. Selanjutnya pengamatan dilakukan sampai dengan 28 hari, dan dihasilkan ekstrak sambiloto mulai dari konsentrasi 6,375 mg/ml tidak menunjukkan pertumbuhan *M. tuberculosis* dalam media MODS. Kontrol positif isoniazid tidak menunjukkan pertumbuhan bakteri, sedangkan kontrol negatif menunjukkan pertumbuhan bakteri yang banyak pada hari ke-12. Penentuan adanya pertumbuhan *M. tuberculosis* didasarkan pada pengamatan mikroskopis terhadap koloni *M. tuberculosis* dalam pembentukan tali (*cord formation*). Kesimpulannya, dalam penelitian ini, penggunaan ekstrak dengan konsentrasi 6,375 mg/ml pada metode MODS menunjukkan tidak ada pertumbuhan *Mycobacterium tuberculosis*, yang mengindikasikan bahwa ekstrak tersebut efektif dalam menghambat pertumbuhan bakteri pada konsentrasi ini. Uji pkCSM online yang dilakukan dalam penelitian ini menunjukkan bahwa ekstrak yang digunakan tidak bersifat sitotoksik, yang berarti ekstrak tersebut aman bagi sel tubuh dan tidak menyebabkan kerusakan sel. sehingga berpotensi sebagai adjuvan terapi pengobatan tuberculosis.

Kata Kunci: *Andrographis paniculata, Antimycobacterial activity, MODS, Pharmacokinetic prediction*

INTRODUCTION

Tuberculosis is a contagious disease caused by the bacteria Mycobacterium tuberculosis. Globally, 3.5% of new TB cases and 18% of previously treated cases have MDR-TB. The highest proportions (>50% in previously treated cases) are found in countries of the former Soviet Union. Among MDR-TB cases in 2017, it was estimated that 8.5% had extensively drug-resistant TB (XDR-TB). Approximately 1.7 billion people, or 23% of the world's population, are estimated to have latent TB infection, and are thus at risk of developing active TB disease during their lifetime. (WHO 2018)

TB treatment requires a long duration, and the long-term use of combination antituberculosis drugs can lead to patient noncompliance with the regimen and irregular medication adherence. This can result in the development of drug resistance in *Mycobacterium tuberculosis*, poor case management, discontinuation of treatment due to side effects, and inadequate drug supply, all of which are factors contributing to resistance (Sandhu 2011). Indonesia has committed to reducing the incidence of tuberculosis cases to 65 per 100,000 population by 2030. Efforts to combat tuberculosis in Indonesia from 2020 to 2024 are directed towards accelerating th2e country's efforts to achieve tuberculosis elimination by 2030. The strategy for tuberculosis control in Indonesia for 2020-2024 is implemented to achieve the target of reducing the incidence of tuberculosis from 319 per 100,000 population in 2017 to 190 per 100,000 population, as well as lowering the mortality rate due to tuberculosis from 42 per 100,000 population in 2017 to 37 per 100,000 population by 2024 (Kementerian Kesehatan Republik Indonesia 2020).

Observed in vitro antimycobacterial activity of 5 mg/ml aqueous extract of *Andrographis paniculata* using Lowenstein-Jensen (L-J) medium, showing 100% inhibition against *M*. *tuberculosis* H37Rv strain and 93.7% inhibition against multidrug-resistant (MDR) strains (Radji et al. 2015).

The sambiloto herb is empirically used to treat various diseases, including the inhibition of *M. tuberculosis* growth. Sambiloto herb refers to all parts of the above-ground plant of *Andrographis paniculata* (Burm.f.) Nees, family Acanthaceae, which contains not less than 0.50% andrographolide (Kementerian Kesehatan Republik Indonesia 2017). The active compounds in sambiloto extract (*Andrographis paniculata*) include Andrographolide (Jayakumar et al. 2013). 13-*dien*-15, 16, 19-*triol Ent- labdane*, 8α-methoxy-14-deoxy-17β- hydroxyandrographolide Ent-labdane, *Andrographolactone*, *Andrograpanin* (Chauhan et al.

2019), *panaculoside*, flavonoids, *andrographonin*, *panicalin, neoandrographolide*, *apigenin 7-4-dimethyl ether* (Joselin and Jeeva 2014), 14- *deoxyandrographolide,* 14- *deoxy* 11, 12- *didehydroandrographolide,* 19-O-β-D-*glucopyranosyl-ent-labda*-8 (Arifullah et al. 2013).

The synthesis of mycolic acid in *Mycobacterium tuberculosis* involves two types of fatty acid synthase systems (FAS), namely FAS-I and FAS-II. FAS-II consists of a series of enzymes responsible for elongating fatty acid chains synthesized by FAS-I. Inactivation or deficiency of any of these enzymes will inhibit mycolic acid biosynthesis, making it a potential target in the development of anti-tuberculosis drugs. (Wulan et al.2022)

The fatty acid biosynthesis of *Mycobacterium tuberculosis* involves the production of long-chain fatty acids (meromycolic acid) and shorter carboxylated fatty acid derivatives, followed by condensation to form the final molecules. The cell wall of *Mycobacterium tuberculosis* consists of three types of mycolic acids: α, methoxy, and keto. The difference between them lies in the functional groups found in the meromycolic acid chain. (Siregar MIT et all.2015). Based on this theory, it is suspected that the andrographolide and others compound in the sambiloto plant interferes with the formation of mycolic acid, leading to bacterial cell wall lysis or inhibiting its growth.

This mechanism of action is similar to isoniazid, as both inhibit the synthesis of mycolic acid in *M. tuberculosis* bacteria. Isoniazid enters the *M. tuberculosis* cell as a prodrug through passive diffusion. INH is then activated by the catalase-peroxidase enzyme expressed by the *KatG* gene of *M. tuberculosis* to its active form. The active INH then inhibits the biosynthesis of mycolic acid (long chain α-branched ß-hydroxylated fatty acids) in the cell wall of *M. tuberculosis*. (Siregar MIT, 2015).

One of the medicinal plants that has been empirically used as a remedy is sambiloto (*Andrographis paniculata*). Some of the benefits of sambiloto include treating itching, vaginal discharge, acting as an antipyretic, and diuretic, as well as treating several degenerative diseases such as diabetes, high blood pressure, and rheumatism (Rachmani et al.2016).

The study by Nugroho et al. (2018) demonstrated that the administration of andrographolide, 14.8% extract of Andrographis paniculata, used orally at a dose of 10 mg/kg body weight, significantly reduced the expression of TNF-α in the lungs of tuberculosis-infected rats, which were injected with 1 mg/kg body weight of Complete Freund's Adjuvant (CFA) via intravenous injection in the tail. The results of the study showed a positive correlation between TNF-α expression in the lungs and the formation of granulomas in the lungs. The higher the expression of TNF-α in the lungs, the greater the number of granulomas formed. In the group treated with andrographolide extract, the average number of granulomas was lower (decreased) compared to the CFA group, with a significance level of 0.013. The administration of andrographolide extract was able to reduce the number of lung granulomas.

From previous studies methanol extract of *Andrographis paniculata* is more effective in inhibiting the growth of pathogenic bacteria, showing around 95% inhibition, compared to chloroform extract with 80% inhibition and hexane extract with 65% inhibition (Joselin and Jeeva 2014).

Andrographolide achieved the highest inhibition (96.38 ± 0.39%) at a concentration of 100 µg/ml. Positive controls for isoniazid and fluoroquinolones showed inhibition of 98.89 ± 0.45% and 94.95 ± 0.73%, respectively, at the same concentration, the turbidometric method showed inhibition against *M. microti,* with the inhibitory effect comparable to standard drugs such as isoniazid and fluoroquinolones. *M. microti* is a Gram-positive, non-motile, acid-fast bacterium within the *M. tuberculosis* complex, sensitive to isoniazid, ethambutol, rifampicin, streptomycin, and pyrazinamide, which causes tuberculosis in rodents (Garg and Shrivastava 2013).

This study is different from previous studies, as it uses various concentrations of 70% ethanol sambiloto extract with concentrations of 0.255; 1.275; 2.55; 6.375; 12.75; 25.5; 50.0; 100.0 mg/ml in 1% DMSO solvent using MODS medium. The observed parameter is the growth of *Mycobacterium tuberculosis* bacteria in liquid medium, rather than in solid medium. Variation in concentration is used to determine at which

concentration *Mycobacterium* bacteria do not grow in MODS medium.

The research conducted differs from previous studies, which used a disk paper with Lowenstein-Jensen (L-J) medium at a concentration of 5mg/ml (Radji et al. 2015). The testing used a well diffusion method with concentrations of 2mg/well and 5mg/well, resulting in inhibition with chloroform extract showing 80% inhibition and hexane extract showing 65% inhibition (Joselin and Jeeva 2014). The difference from previous studies is that the MODS method was chosen because it is the gold standard for examining the growth of *Mycobacterium tuberculosis* in liquid medium. The reason for selecting the concentrations in the test was to start from the lowest concentration up to the highest, until the concentration at which *Mycobacterium tuberculosis* did not grow in the MODS medium was found. This study uses *Mycobacterium tuberculosis* strain H37Rv, which distinguishes it from previous studies, where a solid agar disk diffusion medium was used, while this study utilizes the liquid MODS medium.

This studies conducted with various concentration of extract in MODS medium to inhibit the growth of *M. tuberculosis* bacteria. The objective of this research is to determine whether the tested concentration of sambiloto can inhibit the growth of *M. tuberculosis.*

MATERIALS AND METHODS

Plant determination

Sambiloto Herb was obtained from Balai Penelitian Tanaman Rempah dan Obat (Balittro) in Bogor, West Java. The plant used is an herb, consisting of all parts of the plant above ground. The sambiloto herb is harvested at 3-4 months of age, and it is harvested during the flowering stage in the dry season. Plant determination was carried out by the Herbarium Depokensis (UIDEP), Biota Collection Room at the FMIPA Universitas Indonesia, Depok. The identification result with the number: 12/UN2.F3.11/PDP.02.00/2023.

Extraction of simplisia

The simplicia of sambiloto herb was macerated using 70% ethanol solvent (1:10) and filtered every 24 hours. The re-maceration process was repeated three times, then filtered and evaporated using EYELA rotary vacuum evaporator N-11 to determine the yield of the crude extract. Subsequently, the extract was processed using a EYELA FD-1000 Freeze Dryerfreeze and the yield of the dry extract was calculated. The selection of 70% ethanol as the solvent in the extraction process is because 70% Ethanol solvent is a universal solvent capable of attracting compounds that are soluble in both non-polar and polar solvents.(Dianda TP et all. 2022), The extract is stored in a refrigerator at a temperature below 20°C.

Phytochemical screening

The standardization of the extract conducted includes specific extract standardization such as phytochemical testing, such as test for tannin / polyphenol: To the diluted extract, 3-4 drops of 10% FeCl3 were added, blue color was seen for phenolic and the presence of catechol tannin turned the solution green. Test for flavonoids : extract solution, 1.5 mL of 50% methanol solution a small magnesium chunk were warmed. 5-6 drops of concentrated HCl were added, red color was observed for flavonoids. Test for alkaloids: Meyer's test: extract solution, 1 mL of Meyer's reagent was added. The presence of pale yellow precipitate indicated the presence of alkaloids. Dragendroff's test: extract solution was warmed with 2% H2SO4. Few drops of Dragendroff's reagent were added. Orange-red. Indicated the presence of alkaloids. Test for quinon: the extract sample was dissolved in 70% alcohol, then a few drops of 1 N sodium hydroxide were added. The appearance of a red solution indicates the presence of quinone. Test for saponins: The extract solution was added to distilled water and then shaken vigorously. The appearance of froth indicates the presence of saponins (Harbone 2006; Sharma et al. 2020).

Preparation of microscopic-observation and drug-susceptibility (MODS) culture medium

The first step dissolve 5.9 g of 7H9 powder medium in 900 ml of sterile distilled water containing 3.1 ml of glycerol and 1.25 g of casitone, shake the solution until all components are dissolved (use a magnetic stirrer if available). Next, the solution is placed in the autoclave at 121-124ºC for 15 minutes. Next, cool the medium and aliquot it into sterile plastic tubes, 4.5 ml per tube, for sample preparation. After that Incubate at 37ºC for 48 hours to ensure sterility (the medium should remain clear). Finally, store at 2-8ºC in tightly closed tubes. (Brady MF et all. 2008)

Preparation of *M. tuberculosis* H37Rv bacterial suspension at 0.5 McFarland

M. tuberculosis H37Rv strain from the Central BSL-3 Laboratory at Universitas Padjadjaran. To prepare a bacterial suspension, first take 1-2 colonies of *M. tuberculosis* H37Rv from a solid Ogawa agar tube. Then, transfer the bacterial colonies into a boiling tube containing 3 ml of PBS Tween and vortex the solution until homogeneous. After homogenizing, let it stand for 15 minutes. Next, take approximately 1-2 ml of the suspension and transfer it into a McFarland tube containing 3 ml of PBS Tween. Measure the turbidity using a densitometer and adjust until the concentration reaches 0.5 McFarland. Once the bacterial suspension at 0.5 McFarland is obtained, transfer it to a small tube and label it with the name and date of preparation.

Preparation of sambiloto extract and isoniazid solution

Sambiloto extract was prepared at concentrations of 0.255; 1.275; 2.55; 6.375; 12.75; 25.5; 50.0; 100.0 mg/ml in 1% DMSO solvent using MODS medium. For the isoniazid standard solution (positive control) with a concentration of 0.5 ppm was dissolve in aquabidest. The negative solution contains *M. tuberculosis* bacteria + DMSO 1%.

Testing anti-*M. tuberculosis* activity

To test the activity of *M. tuberculosis* using the MODS medium with a 24-well plate, each well contains 900 µl of MODS solution (consisting of MODS medium + OADC + *M. tuberculosis* bacteria). Then, 100 µl of the extract or test material was added to each well plate. Sambiloto extracts were dissolved in 1% DMSO, as well as isoniazid solution at 0.5 ppm (positive control) and *M. tuberculosis* + 1% DMSO (negative control). All test materials were replicated 3 times. Next, all test materials are incubated at a temperature of 36-37°C. The presence of *M. tuberculosis* was observed daily using Inverted Microscope Upgradable Camera Microscope Carl Zeiss-Primovert at 10x and 40x magnification. The presence of M. tuberculosis growth was indicated by the formation of bacterial cords. The analysis method used is qualitative, by observing whether bacteria grow or do not grow in the MODS medium at which concentration and on which day, as recorded in the study.

Pharmacokinetic and toxicity prediction

pkCSM online application was used to predict the pharmacokinetic activity and toxicity of compounds, which can be downloaded from the website https://biosig.lab.uq.edu.au/pkcsm/. The ligand of the test compound must be in the form of a 3D structure using the 2D Chem Draw 18.1 program, then stabilize the structure using Chem 3D 18.1, selecting the MMFF94 Minimization feature. Next, the compound was saved in SMILES format. For the analysis of the compound, pkCSM application was used to predict pharmacokinetic properties (Absorption, Distribution, Metabolism, Excretion, Toxicity / ADMET) (Pires et al. 2024).

RESULT AND DISCUSSION

This research uses sambiloto herb simplicia, which is then extracted using the re-maceration method with a 70% ethanol solvent (1:10). The 70% ethanol solvent is used to extract all secondary metabolite compounds, both polar to non-polar. Subsequently, the maceration results are concentrated using a rotary evaporator to obtain a crude extract. The crude extract is then dried using a freeze dryer to obtain a dry

extract aimed at preventing degradation and fungal growth, allowing for longer shelf life. The weight of the simplicia and the % yield can be seen in Table 1.

Table 1. Yield of sambiloto extract

Extract	Weight of the Simplicia (g)	Yield of Crude Extract (%)	Yield After Freeze Drying (%)
70% Ethanol Extract of Sambiloto (*Andrographis paniculata*)	5100	15.88 %	13.3 %

Table 2. Phytochemical content of sambiloto extracts

Extract	Phenol	Flavonoid	Alkaloid	Tannin	Quinone	Saponin
70% Ethanol Extract of Sambiloto (*Andrographis paniculata*)	+	+	+	+	-	+

+ : positive, **-** : negative

The results of the phytochemical screening test of sambiloto extract show the presence of secondary metabolites including phenols, flavonoids, alkaloids, tannins, and saponins (Table 2). The analysis of the identification of andrographolide compounds in sambiloto extract using HPLC with PDA Detector (Figure 1).

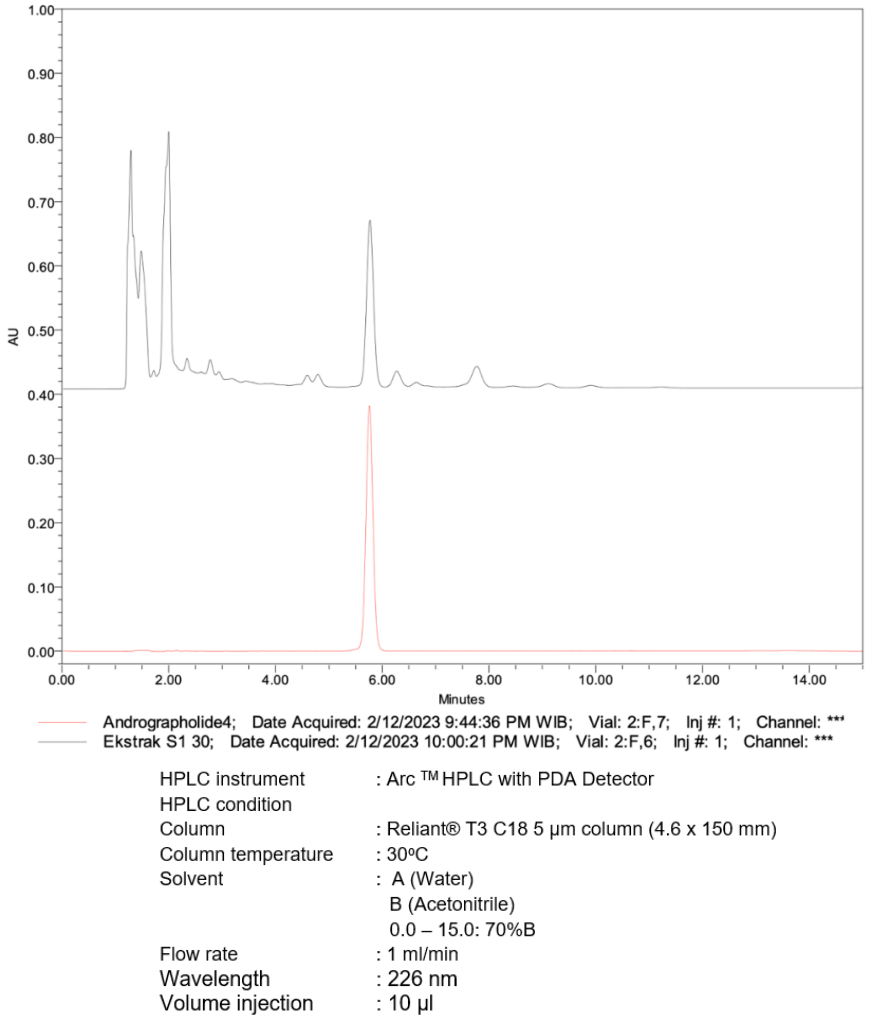


Figure 1. Overlay of the chromatogram of the Andrographis paniculata sample (top) and the standard andrographolide compound (bottom).

Flavonoid compounds possess antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anti-cancer activities (Nugraha et al. 2017). Flavonoids, tannins, and phenols are classified as phenolic compounds. Saponin compounds have antibacterial activity that disrupts the permeability of bacterial cell membranes, resulting in membrane damage and the release of various essential components from within the bacterial cell, such as proteins, nucleic acids, and nucleotides. Quinones act as antibacterial agents by inhibiting bacterial growth through the formation of irreversible complex compounds with the nucleophilic amino acid residues on transmembrane proteins in the plasma membrane, cell wall polypeptides, and enzymes present on the cell membrane surface, thus disrupting bacterial cell life (Hadi et al. 2019). Based on the results of this phytochemical test, sambiloto extract shows potential in inhibiting bacterial growth.

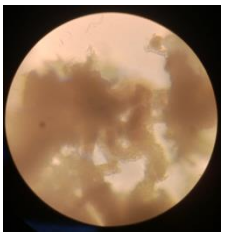
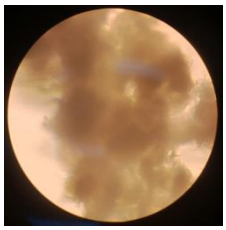
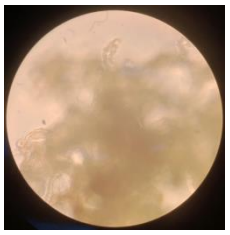
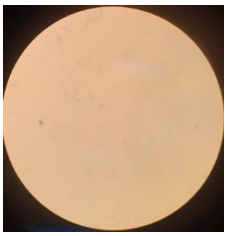
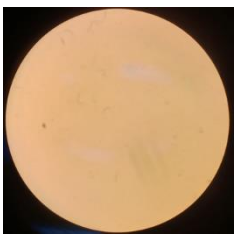
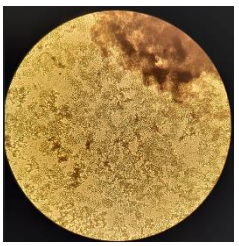
In the in vitro study no growth of *M. tuberculosis* was observed in any of the test materials, including Sambiloto extracts at concentrations of 6.375; 12.75; 25.5; 50.0; 100.0 mg/ml in 1% DMSO solvent using MODS medium, and isoniazid (INH) standard. After incubation at 37°C in MODS medium for 30 days. No growth of *M. tuberculosis* was detected in the test extracts, indicating that the test extracts are capable of inhibiting the growth of *M. tuberculosis* (Table 3 and table 4).

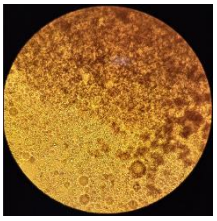
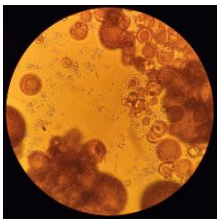
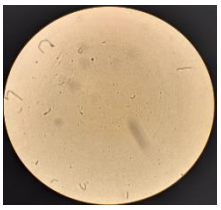
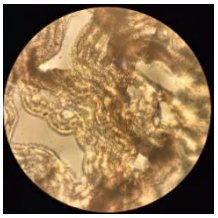
Table 3. The concentration of sambiloto extract in inhibiting the growth of M. tuberculosis bacteria in the MODS medium:

The concentration of sambiloto extract	Replication	Growth in the MODS medium
0,255 mg/ml in DMSO 1%	1	**+**
	2	**+**
	3	**+**
1,275 mg/ml in DMSO 1%	1	**+**
	2	**+**
	3	**+**
2,55 mg/ml in DMSO 1%	1	**+**
	2	**+**
	3	**+**
6,375 mg/ml in DMSO 1%	1	-
	2	-
	3	-
12,75 mg/ml in DMSO 1%	1	-
	2	-
	3	-
25,5 mg/ml in DMSO 1%	1	-
	2	-
	3	-
50,0 mg/ml in DMSO 1%	1	-
	2	-
	3	-
100 mg/ml in DMSO 1%	1	-
	2	-
	3	-
Positive Control: Isoniazid 0,5 ppm + *M. tuberculosis*	1	-
	2	-
	3	-
Negative Control: *M. Tuberculosis* Bacteria + DMSO 1%	1	+
	2	+
	3	+

Growth = (+) : No Growth = (-)

Table 4. Cord formation of *M. tuberculosis* in MODS,

No	Test Material	MODS Method	Growth / No Growth
1	Sambiloto Extract 0,255 mg/ml in DMSO 1% + *M. tuberculosis*		*M. tuberculosis* Grows Abundantly
2	Sambiloto Extract 1,275 mg/ml in DMSO 1% + *M. tuberculosis*		*M. tuberculosis* Grows Abundantly
3	Sambiloto Extract 2,55 mg/ml In DMSO 1% + *M. tuberculosis*		*M. tuberculosis* Grows Abundantly
4	Sambiloto Extract 6,375 mg/ml in DMSO 1% + *M. tuberculosis*		No Growth
5	Sambiloto Extract 12,75 mg/ml in DMSO 1% + *M. tuberculosis*		No Growth
6	Sambiloto Extract 25 mg/ml + DMSO 1% +*M. tuberculosis*		No Growth

No	Test Material	MODS Method	Growth / No Growth
7	Sambiloto Extract 50 mg/ml + DMSO 1% + *M. tuberculosis*		No Growth
8	Sambiloto Extract 100 mg/ml + DMSO 1% + *M. tuberculosis*		No Growth
9	Positive Control: Isoniazid 0,5 ppm + *M. tuberculosis*		No Growth
10	Negative Control: *M. tuberculosis* Bacteria + DMSO 1%		*M. tuberculosis* Grows Abundantly

In the observation of the MODS well plates from days 1 to 7, none of the test materials showed growth of M. tuberculosis. From days 8 to 14, sambiloto extract with concentrations of 0.255; 1.275; 2.55 mg/ml and the negative control well plate began to develop bacterial colonies by day 12. This is consistent with the study which reported that TB colonies will appear between days 7 and 10 on MODS medium (Alva et al. 2013). From days 15 to 21, neither the test materials (sambiloto extract at concentrations of 6.375; 12.75; 25.5; 50.0; 100.0 mg/ml) nor the positive control showed bacterial growth. From days 22 to 28, the test materials and positive control still did not exhibit growth of M. tuberculosis. No cord formation of *M. tuberculosis* was observed in the test extracts and positive control. Next, examinations were performed using Ziehl-Neelsen staining and the rapid TB Ag MPT64 test, the presence of two red strips would indicate the identification of *M. tuberculosis*. Finally, the liquid from the well plates was cultured on Ogawa solid agar medium and incubated for 2 months. No growth of *M. tuberculosis* was found in the test materials.

Microscopic Observation Drug Susceptibility (MODS) is a cost-effective and simple tool for high-performance TB detection. This study uses the MODS method because *M. tuberculosis* grows faster in liquid media compared to solid media. Microscopic detection of *M. tuberculosis* growth in liquid media occurs earlier than waiting for the appearance of macroscopic colonies in solid media, and this growth is characteristic of *M. tuberculosis*, distinguishing it from atypical mycobacteria or fungal or bacterial contaminants. Isoniazid and rifampicin can be incorporated into the MODS test to allow for the simultaneous direct detection (Brady et al. 2008). MODS is based on the visual identification of the characteristic cord-like pattern of *M. tuberculosis* colonies during growth in the liquid phase (Alva et al. 2013).

From previous studies of the 63 samples that tested positive with MODS, 43 samples were confirmed by GeneXpert and 22 samples by ZN staining. This study indicates that MODS remains the most effective diagnostic method for TB meningitis (Paramitha et al. 2018). Other studies about evaluating the effectiveness of MODS method for determining how quickly and accurately tuberculosis diagnosis can detect drug resistance, it was found that the MODS assay had a sensitivity of 94.12% and specificity of 85.71% in detecting TB compared to the reference culture MGIT/7H11. The MODS assay demonstrated excellent capability in detecting MDR-TB, with perfect sensitivity and very high specificity (Sanogo et al. 2017). Other studies reported that the sensitivity of the MODS assay for detecting *M. tuberculosis* ranged from 87.8% to 94.3%, with specificity ranging from 96.8% to 100%. The MODS assay successfully detected *M. tuberculosis* and drug resistance with high sensitivity and a faster time to positivity compared to standard culture methods and traditional drug susceptibility testing (DST) (Huang et al. 2015). Based on the data above, andrographolide, which is the main component of andrographis extract, has an inhibitory effect on the growth of *M. tuberculosis* bacteria by damaging the bacterial cell wall, which contains a high amount of mycolic acid, leading to cell wall lysis. This plant has the potential to treat diseases caused by *M. tuberculosis* infection.

Related to its clinical potential, pkCSM analysis was done to assess its safety for clinical use. Based on the predicted values from pkCSM (Table 5), Caco-2 consists of human colorectal epithelial adenocarcinoma cells. The Caco-2 monolayer cells are widely used as an in vitro model of the human intestinal mucosa to predict the absorption of orally administered drugs. a compound is considered to have high Caco-2 permeability if it has a Papp > 8 x 10-6 cm/s. For the pkCSM predictive model, high Caco-2 permeability will be translated into a predictive value > 0.90. According to the predicted results for Caco-2 permeability using pkCSM, the compound andrographolide (the active component of sambiloto) has a Caco-2 cell permeability value of 1.145 > 0.90. Thus, it is stated that sambiloto herb is well absorbed in the digestive system (Nursanti et al. 2022). Based on the predicted distribution permeability values, the extract is below the standard, which means that the ligand is less distributed into the Blood-Brain Barrier (BBB) and Central Nervous System (CNS) permeability. The predicted metabolism indicates that the ligand is metabolized by the CYP3A4 enzyme, which is part of the cytochrome P450 enzyme family. This enzyme plays an important role in drug metabolism in the body and can affect drug levels in the blood and its effectiveness. Based on the predicted total clearance values using pkCSM, the result obtained is 1.176 Log ml/mm/kg. Based on the predicted Ames Toxicity using pkCSM, the results as shown in the table above indicate that the sambiloto extract is non-mutagenic and does not exhibit hepatotoxicity.

Table 5. The prediction results of ADMET using pkCSM for Sambiloto

Property	Model Name	Unit	Sambiloto (Andrographolide)	Requirement value
Absorption	Water solubility	log mol/l	-3.416	-
	CaCo2 permeability	logPapp in 10^{-6} cm/s	1.145	High Caco-2 permeability would translate in predicted value > 0.90
	Intestinal absorption (human)	% absorbed	93.24	> 30%
	Skin permeability	Log Kp	-3.643	≥ -2.5
	P-glycoprotein substrate (A5)	Yes/No	No	-
	P-glycoprotein I inhibitor (A6)	Yes/No	Yes	-

Property	Model Name	Unit	Sambiloto (Andrographolide)	Requirement value
	P-glycoprotein II inhibitor (A7)	Yes/No	No	-
Distribution	VDss (human)	Log L/kg	-0.113	≥ -0.15
	Fraction unbound (human)	Fu	-0.251	-
	BBB permeability	Log BB	-,055	≥ -1
	CNS permeability	Log PS	-2.6	≥ -3
Metabolism	CYP2D6 substrate	Yes / No	No	-
	CYP3A4 substrate	Yes / No	Yes	-
	CYP1A2 substrate	Yes / No	No	-
	CYP2C19 substrate	Yes / No	No	-
	CYP2C9 substrate	Yes / No	No	-
Excretion	Total Clearence	Log ml/mm/kg	1.179	Higher is better
	Renal OCT2 substrate	Yes / No	No	-
Toxicity	AMES toxicity	Yes / No	No	No
	Max Tolerated dose (Human)	Log (mg/kg/day)	-0.33	Less than or equal to 0.477 Log (mg/kg/day) is considered low, if high than greater than 0.477 Log (mg/kg/day)
	Hepatotoxicity	**Yes / No**	**No**	**No**

In this study, the use of an extract with a concentration of 6.375 mg/ml in the MODS method showed no growth of *Mycobacterium tuberculosis*, indicating that the extract is effective in inhibiting bacterial growth at this concentration. The online pkCSM test conducted in this study showed that the extract used is non – mutagenic and does not exhibit hepatotoxicity, meaning that the extract is safe for body cells and does not cause cellular damage. Clinical implication in society is that this extract has the potential to be an adjunct therapy for TB patients, which can help enhance the effectiveness of treatment and provide an additional alternative in managing TB infections.

Limitation

In this study, the andrographolide content in the *Andrographis paniculata* extract was not measured, but the concentration of the overall extract was determined.

CONCLUSION

In conclusion, this study, the use of an extract with a concentration of 6.375 mg/ml in the MODS method showed no growth of *Mycobacterium tuberculosis*, indicating that the extract is effective in inhibiting bacterial growth at this concentration. The online pkCSM test conducted in this study showed that the extract used is not cytotoxic, meaning that the extract is safe for body cells and does not cause cellular damage. Therefore, it has the potential to be an adjunct therapy in the treatment of tuberculosis.

RECOMENDATION

Further research is needed to determine the andrographolide content in the extract, which is suspected to have the potential to inhibit the growth of *Mycobacterium tuberculosis*, as well as in vivo testing to measure the treatment effectiveness and toxicity.

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REFERENCES

Alva A, Aquino F, Gilman RH, Olivares C, Requena D, Gutiérrez AH, Caviedes L, Coronel J, Larson S, Sheen P, Moore DAJ, Zimic M (2013) Morphological characterization of *Mycobacterium tuberculosis* in a MODS culture for an automatic diagnostics through pattern recognition. Plos One 8:e82809. doi: 10.1371/journal.pone.0082809

Arifullah M, Namsa ND, Mandal M, Chiruvella KK, Vikrama P, Gopal GR (2013) Evaluation of anti–bacterial and anti–oxidant potential of andrographolide and echiodinin isolated from callus culture of *Andrographis paniculata* Nees. Asian Pac J Trop Biomed 3:604–610. doi: 10.1016/S2221-1691(13)60123-9

Brady MF, Coronel J, Gilman RH, Moore DA (2008) The MODS method for diagnosis of tuberculosis and multidrug resistant tuberculosis. J Vis Exp JoVE 845. doi: 10.3791/845

Brady MF, Coronel J, Gilman RH, Moore DAJ. The MODS method for diagnosis of tuberculosis and multidrug resistant tuberculosis. J Vis Exp. 2008;(18):1–5

Chauhan ES, Sharma K, Bist R (2019) *Andrographis paniculata* : A review of its phytochemistry and pharmacological activities. Res J Pharm Technol 12:891–900. doi: 10.5958/0974-360X.2019.00153.7

Garg HK, Shrivastava A (2013) Clinical use of andrographolide as a potential drug against vole tuberculosis. Int J Pure Appl Zool 1:223–226

Hadi DK, Erina E, Rinidar R, Fakhrurrazi F, Rasmaidar R, Sayuthi A (2019) Daya hambat ekstrak etanol daun mengkudu *(Morinda citrifolia* L.) terhadap pertumbuhan *Salmonella sp*. dan *Escherichia coli* (Inhibition effect of ethanol extract of noni leaf (*Morinda citrifolia* L.) on the growth of *Salmonella sp*. and *Escherichia coli*). J Ilm Mhs Vet 3:87–97

Harbone JB (2006) Metode fitokimia: Penuntun cara modern menganalisis tumbuhan. Penerbit ITB, Bandung

Huang Z, Qin C, Du J, Luo Q, Wang Y, Zhang W, Zhang X, Xiong G, Chen J, Xu X, Li W, Li J (2015) Evaluation of the microscopic observation drug susceptibility assay for the rapid detection of MDR-TB and XDR-TB in China: A prospective multicentre study. J Antimicrob Chemother 70:456–462. doi: 10.1093/jac/dku384

Jayakumar T, Hsieh C-Y, Lee J-J, Sheu J-R (2013) Experimental and clinical pharmacology of *Andrographis paniculata* and its major bioactive phytoconstituent andrographolide. Evid Based Complement Alternat Med 2013:846740. doi: 10.1155/2013/846740

Joselin J, Jeeva S (2014) *Andrographis paniculata*: A review of its traditional uses, Phytochemistry and Pharmacology. Med Aromat Plants 3:1–16. doi: 10.4172/2167-0412.1000169

Kementerian Kesehatan Republik Indonesia (2017) Formularies. In: Farmakope herbal indonesia edisi II tahun 2017. Direktorat Jenderal Kefarmasian dan Alat Kesehatan, Kementerian Kesehatan Republik Indonesia, Jakarta, pp 213–218

Kementerian Kesehatan Republik Indonesia (2020) Strategi nasional penanggulangan tuberkulosis di Indonesia 2020-2024. Kementerian Kesehatan Republik Indonesia, Jakarta

Nugroho YS, Reviono R, Suradi S, Prasetyo DH. Effect of Andrographolide on The Expression of TNF-α and Pulmonary Tuberculosis in Rats Granulomas are Infected With Mycobacterium tuberculosis. J Respirologi Indones. 2018;38(2):75–82.

Nugraha AC, Prasetya AT, Mursiti S (2017) Isolasi, identifikasi, uji aktivitas senyawa flavonoid sebagai antibakteri dari daun mangga. Indones J Chem Sci 6:91–96. doi: 10.15294/ijcs.v6i2.12087

Nursanti O, Aziz A, Hadisoebroto G (2022) Prediksi toksisitas dan farmakokinetika untuk mendapatkan kandidat obat analgesik. J Noncommunicable Dis 3:34–46. doi: 10.5236/jond.v3i1.654

Paramitha NA, Sribudiani Y, Rizal A (2018) Perbandingan positivitas metode MODS, pewarnaan ZN, dan GeneXpert untuk mendeteksi *M. tuberculosis* pada pasien meningitis TB. Maj Kedokt Bdg 50:240–246

Pires DEV, Blundell TL, Ascher DB (2024) pkCSM: Theory. https://biosig.lab.uq.edu.au/pkcsm/theory. Accessed 29 July 2024

Rachmani, E. P. N. (2016). Aktivitas Antioksidan Ekstrak dan Fraksi Herba Sambiloto (Andrographis paniculata). MPI (Media Pharmaceutica Indonesiana), 1(2), 100–105

Radji M, Kurniati M, Kiranasari A (2015) Comparative antimycobacterial activity of some Indonesian medicinal plants against multi-drug resistant *Mycobacterium tuberculosis*. J Appl Pharm Sci 5:19–22. doi: 10.7324/JAPS.2015.50104

Sandhu GK (2011) Tuberculosis: Current situation, challenges and overview of its control programs in India. J Glob Infect Dis 3:143–150. doi: 10.4103/0974-777X.81691

Sanogo M, Kone B, Diarra B, Maiga M, Baya B, Somboro AM, Sarro YS, Togo ACG, Dembele BPP, Goita D, Kone A, M'Baye O, Coulibaly N, Diabate S, Traore B, Diallo MH, Coulibaly YI, Saleeb P, Belson M, Orsega S, Siddiqui S, Polis MA, Dao S, Murphy RL, Diallo S (2017) Performance of microscopic observation drug susceptibility for the rapid diagnosis of tuberculosis and detection of drug resistance in Bamako, Mali. Clin Microbiol Infect 23:408.e1-408.e6. doi: 10.1016/j.cmi.2017.01.004

Sharma T, Pandey B, Shrestha BK, Koju GM, Thusa R, Karki N (2020) Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. Tribhuvan Univ J 35:1–11. doi: 10.3126/tuj.v35i2.36183

Siregar MIT. Mekanisme Resistensi Isoniazid &Mutasi Gen KatG Ser315Thr (G944C) *Mycobacterium tuberculosis* Sebagai Penyebab Tersering Resistensi Isoniazid. Jambi Med J. 2015;3(2):119–31.

Tirza Putri Dianda dan Profiyanti Hermien Suharti (2022) Pengaruh Waktu dan Kadar Etanol Pada Maserasi Lidah Buaya Terhadap Antiseptik Hand Sanitizer Gel. *Distilat. 2022, 8 (4), 1000-1008* p-ISSN : 1978-8789, e-ISSN: 2714-7649. http://distilat.polinema.ac.id

WHO. (2018). TB burden report 2018. In World Health Organization (Vol. 63, Issue 10). https://apps.who.int/iris/handle/10665/274453

Wirda Wulan, A. Mu'nisa, A. Irma Suryani (2022) Uji Efektivitas Ekstrak Daun Sambiloto (Andrographis paniculata) dan Rumput Mutiara (Oldenlandia corymbosa) sebagai Antituberkulosis, Prosiding Seminar Nasional Biologi FMIPA UNM