Trop J Nat Prod Res, March 2024; 8(3):6516-6518 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Short Communication

Anti-Tuberculosis Study of *Mycobacterium tuberculosis* H37Rv *of Aspilia pluriceta* Extract and Fractions

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ARTICLE INFO

Article history:
Received 17 October 2023
Revised 22 March 2024
Accepted 22 March 2024
Published online 01 April 2024

ABSTRACT

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis (Mtb)*. Mtb affects not only the respiratory system but also other body organs, such as the lungs, leading to pulmonary and extrapulmonary TB, the brain, and the spine. *Aspilia pluriceta* from Meru Betiri National Park in Indonesia is one plant with potential for *Mycobacterium tuberculosis*. This study aimed to determine the antituberculosis activity of extracts and fractions of *Aspilia pluriceta* against *Mycobacterium tuberculosis H37Rv*. *Aspilia pluriceta* was extracted by maceration with methanol, and the crude methanol extract was fractionated successively with hexane, dichloromethane (DCM), and ethyl acetate. The phytochemical of the crude extract was determined by TLC and visualised by spraying with appropriate staining reagents. The crude extract and fractions of *Aspilia pluriceta* at concentrations 25, 100, 500, and 1000 µg/mL, respectively, were tested against the H37Rv strain of *Mycobacterium tuberculosis* using the resazurin method and analysed by an ELISA reader. Isoniazid, a first-line drug used in the treatment of tuberculosis, was used as the positive control agent. The phytochemical screening test revealed the presence of saponins, alkaloids, tannins, flavonoids, and terpenes. The results of anti-TB showed that the ethyl acetate fraction exhibited the most potent activity against *Mycobacterium tuberculosis* H37Rv with an IC_{50} value of 0.48 µg/mL. The crude extract and other fractions exhibited low activity against the tested organism. The findings showed that the plant holds potential for phytochemicals with antituberculosis activity, which could be further explored through bioactivity-guided isolation for leads against Mycobacterium tuberculosis.

***Keywords*:** Anti-tuberculosis, *Aspilia pluriceta*, phytochemicals , *Mycobacterium tuberculosis H27Rv*..

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis (Mtb*). The various strains of *Mycobacteria* are *M. tuberculosis, M. bovis, M. africanum, M. microti*, and *M. cannettii*. Among these, *M. tuberculosis H37Rv* is often found in TB infections. WHO reported that around 1.5 million people died from TB in 2020. This disease is ranked the 13th disease with the most deaths. *M. tuberculosis* is a rod-shaped acid-fast bacterial infection that spreads through the air and is transmitted by coughs, sneezes, or talks that can be inhaled by people nearby. When TB bacilli are inhaled, they quickly pass through the mouth and nose and usually enter the smallest and lowest parts of the bronchioles and pulmonary alveoli.[1]
Several plants have been reported to have phytochemical compounds with anti-TB properties. One such plant with the potential for *Mycobacterium tuberculosis* is *Aspilia pluriseta*, Schweinf, shown in Figure 1. Initial examination of the *A. pluriseta* methanol extract showed broad-spectrum activity (inhibiting the growth of Gram-negative, Gram-positive, acid-fast, and fungal bacteria).

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Citation: Puspaningtyas AP, Nugraha AS, Retnaningtyas Y, Zulaiha S. Anti-Tuberculosis Study of *Mycobacterium tuberculosis* H37Rv *of Aspilia pluriceta* Extract and Fractions. Trop J Nat Prod Res. 2024; 8(3):6516-6518. https://doi.org/10.26538/tjnpr/v8i3.7

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Phytochemical screening of the fractions of *A.pluriseta* (hexane, Dichloromethane, Ethyl Acetate, and Methanol) showed the presence of terpenoids, alkaloids, phenolics, flavonoids, and anthraquinones. These bioactive phytoconstituents may have been responsible for the anti-TB agent activity of this plant.[2] *Aspilia pluriseta* also demonstrated potent *in vitro* antibacterial activity with a MIC value of 25 µg/mL (Dichloromethane fraction), ethyl acetate fraction (6.25 µg/mL), and methanol fraction (12.5 µg/mg).[3] Therefore, this research was carried out to determine the phytochemicals present in the crude extract and fractions of the *Aspilia pluriseta* plant and to determine the extract or fractions that have the most significant *in vitro* antituberculosis potential against *Mycobacterium tuberculosis* H37Rv using the resazurin colourimetric method.

Figure 1: *Aspilia puriseta*, Schweinf

Material and Methods

Plant collection and preparation

The whole plant (flowers, leaves, stems, and roots) of *Aspilia pluriseta* was obtained from Meru Betiri National Park in East Java, Indonesia, on June 14th, 2022. The plants were determined at the Department of Integrated Agricultural Development, Jember State Polytechnic, Ministry of Education, Culture, Research and Technology in Indonesia, and voucher specimen number No.105/PL17.8/PG was assigned.

Extraction and fractionation

Dried and powdered (Simplicia) whole plant of *Aspilia pluriceta* was extracted by maceration with methanol and fractionated with solvents of increasing polarity (hexane, dichloromethane (DCM), and ethyl acetate). The extract and fractions were evaporated to dryness under reduced pressure using a rotary evaporator at 40°C.[4]

Phytochemical Screening

The TLC method was adopted for the phytochemical screening. *Aspilia pluriseta* extract and fractions were dissolved in each solvent. 2 µL of sample solution and the standard solution were spotted on the TLC plate. The TLC plate was evaluated and observed by UV lamps with wavelengths of 254 and 365 nm.[4] The plate was sprayed with a staining reagent, and the appearance of visible stains was analysed.

In vitro Antituberculosis Test

In vitro, anti-TB activity was tested by the resazurin colourimetric method. This method has high sensitivity, is faster, and can also be used to detect *Mtb* drug resistance strains. In this method, *M. tuberculosis* strain H37Rv was used. The positive control was isoniazid as a first-line antituberculosis drug. The assay used modified 7H9 liquid media. This medium contained 10% oleic acid-Albumin Dextrose-Catalase (OADC), 0.1% calcitone, and 0.5% glycerol. Inoculum preparation or bacterial growth proceeded in liquid growth media in an incubator at 37°C for 5-10 days before testing. The bacterial suspension was diluted in the liquid media in a micro-well plate until 5 x 10^7 CFU/mL or 100 Klett unit/mL was achieved. The inoculum size was verified by placing serially diluted bacterial suspensions on 7H9 agar plates supplemented with 10% OADC. Then, the plates were incubated at 37°C for four weeks before counting *M. tuberculosis* colonies. The growth indicator used resazurin reagent from resazurin sodium salt powder (Hi-Media). This reagent (0.01% w/v) was prepared in sterile distilled water and stored at 4°C for a week. The *in vitro* anti-TB activity was tested using a 1:10 dilution of the highest concentration in a modified 7H9 liquid medium. Aliquot concentrations (25, 100, 500, and 1000 µg/mL, respectively) of the test samples of *Aspilia pluriseta* extract/fractions were transferred into the 96 well plates containing 7H9 media and *M. tuberculosis* H37Rv suspension. The plates were incubated at 37°C for five days. After that, 20 µL of resazurin was added and incubated at 37°C for 24 hours. Resazurin reagent was also added to the control/blank plate, incubated overnight, and then observed. The well plates were observed for colour changes. Blue colour indicated no mycobacterial growth, while pink colour indicated mycobacterial growth. Finally, the IC_{50} value was determined by calculating the percent viability of mycobacterial.[5,6,7,8,9,10] The percentage viability of mycobacteria was calculated using the following equation.

$$\% \text{ viability} = \frac{(\text{abs sample} - \text{abs blank})}{(\text{abs Mtb} - \text{abs blank})} \times 100\%$$

Data analysis

The IC_{50} (the concentration required to inhibit 50% of *Mycobacterium tuberculosis*) was computed from the linear regression and equation y= bx + a (y=50 and x = IC_{50} value) of the plot of the percentage mycobacteria viability against concentration.[6]

Results and Discussion

The extract and fractions yield are shown in Table 1. The crude methanol extract has a yield of 137.5 g (13.75 % w/w) computed from the dried powdered plant sample. The hexane fraction gave the highest yield (37.83 ± 7.78 w/w), followed by DCM (31.67 ± 6.45 % w/w) and ethyl acetate. The qualitative phytochemical study was carried out on extract and fractions of the *Aspilia pluriseta* plant using the TLC method with several solvents as eluents and sprayed with staining reagents. The results of the phytochemical study are shown in Table 2. The result indicates alkaloids, flavonoids, saponins, and tannins. Terpenoids were present in the hexane fraction, the non-polar solvent.

The IC_{50} value was obtained from the linear regression equation of the plot between percentage Mycobacteria viability versus concentration. The IC_{50} value of each sample is shown in Table 3. Specifically, the IC_{50} can be categorised as follows: strong (< 50 µg/mL), moderate (50-100 µg/mL), and weak (>100 µg/mL).[11]

The *in vitro* antituberculosis test used the resazurin colourimetric method. The IC_{50} value of isoniazid as a positive control was 25.30 µg/mL. The plant sample's methanol extract, hexane, and DCM fraction showed higher IC_{50} values than isoniazid (positive control agent), indicating weak activity against Mtb. In contrast, the ethyl acetate fraction exhibited a significant IC_{50} value of 0.48 µg/mL, even smaller than the positive control agent. The Ethyl acetate fraction showed potent activity against *Mycobacterium tuberculosis* H37Rv and hence was categorised as possessing very strong activity. *Aspilia pluriseta* extract has strong antituberculosis activity, as demonstrated by previous *in vitro* studies. The MIC values of *Aspilia pluriseta* of dichloromethane, ethyl acetate, and methanol fraction were 25 µg/mL, 6.25 µg/ mL, and 12.5 µg/mL, respectively.[2,3] The results of the phytochemical study showed that the ethyl acetate fraction contained flavonoids, saponins, and terpenoids. Flavonoids exhibit antibacterials by inhibiting oxygen, leading to the interruption of energy metabolism. Insufficient energy hampers the biosynthesis of bacterial macromolecules. On the other hand, Saponins induce proteins and enzyme leakages from bacteria cells. They can bind to the cytoplasmic membrane, so the stability of the cell membrane is disturbed, the cell cytoplasm leaks out, and finally, the cell dies.[11] The IC_{50} value of the positive control isoniazid (INH) is included in the strong category.

Table 1: Percentage yield of *Aspilia pluriseta* fractionation results

Sample	Average Yield (%w/w) ± SD
Hexane fraction	37.83 ± 7.78
DCM Fraction	31.67 ± 6.45
Ethyl acetate fraction	23.67 ± 4.19

Table 2: Results of Phytochemical Study of *Aspilia pluriseta* Extracts/fraction

Sample	Phytochemicals				
	Alkaloid	Flavonoid	Tannin	Saponin	Terpenoid
Methanol extract	-	+	+	+	+
Hexane fraction	-	-	-	+	+
DCM Fraction	+	+	-	+	+
Ethyl acetate fraction	-	+	-	+	+

Note : (+): Presence, (-) : Absence

Table 3: IC_{50} value of *Aspilia pluriseta* extracts/fraction

Sample	IC_{50} value (µg/mL)
Methanol extract	221.85
Hexane fraction	218.55
Dichloromethane fraction	238.41
Ethyl acetate fraction	0.48
Isoniazid (positive control)	25.30

INH is one of the most effective antituberculosis agents for treating and preventing TB disease caused by *M. tuberculosis*.[5] The IC_{50} values of ethyl acetate, hexane, dichloromethane (DCM) fractions, and methanol extract of *Aspilia pluriseta* from this study were 0.48 µg/mL, 218.55 µg/mL, 238.41 µg/mL, and 221.85 µg/mL, respectively. The antituberculosis activity of the extract and fractions were ranked in the following order: ethyl acetate > hexane fraction, > methanol extract, > dichloromethane fraction.

Conclusion

The presence of different bioactive phytochemicals in *Aspilia pluriseta* may have been responsible for its various medicinal uses in treating various infectious diseases, including tuberculosis. In particular, the ethyl acetate fraction significantly inhibited the *Mycobacterium tuberculosis* H37Rv strain implicated in most cases of tuberculosis infection, with an IC_{50} value of 0.48 µg/mL, which was more potent in this study than INH (25.30 µg/mL). Bioactivity-guided isolation of this fraction may lead to a new chemical entity that could be developed into a pharmaceutical product for treating tuberculosis. This study validates the ethnomedicinal claims of the Meru Betiri people, Indonesia, regarding using different parts of *Aspilia pluriseta* to treat various diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to acknowledge the financial assistance from the University of Jember on the Post-Doctoral Grant scheme.

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