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Phytochemical Profile and Preliminary Antituberculosis Activity of *Tabernaemontana divaricata* Extract and Fractions Against *Mycobacterium tuberculosis* H37Rv

Profil Fitokimia dan Aktivitas Awal Antituberkulosis Ekstrak dan Fraksi Mondokaki terhadap *Mycobacterium tuberculosis* H37Rv

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ABSTRACT

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. This study evaluated the phytochemical profile and preliminary antituberculosis activity of a methanol extract and solvent fractions of *Tabernaemontana divaricata*. The dried plant material was macerated with methanol and sequentially fractionated using n-hexane, dichloromethane, and ethyl acetate. Phytochemical screening was performed by thin-layer chromatography. Antimycobacterial activity against *M. tuberculosis* H37Rv was assessed using a resazurin-based assay at 25, 100, 500, and 1,000 µg/mL. Alkaloids, flavonoids, saponins, and tannins were detected in all samples, whereas terpenoids were not detected. The methanol extract and all solvent fractions inhibited mycobacterial growth, with the dichloromethane and ethyl acetate fractions showing consistently high inhibition across the tested concentrations. These findings indicate that *T. divaricata* is a promising source of antimycobacterial constituents and support further bioassay-guided fractionation, compound isolation, and confirmatory antituberculosis testing.

Kata kunci:

Aktivitas antituberkulosis
Mycobacterium tuberculosis H37Rv
Penapisan fitokimia
Tabernaemontana divaricata

ABSTRAK

Tuberkulosis merupakan penyakit infeksi yang disebabkan oleh *Mycobacterium tuberculosis*. Penelitian ini mengevaluasi profil fitokimia dan aktivitas awal antituberkulosis ekstrak metanol serta fraksi pelarut *Tabernaemontana divaricata*. Bahan tanaman kering dimaserasi dengan metanol, kemudian difraksinasi secara bertingkat menggunakan n-heksana, diklorometana, dan etil asetat. Penapisan fitokimia dilakukan dengan kromatografi lapis tipis. Aktivitas terhadap *M. tuberculosis* H37Rv diuji menggunakan metode berbasis resazurin pada konsentrasi 25, 100, 500, dan 1.000 µg/mL. Alkaloid, flavonoid, saponin, dan tanin terdeteksi pada seluruh sampel, sedangkan terpenoid tidak terdeteksi. Ekstrak metanol dan seluruh fraksi pelarut menghambat pertumbuhan mikobakteri, dengan fraksi diklorometana dan etil asetat menunjukkan penghambatan yang relatif tinggi pada konsentrasi yang diuji. Temuan ini menunjukkan bahwa *T. divaricata* berpotensi sebagai sumber senyawa antimikobakteri dan mendukung fraksinasi terpandu bioaktivitas, isolasi senyawa, serta pengujian antituberkulosis konfirmatif.

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1. INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. Although the disease primarily affects the lungs, it may also involve other organs. Persistent transmission, prolonged treatment, and its disproportionate impact on socioeconomically disadvantaged populations make TB not only a clinical concern but also a major social and public health challenge. In 2024, an estimated 10.7 million people developed TB worldwide, and approximately 1.23 million died from the disease. Indonesia contributed a substantial proportion of the global TB burden and remained among the countries with the highest numbers of incident cases (World Health Organization [WHO], 2025). Effective TB control depends on early diagnosis, appropriate multidrug therapy, and adherence throughout the treatment period. Drug-susceptible TB is generally treated for six months using a combination of first-line medicines, including isoniazid, rifampicin, pyrazinamide, and ethambutol. Treatment interruption or inappropriate drug use may lead to therapeutic failure, continued transmission, and the development of drug-resistant TB. Adverse drug reactions, particularly hepatotoxicity, may also compromise treatment adherence and clinical outcomes (Ministry of Health of the Republic of Indonesia, 2020).

The continuing burden of TB and the limitations of current therapy have encouraged the search for new antimycobacterial agents, including compounds derived from medicinal plants. Indonesia has approximately 30,000 plant species, more than 10,000 of which have reportedly been used in traditional medicine for disease prevention, treatment, and health maintenance (Mursito, 2003; Ratnawati & Supriyati, 2019). This extensive biological diversity provides considerable opportunities for natural-product research. Nevertheless, traditional use alone does not establish efficacy or safety. Phytochemical characterization and controlled pharmacological evaluation are therefore required to assess the therapeutic potential of medicinal plants.

Previous studies have indicated that species of the genus *Tabernaemontana* may possess antimycobacterial activity. Mohamad et al. (2018) reported that a root extract identified as *Tabernaemontana coronaria* inhibited *M. tuberculosis*H37Rv in vitro, with minimum inhibitory and minimum bactericidal concentrations of 100 and 200 µg/mL, respectively. Among the preparations examined, the n-hexane extract showed the strongest inhibitory activity. Gas chromatography–mass spectrometry analysis identified several lipophilic constituents, while further observations showed that the extract affected mycobacterial growth kinetics and cellular integrity (Mohamad et al., 2018). *Tabernaemontana divaricata*, locally known as mondokaki, has been used in several Asian traditional medicine systems. Different parts of the plant have traditionally been used for conditions associated with infection, inflammation, pain, and oxidative stress (Pratchayasakul et al., 2008). Experimental studies have also demonstrated the anti-inflammatory activity of its aqueous and methanol leaf extracts in vivo (Jain et al., 2013). These findings provide a pharmacological basis for further investigation but do not establish activity against *M. tuberculosis*.

Despite the reported biological properties of *T. divaricata*, comparative evidence regarding the antimycobacterial activity of fractions separated according to solvent polarity remains limited. This study therefore aimed to characterize the major phytochemical groups present in the methanol extract and its n-hexane, dichloromethane, and ethyl acetate fractions and to compare their in vitro activity against *M. tuberculosis* H37Rv using a resazurin-based assay. Sequential solvent fractionation was used as a preliminary approach to examine the distribution of phytochemical groups and identify promising fractions for subsequent chemical isolation and confirmatory antimycobacterial investigation.

2. METHODS

2.1. Plant Collection and Preparation

Whole plants of *T. divaricata*, including the flowers, leaves, stems, and roots, were collected from Meru Betiri National Park, East Java, Indonesia, on 14 June 2022, at the geographic coordinates 8.4019038° S and 113.7447166° E. A representative photograph of the collected plant is presented in Figure 1. The plant specimen was authenticated by Ir. Budi Prasetyo, S.Pt., M.P., IPM, a botanist at the Department of Integrated Agricultural Development, Jember State Polytechnic, Indonesia. A voucher specimen was deposited in the institutional collection under accession number 106/PL17.8/PG/2022.

The collected plant materials were thoroughly cleaned to remove adhering soil and other contaminants. They were subsequently oven-dried at 40–50 °C during working hours for five days until the final moisture content was ≤10%. The dried materials were then ground into a fine powder and stored under appropriate conditions until extraction.

2.2. Materials and Instruments

Methanol, dichloromethane, ethyl acetate, and *n*-hexane of analytical grade were purchased from Merck (Darmstadt, Germany). Silica gel 60 GF_{254} plates for thin-layer chromatography were also obtained from Merck (Darmstadt, Germany). Isoniazid of analytical grade was purchased from Sigma-Aldrich (St. Louis, MO, USA), while resazurin sodium salt was supplied by HiMedia Laboratories (Mumbai, India). The reference strain *Mycobacterium tuberculosis* H37Rv (ATCC 27294) was tested at the Tuberculosis Laboratory, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia. The principal instruments used in this study included a PhoenixSpec nephelometer (Becton, Dickinson and

Company, Sparks, MD, USA) and an Infinite F50 microplate reader (Tecan, Männedorf, Switzerland).



Figure 1. Morphological appearance of *Tabernaemontana divaricata* L. collected from Meru Betiri National Park, East Java, Indonesia.

2.3. Extraction and Fractionation

The dried and powdered plant material was extracted by maceration with 2.5 L of methanol at a material-to-solvent ratio of 1:5 (w/v). The extraction was performed in three successive maceration cycles. After each cycle, the mixture was filtered through filter paper under vacuum, and the filtrates were combined. The combined methanolic filtrates were concentrated under reduced pressure at 40 ˚C and approximately 200 mbar using a DLAB rotary evaporator to obtain the crude methanolic extract. The methanolic extract was then sequentially fractionated using solvents of increasing polarity, namely *n*-hexane, dichloromethane, and ethyl acetate. Each fraction was collected separately and concentrated under reduced pressure at 40 ˚C and approximately 200 mbar. The concentrated fractions were further dried to a constant mass to confirm complete solvent removal, and the yield of each fraction was subsequently determined.

2.4. Phytochemical Screening

Qualitative phytochemical screening of the methanolic extract and its fractions was performed using thin-layer chromatography. Each sample was dissolved in a suitable solvent, and 2 µL of the resulting solution was applied to a silica gel 60 GF_{254} TLC plate. The plates were developed using appropriate mobile phases, dried, and examined under ultraviolet light at 254 and 365 nm. The developed plates were then treated with compound-specific visualization reagents. Alkaloids were detected using Dragendorff's reagent and identified by the appearance of orange spots. Flavonoids were detected using a glacial acetic acid–*n*-butanol–water mobile phase at a ratio of 1:4:5 and identified by yellow spots. Tannins were detected using ferric chloride reagent and identified by grey spots. Saponins were detected using Liebermann–Burchard reagent and identified by yellow-brown spots, while terpenoids were detected using anisaldehyde–sulfuric acid reagent and identified by red-purple spots. Compound classes were assigned based on their chromatographic behavior and characteristic colors after visualization.

2.5. In Vitro Antituberculosis Assay

Antimycobacterial activity was evaluated against *M. tuberculosis* H37Rv (ATCC 27294) using a resazurin-based microplate assay, with isoniazid serving as the positive control. All procedures involving viable *M. tuberculosis*were conducted in a biosafety level 3 laboratory at the Tuberculosis Laboratory, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia, in accordance with institutional biosafety requirements. The assay was performed in Middlebrook 7H9 broth supplemented with 10% oleic acid–albumin–dextrose–catalase, 0.1% Casitone, and 0.5% glycerol. The bacterial culture was incubated at 37 ˚C for 5–10 days. The inoculum density was adjusted to approximately 5×10^7 CFU/mL using a nephelometer and verified by serial dilution and colony counting after four weeks of incubation. A 0.01% (w/v) resazurin solution was prepared by dissolving resazurin sodium salt in sterile distilled water, stored at 4 ˚C, and used within one week. The methanolic extract and its fractions were tested at final concentrations of 25, 100, 500, and 1,000 µg/mL. Each sample concentration was dispensed into a 96-well microplate containing Middlebrook 7H9 medium and the bacterial suspension. The plates were incubated at 37 ˚C for five days, after which 20 µL of resazurin solution was added to each well. Following an additional 24 h of incubation, resazurin reduction was quantified using an Infinite F50 microplate reader with reference-wavelength correction. Wells that remained blue indicated inhibition of bacterial metabolic activity, whereas a color change to pink indicated bacterial growth. The assay included isoniazid-positive, untreated bacterial

growth, medium sterility, solvent, and sample-background controls. Each concentration was tested in two technical replicates. After blank correction, the percentage of mycobacterial growth inhibition was calculated using the following equation:

$$\text{Growth inhibition (\%)} = \left[1 - \frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{growth control}} - A_{\text{medium blank}}}\right] \times 100$$

where A_{sample} is the absorbance of the bacterial suspension treated with the extract or fraction, $A_{\text{sample blank}}$ is the absorbance of the corresponding sample without bacteria, $A_{\text{growth control}}$ is the absorbance of the untreated bacterial suspension, and $A_{\text{medium blank}}$ is the absorbance of the sterile culture medium. Results were expressed as the mean ± standard deviation of two technical replicates.

2.6. Data Analysis

Extract and fraction yields were calculated as percentages of the initial material and are presented as mean ± standard deviation from three determinations. The coefficient of variation was calculated by dividing the standard deviation by the mean and multiplying by 100. Antimycobacterial activity was expressed as percentage growth inhibition and reported as mean ± standard deviation from two technical replicates. Because of the limited number of replicates, no inferential statistical tests or post hoc comparisons were performed.

3. RESULTS AND DISCUSSION

3.1. Extract and Fraction Yields

Methanol extraction of 1,000 g of dried whole-plant *T. divaricata* yielded 116.4 g of crude extract, corresponding to an extraction yield of 11.64% (w/w). Subsequent solvent partitioning produced different recovery yields among the fractions (**Table 1**), with the dichloromethane (DCM) fraction showing the highest yield (14.56 ± 2.41%), followed by the n-hexane (11.20 ± 1.94%) and ethyl acetate (9.98 ± 3.60%) fractions. The greater recovery in the DCM fraction suggests that a considerable proportion of extractable constituents possessed intermediate polarity, whereas fewer compounds partitioned into the more polar ethyl acetate fraction. However, extraction yield reflects only the quantity of recovered material and should not be interpreted as an indicator of biological potency or phytochemical abundance. Compared with the study by Rathaur et al. (2020), the methanol extract and ethyl acetate fraction obtained in the present work exhibited higher extraction yields (11.64% vs. 4.0% and 9.98% vs. 2.5%, respectively), which may be attributed to differences in plant materials, extraction conditions, solvent partitioning procedures, and environmental factors affecting metabolite accumulation. These findings demonstrate that solvent polarity markedly influenced the distribution of extractable constituents during fractionation and provided sufficient material for subsequent phytochemical characterization and antimycobacterial screening (Rathaur et al., 2020).

3.2. Phytochemical Screening

TLC-based phytochemical screening indicated the presence of alkaloids, flavonoids, tannins, and saponins in the methanol extract and all solvent fractions of *T. divaricata*, whereas terpenoids were not detected under the applied chromatographic conditions (**Figure 2** and **Table 2**). The methanol extract, n-hexane fraction, dichloromethane fraction, and ethyl acetate fraction showed different chromatographic band patterns under UV light at 254 and 365 nm and after derivatization with compound-specific reagents. These findings are generally consistent with previous reports identifying alkaloids, flavonoids, tannins, and saponins in *T. divaricata* extracts (Rathaur et al., 2020; Shanmugapriya et al., 2021). The detection of the same phytochemical classes in fractions of different polarities may reflect differences in compound polarity within each broad chemical group, incomplete separation during liquid-liquid partitioning, or the limited specificity of qualitative TLC screening. However, the present results only indicate the qualitative presence of these compound classes because retention factor values, band intensities, and quantitative phytochemical contents were not determined. The absence of terpenoid-specific coloration should therefore be interpreted as "not detected under the applied TLC conditions" rather than evidence of complete absence. Furthermore, the qualitative screening does not establish which compounds were responsible for the observed antimycobacterial activity.

3.3. Antimycobacterial Activity

The antimycobacterial activity of the methanol extract and solvent fractions of *T. divaricata* against *M. tuberculosis* H37Rv was evaluated at concentrations of 25, 100, 500, and 1000 µg/mL using the resazurin microplate assay. The percentage inhibition was recalculated from the raw absorbance values after blank correction, and the results are presented in **Figure 3**. Overall, the methanol extract and all solvent fractions inhibited *M. tuberculosis* growth at one or more tested concentrations, indicating that *T. divaricata* contains constituents with antimycobacterial potential. The methanol extract produced inhibition values ranging from 46.82 ± 4.95% to 90.22 ± 23.52%, with the lowest activity observed at 25 µg/mL. Among the solvent fractions, the dichloromethane and ethyl acetate fractions generally maintained high inhibition across the tested concentrations, while the n-hexane fraction also showed substantial activity but with greater variation between concentrations. The ethyl acetate fraction produced inhibition values of 78.59 ± 9.89%, 82.58 ± 22.01%, 89.44 ± 24.02%, and 100.43 ± 7.27% at 25, 100, 500, and 1000 µg/mL, respectively. The dichloromethane fraction produced corresponding inhibition values of 83.87 ± 24.02%, 107.00 ± 3.43%, 74.45 ± 24.02%, and 99.36 ± 14.64%. These findings suggest that constituents with antimycobacterial activity were distributed across fractions of different polarities, although the consistently high responses of the dichloromethane and ethyl acetate fractions indicate that medium-polarity constituents may contribute substantially to the observed activity.

Table 1. Extraction and fraction yields of *Tabernaemontana divaricata*

Sample	Yield (%)	Mean ± SD (%)	CV (%)
Methanol extract	11.64	Not applicable	Not applicable
n-Hexane fraction	12.65, 10.00, 9.10	11.20 ± 1.94	17.29
Dichloromethane fraction	19.35, 13.90, 12.50	14.56 ± 2.41	16.56
Ethyl acetate fraction	12.55, 12.15, 8.75	9.98 ± 3.60	36.11

Values for solvent fractions are presented as the individual yields of three fractionation replicates and mean ± SD. CV, coefficient of variation.

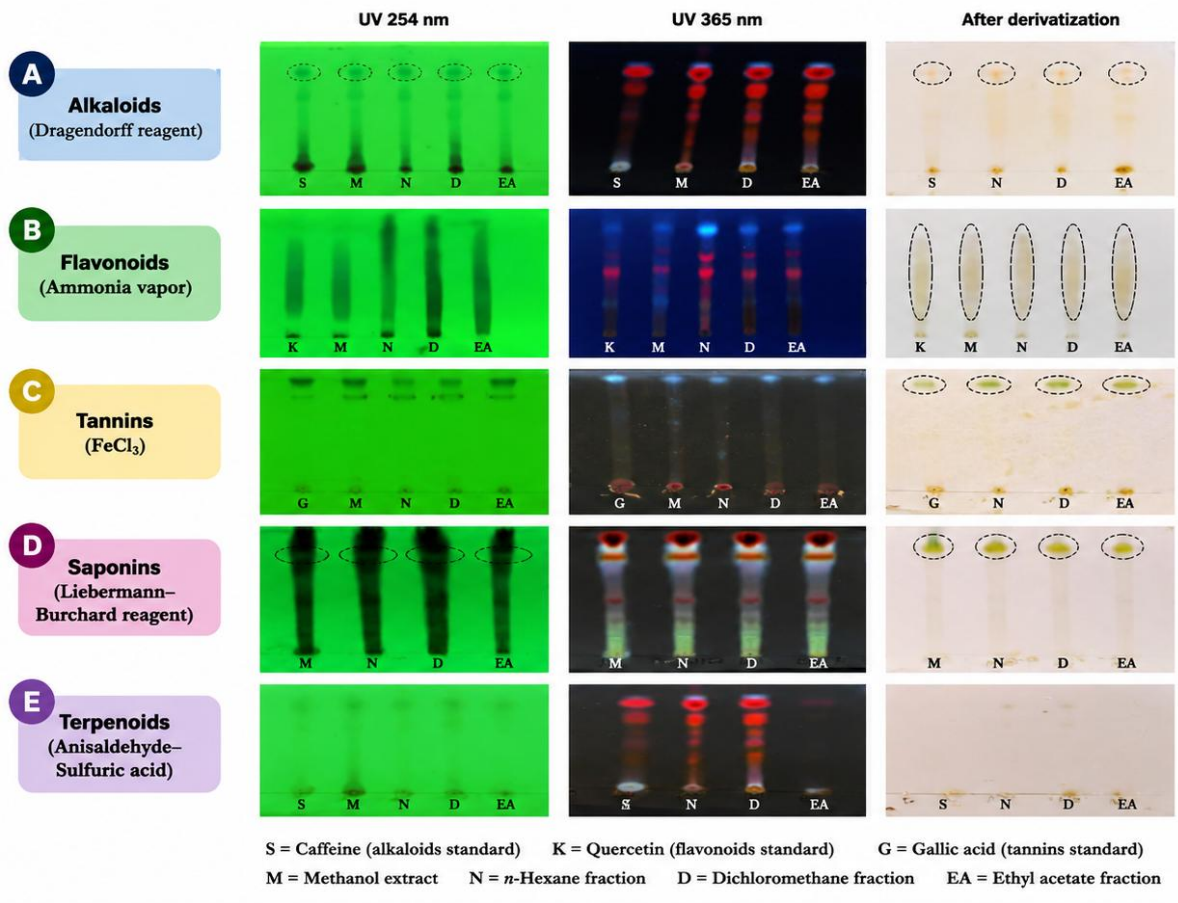

Figure 2. TLC-based phytochemical profiles of the methanol extract and solvent fractions of *T. divaricata*. The chromatograms represent screening for (A) alkaloids, (B) flavonoids, (C) tannins, (D) saponins, and (E) terpenoids under UV light at 254 and 365 nm and after derivatization with the corresponding detection reagents. M, methanol extract; NH, n-hexane fraction; DCM, dichloromethane fraction; EA, ethyl acetate fraction.

Table 2. TLC-based phytochemical profile of the methanol extract and solvent fractions of *T. divaricata*

Sample	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids
Methanol extract	+	+	+	+	−
n-Hexane fraction	+	+	+	+	−
Dichloromethane fraction	+	+	+	+	−
Ethyl acetate fraction	+	+	+	+	−

Note: (+), detected; (−), not detected under the applied TLC conditions.

As shown in **Figure 3**, the concentration-response patterns were not strictly monotonic for all samples. The dichloromethane fraction showed greater inhibition at 100 µg/mL than at 500 µg/mL, while the n-hexane fraction exhibited higher inhibition at 25 µg/mL than at 100 µg/mL. The methanol extract also showed a decrease in mean inhibition between 100 and 500 µg/mL. These fluctuations indicate that the responses cannot be adequately described by a conventional sigmoidal dose-response curve. The variation may reflect the complexity of crude extracts and fractions, in which multiple compounds can interact through synergistic, antagonistic, or concentration-dependent effects. However, the cited studies by Carvalho et al. (2011) and Franzblau et al. (1998) primarily describe antimycobacterial screening methods rather than directly demonstrating such phytochemical interactions. Therefore, this explanation should be regarded as a possible interpretation rather than a confirmed mechanism.

Several calculated inhibition values exceeded 100%, including those observed for selected fractions and the isoniazid control. These values occurred because the corrected absorbance of some treated wells was lower than the mean blank absorbance. They should therefore be interpreted as analytical artifacts arising from the blank-correction procedure rather than as biological inhibition greater than 100%. The 100% reference line in **Figure 3** is included to indicate the theoretical limit of complete inhibition and to highlight values affected by this correction artifact. Consequently, the current findings are best interpreted as qualitative to semi-quantitative screening results rather than definitive measurements of antimycobacterial potency.

Because the assay included only four concentrations and two technical replicates, the dataset did not provide sufficient resolution to establish a robust concentration-response model or determine reliable IC_{50} values. Most fractions already produced more than 50% inhibition at the lowest tested concentration, while their responses did not increase consistently with concentration. Estimating IC_{50} values under these conditions would require extrapolation beyond the experimentally tested range and could produce unstable or misleading estimates. Therefore, the present results should be positioned as preliminary antimycobacterial screening that identifies the dichloromethane and ethyl acetate fractions as candidates for further investigation. Future experiments should include concentrations below 25 µg/mL, sample-specific blanks, and at least three independent biological replicates to support validated nonlinear concentration-response analysis (Franzblau et al., 1998; Palomino & Portaels, 1999).

3.4. Relationship Between Phytochemical Profile and Antimycobacterial Activity

The antimycobacterial activity observed in the methanol extract and solvent fractions may be associated with the presence of alkaloids, flavonoids, tannins, and saponins detected by TLC. However, because the phytochemical analysis was qualitative, the contribution of each compound class to the observed inhibition could not be determined directly. Alkaloids are particularly relevant because species of *Tabernaemontana* are known to contain structurally diverse indole alkaloids with antimicrobial potential. Flavonoids and tannins may also contribute through interactions with microbial proteins, enzymes, and membrane-associated targets, whereas saponins may affect membrane permeability. These possible mechanisms remain interpretative because no isolated compounds or target-specific assays were evaluated in the present study. Previous research on *T. coronaria* demonstrated that plant extracts could inhibit *M. tuberculosis*H37Rv and alter mycobacterial growth kinetics and cellular integrity, supporting the potential of the genus as a source of antimycobacterial constituents (Mohamad et al., 2018). Shanmugapriya et al. (2021) also reported antibacterial activity in *T. divaricata* extracts containing several phytochemical classes, although their study involved non-mycobacterial ocular pathogens and therefore provides only indirect support for the present findings. The relatively high inhibition shown by the dichloromethane and ethyl acetate fractions suggests that medium-polarity compounds may contribute substantially to the activity. Nevertheless, the current data do not support assigning the effect to a specific compound class or claiming that either fraction is definitively more potent. Bioassay-guided fractionation, chromatographic isolation, and structural identification are required to determine the active constituents and confirm their antimycobacterial mechanisms.

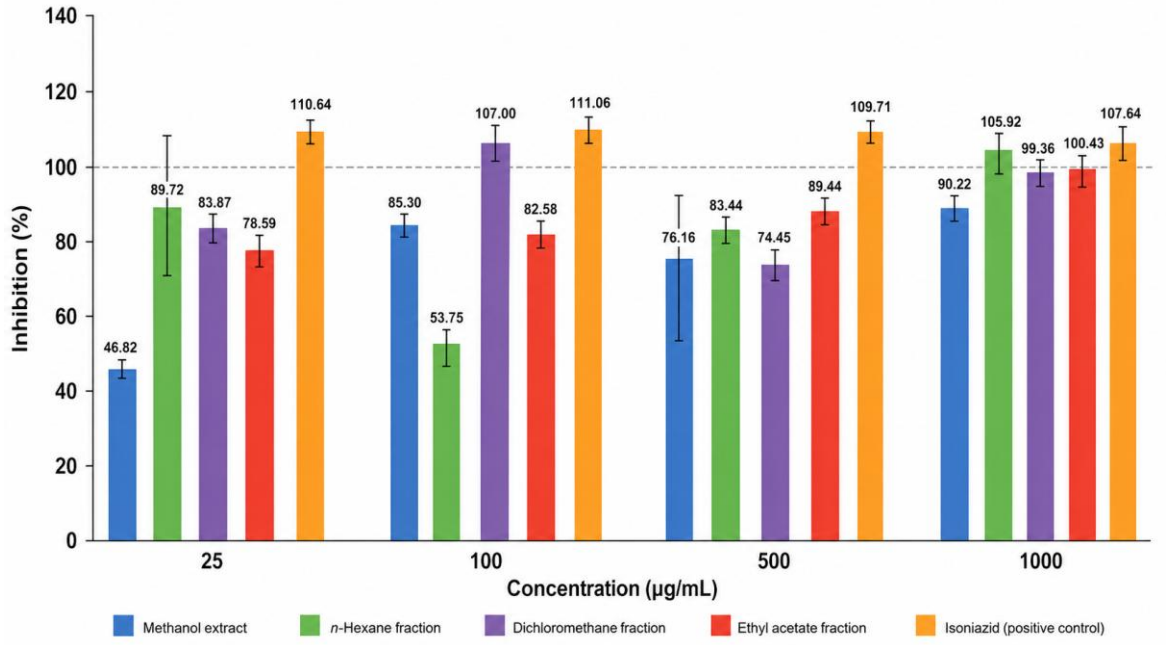

Figure 3. Antimycobacterial activity of the methanol extract and solvent fractions of *T. divaricata* against *M. tuberculosis* H37Rv. Percentage inhibition was determined at concentrations of 25, 100, 500, and 1000 µg/mL using the resazurin microplate assay. Values are presented as mean ± SD from two technical replicates. Isoniazid was used as the positive control. The dashed horizontal line indicates 100% inhibition. Values above 100% resulted from corrected sample absorbance values below the mean blank absorbance and should not be interpreted as biological inhibition exceeding 100%.

3.5. Study Implications and Limitations

The present findings provide preliminary evidence that *T. divaricata* contains constituents capable of inhibiting *M. tuberculosis* H37Rv in vitro. The relatively high inhibition observed in the dichloromethane and ethyl acetate fractions supports their selection for further bioassay-guided fractionation and chemical characterization. This step is important because qualitative TLC screening only confirms the presence of broad phytochemical classes and does not identify the specific compounds responsible for the activity. Further work should therefore combine chromatographic separation with spectroscopic identification and repeated antimycobacterial testing of isolated subfractions or compounds. Similar approaches have been used to connect phytochemical composition with antimycobacterial effects in other medicinal plants and *Tabernaemontana* species (Mohamad et al., 2018; Pavan et al., 2009). However, several limitations must be considered. The assay used only four concentrations and two technical replicates, which limited the precision of the estimates and prevented reliable statistical comparison or valid IC_{50} determination. The non-monotonic concentration-response patterns and several corrected inhibition values above 100% also indicate possible analytical interference, particularly from sample color, precipitation, or inadequate sample-specific blank correction. In addition, the present study evaluated only one laboratory strain and did not include clinical or drug-resistant *M. tuberculosis* isolates. Therefore, the findings should be interpreted as preliminary screening results rather than evidence of therapeutic efficacy. Future studies should use a wider concentration range, at least three independent biological replicates, sample-specific blanks, validated nonlinear dose-response analysis, and confirmatory testing against drug-sensitive and resistant strains before stronger conclusions regarding potency or lead-compound potential are made.

4. CONCLUSION

This study provides preliminary evidence that *T. divaricata* is a promising source of antimycobacterial constituents. The methanol extract and all solvent fractions inhibited the growth of *M. tuberculosis* H37Rv, with the dichloromethane and ethyl acetate fractions showing consistently high activity across the tested concentrations. TLC analysis also confirmed the presence of alkaloids, flavonoids, tannins, and saponins, supporting the chemical diversity of the active fractions. Although the current data did not support reliable IC_{50} estimation, the findings establish a clear basis for further bioassay-guided fractionation, compound isolation, and confirmatory testing using broader concentration ranges and independent biological replicates. These results are useful for researchers working on medicinal plants, natural-product-based antimycobacterial agents, and early-stage tuberculosis drug discovery because they identify priority fractions for deeper chemical and pharmacological investigation.

AUTHOR CONTRIBUTIONS

Conceptualization, A.R.P., and I.P.S.; methodology, A.R.P., I.P.S., U.E., and A.D.S.P.; validation, I.P.S., U.E., and A.D.S.P.; formal analysis, A.R.P. and I.P.S.; investigation, A.R.P. and A.D.S.P.; resources, I.P.S. and A.D.S.P.; data curation, A.R.P.; writing, original draft preparation, A.R.P.; writing, review and editing, A.R.P., I.P.S., and A.D.S.P.; visualization, A.R.P.; supervision, U.E., and A.D.S.P.; project administration, U.E.; funding acquisition, A.R.P. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study involved plant materials and an established laboratory strain of *Mycobacterium tuberculosis*and did not involve human participants or experimental animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The raw absorbance data, phytochemical screening records, and supporting calculations generated in this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funder had no role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the revision of this manuscript, the authors used ChatGPT (OpenAI) to assist with improving the clarity, organization, and readability of the text. The authors subsequently reviewed, edited, and verified the entire manuscript to ensure that it accurately represented the original study data, methods, interpretations, and conclusions. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

REFERENCES

Carvalho, R., de Sonneville, J., Stockhammer, O. W., Savage, N. D. L., Veneman, W. J., Ottenhoff, T. H. M., Dirks, R. P., Meijer, A. H., & Spaink, H. P. (2011). A high-throughput screen for

tuberculosis progression. *PLOS ONE, 6*(2), e16789. https://doi.org/10.1371/journal.pone.0016789

Franzblau, S. G., Witzig, R. S., McLaughlin, J. C., Torres, P., Madico, G., Hernandez, A., Degnan, M. T., Cook, M. B., Quenzer, V. K., Ferguson, R. M., & Gilman, R. H. (1998). Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. *Journal of Clinical Microbiology, 36*(2), 362–366. https://doi.org/10.1128/JCM.36.2.362-366.1998

Jain, S., Sharma, P., Ghule, S., Jain, A., & Jain, N. (2013). In vivo anti-inflammatory activity of *Tabernaemontana divaricata* leaf extract on male albino mice. *Chinese Journal of Natural Medicines, 11*(5), 472–476. https://doi.org/10.1016/S1875-5364(13)60086-2

Ministry of Health of the Republic of Indonesia. (2020). *Pedoman nasional pelayanan kedokteran tata laksana tuberkulosis*. https://repository.kemkes.go.id/book/124

Mohamad, S., Ismail, N. N., Parumasivam, T., Ibrahim, P., Osman, H., & Wahab, H. A. (2018). Antituberculosis activity, phytochemical identification of *Costus speciosus* (J. Koenig) Sm., *Cymbopogon citratus* (DC. ex Nees) Stapf., and *Tabernaemontana coronaria* (L.) Willd. and their effects on the growth kinetics and cellular integrity of *Mycobacterium tuberculosis* H37Rv. *BMC Complementary and Alternative Medicine, 18*, Article 5. https://doi.org/10.1186/s12906-017-2077-5

Mursito, B. (2003). *Ramuan tradisional untuk pelangsing tubuh*. Penebar Swadaya.

Palomino, J. C., & Portaels, F. (1999). Simple procedure for drug susceptibility testing of *Mycobacterium tuberculosis* using a commercial colorimetric assay. *European Journal of Clinical Microbiology & Infectious Diseases, 18*(5), 380–383. https://doi.org/10.1007/s100960050298

Pavan, F. R., Leite, C. Q. F., Coelho, R. G., Coutinho, I. D., Honda, N. K., Cardoso, C. A. L., Vilegas, W., Andrade Leite, S. R., & Sato, D. N. (2009). Evaluation of anti-*Mycobacterium tuberculosis* activity of *Campomanesia adamantium* (Myrtaceae). *Química Nova, 32*(5), 1222–1226. https://doi.org/10.1590/S0100-40422009000500026

Pratchayasakul, W., Pongchaidecha, A., Chattipakorn, N., & Chattipakorn, S. (2008). Ethnobotany and ethnopharmacology of *Tabernaemontana divaricata*. *Indian Journal of Medical Research, 127*(4), 317–335. https://pubmed.ncbi.nlm.nih.gov/18577786/

Rathaur, B., Ali, M., Kumar, S., & Nishad, U. (2020). Phytochemical analysis of *Tabernaemontana divaricata*. *Journal of Pharmacognosy and Phytochemistry, 9*(2), 1283–1291. https://www.phytojournal.com/archives/view-pdf/11029/9-2-23

Ratnawati, G., & Supriyati, N. (2019). Skrining aktivitas antituberkulosis tumbuhan obat Riset Tumbuhan Obat dan Jamu 2012. *Jurnal Jamu Indonesia, 4*(2), 69–75. https://doi.org/10.29244/jji.v4i2.161

Shanmugapriya, R., Babu, S., Ambikapathy, V., Panneerselvam, A., Prakash, P., & Kanmani. (2021). Phytochemical and antibacterial properties of *Tabernaemontana divaricata* L. against ocular infection. *International Journal of Botany Studies, 6*(6), 754–758. https://www.botanyjournals.com/assets/archives/2021/vol6issue6/6-6-68-534.pdf

World Health Organization. (2025). *Global tuberculosis report 2025*. https://www.who.int/publications/i/item/9789240116924

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