e-ISSN: 3030-878X

The 4th International Conference of Environmental Health (ICOEH)

Bidara Upas (Merremia Mammosa) as a Traditional Medicine for Tuberculosis

Khambali[1*], Rachmaniyah[1], Fitri Rokhmalia[1], Rosidi Roslan[2]
[1]Department of Environmental Health, Poltekkes Kemenkes Surabaya, Indonesia
[2]Surabaya Health Quarantine Agency
Corresponding author: khambali007@poltekkesdepkes-sby.ac.id

ABSTRACT
Tuberculosis (TB) is a disease that affects the lungs caused by the bacterium *Mycobacterium tuberculosis*, which is acid-fast but will die upon direct exposure to sunlight. TB is the disease with the highest prevalence worldwide. In Indonesia, the eastern region has the highest prevalence, reaching 210 per 100,000 people. Despite the availability of Anti-Tuberculosis Drugs (ATDs), TB eradication efforts remain suboptimal. Bidara upas (*Merremia mammosa*), which contains bioactive compounds such as alkaloids, tannins, flavonoids, and polyphenols, has shown anti-inflammatory, anti-cancer, detoxifying, and analgesic properties. The aim of this study is to elucidate the mechanisms and the compounds present in Bidara upas extract that affect macrophage cells in mice injected with *Mycobacterium tuberculosis*. This is an experimental study. The research sample consisted of white mice injected with Mycobacterium tuberculosis and subsequently treated with bidara upas extract at concentrations of 50 ppm, 100 ppm, and 150 ppm. The results showed the minimum inhibitory concentration (MIC) of bidara upas extract at 100 mg/ml, supported by the Acid-Fast Bacilli (AFB) test using Ziehl-Neelsen staining and niacin testing. The 100 ppm bidara upas extract was relatively safe as it did not cause death in the mice, and there were no significant differences in liver and lung organs between the treated and control mice. Based on these results, further testing is recommended to investigate the fractionation and isolation of active compounds with anti-tuberculosis properties.

Keywords: *Merremia mammosa*, TB, Anti tuberculosis

INTRODUCTION
Tuberculosis, also known as TB, is a disease that can be transmitted through the air via droplets containing the bacterium *Mycobacterium tuberculosis*. The primary organ affected by this bacterium is the lungs. *Mycobacterium tuberculosis* is distinctive in that it is acid-fast during staining, which is why it is referred to as BTA (Bacillus Acid-Fast). This bacterium can easily die upon direct exposure to sunlight but can survive for several hours in dark and humid environments. Generally, this bacterium can exhibit dormancy (having inactive body tissues) (Ravimohan *et al*., 2018). The WHO reports that there are 22 countries in the world with the highest prevalence of TB. Among them, 10 countries are located in Asia, with Indonesia ranked third based on the highest prevalence rates of TB (Aini & Hatta, 2017) (Aslam *et al*., 2018).

In Indonesia, the number of new TB cases reaches 539,000 with 101,000 deaths per year. The incidence of TB varies across Indonesia, with the highest cases found in the eastern region of the country, having a prevalence of 210 per 100,000 people. The high number of TB cases is estimated to be due to incomplete treatment of TB patients and non-compliance with medication, dosage, regimen, and administration. Non-compliance with medication can lead to resistance to anti-tuberculosis drugs (ATDs), including mono-resistant, poly-resistant, multi-drug resistant (MDR-TB),

extensively drug-resistant (XDR-TB), and total drug-resistant (Total DR) TB (Purwitasari & Agil, 2022), (de Souza *et al*., 2023).

In addition, other factors contribute to the increase in resistance, such as the side effects of medication consumption. All anti-tuberculosis drugs (ATDs) consumed by TB patients have the potential to cause side effects, which can range from severe to moderate and even mild. When patients experience side effects from ATDs, they may stop taking the medication unilaterally. This non-compliance can lead to higher prevalence rates of TB, as treatment typically requires 6 months. However, patients who develop resistance must use second-line ATDs, which are very expensive (Kaihena, 2013), (Wahyudi, 2022).

Based on this background, there is a need for alternative treatments for TB using natural ingredients. Such treatments have long been used by communities. Moreover, 75–80% of people do not receive clinical treatment for their ailments. Medicinal plants with anti-tuberculosis properties encourage researchers and pharmacists to explore and develop these treatments further (Xu et al., 2021). This highlights the importance of alternative natural medicines in improving public health. One such plant that can be used for alternative treatment is Bidara upas (*Merremia mammosa*) (Collin *et al*., 2018), (Dalimartha, 2009).

Merremia mammosa is a climbing plant that can grow to a length of 3 to 6 meters and has tubers clustered in the soil, weighing up to 5 kg. When consumed raw, it has a bitter, bland, and astringent taste. According to research, compounds such as polyphenols, flavonoids, and terpenoids in this plant exhibit antibacterial activity against *Mycobacterium tuberculosis* .(Lestari *et al*., 2023).

Based on the background described above, the objectives of this study are to demonstrate that Bidara upas extract can improve macrophage cells in the lungs infected with *Mycobacterium tuberculosis* and to establish whether increasing doses of Bidara upas extract have an effect on enhancing macrophage cells in the lungs infected with *Mycobacterium tuberculosis*.

RESEARCH METHOD

Preparation of Plant Material

The tubers of Merremia mammosa were sourced from Sumenep, Madura, Indonesia. The prepared materials were then washed (cleaned), sliced thinly, and dried without direct sunlight until they were completely dry. After drying, the tubers were ground into a fine powder, making them ready for extraction.

Figure 1. Drying of *Merremia mammosa*

Extraction

The dried and powdered material was then subjected to extraction three times for 2 minutes each using methanol.

Figure 2. Exstraction *Merremia mammosa*

Test Bacteria and Treatment of Experimental Animals

The bacteria used in this study were *Mycobacterium tuberculosis* from the laboratory, and the experimental animals were mice (*Mus musculus*). The mice were injected with *Mycobacterium tuberculosis* and Bidara upas (*Merremia mammosa*) extract, then housed in the same cage. Subsequently, their lungs and livers were collected for microscopic examination.

The treatment groups were divided into 5 groups, with each group consisting of 7 mice:

1. Group 1: Mice injected only with *Mycobacterium tuberculosis*.
2. Group 2: Mice injected with *Mycobacterium tuberculosis* and Bidara upas extract at 50 ppm.
3. Group 3: Mice injected with *Mycobacterium tuberculosis* and Bidara upas extract at 100 ppm.
4. Group 4: Mice injected with *Mycobacterium tuberculosis* and Bidara upas extract at 150 ppm.
5. Group 5: Control group not injected with *Mycobacterium tuberculosis.*

Figure 3. Injection in Test Animals

RESULT AND DISCUSSION

Bidara Upas (*Merremia mammosa) Extraction*

The Bidara upas (*Merremia mammosa*) tubers were obtained from Sumenep, Madura. The tubers were sorted, washed, and sliced thinly. They were then air-dried in a shaded area, away from direct sunlight. Once thoroughly dried, the tubers were ground into a powder. The powdered material was weighed and mixed with methanol for extraction. (Agil *et a*l., 2021).

Tab1e 1. Bidara Upas Extract Yield

Extraction	Weight of the Powder	Yield
Metanol	*10 gr*	*1.794,25 gr*

Test of *Mycobacterium tuberculosis* Growth

Based on the results of the extraction of Bidara upas tubers using methanol, the extract was found to inhibit the growth of Mycobacterium tuberculosis, with a minimum inhibitory concentration (MIC) of 100 ppm.

Table 2. Antibacterial test results from bidara upas extract

Material Test	Consentration mg/ml	Yield
Bidara upas methanol extract	*1500*	+
	1000	+
	500	+
	400	+
	300	+
	200	+
	100	+
	50	-
	25	-
Positivr control	*10*	+
Negative cotrol	-	-

Description :

1. Sign (+) : Inhibition of Mycobacterium tuberculosis growth
2. Sign (-) : No inhibition of Mycobacterium tuberculosis growth
3. Control (+) : Not injected with Mycobacterium tuberculosis
4. Control (-) : injected with Mycobacterium tuberculosis

Based on the table, it can be observed that the minimum inhibitory concentration (MIC) of Bidara upas extract using methanol is 100 mg/ml. Additionally, the Acid-Fast Bacilli (AFB) test showed negative results, indicating no growth of Mycobacterium tuberculosis at concentrations ranging from 1500 to 100 mg/ml. The AFB test, which indicates the presence of Mycobacterium tuberculosis, was marked by the appearance of red dots at concentrations of 50 to 25 mg/ml. This is consistent with the findings of the study (Agil et al., 2021) The Acid-Fast Bacilli (AFB) test showed red dots at a concentration of 25 mg/ml, indicating the presence of Mycobacterium tuberculosis.

Acute Toxicity Test Results

Test animals (*Mus musculus*) were administered methanol extract of Bidara upas at doses deemed safe. To determine the LD50, the number of mice that died within 24 hours after receiving the Bidara upas extract was observed. This observation helps in calculating the LD50, which indicates the dose required to cause death in 50% of the test animals. The results provide important information on the safety and toxicity of the Bidara upas extract (Ayun et al., 2021). Results of the acute toxicity test using bidara upas extract on mice:

Table 3. Results of the Acute Toxicity Test of Bidara Upas (*Merremia mammosa*) Extract on Mice

Group	Number of Mice	
	Survived	Died
1	7	0
2	7	0
3	7	0
4	7	0
5	7	0

Description :

1. Group 1: Mice injected only with *Mycobacterium tuberculosis*.
2. Group 2: Mice injected with *Mycobacterium tuberculosis* and Bidara upas extract at 50 ppm.
3. Group 3: Mice injected with *Mycobacterium tuberculosis* and Bidara upas extract at 100 ppm.
4. Group 4: Mice injected with *Mycobacterium tuberculosis* and Bidara upas extract at 150 ppm.
5. Group 5: Control group not injected with *Mycobacterium tuberculosis.*

Microscopic examination of the organs, such as the lungs of mice, showed differences between those injected with *Mycobacterium tuberculosis* alone and those injected with both *Mycobacterium tuberculosis* and Bidara upas extract. The liver of mice injected with *Mycobacterium tuberculosis* alone appeared hollow with widespread white discoloration extending into the liver. In contrast, the liver of mice injected with both *Mycobacterium tuberculosis* and Bidara upas extract (100–150 ppm) appeared red with only a few white spots and no hollow areas.

The study on the inhibitory effect of Bidara upas (Merremia mammosa) extract on the growth of *Mycobacterium tuberculosis* confirms that this tuber has been traditionally used in Indonesia, particularly in Madura. The methanol extract of Bidara upas was prepared by infusing 15 grams of the powdered tuber and drying it by air-drying. The testing aimed to validate that the Bidara upas tuber can act as an inhibitor of *Mycobacterium tuberculosis* growth. The research results indicate that the Bidara upas extract has a Minimum Inhibitory Concentration (MIC) of 100 mg/ml, which effectively inhibits the growth of *Mycobacterium tuberculosis* (Bains et al., 2020).

Previous research has demonstrated that antibacterial activity against *Mycobacterium tuberculosis* is associated with several compounds, including polyphenols, terpenoids, and flavonoids. This is consistent with findings from (Huang et al., 1980) ho reported that benzenoid polyphenols exhibit antibacterial activity against *Mycobacterium tuberculosis*. Polyphenols are metabolic

compounds produced by higher plants and have multiple functions for humans. Research indicates that polyphenols provide antibacterial activity against pathogens, reinforcing their potential as effective components in natural antibacterial treatments (Bae et al., 2022). Additionally (Thongtan et al., 2003) have explained that triterpenes also possess the ability to inhibit the growth of *Mycobacterium tuberculosis*.

CONCLUSION AND RECOMMENDATION

Based on the conducted tests, the extract of Bidara upas (Merremia mammosa) is capable of inhibiting the growth of Mycobacterium tuberculosis with a Minimum Inhibitory Concentration (MIC) of 100 mg/ml. The results of the acute toxicity test indicate that the Bidara upas extract is categorized as non-toxic.

Further research is needed to eventually isolate compounds that can be utilized as anti-tuberculosis agents, potentially serving as alternative treatments for tuberculosis.

REFERENCES

Agil, M., Studiawan, H., & Purwitasari, N. (2021). Efficacy of Merremia mammosa Hall Terpenoid Fraction againts Mycobacterium tuberculosis. *Research Journal of Pharmacy and Technology*, *14*(12), 6617–6620.

Aini, N., & Hatta, H. R. (2017). *Sistem Pakar Pendiagnosa Penyakit Tuberkulosis*.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., & Qamar, M. U. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, 1645–1658.

Ayun, A. Q., Faridah, D. N., Yuliana, N. D., & Andriyanto, A. (2021). Pengujian toksisitas akut LD50 infusa benalu teh (Scurrula sp.) dengan menggunakan mencit (Mus musculus). *Acta Veterinaria Indonesiana*, *9*(1), 53–63.

Bae, J.-Y., Seo, Y.-H., & Oh, S.-W. (2022). Antibacterial activities of polyphenols against foodborne pathogens and their application as antibacterial agents. *Food Science and Biotechnology*, *31*(8), 985–997.

Bains, S., Kaur, R., & Sethi, M. (2020). Phytochemical analysis of medicinal plants and their antimicrobial activity. *Agricultural Research Journal*, *57*(3), 444–448.

Collin, S. M., De Vries, G., Lönnroth, K., Migliori, G. B., Abubakar, I., Anderson, S. R., & Zenner, D. (2018). Tuberculosis in the European Union and European Economic Area: a survey of national tuberculosis programmes. *European Respiratory Journal*, *52*(6).

Dalimartha, S. (2009). *Atlas Tumbuhan Obat Indonesia Jilid 6.* Puspa Swara.

de Souza, R. A., Barreto, F. R., Santos, C. A. de S. T., Nery, J. S., de Jesus Lima, C. C. O., Castelo, I. S., Natividade, M., de Andrade, J. A. F., Barreto, M. L., & Pereira, S. M. (2023). Evolution of the Bacillus Calmette-Guérin scar Russia and Moreau strains in newborns: A brazilian cohort. *Vaccine: X*, *14*, 100323.

Huang, P. H., Chen, W. S., & Hu, Y. (1980). Studies on antituberculosis constituents from Ardisia japonica. *Yaoxue Tongbao*, *15*, 39.

Kaihena, M. (2013). Propolis sebagai imunostimultor terhadap infeksi Micobacterium tuberculosis. *Prosiding FMIPA Universitas Pattimura*, 69–78.

Lestari, T., Fuady, A., Yani, F. F., Putra, I. W. G. A. E., Pradipta, I. S., Chaidir, L., Handayani, D., Fitriangga, A., Loprang, M. R., & Pambudi, I. (2023). The development of the national tuberculosis research priority in Indonesia: A comprehensive mixed-method approach. *PloS One*, *18*(2), e0281591.

Purwitasari, N., & Agil, M. (2022).

Metabolite profiling of extract and fractions of bidara upas (Merremia mammosa (Lour.) Hallier F.) tuber using UPLC-QToF-MS/MS. *Biomedical and Pharmacology Journal*, *15*(4), 2025–2041.

Ravimohan, S., Kornfeld, H., Weissman, D., & Bisson, G. P. (2018). Tuberculosis and lung damage: from epidemiology to pathophysiology. *European Respiratory Review*, *27*(147).

Thongtan, J., Kittakoop, P., Ruangrungsi, N., Saenboonrueng, J., & Thebtaranonth, Y. (2003). New Antimycobacterial and Antimalarial 8, 9-Secokaurane Diterpenes from Croton k ongensis. *Journal of Natural Products*, *66*(6), 868–870.

Wahyudi, D. (2022). *Hubungan Kadar Interleukin 6 Terhadap Konversi Sputum Pada Pengobatan Tuberkulosis Paru= Relation Of Interleukin 6 Levels With Sputum Conversion In The Pulmonary Tuberculosis Treatment*. Universitas Hasanuddin.

Xu, Y., Liang, B., Kong, C., & Sun, Z. (2021). Traditional medicinal plants as a source of antituberculosis drugs: a system review. *BioMed Research International*, *2021*(1), 9910365.